

Edited by
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Encyclopedia of Human Nutrition

Third Edition



ENCYCLOPEDIA OF HUMAN NUTRITION

THIRD EDITION

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THIRD EDITION

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VOLUME 1



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Dr. Caballero is Professor of International Health at the Bloomberg School of Public Health and Professor of Pediatrics at the School of Medicine, Johns Hopkins University, Baltimore, MD, USA. He has over 20 years of experience as a scholar, researcher, and leader in the area of child health and nutrition. He obtained his MD from the University of Buenos Aires, Argentina and his PhD (in neuroendocrine regulation) from MIT, Cambridge, MA, USA. He started his faculty career at Harvard Medical School, and moved to Johns Hopkins in 1990 to found the Center for Human Nutrition.

Dr. Caballero is a recognized expert on the nutritional needs of children and adults, and on nutrient requirements in undernourished populations. For the past 10 years, he has focused on the problem of childhood obesity in the US and in developing countries, and explored the impact of dietary transition and globalization on health indicators. He is an active participant in key scientific committees advising the US government on issues of diet and health, including the Dietary Reference Intakes (DRI) Committee; the Expert Panel on Macronutrient Requirements; and the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences. He was a member of the Dietary Guidelines for Americans Advisory Committee, and is currently a member of the Scientific Advisory Board of the Food and Drug Administration (FDA) and of the International Life Sciences Institute (ILSI).

Dr. Caballero is an active leader in the area of global health, specifically on diet, lifestyle, and disease risk. He is Chairman of the Board of the Pan American Health and Education Foundation, in Washington, DC, USA and member of the Board of Directors of the International Nutrition Foundation, Boston, MA, USA. Recent awards include the Ancel Keys Prize for achievements in international public health and the Thompson–Beaudette Lectureship from Rutgers University. In 2011, he was named to the Spanish Academy of Nutritional Sciences.

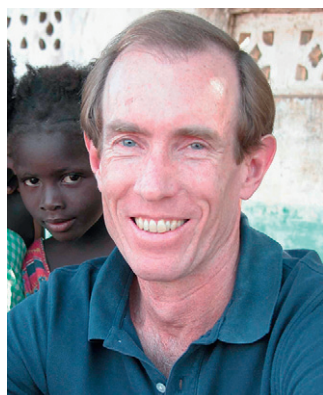
Dr. Caballero is the Editor-in-Chief of the *Encyclopedia of Food Sciences and Nutrition*, a 10-volume work on food production, consumption and biological effects. He is also Editor-in-Chief of the *Encyclopedia of Human Nutrition*, which received the Book of the Year Award from the British Medical Association. His *Guide to Dietary Supplements* summarizes the current scientific basis for the use of mineral and vitamin supplements. His book *The Nutrition Transition: Diet and Disease in the Developing World* explored the impact of demographic and economic development on diet- and lifestyle-related diseases in developing countries. His book *Obesity in China* summarizes research conducted in rural and urban China to track the impact of socioeconomic development on health outcomes. He is also co-editor of the most widely used textbook in human nutrition, *Modern Nutrition in Health and Disease*.

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Andrew Prentice studied biochemistry before a PhD in Nutritional Science at Darwin College, University of Cambridge. Animal and laboratory work for his thesis on 'The Biochemical Effects of Riboflavin Deficiency' was conducted at the Dunn Nutrition Unit. His early post-graduate studies were conducted in The Gambia with a focus on malnutrition in mothers and babies. He subsequently headed the Regulation of Human Energy Metabolism Group at the Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Cambridge, UK, conducting detailed metabolic studies using whole-body calorimetry and stable isotopic tracer methods. In 1999, Professor Prentice established the MRC International Nutrition Group at the London School of Hygiene & Tropical Medicine inheriting the permanent field laboratories at MRC Keneba in rural Gambia. His team now researches key areas of diet-disease interactions in The Gambia, Tanzania, and Kenya, with the ultimate aim of improving nutritional interventions in poor populations.

Prof Prentice has received the 1989 Peter Debye Science Prize, the 1998 Gunnar-Levin Nutrition Medal from the Swedish Medical Association, the 1999 BNF Science Prize, the 1999 FENS Medal from the Federation of European Nutrition Societies, the 2001 Kellogg's International Nutrition Prize, the 2003 Edna and Robert Langholz International Nutrition Award, the 2006 Lucille Hurley Distinguished Lecturer Award, the 2010/11 EV McCollum International Nutrition Lectureship from the American Society of Nutrition, and the 2011 George G Graham Lectureship from Johns' Hopkins University. He has been a member of numerous national and international advisory bodies and held senior leadership positions in both the UK and American Societies for Nutrition.

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PREFACE

By the turn of the twentieth century, nutrition science had completed a slow but remarkable historical transition, from a discipline focused on preventing nutrient deficiencies (and hence focused on identifying minimum nutrient needs) to one aimed at reducing disease risk and optimizing health, seeking to define an elusive optimal diet. But progress in our knowledge has not yet caught-up with that transition in focus, and our understanding of how diet constituents affect long-term disease risks is still not on a par with our knowledge of essential nutrients, their metabolism, and required intake levels. One reason is that the experiments needed to unravel diet–health interrelationships are more complex, costly, and in some cases unfeasible, compared with the classical studies that identified vitamins and other essential nutrients. Another reason is that, although the discovery of essential nutrients was based on a strong, unifying scientific paradigm (the concept of a compound essential for human life but which humans are unable to make), there is no single or unifying paradigm from which to explore diet–health relationships. In addition, our ability to timely process and integrate scientific discoveries is now continuously challenged by the massive volume of information of the digital era.

In that context, the need to provide accurate, succinct, and up-to-date information on a wide range of topics is more important than ever, and is the aim of this Encyclopedia. Currently, nutrition research and practice is fundamentally a multidisciplinary endeavor, so we aim to offer scientific information to a wide audience of researchers and professionals. In addition, the information revolution of the internet has

changed the consumer from a passive recipient of advice to an active participant in decisions involving health and related issues. Thus, although this work is not specifically targeted to the general public, we hope that the educated readers with a minimum scientific background should also be able to obtain from this book useful (and reliable) information on their topic of interest.

This third edition builds on the success of the previous one. We have included new articles or made extensive updates when needed, while keeping the proven core of established knowledge. The comprehensive index and extensive cross-referencing will allow readers to quickly identify specific topics, and to move deeper into related areas if desired.

We have a great debt of gratitude to the hundreds of authors who contributed to the large body of knowledge represented here. In turn, authors benefited from the valuable feedback of our distinguished Editorial Advisory Board. Of course, as editors we are ultimately responsible for the content, particularly for any errors. Finally, both the print and electronic version have the unmistakable production quality of the Major Reference Works division of Elsevier, and this is the result of the unrelenting enthusiasm and hard work of our editorial team.

We hope this work will be a valuable addition to the knowledge base of any person interested in the critical area of nutrition, diet, and human health.

Benjamin Caballero
Editor-in-Chief

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GUIDE TO USE OF THE ENCYCLOPEDIA

Structure of the Encyclopedia

The Encyclopedia is arranged as a series of entries in alphabetical order. Some entries comprise a single article, whilst entries on more diverse subjects consist of several articles that deal with various aspects of the topic. In the latter case the articles are arranged in a logical sequence within an entry.

To help you realize the full potential of the material in the Encyclopedia we have provided three features to help you find the topic of your choice.

Contents Lists

Your first point of reference will probably be the contents list. The complete contents list appearing in each volume will provide you with the volume number and page number of the entry. On the opening page of an entry a mini-contents list is provided so that the full details of the articles within the entry are immediately available.

Alternatively you may choose to browse through a volume using the alphabetical order of the encyclopedia as your guide. To assist you in identifying your location within the Encyclopedia a running headline indicates the current entry and article within that entry. Please see an example below:

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Cross References

All of the articles in the Encyclopedia have been cross referenced. The cross references, appear at the end the articles and they link together related articles.

Example

The following list of cross references appear at the end of the entry entitled Nutritional Assessment: Clinical examination.

See also: Dietary Intake Measurement: Methodology. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Nutritional Assessment: Anthropometry; Biochemical Indices

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A

ADIPOSE TISSUE

Structure, Function and Metabolism

G Frühbeck and J Gómez-Ambrosi, Instituto de Salud Carlos III, Universidad de Navarra, Pamplona, Spain

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Abbreviations

ALBP/FABP4/aP2	Adipocyte fatty acid binding protein	PGAR/FAF	Peroxisome proliferator-activated receptor angiopoietin related protein/ fasting-induced adipose factor
BAT	Brown adipose tissue	PGC-1 α	Peroxisome proliferator-activated receptor- γ coactivator-1 α
BMP	Bone morphogenetic protein	PPAR	Peroxisome proliferator-activated receptor
C/EBPs	CCAAT/enhancer binding proteins	PRDM16	Positive regulatory domain containing 16
CETP	Cholesteryl ester transfer protein	Pref-1	Preadipocyte factor-1
CRP	C-reactive protein	RBP4	Retinol binding protein-4
HSL	Hormone-sensitive lipase	UCP	Uncoupling protein
IL	Interleukin	WAT	White adipose tissue
LPL	Lipoprotein lipase		
MCP-1	Monocyte chemoattractant protein-1		

Glossary

Adipogenesis Development or formation of fat.

Angiogenesis Formation of new blood vessels by branching morphogenesis.

Autocrine A secreted substance which acts on surface receptors of the same cell.

Catecholamines Any of various amines (e.g., epinephrine, norepinephrine, and dopamine) that contain a dihydroxy benzene ring, are derived from tyrosine and operate as hormones and/or neurotransmitters.

Fatty-acid-binding proteins (FABPs) Family of carrier proteins for fatty acids and other lipophilic substances such as eicosanoids and retinoids. These proteins are thought to facilitate the transfer of fatty acids between extra- and intracellular membranes. Some family members are also believed to transport lipophilic molecules from the plasma membrane to certain intracellular receptors such as PPAR.

Lipogenesis Normal deposition of fat or the conversion of carbohydrate or protein to fat.

Lipolysis Triacylglycerol breakdown to yield fatty acids and glycerol.

Paracrine Mode of hormone action in which a hormone binds to receptors on and affects the function of cells near to the cell that produced it.

Peroxisome proliferator-activated receptors (PPARs) Group of nuclear receptor proteins that function as transcription factors regulating the expression of genes. PPARs play essential roles in the regulation of cellular differentiation, development, and metabolism (carbohydrate, lipid and protein) of higher organisms.

Pleiotropic Producing many effects; multiple effects from a single gene; the control by a single gene of several distinct and seemingly unrelated phenotypic effects.

Introduction

The role of white adipose tissue (WAT) in storing and releasing lipids for oxidation by skeletal muscle and other tissues became so firmly established decades ago that a persistent lack of interest hindered the study of the extraordinarily dynamic behavior of adipocytes. However, disentangling the neuroendocrine systems, which regulate energy homeostasis and adiposity has jumped to a first-priority challenge, with the recognition of obesity as one of the major public health problems. Strictly speaking, obesity is not defined as an excess of body weight but as an increased adipose tissue accretion, to the extent that health may be adversely affected. Therefore, in the last decades, adipose tissue has become the research focus of biomedical scientists for epidemiological, pathophysiological, and molecular reasons. Although the primary role of adipocytes is to store triglycerides during periods of caloric excess and to mobilize this reserve when expenditure exceeds intake, it is now widely recognized that adipose tissue lies at the heart of a complex network participating in the regulation of a variety of quite diverse biological functions (Figure 1).

Development

During fetal development WAT emerges at midgestation in humans or postnatally in rodents. Although the evolutionary and developmental features of WAT and brown adipose tissue (BAT) already suggested that they are quite distinct tissues, until recently, white and brown adipocytes were thought to be derived from the same precursor cell. Elegant *in vivo* fate mapping experiments in mice have recently provided a clear evidence that brown adipocytes arise from a separate and distinct population of progenitors (Figure 2). Brown fat cells are now known to exhibit a 'myogenic' signature and share a

common mesenchymal origin with skeletal muscle. In brief, mesenchymal stem cells can enter several cell lineages which culminate in the formation of bone, muscle, and adipose tissue, among others. The precursor cells destined to become white adipocytes first differentiate into adipoblasts and then preadipocytes through carefully-timed exposure to key regulators. Two members of the family of bone morphogenetic proteins (BMP), specifically BMP2 and BMP4, as well as PPAR γ and C/EBPs are pivotal to drive these different phases. *Myf5*-expressing precursors give rise to skeletal muscle and brown adipose tissue, but not white adipose tissue (Figure 2). BMP7 singularly drives the brown fat cell fate in both mesenchymal progenitor cells and committed brown preadipocytes suppressing early adipogenic inhibitors, such as *neccin*, *Pref-1*, and *WNTs*, at the same time inducing the key molecular determinant positive regulatory domain containing 16 (PRDM16) that triggers the activation of the complete brown adipogenesis program and blocks the induction of myotube-specific genes such as *Myf5*, *MyoD* and *myogenin*. PRDM16 binds and coactivates PPAR γ with subsequent induction of key features to specify a brown fat fate, i.e., increased mitochondrial biogenesis and expression of UCP1, among others.

Pericytes, the cells that surround the endothelium tubes of the microvasculature, have been also recently identified as progenitor cells that become committed to the white adipocyte lineage either prenatally or in the early postnatal period. Adipose tissue has long been recognized to expand in conjunction with its vasculature, but these new findings suggest that the blood vessels may actively direct the process. Thus, as well as serving as a progenitor niche, blood vessels may also produce signals for adipocyte development.

The determination phase results in the conversion of the stem cell to a preadipocyte, which still shares some morphological features with its precursor cell but has lost the potential to differentiate into other cell types (Figure 3). In the

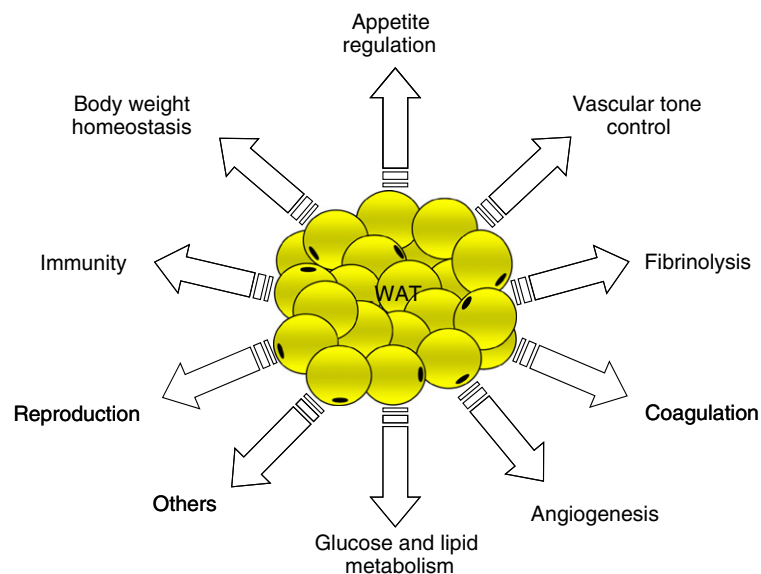


Figure 1 Dynamic view of white adipose tissue based on the pleiotropic effects on quite diverse physiological functions. WAT, white adipose tissue.

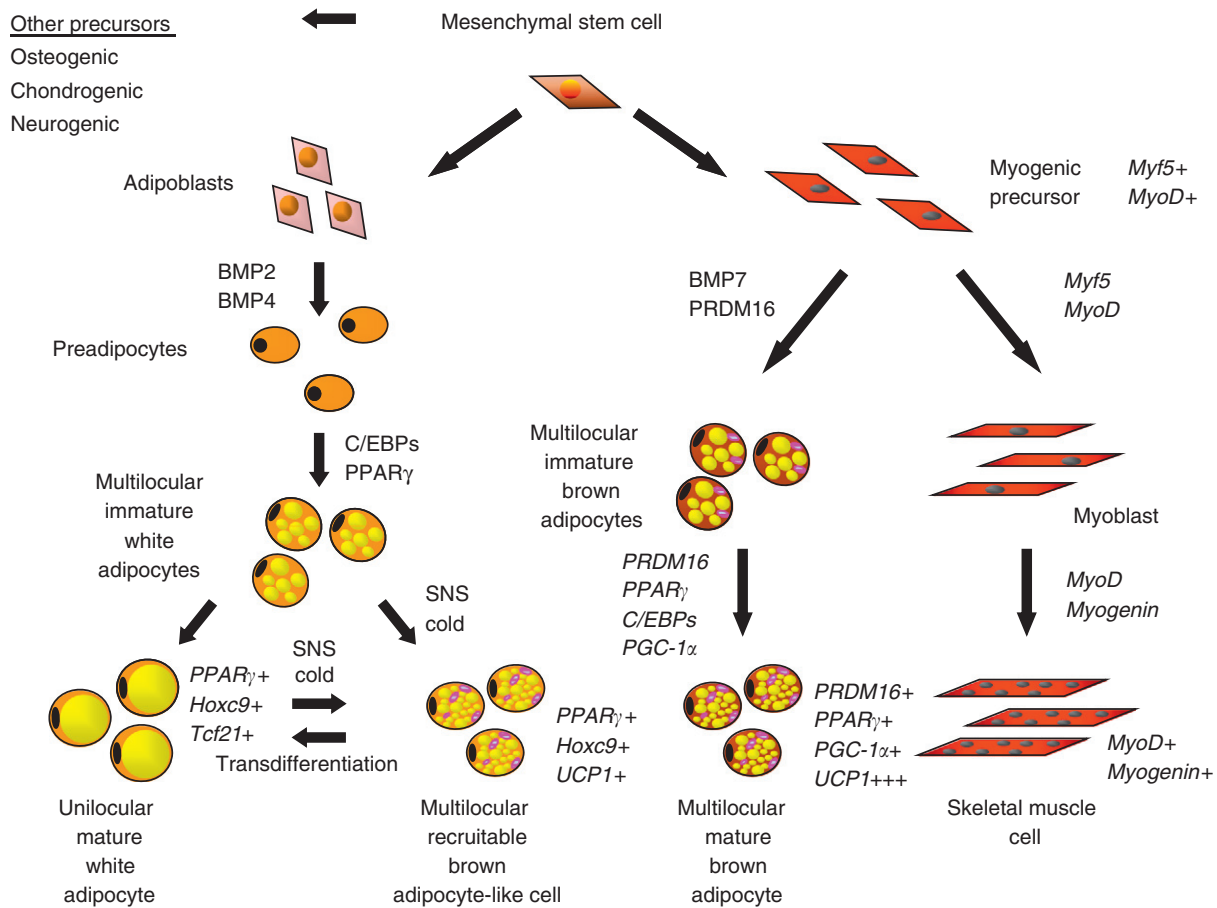


Figure 2 Schematic diagram of the histogenesis of white and brown adipocytes. BMP, bone morphogenetic protein; C/EBPs, CCAAT/enhancer binding proteins; PGC-1 α , peroxisome proliferator-activated receptor- γ coactivator-1 α ; PPAR, peroxisome proliferator-activated receptor; PRDM16, positive regulatory domain containing 16; UCP1, uncoupling protein 1.

subsequent phases of terminal differentiation, the committed preadipocyte takes on the characteristics of the white mature adipocyte by acquiring all the machinery needed for lipid transport, synthesis, and mobilization, hormonal responsiveness and the secretion of adipocyte-specific proteins. The morphological and functional changes that take place in the course of adipogenesis represent a shift in transcription factor expression and activity leading from a primitive, multipotent state to a final phenotype characterized by alterations in cell shape and lipid accumulation. Various redundant signaling pathways and transcription factors directly influence fat cell development by converging the upregulation of PPAR γ , which embodies a common and essential regulator of adipogenesis as well as of adipocyte hypertrophy. Among the broad panoply of transcription factors C/EBPs and the basic helix-loop-helix family (ADD1/SREBP-1c, adipocyte determination and differentiation factor-1/sterol regulatory element binding protein-1c) also stand out together with their link with the existing nutritional status. The transcriptional repression of adipogenesis includes both active and passive mechanisms. The former directly interferes with the transcriptional machinery, whereas the latter is based on the binding of negative regulators to yield inactive forms of known activators.

Adipose tissue develops extensively in homeotherms with the proportion to body weight varying greatly between species. There are two processes of adipose tissue formation. In the primary fat formation, which takes place relatively early (in human fetuses the first traces of a fat organ are detectable between 14 and 16 weeks of prenatal life), gland-like aggregations of epitheloid precursor cells, called lipoblasts or preadipocytes are laid down in specific locations and accumulate multiple lipid droplets. The secondary fat formation takes place later in fetal life (after the 23rd week of gestation) as well as in the early postnatal period, whereby the differentiation of other fusiform precursor cells that accumulate lipid to ultimately coalesce into a single large drop per cell leads to the dissemination of fat depots formed by unilocal white adipocytes in many areas of connective tissue. Adipose tissue may be partitioned by connective tissue septa into lobules. The number of fat lobules remains constant, whereas in the subsequent developmental phases the lobules' size grows continuously. At the sites of early fat development, a multilocal morphology of adipocytes predominates, reflecting the early developmental stage. Microscopic studies have shown that the second trimester may be a critical period for the development of obesity in later life. At the beginning of the third trimester, adipocytes are present in

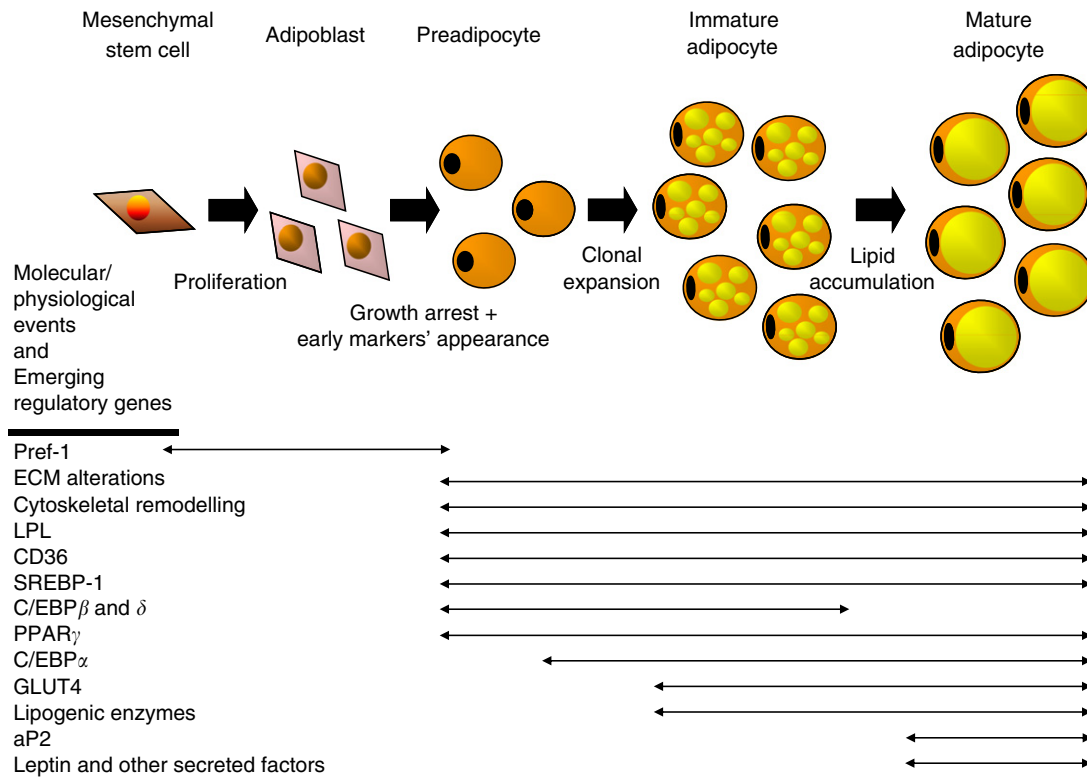


Figure 3 Multistep process of adipogenesis together with events and participating regulatory elements. aP2, adipocyte fatty acid binding protein; CD36, fatty acid translocase; C/EBPs, CCAAT/enhancer binding proteins; ECM, extracellular matrix; GLUT4, glucose transporter type 4; LPL, lipoprotein lipase; PPAR, peroxisome proliferator-activated receptor; SREBP-1, sterol regulatory element binding protein-1.

the main fat depots but are still relatively small. During embryonic development it is important to emphasize the temporo-spatial tight coordination of angiogenesis with the formation of fat cell clusters. At birth, body fat has been reported to account approximately for 16% of total body weight (with brown fat constituting 2–5%) with an increase in body fat from approximately 0.7 to 2.8 kg during the first year of life.

Adipogenesis, i.e., the development of adipose tissue, varies according to sex and age. Two sensitive periods for changes in adipose tissue cellularity with peaks of accelerated adipose mass enlargement have been established, namely after birth and from 9–13 years. The capacity for cell proliferation and differentiation is highest during the first year of life, whereas it is less pronounced in the years before puberty. Childhood-onset obesity is characterized by a combination of fat cell hyperplasia and hypertrophy, whereas in adult-onset obesity a hypertrophic growth predominates. Initially, excess energy storage starts as hypertrophic obesity, resulting from the accumulation of excess lipid in a normal number of unilocular adipose cells. In this case, adipocytes may be four times their normal size. If the positive energy balance is maintained, a hyperplasic or hypercellular obesity characterized by a greater than normal number of cells is developed. It has been recently shown that adult humans are capable of new adipocyte formation with samples containing a significant proportion of cells with the ability of undergoing differentiation. Multipotent stem cells and adipoblasts, which are

found during embryonic development, are still present postnatally.

Hormones, cytokines, growth factors, and nutrients influence the dynamic changes related to adipose tissue mass as well as its pattern of distribution (**Figure 4**). The responsiveness of fat cells to neurohumoral signals may vary according to peculiarities in the adipose lineage stage at the moment of exposure. Moreover, the simultaneous presence at specific threshold concentrations of some adipogenic factors may be a necessary requirement to trigger terminal differentiation.

By measuring the relative abundance of ^{14}C in genomic DNA from adipocytes it has been recently clarified that in individuals approximately 10% of adipocytes experience apoptosis, whereas a comparable proportion are renewed, each year. Thus, WAT turns out to be a more dynamic tissue than was previously assumed. These findings are consistent across a wide range of body mass index (BMI), including subjects with early-onset obesity, and following weight loss. The adipocyte number has been shown to be a major determinant of fat mass in the adult; the number of adipocytes in both lean and obese subjects appears to be set during childhood. However, it remains possible that the common scenario of gradual but significant weight gain throughout adult life may be underpinned by an initial increase in triacylglycerol loading until an adipocyte size threshold is reached, when additional new adipocytes are recruited from committed precursor cells or mesenchymal stem cells.

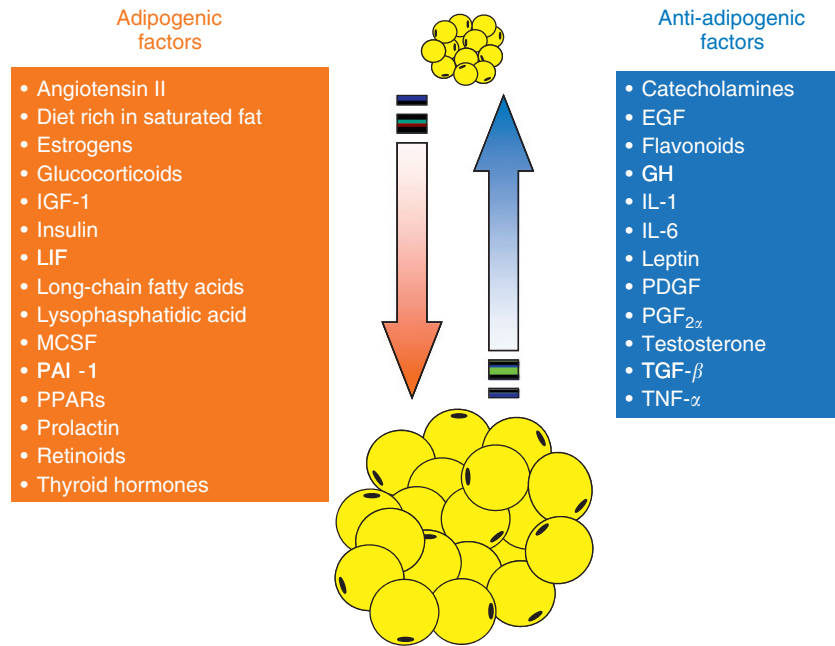


Figure 4 Factors exerting a direct effect on adipose mass. EGF, epidermal growth factor; GH, growth hormone; IGF-1, insulin-like growth factor 1; IL-1, interleukin-1; IL-6, interleukin-6; LIF, leukemia inhibitory factor; MCSF, macrophage colony stimulating factor; PAI-1, plasminogen activator inhibitor-1; PDGF, platelet-derived growth factor; PGF_{2α}, prostaglandin F_{2α}; PPARs, peroxisome proliferator-activated receptors; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

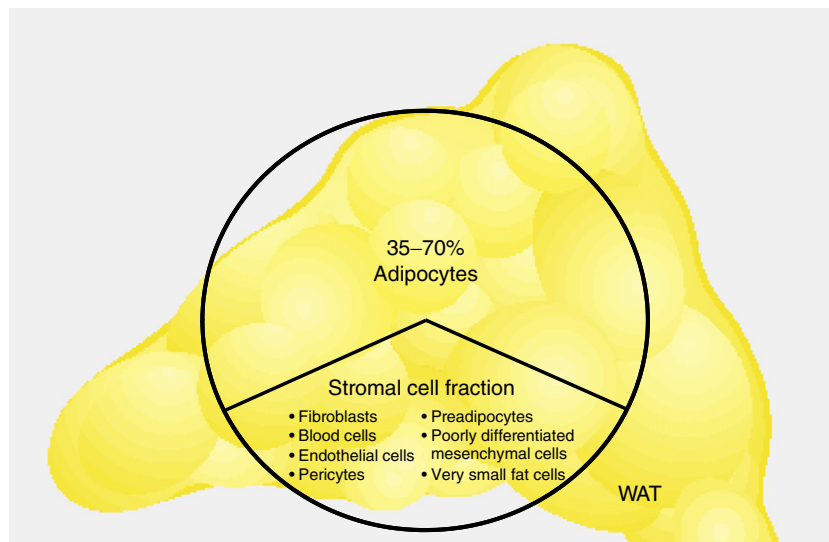


Figure 5 Schematic representation of cell types present in adipose tissue.

Structure

Adipose tissue is a special loose connective tissue dominated by adipocytes. The name of these cells is based on the cell's content of a large lipid droplet with "adipo" being a combining form derived from the Latin *adeps* meaning "pertaining to fat". In adipose tissue, fat cells are individually held in place by delicate reticular fibers clustering in lobular masses bounded by fibrous septa surrounded by a rich capillary network. In adults adipocytes may comprise approximately 90% of

adipose mass accounting only for roughly 25% of the total cell population. Thus, adipose tissue itself is composed of not only adipocytes, but also by other cell types, termed as the stroma-vascular fraction, comprising blood cells, endothelial cells, pericytes as well as adipose precursor cells among others (**Figure 5**), which account for the remaining 75% of the total cell population, representing a wide range of targets for an extensive autocrine–paracrine cross-talk.

Adipocytes, which are typically spherical and vary enormously in size (20–200 μm in diameter, with variable volumes

ranging from a few picoliters to approximately 3 nl), are embedded in a connective tissue matrix and are uniquely adapted to store and release energy. Surplus energy is assimilated by adipocytes and stored as lipid droplets. The stored fat is composed of mainly triacylglycerols (approximately 95% of the total lipid content comprised principally by oleic and palmitic acids) and to a smaller degree diacylglycerols, phospholipids, unesterified fatty acids, and cholesterol. To accommodate the lipids adipocytes are capable of changing their diameter 20-fold and their volumes by several thousand-fold. However, the increase in size of fat cells is not indefinite. Once a maximum capacity is attained, which in humans averages 1000 pl, the formation of new adipocytes from the precursor pool takes place. The interior of adipocytes appears unstained since the histological techniques of standard tissue preparation dissolve the lipids, leaving a thin rim of eosinophilic cytoplasm that typically loses its round shape during tissue processing, thus contributing to the sponge-like appearance of WAT in routine preparations for light microscopy (Figures 6 and 7). Owing to the fact that approximately 90% of the cell volume is a lipid droplet, the small dark nucleus becomes a flattened semilunar structure pushed against the edge of the cell and the thin cytoplasmic rim is also pushed to the periphery of the adipocytes. Mature white adipose cells contain a single large lipid droplet and are described as unilocular. However, developing white adipocytes are transiently multilocular containing multiple lipid droplets before these finally coalesce into a single large drop (Figure 8). The nucleus is round or oval in young fat cells, but is cup-shaped and peripherally displaced in mature adipocytes. The cytoplasm is stretched to form a thin sheath around the fat globule, although a relatively large volume is concentrated around the nucleus. A thin external lamina called basal lamina surrounds the cell. The smooth cell membrane shows no microvilli but has abundant smooth micropinocytotic invaginations that often fuse to form small vacuoles appearing as rosette-like configurations (Figure 9). Mitochondria are few in number with loosely arranged membranous cristae. The Golgi zone is small and the cytoplasm is filled with free ribosomes, but contains only a limited number of short profiles of the granular endoplasmic reticulum. Occasional lysosomes can also be found. The coalescent lipid droplets contain a mixture of neutral fats, triglycerides, fatty acids, phospholipids, and cholesterol. A thin interface membrane separates the lipid droplet from the cytoplasmic matrix. Peripheral to this membrane is a system of parallel meridional thin filaments. Because of the size of these cells, relative to the thickness of the section, the nucleus (accounting for only one-fortieth of the cell volume) may not always be present in the section. Unilocular adipocytes usually appear in clumps near blood vessels, which is reasonable because the source and dispersion of material stored in fat cells depends on transportation by the vascular system.

Brown fat is a specialized type of adipose tissue that plays an important role in body temperature regulation. It is present in significant amounts in rodents and hibernating animals. In the newborn brown fat is well developed in the neck and interscapular region. Until recently, it was generally accepted that BAT involutes steadily during the first few months, with clearly recognizable depots having

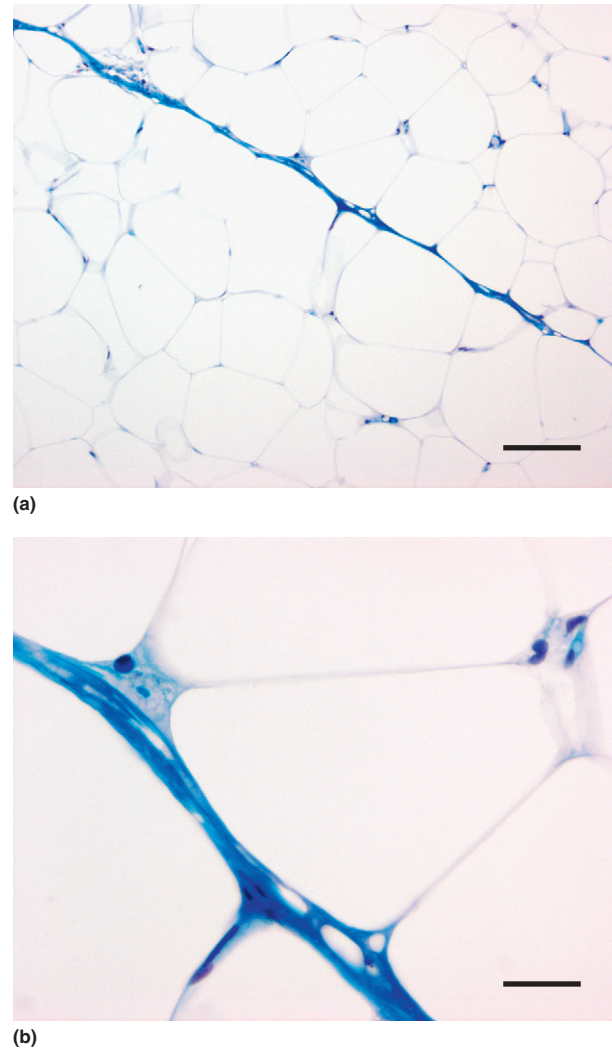
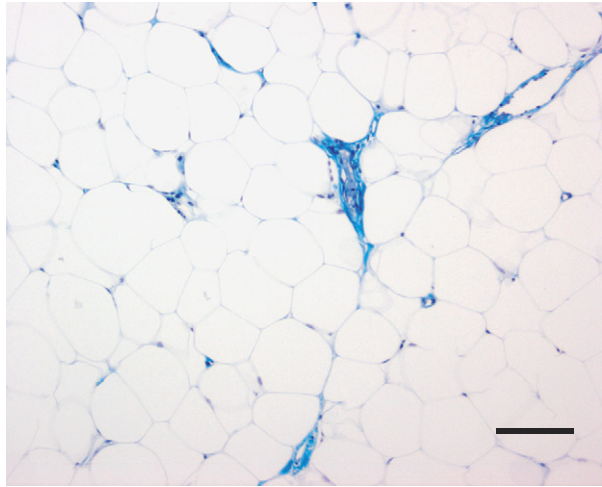


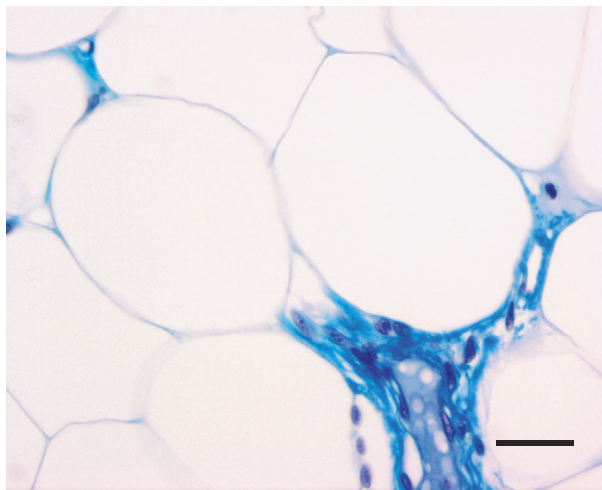
Figure 6 (a) Human subcutaneous white adipose tissue with a Masson trichrome staining (10 × ; bar = 100 μm). (b) Same tissue at a higher magnification (40 × ; bar = 25 μm). (Courtesy of Dr. MA Burrell and Dr. M Archanco, University of Navarra).

essentially disappeared within the first years after birth. In normal adults, only occasional brown adipocytes were thought to be scattered through white fat masses. However, recent findings using positron emission tomography (PET) with fluorodeoxyglucose (a marker of metabolic activity) have shown in adults symmetrical areas of increased tracer uptake in the upper parts of the body, broadly corresponding to the distribution of BAT in lower mammals and in human neonates. The main BAT depots in adults are found in the supraclavicular region and neck, with additional activity in the paravertebral, mediastinal, para-aortic, and suprarenal areas. The activity of this BAT can be acutely enhanced by cold exposure and stimulated by the sympathetic nervous system.

The brown color of BAT is derived from a rich vascular network and abundant mitochondria and lysosomes. The individual multilocular adipocytes are frothy-appearing cells due to the fact that the lipid, which does not coalesce as readily as in white fat cells, is normally stored in multiple small droplets,



(a)



(b)

Figure 7 (a) Human omental white adipose tissue with a Masson trichrome staining ($10\times$; bar = $100\text{ }\mu\text{m}$). (b) Same tissue at a higher magnification ($40\times$; bar = $25\text{ }\mu\text{m}$). (Courtesy of Dr. MA Burrell and Dr. M Archanco, University of Navarra).

has been leached out during tissue processing (Figure 10). The spherical nuclei are centrally or eccentrically located within the cell. Compared to the unilocular white adipocytes, the cytoplasm of the multilocular brown fat cell is relatively abundant and strongly stained because of the numerous mitochondria present. The mitochondria are involved in the oxidation of the stored lipid, but because they exhibit a reduced potential to carry out oxidative phosphorylation, the energy produced is released in the form of heat due to the uncoupling activity of UCP and not captured in adenosine triphosphate (ATP). Therefore, brown adipose tissue is extremely well vascularized so that the blood is warmed when it passes through the active tissue.

Distribution

WAT may represent the largest endocrine tissue of the whole organism especially in overweight and obese patients. The

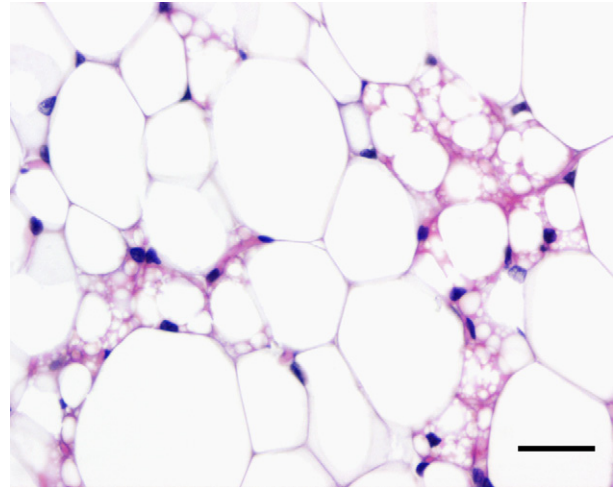
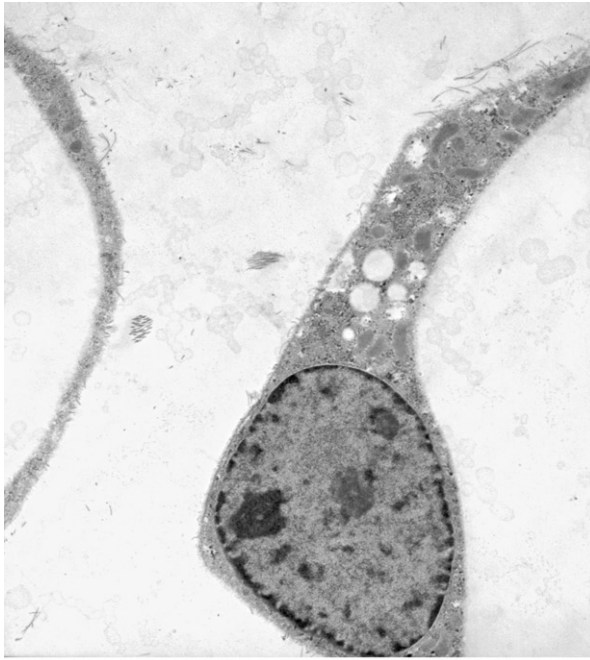


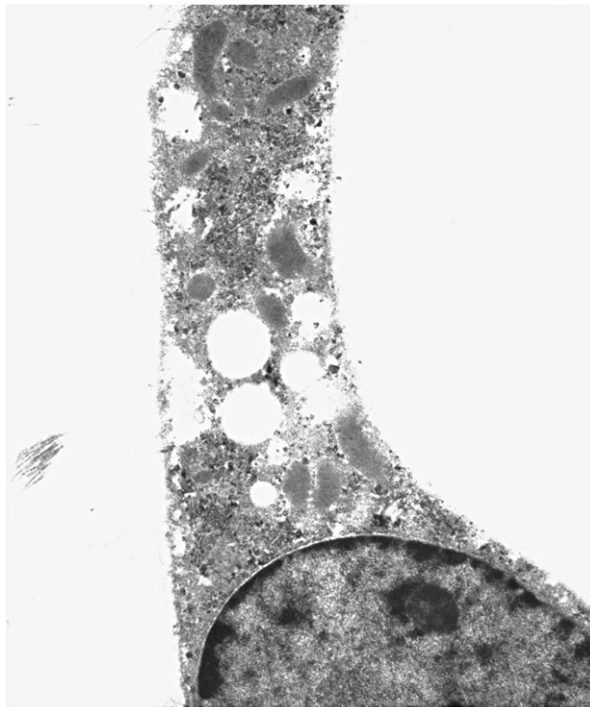
Figure 8 Paraffin section of rat abdominal white adipose tissue with a hematoxylin and eosin stain showing the simultaneous presence of uni- and multilocular adipocytes ($40\times$; bar = $25\text{ }\mu\text{m}$). (Courtesy of Dr. MA Burrell and Dr. M Archanco, University of Navarra).

anatomical distribution of individual fat pads dispersed throughout the whole body and not connected to each other collides with a classic organ-specific localization. WAT exhibits clear, regional differences in its sites of predilection (Table 1). The hypodermal region invariably contains fat, except in a few places such as the eyelids and the scrotum. Adipocytes also accumulate around organs like kidneys and adrenals, in the coronary sulcus of the heart, in bone marrow, mesentery, and omentum. Unilocular fat is widely distributed in the subcutaneous tissue of humans but exhibits quantitative regional differences that are influenced by age and sex. In infants and young children there is a continuous subcutaneous fat layer – the panniculus adiposus, over the whole body. This layer thins out in some areas in adults but persists and grows thicker in certain other regions. The sites differ in their distribution among sexes being responsible for the characteristic body form of males and females, termed android and gynecoid fat distribution. In males, the main regions include the nape of the neck, the subcutaneous area over the deltoid and triceps muscles, and the lumbosacral region. In females, subcutaneous fat is most abundant in the buttocks, epitrochanteric region, anterior and lateral aspects of the thighs as well as the breasts. Additionally, extensive fat depots are found in the omentum, mesenteries, and the retroperitoneal area of both sexes. In well-nourished, sedentary individuals, the fat distribution persists and becomes more obvious with advancing age with males tending to deposit more fat in the visceral compartment. Depot-specific differences may be related not only to the metabolism of fat cells but also to their capacity to form new adipocytes. Additionally, regional differences may result from variations in hormone receptor distribution as well as from specific local environmental characteristics as a consequence of differences in innervation and vascularization.

Regional distribution of body fat is known to be an important indicator for metabolic and cardiovascular alterations in some individuals. The observation that the topographic distribution of adipose tissue is relevant to understanding the relation of obesity to disturbances in glucose and lipid

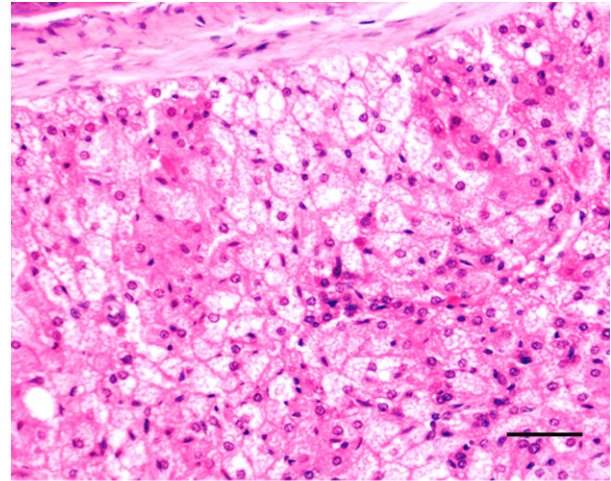


(a)

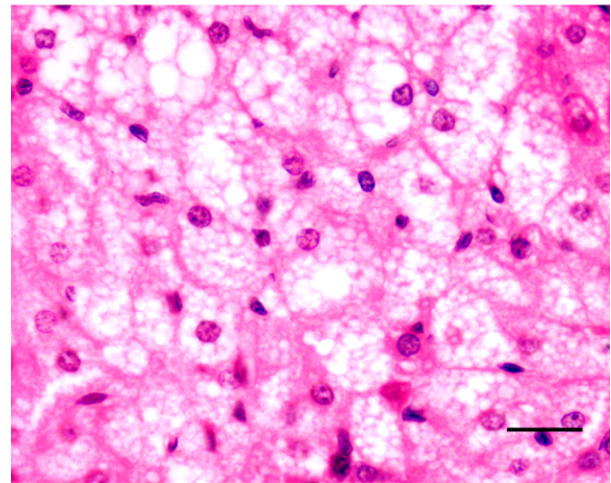


(b)

Figure 9 (a) Transmission electron micrographs with the characteristically displaced nucleus to one side and slightly flattened by the accumulated lipid. The cytoplasm of the fat cell is reduced to a thin rim around the lipid droplet ($7725\times$). (b) The cytoplasm contains several small lipid droplets that have not yet coalesced. A few filamentous mitochondria, occasional cisternae of endoplasmic reticulum and a moderate number of free ribosomes are usually visible ($15000\times$). (Courtesy of Dr. MA Burrell and Dr. M Archanco, University of Navarra).



(a)



(b)

Figure 10 (a) Paraffin section of rat brown adipose tissue with a hematoxylin and eosin stain ($20\times$; bar = $50\mu\text{m}$). (b) Same tissue at a higher magnification ($40\times$; bar = $25\mu\text{m}$). (Courtesy of Dr. MA Burrell and Dr. M Archanco, University of Navarra).

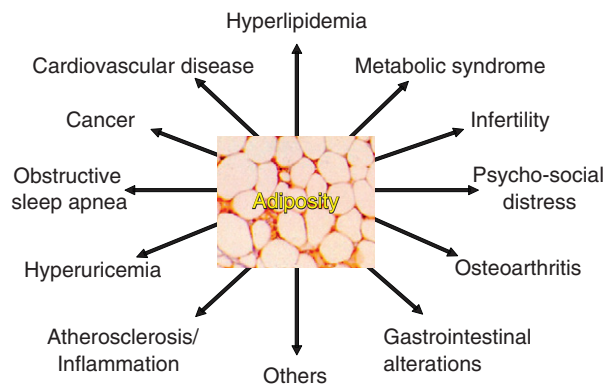
metabolism was formulated before the 1950s. Since then numerous prospective studies have revealed that android or male-type obesity correlates more often with an elevated mortality and risk for the development of type 2 diabetes mellitus, dyslipidemia, hypertension, and atherosclerosis than gynoid or female-type obesity. Obesity has been reported to cause or exacerbate a large number of health problems with a known impact on both life expectancy and quality of life. In this respect, the association of increased adiposity is accompanied by important pathophysiological alterations, which lead to the development of a wide range of co-morbidities (Figure 11).

Function

Although many cell types contain small reserves of carbohydrate and lipid, the adipose tissue is the body's most capacious energy reservoir. Because of the high energy content

Table 1 Distribution of main human adipose tissue depots

<i>Subcutaneous (approx. 80%; deep + superficial layers)</i>	
Truncal	
Cervical	
Dorsal	
Lumbar	
Abdominal	
Gluteofemoral	
Mammary	
<i>Visceral (approx. 20%; thoracic–abdominal–pelvic)</i>	
Intrathoracic (extra-intrapericardial)	
Intraabdominopelvic	
Intraperitoneal	
Omental (greater and lesser omentum)	
Mesenteric (epiploon, small intestine, colon, rectum)	
Umbilical	
Extraperitoneal	
Peripancreatic (infiltrated with brown adipocytes)	
Perirenal (infiltrated with brown adipocytes)	
Intrapelvic	
Gonadal (parametrial, retrouterine, retropubic)	
Urogenital (paravesical, para-retrorectal)	
<i>Intraparenchymatous (physiologically or pathologically)</i>	
Inter-intramuscular and perimuscular (inside the muscle fascia)	
Perivascular	
Paraosseal (interface between bone and muscle)	
Ectopic (steatosis, intramyocardial, lipodistrophy, etc.)	

**Figure 11** Main comorbidities associated with increased adiposity.

per unit weight of fat as well as due to its hydrophobicity, the storage of energy in the form of triglycerides is a highly efficient biochemical phenomenon (1 g of adipose tissue contains approximately 800 mg triacylglycerol and only approximately 100 mg of water). It represents quantitatively the most variable component of the organism, varying from a few percent of body weight in elite athletes to more than half of the total body weight in morbidly obese patients. The normal range is approximately 10–20% body fat for males and approximately 20–30% for females, accounting approximately for an energy reserve of 25–50 days in men and 40–60 days in women. During pregnancy most species accrue additional reserves of adipose tissue to help support the development of the fetus and to further facilitate the lactation period.

Energy balance regulation is an extremely complex process composed of multiple interacting homeostatic and behavioral pathways aimed at maintaining constant energy stores. It is now evident that body weight control is achieved through highly orchestrated interactions between nutrient selection, organoleptic influences, and neuro-endocrine responses to diet as well as being influenced by genetic and environmental factors. The concept that circulating signals generated in proportion to body fat stores influence appetite and energy expenditure in a coordinated manner to regulate body weight was proposed almost 50 years ago. According to this model, changes in energy balance sufficient to alter body fat stores were signaled via one or more circulating factors acting in the brain to elicit compensatory changes in order to match energy intake to energy expenditure. This was formulated as the "lipostatic theory" assuming that as adipose tissue mass enlarges, a factor that acts as a sensing hormone or "lipostat" in a negative feedback control from adipose tissue to hypothalamic receptors informs the brain about the abundance of body fat, thereby allowing feeding behavior, metabolism, and endocrine physiology to be coupled to the nutritional state of the organism. The existing body of evidence gathered in the last decades through targeted expression or knockout of specific genes involved in different steps of the pathways controlling food intake, body weight, adiposity, or fat distribution has clearly contributed to unraveling the underlying mechanisms of energy homeostasis. The findings have fostered the notion of a far more complex system than initially thought, involving the integration of a plethora of factors.

The identification of adipose tissue as a multifunctional organ as opposed to a passive organ for the storage of excess energy in the form of fat has been brought about by the emerging body of evidence gathered during the last decades. This pleiotropic nature is based on the ability of fat cells to secrete a large number of hormones, growth factors, enzymes, cytokines, complement factors, and matrix proteins, collectively termed as adipokines or adipocytokines (Table 2, Figure 12), at the same time as expressing receptors for most of these factors (Table 3), which warrants an extensive cross-talk at a local and systemic level in response to specific external stimuli or metabolic changes. The vast majority of adipocyte-derived factors have been shown to be dysregulated in alterations accompanied by changes in adipose tissue mass such as overfeeding and lipodistrophy, thus providing evidence for their involvement in the etiopathology and comorbidities associated with obesity and cachexia.

WAT is actively involved in cell function regulation through a complex network of endocrine, paracrine, and autocrine signals, which influence the response of many tissues, including the hypothalamus, pancreas, liver, skeletal muscle, kidneys, endothelium, and immune system. Adipose tissue serves the functions of being a store for energy reserve, insulation against heat loss through the skin, and a protective padding of certain organs. A rapid turnover of stored fat can take place, and with only a few exceptions (orbit, major joints as well as palm, and foot sole), the adipose tissue can be used up almost completely during starvation. Adipocytes are uniquely equipped to participate in the regulation of other functions such as reproduction, immune response, blood pressure control, coagulation, fibrinolysis, and angiogenesis.

Table 2 Relevant factors secreted by adipose tissue to the bloodstream

<i>Molecule</i>	<i>Function/effect</i>
Adiponectin/ACRP30/AdipoQ/apM1/GBP28	Plays a protective role in the pathogenesis of type 2 diabetes and cardiovascular diseases
Adipsin	Possible link between the complement pathway and adipose tissue metabolism
Angiotensinogen	Precursor of angiotensin II; regulator of blood pressure and electrolyte homeostasis
ASP	Influences the rate of triacylglycerol synthesis in adipose tissue
Chemerin	Regulates adipocyte differentiation and glucose uptake. It is potentially involved in the inflammatory response
FFA	Oxidized in tissues to produce local energy. Serve as a substrate for triglyceride and structural molecules synthesis. Involved in the development of insulin resistance
Glycerol	Structural component of the major classes of biological lipids and gluconeogenic precursor
IGF-1	Stimulates proliferation of a wide variety of cells and mediates many of the effects of growth hormone
IL-6	Implicated in host defense, glucose and lipid metabolism, and regulation of body weight
Leptin	Signals to the brain about body fat stores. Regulation of appetite and energy expenditure. Wide variety of physiological functions
NO	Important regulator of vascular tone. Pleiotropic involvement in pathophysiological conditions
Omentin	Enhances insulin-stimulated glucose uptake
PAI-1	Potent inhibitor of the fibrinolytic system
PGI ₂ & PGF _{2α}	Implicated in regulatory functions such as inflammation and blood clotting, ovulation, menstruation and acid secretion
Resistin	Putative role in insulin resistance. May participate in inflammation
TNF-α	Interferes with insulin receptor signaling and is a possible cause of the development of insulin resistance in obesity
Vaspin	Exhibits insulin-sensitizing effects
VEGF	Stimulation of angiogenesis
Visfatin/PBEF/NAMPT	Catalyzes the biosynthesis of nicotinamide adenine dinucleotide. Regulates vascular smooth muscle and immune cell function. Potentially involved in the regulation of insulin sensitivity

The advent of microarray technology has dramatically changed the study of the pattern of gene expression by enabling the simultaneous analysis of thousands of genes in a single experiment. Interestingly, the high number and ample spectrum of genes found to be expressed in WAT together with the changes observed in samples from obese patients substantiates the view of an extraordinarily active and plastic tissue. The complex and complementary nature of the expression profile observed in obese adipose tissue reflects a pleiad of adaptive changes affecting crucial physiological functions that may need to be further explored through genomic and proteomic approaches.

The endocrine activity of WAT was postulated almost 20 years ago alluding to the tissue's ability for steroid hormone interconversion. In recent years, especially since the discovery of leptin, the list of adipocyte-derived factors has been increasing at a phenomenal pace. Another way of addressing the production of adipose-derived factors is by focusing on the functions in which they are implicated (**Figure 12**). One of the best known aspects of WAT physiology relates to the synthesis of products involved in lipid metabolism such as perilipin, adipocyte lipid binding protein (ALBP, FABP4 or aP2), CETP, and retinol binding protein (RBP). Adipose tissue has been also identified as a source of production of factors with immunological properties participating in immunity and stress responses as is the case of ASP and metallothionein. More recently, the pivotal role of adipocyte-derived factors implicated in cardiovascular function control such as angiotensinogen, adiponectin, peroxisome proliferator-activated receptor γ angiopoietin related protein/fasting-induced adipose factor (PGAR/FIAF), and C-reactive protein (CRP) has been established. A further subsection of proteins produced by

adipose tissue concerns other factors with an autocrine–paracrine function like PPAR γ , IGF-1, monobutyrin, and the UCPs.

BAT is specialized for heat production; its lipid stores turn over rapidly, and the liberated fatty acids are oxidized by the brown adipocyte's mitochondria in a process that generates heat directly. In neonatal mammals, hibernators, and rodents, a crucial function of BAT is the maintenance of body temperature through cold-induced thermogenesis. In addition, BAT thermogenesis is activated during overeating – an important aspect of diet-induced thermogenesis. In humans, as is the case in other larger mammals, the functional capacity of brown adipose tissue decreases because of the relatively higher ratio between heat production from basal metabolism and the smaller surface area encountered in adults. In addition, clothing and indoor life have reduced the need for adaptive nonshivering thermogenesis. However, it has been recently shown that human WAT can be infiltrated with brown adipocytes expressing UCP-1. The prevalence of active BAT in normal adults can be only estimated indirectly, but is thought to be present in approximately 10% of the general population. BAT, therefore, has the potential to play a role in normal energy balance and could become a pharmacological target for new drugs to treat obesity.

Regulation of Metabolism

The control of fat storage and mobilization has been marked by the identification of a number of regulatory mechanisms in the last decades. Isotopic tracer studies have clearly shown that lipids are continuously being mobilized and renewed even in

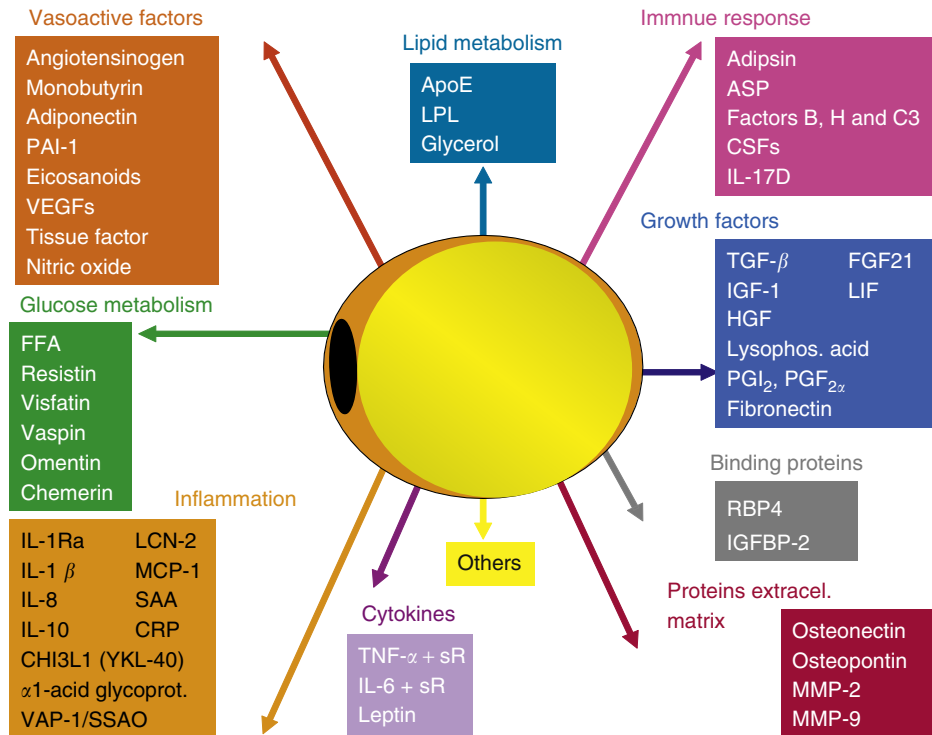


Figure 12 Factors secreted by white adipose tissue, which underlie the multifunctional nature of this endocrine organ. Although owing to their pleiotropic effects some of the elements might play more than one physiological role, they have been included only under one function for clarity of the figure. ApoE, apolipoprotein E; ASP, acylation-stimulating protein; CHI3L1 (YKL-40), chitinase-3-like protein 1; CSF, colony-stimulating factor; FGF21, fibroblast growth factor 21; HGF, hepatocyte growth factor; IGF-1, insulin-like growth factor-1; IGFBP-2, insulin-like growth factor binding protein-2; IL-1, interleukin-1; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; IL-17, interleukin-17; IL-1Ra, interleukin-1 receptor antagonist; LCN-2, lipocalin-2; LIF, leukemia inhibitory factor; LPL, lipoprotein lipase; MCP-1, monocyte chemoattractant protein-1; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; PAI-1, plasminogen activator inhibitor-1; PGF_{2 α} , prostaglandin F_{2 α} ; PGI₂, prostacyclin; RBP4, retinol binding protein-4; SAA, serum amyloid A; TGF- β , transforming growth factor- β ; TNF- α , tumor necrosis factor- α ; VAP-1/SSAO, vascular adhesion protein-1/semicarbazide-sensitive amine oxidase; VEGF, vascular endothelial growth factor.

individuals in energy balance. Fatty acid esterification and triglyceride hydrolysis take place continuously. The half-life of depot lipids in rodents is approximately 8 days, meaning that almost 10% of the fatty acid stored in adipose tissue is replaced daily by new fatty acids. The balance between lipid loss and accretion determines the net outcome on energy homeostasis.

The synthesis of triglycerides, also termed lipogenesis, requires the supply of fatty acids and glycerol. The main sources of fatty acids are the liver and the small intestine. Fatty acids are esterified with glycerol phosphate in the liver to produce triglycerides. Since triglycerides are bulky polar molecules that do not cross cell membranes well, they must be hydrolyzed to fatty acids and glycerol before entering fat cells. Serum very low-density lipoproteins (VLDLs) are the major form in which triacylglycerols are carried from the liver to WAT. Short-chain fatty acids (16 carbons or less) can be absorbed from the gastrointestinal tract and carried in chylomicra directly to the adipocyte. Inside fat cells glycerol is mainly synthesized from glucose. In WAT fatty acids can be synthesized from several precursors, such as glucose, lactate, and certain amino acids, with glucose being quantitatively the most important in humans. In the case of glucose, GLUT4, the principal glucose transporter of adipocytes, controls the entry of the substrate

into the adipocyte. Insulin is known to stimulate glucose transport by promoting GLUT4 recruitment as well as increasing its activity. Inside the adipocyte, glucose is initially phosphorylated and then metabolized both in the cytosol and in the mitochondria, to produce cytosolic acetyl-CoA with the flux being influenced by phosphofructokinase and pyruvate dehydrogenase. Glycerol does not readily enter the adipocyte, but the membrane-permeable fatty acids do. Once inside the fat cells, fatty acids are re-esterified with glycerol phosphate to yield triglycerides. Lipogenesis is favored by insulin, which activates pyruvate kinase, pyruvate dehydrogenase, acetyl-CoA carboxylase, and glycerol phosphate acyltransferase. When excess nutrients are available insulin decreases acetyl-CoA entry into the tricarboxylic acid cycle while directing it towards fat synthesis. This insulin effect is antagonized by growth hormone. The gut hormones glucagon-like peptide 1 and gastric inhibitory peptide also increase fatty acid synthesis, whereas glucagon and catecholamines inactivate acetyl-CoA carboxylase, thus decreasing the rate of fatty acid synthesis.

The release of glycerol and free fatty acids by lipolysis plays a critical role in the ability of the organism to provide energy from triglyceride stores. In this sense, the processes of lipolysis and lipogenesis are crucial for the attainment of body weight control. For this purpose adipocytes are equipped with a well

Table 3 Main receptors expressed by adipose tissue

<i>Receptor</i>	<i>Main effect of receptor activation on adipocyte metabolism</i>
<i>Hormone-cytokine receptors</i>	
Adenosine	Inhibition of lipolysis
Adiponectin (AdipoR1 & AdipoR2)	Regulation of insulin sensitivity and fatty acid oxidation
Angiotensin II	Increase of lipogenesis. Stimulation of prostacyclin production by mature fat cells. Interaction with insulin in regulation of adipocyte metabolism
Chemerin	Regulation of adipocyte differentiation and glucose uptake. Potential role in inflammatory response
GH	Induction of leptin and IGF-I expression. Stimulation of lipolysis
Ghrelin	Stimulation of adipogenesis and lipogenesis. Induction of glucose uptake
IGF-I and -II	Inhibition of lipolysis. Stimulation of glucose transport and oxidation
IL-6	LPL activity inhibition. Induction of lipolysis
Insulin	Inhibition of lipolysis and stimulation of lipogenesis. Induction of glucose uptake and oxidation. Stimulation of leptin expression
Leptin (OB-R)	Stimulation of lipolysis. Autocrine regulation of leptin expression
NPY-Y1 and Y5	Inhibition of lipolysis. Induction of leptin expression
Prostaglandin	Strong antilipolytic effects (PGE ₂). Modulation of preadipocyte differentiation (PGF _{2α} and PGI ₂)
TGF-β	Potent inhibition of adipocyte differentiation
TNF-α	Stimulation of lipolysis. Regulation of leptin secretion. Potent inhibition of adipocyte differentiation. Involvement in development of insulin resistance
VEGF	Stimulation of angiogenesis
<i>Catecholamine-nervous system receptors</i>	
Endocannabinoids CB ₁	Stimulation of adiponectin expression. Induction of glucose uptake and GLUT4 translocation. Inhibition of lipogenesis
Muscarinic	Inhibition of lipolysis
Nicotinic	Stimulation of lipolysis
α ₁ -AR	Induction of inositol phosphate production and PKC activation
α ₂ -AR	Inhibition of lipolysis. Regulation of preadipocyte growth
β ₁ -, β ₂ - and β ₃ -AR	Stimulation of lipolysis. Induction of thermogenesis. Reduction of leptin mRNA levels
<i>Nuclear receptors</i>	
Androgen	Control of adipose tissue development (antiadipogenic signals). Modulation of leptin expression
Estrogen	Control of adipose tissue development (proadipogenic signals). Modulation of leptin expression
Glucocorticoids	Stimulation of adipocyte differentiation
PPARδ	Regulation of fat metabolism. Plays a central role in fatty acid-controlled differentiation of preadipose cells
PPARγ	Induction of adipocyte differentiation and insulin sensitivity
RAR/RXR	Regulation of adipocyte differentiation
T ₃	Stimulation of lipolysis. Regulation of leptin secretion. Induction of adipocyte differentiation. Regulation of insulin effects
<i>Lipoprotein receptors</i>	
HDL	Clearance and metabolism of HDL
LDL	Stimulation of cholesterol uptake
VLDL	Binding and internalization of VLDL particles. Involvement in lipid accumulation

ACRP30, adipocyte complement-related protein of 30 kDa; apM1, adipose most abundant gene transcript 1; ASP, acylation-stimulating protein; FFA, free fatty acids; GBP28, gelatin-binding protein 28; GH, growth hormone; HDL, high density lipoprotein.; IGF, insulin-like growth factor; IL-6, interleukin 6; LDL, low density lipoprotein; LPL, lipoprotein lipase; NO, nitric oxide; NPY-Y1, and -Y5, neuropeptide receptors Y-1 and -5; OB-R, leptin receptor; PAI-1, plasminogen activator inhibitor-1; PGE₂, prostaglandin E₂; PGF_{2α}, prostaglandin F_{2α}; PGI₂, prostacyclin; PPAR, peroxisome proliferator-activated receptor; RAR, retinoic acid receptor; RXR, retinoid x receptor; T₃, triiodothyronine; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; VLDL, very low density lipoprotein; α₁- and α₂-AR, α₁-, and α₂-adrenergic receptors; β₁-, β₂- and β₃-AR, β₁-, β₂-, and β₃ adrenergic receptors.

developed enzymatic machinery, together with a number of non-secreted proteins and binding factors directly involved in the regulation of lipid metabolism. The hydrolysis of triglycerides from circulating VLDL and chylomicrons is catalyzed by lipoprotein lipase (LPL). This rate-limiting step plays an important role in directing fat partitioning. Although LPL controls fatty acid entry into adipocytes, fat mass has been shown to be preserved by endogenous synthesis. From observations made in patients with total LPL deficiency it can

also be concluded that fat deposition can take place in the absence of LPL. A further key enzyme catalyzing a rate-limiting step of lipolysis is hormone-sensitive lipase (HSL), which cleaves triacylglycerol to yield glycerol and fatty acids within adipocytes. Some fatty acids are re-esterified, so that the fatty acid-glycerol ratio leaving the cell is usually less than the theoretical 3:1. Increased concentrations of cAMP activate HSL as well as promote its movement from the cytosol to the lipid droplet surface. Catecholamines and glucagon are known

inducers of the lipolytic activity, whereas the stimulation of lipolysis is attenuated by adenosine and prostaglandin E₂. Interestingly, HSL deficiency leads to male sterility and adipocyte hypertrophy, but not to obesity, with an unaltered basal lipolytic activity suggesting that other lipases may also play a relevant role in fat mobilization.

The lipid droplets contained in adipocytes are coated by structural proteins, such as perilipin, that stabilize the single fat drops and prevent triglyceride hydrolysis in the basal state. The phosphorylation of perilipin following adrenergic stimulation or other hormonal inputs induces a structural change of the lipid droplet that allows the hydrolysis of triglycerides. After hormonal stimulation, HSL and perilipin are phosphorylated and HSL translocates to the lipid droplet. ALBP, also termed aP2, then binds to the N-terminal region of HSL, preventing fatty acid inhibition of the enzyme's hydrolytic activity.

Adipose tissue has been shown to contain 0.6–1.6 mg of cholesterol per gram wet weight. When expressed per unit of protein or organ mass, fat tissue contains more cholesterol than most other organs or membranes. The cholesterol content of adipose tissue increases with age and weight. The specific activity of adipose cholesterol exceeds that of plasma three- to five-fold. The half-life disappearance time of adipose tissue cholesterol is approximately 1 month, which is consistent with its function as a slowly turning over storage pool. The function of CETP is to promote the exchange of cholesterol esters and triglycerides between plasma lipoproteins. Fasting, high cholesterol diets as well as insulin stimulate CETP synthesis and secretion in WAT. In plasma CETP participates in the modulation of reverse cholesterol transport by facilitating the transfer of cholesterol esters from HDL to triglyceride-rich apoB containing lipoproteins. VLDLs, in particular, are converted to low-density lipoproteins (LDLs), which are subjected to hepatic clearance by the apoB/E receptor system. Adipose tissue probably represents one of the major sources of CETP in humans. In obesity the activity and protein mass of circulating CETP is increased showing a negative correlation with HDL concentrations at the same time as a positive correlation with fasting glycemia and insulinemia suggesting a potential link with insulin resistance.

Synthesis and secretion of RBP by adipocytes is induced by retinoic acid and shows that WAT plays an important role in retinoid storage and metabolism. In fact, RBP mRNA is one of the most abundant transcripts present in both rodent and human adipose tissue. Hepatic and renal tissues have been regarded as the main sites of RBP production, whereas the quantitative and physiological significance of the WAT contribution remains to be fully established.

The processes participating in controlling energy balance as well as the intermediary lipid and carbohydrate metabolism are intricately coupled by neurohumoral mediators. The coordination of the implicated molecular and biochemical pathways underlies, at least in part, the large number of intracellular and secreted proteins produced by WAT with autocrine, paracrine, and endocrine effects. The realization that WAT secretes a plethora of pleiotropic adipokines at the same time as expressing receptors for a huge range of compounds has led to the development of new insights into

the functions of adipose tissue at both the basic and clinical level. At this early juncture in the course of adipose tissue research, much has been discovered. However, much more remains to be learned about its physiology and clinical relevance. Given the adipocyte's versatile and ever-expanding list of secretory proteins, additional, and unexpected consequences are sure to emerge. The growth, cellular composition and gene expression pattern of adipose tissue is under the regulation of a large selection of central mechanisms and local effectors. The exact nature and control of this complex cross-talk has not been fully elucidated representing an exciting research topic.

See also: Appetite: Physiological and Neurobiological Aspects. Body Composition. Coronary Heart Disease: Lipid Theory. Cytokines: Nutritional Aspects. Diabetes Mellitus: Etiology and Epidemiology. Energy: Balance; Metabolism. Fatty Acids: Metabolism. Glucose: Metabolism and Maintenance of Blood Glucose Level. Growth and Development: Physiological Aspects. Nutrition Transition, Diet Change, and its Implications. Obesity: Childhood Obesity; Complications; Definition, Etiology, and Assessment; Prevention; Treatment. Physical Activity: Beneficial Effects. Starvation and Fasting: Biochemical Aspects. Weight Management: Approaches; Weight Cycling/Weight Change; Weight Maintenance

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ADOLESCENTS

Contents

Nutritional Problems of Adolescents

Requirements for Growth and Optimal Health

Nutritional Problems of Adolescents

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Introduction: Normal Adolescent Growth and Diet

Adolescence is a time of profound biological and psychosocial change. During the period of life from 10 to 21 years, adolescents experience rapid growth, with half of eventual adult weight and most of peak bone mass accumulated during this time. This dramatic physical growth increases an adolescent's energy, protein, vitamin, and mineral needs. Given the struggle for independence that often occurs as part of adolescent cognitive and social development, adolescent diets are often characterized by health-compromising eating patterns such as skipping meals, dieting inappropriately, and relying on sugar-sweetened beverages, fast foods, and energy-dense snacks which put them at increased risk for eating disorders, obesity, and chronic diseases such as type II diabetes, metabolic syndrome, and heart disease. Adolescence is therefore a critical period in the life course for both nutrition education and intervention to establish healthy eating patterns and reduce disease risk. Important nutritional problems of adolescents include obesity, eating disorders, anemia, and bone health.

Obesity

Obesity, which results from dietary energy intake exceeding metabolic basal needs and activity, has become the most common disease in adolescence. In fact, the prevalence of pediatric obesity in the US has tripled over the past three decades from 4% to 5% in the 1960s to 16.9% in 2008 according to the most recent National Health and Nutrition Examination Survey (NHANES). In adolescents, the prevalence of obesity is even higher at 18.1%, with 12.6% of adolescents meeting the adult definition of obesity (body mass index (BMI) $>30 \text{ kg m}^{-2}$). There is every indication that pediatric obesity is becoming a problem globally, as a recent review of published studies in child overweight and obesity between 1980 and 2005 suggested that pediatric overweight and obesity have increased in almost all countries for which data are available. Further, estimates from the International

Obesity Task Force (IOTF) suggest that 10% of the world's school-age children are overweight and that at least a quarter of these children are obese. Pediatric obesity is no longer a problem unique to the Western world, as prevalence is even increasing in many developing countries including China and India, and overtaking undernutrition as the major nutritional problem.

Although obesity affects children in all socioeconomic classes, it is more prevalent in those of lower socioeconomic status in the US and developed countries. This suggests that food insecurity and poor food choices are the bigger problems than food unavailability because of poverty. Although only 30% of obesity begins in adolescence, some estimate that up to 80% of obese adolescents will become obese adults, and obese adolescents are at much more risk for diabetes and major medical complications later in life. Because long-term weight loss is usually very difficult and often unsuccessful despite widespread attempts at dieting, efforts to prevent obesity in early life are important.

At the most basic level, obesity arises from an imbalance between calories consumed and those expended. For example, a small increase in dietary intake of 200 calories per day without a corresponding increase in activity could theoretically result in a weight gain of 8 kg over the course of a year. Obesity is rarely due to some identifiable disorder of basal metabolic expenditure such as hypothyroidism. The etiology of obesity is likely more complex arising from a combination of genetics and environmental exposures. Although the heritability of obesity has been estimated to be on the order of 60–80% on the basis of twin studies and family histories, the genetics of obesity are complex and just beginning to be understood. Adult weight is much more reflective of biological parents rather than adoptive parents in twin studies. Known genetic syndromes producing obesity in humans are rare (on the order of 1–2% of obese patients) but should be considered, such as trisomy 21 (Down syndrome), Prader–Willi, Bardet–Biedl, Beckwith–Wiedemann, hypothyroidism, and polycystic ovary syndrome. Therefore, when considering the causes of obesity at all stages of the life course, providers must

consider environmental exposures such as dietary patterns, portion sizes, physical inactivity, and screen time.

Obesity is characterized by an excess amount of body fat. The adipose fat cell is not only a passive storage site but also an endocrinologically active secretor of many substances like leptin, adiponectin, and cytokines which participate in an inflammatory response and may mediate a host of adverse consequences, including insulin resistance and diabetes. Therefore, obesity is related to an increased risk of developing several comorbidities including type II insulin-resistance diabetes mellitus, hyperlipidemia, heart disease, obstructive sleep apnea, asthma and other respiratory problems, back pain and orthopedic problems, fatty liver (nonalcoholic steato-hepatitis (NASH)), gallstones, and depression. The increasing incidence of type II diabetes in obese adolescents is already being noticed, with estimates of 200 000 diabetics under the age of 20 in the US predicted to rise to a lifetime risk of developing diabetes of 33–39% for those born in the year 2000.

Body fat can be measured using numerous methods including waist size, mid-arm circumference, and triceps skin-fold thickness, as well as other more expensive research methods such as underwater weighing, bioelectrical impedance, and dual-energy X-ray absorptiometry (DEXA). Although some of these measures are very accurate, they have limited use in large population studies and the clinical setting due to their lack of availability, invasiveness, and expense. Therefore, BMI, defined as weight (in kilograms) divided by height (in meters) squared, is the most commonly used surrogate measure for determining body fat and identifying obesity. BMI is an imperfect measure of excess adiposity, as it does not distinguish between excess body fat and lean mass; however, it is useful in identifying obesity, as the majority of individuals with high BMI have excess body fat. To identify obesity in children and adolescents in the US, BMI is compared with sex- and age-specific reference values using the Center for Disease Control and Prevention (CDC) BMI-for-age growth charts. Using these BMI charts, physicians can identify overweight (>85th to <95th percentiles) and obesity (>95th percentile) and track BMI standards for adolescents. It should be noted that the rapid increase in obesity prevalence has made standards based on population percentiles meaningless as the physiological side effects of obesity are present in more than just the top 5% of weight-for-age graphs. Instead of just relying on cross-sectional height- and weight-for-age graphs, there is a need for a more valid indicator of obesity (Figures 1 and 2).

Physical examination should include blood pressure measurement because of the high percentage of comorbidity associated with the metabolic syndrome (obesity, hypertension, dyslipidemia, or diabetes). The metabolic syndrome is defined as three or more of the following: abdominal obesity (waist circumference greater than 40 inches in men or 35 inches in women), fasting hypertriglyceridemia (<150 mg dl⁻¹), high fasting glucose greater than 110 mg dl⁻¹, low high-density cholesterol (<40 mg dl⁻¹), and high blood pressure (>135/85 mmHg). So far, it is mostly seen in later life (>40% of those over 60), but is increasingly seen at younger ages (7% of 20–29 years old). Acanthosis nigricans is a skin hyperpigmentation especially around the neck seen in approximately 20% of obese patients, especially

African-Americans, which reflects insulin resistance, and this finding should provoke screening tests for type 2 diabetes. Laboratory screening tests might include thyroid stimulating hormone for hypothyroidism, fasting glucose, insulin, and glycosylated hemoglobin (HbA1C) for type 2 diabetes (Figures 3 and 4).

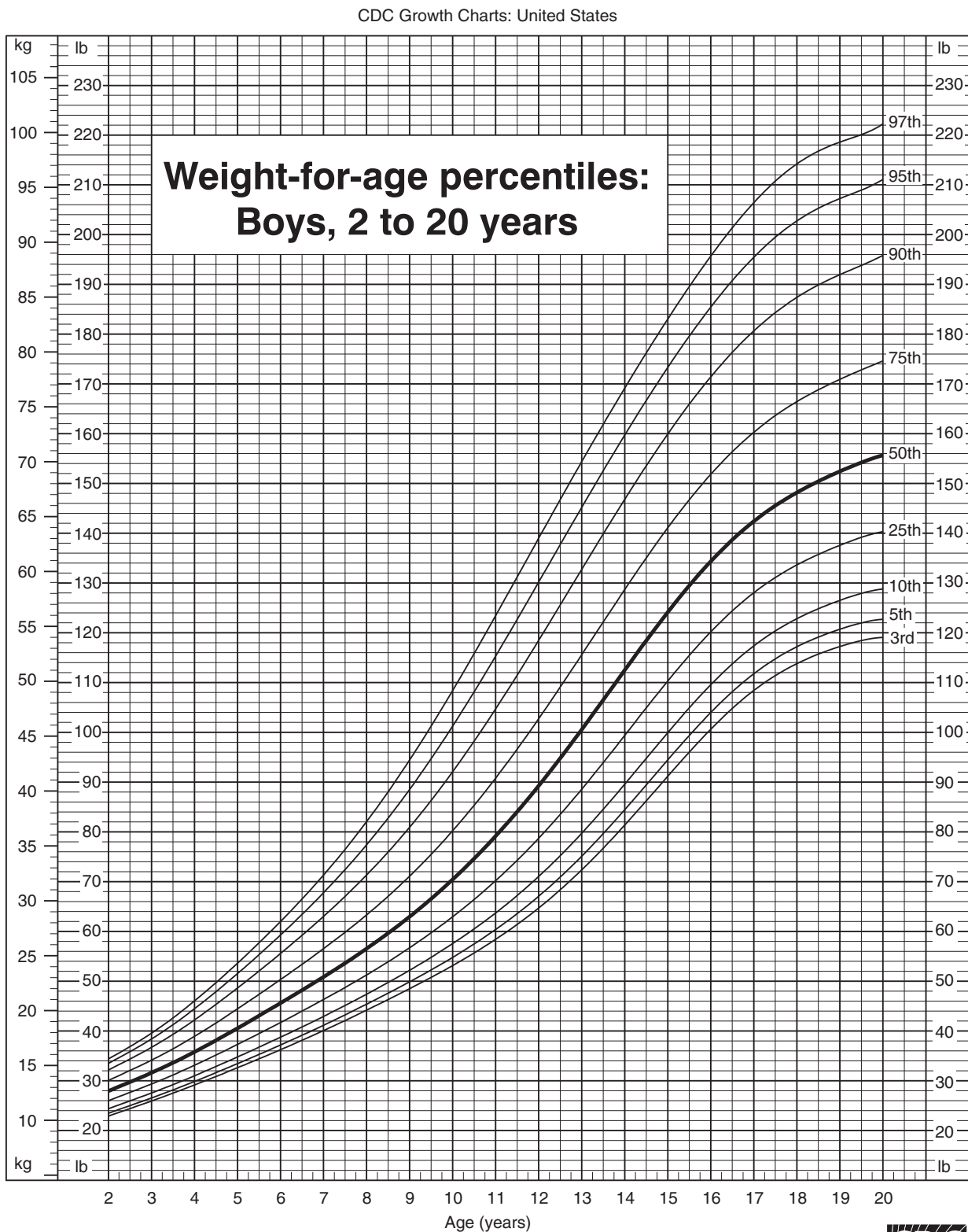
Treatment should ideally involve a multidisciplinary team with a dietician, social worker, physical therapist, and physician, concentrating on lifestyle modification, moderate caloric restriction, and regular exercise, with frequent follow-up and compliance a good indicator of likelihood of success. Diet histories and diet recalls are particularly important in nutritional assessments, but quantitative calorie counts are often unreliable in obese patients because of widespread conscious and subconscious underreporting of 20% or more. Regular meetings with a dietician should involve counseling on healthy eating choices including avoidance of sugar-sweetened beverages, increased fruit and vegetable consumption, and decreasing portion size. Interview of activity should include hours of television watching per day or per week because this is well correlated with obesity, not only because of decreased activity but also because of commercial snack food advertising.

Recent success with low-carbohydrate diets rather than the traditional low-fat diet advice suggests the importance of the role of satiety in maintaining caloric restriction. Most commercial diet plans promise short-term weight loss, but very few long-term studies have shown much widespread success with keeping weight off for more than 6–12 months. As adolescents naturally gain weight with height as they progress through puberty, it is probably more important that they learn healthy eating and activity habits over the long term rather than losing weight quickly only to gain it back within a few months.

Medications such as phentermine-fenfluramine and stimulants have gained recent notoriety with unforeseen side effects. Possible treatment with leptin and other hormones or antagonists has much future promise, but so far has been effective only in rare patients with specific defects. Surgical gastroplasty has proven to be the most successful long-term therapy for massively obese adults, possibly because of suppression of ghrelin, increased satiety, and reduced hunger, but morbidity and mortality are variable and the option of major surgery should be carefully considered only as a last resort before offering it to adolescents.

Eating Disorders

Eating disorders affect 3–5 million in the US, or as many as 11% of high school students, because 86% are diagnosed before age 20. More than 90% are female, 95% Caucasian, and 75% have an onset in adolescence. Eating disorders are probably the most frequent causes of undernutrition in adolescents in developed countries, but only a relatively small percentage meet the full *Diagnostic and Statistical Manual* (DSM) IV criteria for anorexia nervosa, more often falling into the more general category eating disorder not otherwise specified (EDNOS) (see Table 1). Anorexia nervosa is characterized by weight loss or failure to gain weight during a period of growth, leading to a weight that is <85% of expected weight



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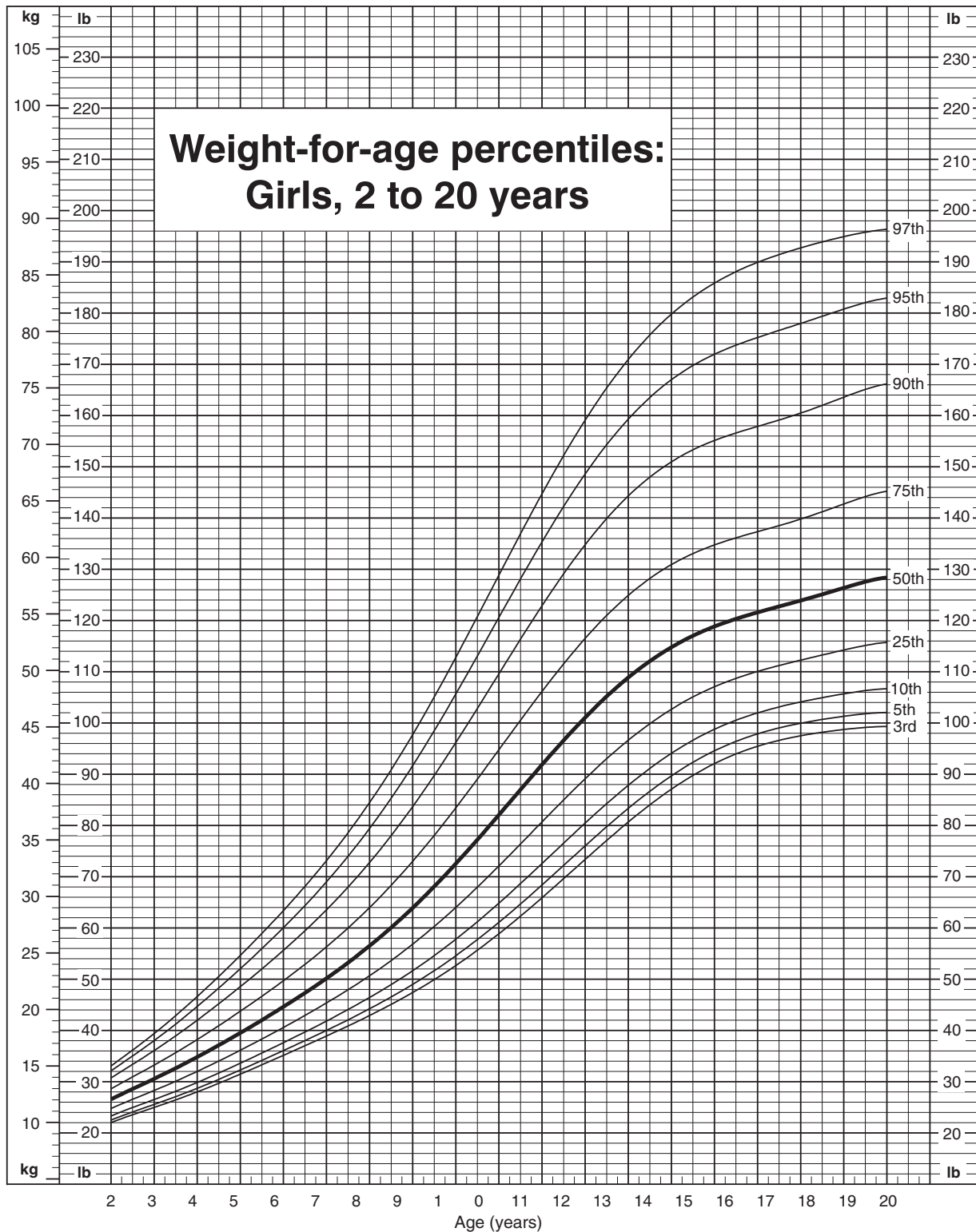
Source: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).



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Figure 1 Weight-for-age percentiles: Boys, 2–20 years.

CDC Growth Charts: United States



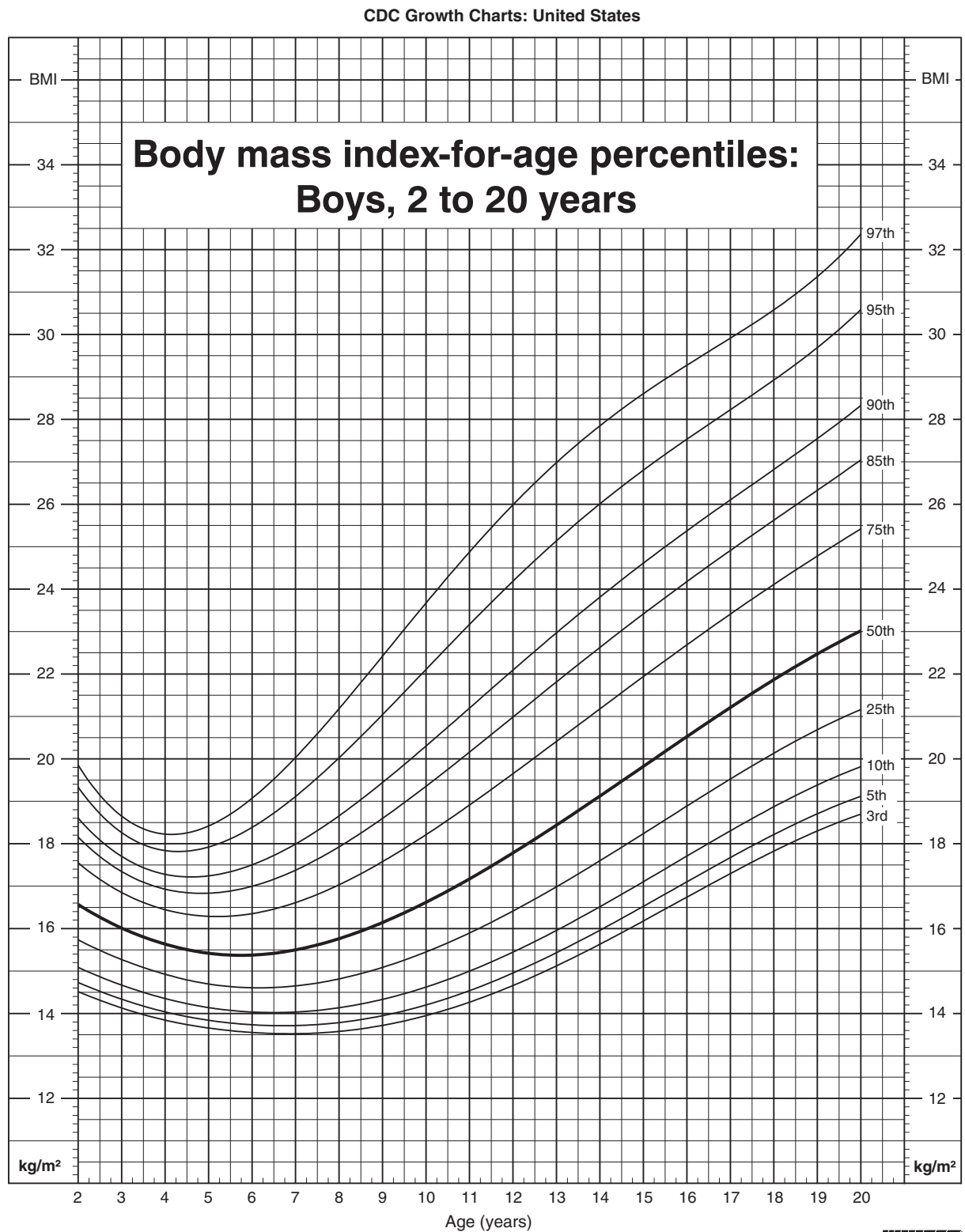
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Source: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).



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Figure 2 Weight-for-age percentiles: Girls, 2–20 years.



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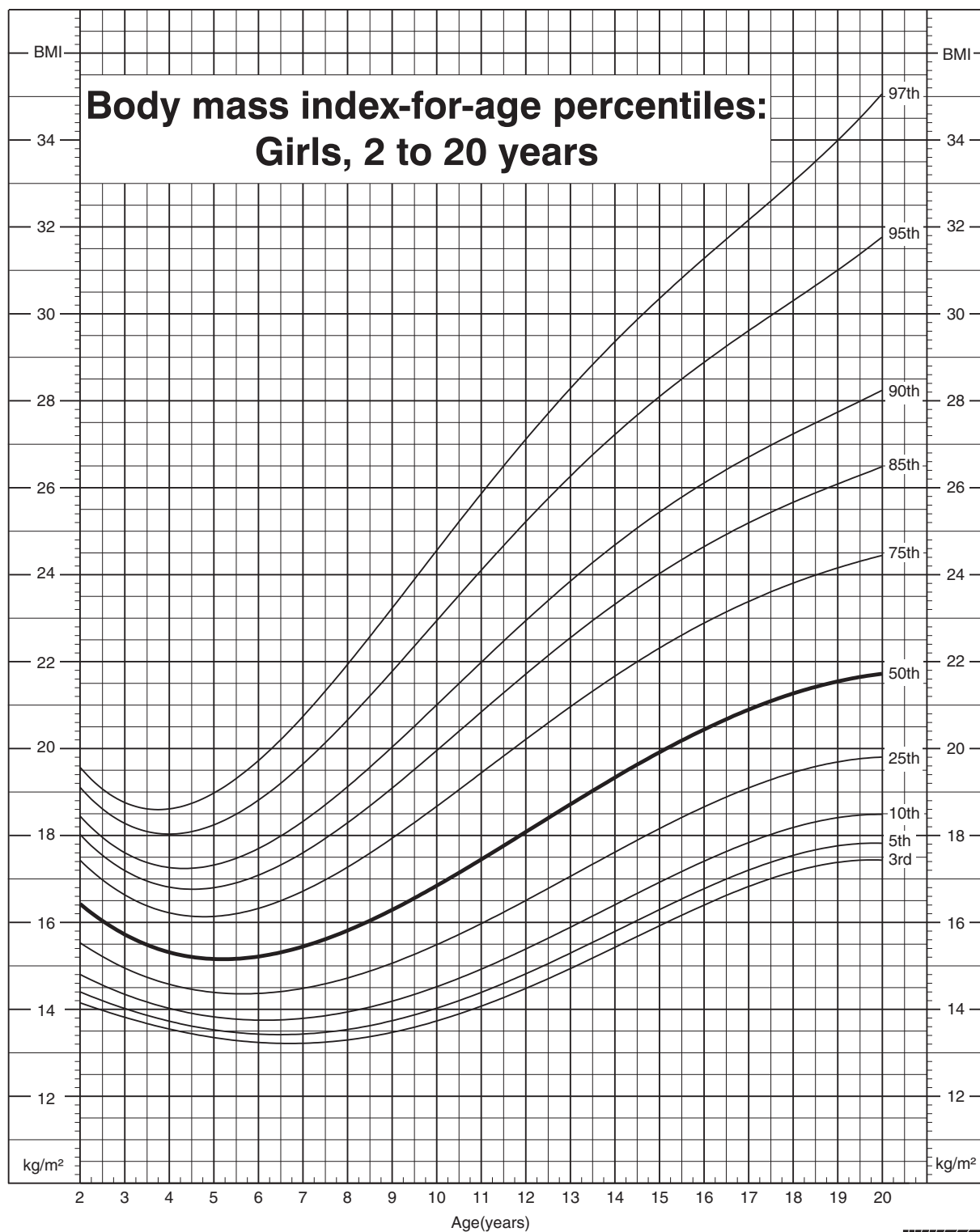
Source: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).



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Figure 3 Body mass index-for-age percentiles: Boys, 2–20 years.

CDC Growth Charts: United States



Published May 30, 2000.

Source: Developed by the National Center for Health Statistics in collaboration with the National Center for Chronic Disease Prevention and Health Promotion (2000).



Figure 4 Body mass index-for-age percentiles: Girls, 2–20 years.

Table 1 DSM IV-TR Criteria*DSM IV-TR Criteria for Anorexia Nervosa Criteria***Criteria**

Refusal to maintain body weight at or above a minimally normal weight for age and height: Weight loss leading to maintenance of body weight <85% of that expected or failure to make expected weight gain during period of growth, leading to body weight less than 85% of that expected.

Intense fear of gaining weight or becoming fat, even though under weight.

Disturbance in the way one's body weight or shape are experienced, undue influence of body weight or shape on self evaluation, or denial of the seriousness of the current low body weight.

Amenorrhea (at least three consecutive cycles) in postmenarchal girls and women.

Amenorrhea is defined as periods occurring only following hormone (e.g., estrogen) administration.

Type

Restricting type: During the current episode of anorexia nervosa, the person has not regularly engaged in binge-eating or purging behavior (self-induced vomiting or misuse of laxatives, diuretics, or enemas).

Binge-eating-purging type: During the current episode of anorexia nervosa, the person has regularly engaged in binge-eating or purging behavior (self-induced vomiting or the misuse of laxatives, diuretics, or enemas).

DSM IV-TR Criteria for Eating Disorder Not Otherwise Specified

Eating disorder not otherwise specified includes disorders of eating that do not meet the criteria for any specific eating disorder.

1. For female patients, all of the criteria for anorexia nervosa are met except that the patient has regular menses.
2. All of the criteria for anorexia nervosa are met except that, despite significant weight loss, the patient's current weight is in the normal range.
3. All of the criteria for bulimia nervosa are met except that the binge eating and inappropriate compensatory mechanisms occur less than twice a week or for less than 3 months.
4. The patient has normal body weight and regularly uses inappropriate compensatory behavior after eating small amounts of food (e.g., self-induced vomiting after consuming two cookies).
5. Repeatedly chewing and spitting out, but not swallowing, large amounts of food.

for weight and age, an intense fear of weight gain, distorted body image, and loss of at least three consecutive menstrual cycles. Bulimia, which is characterized by recurrent episodes of binge eating along with compensatory behaviors such as purging, use of laxatives, or excessive exercise, is more common than anorexia nervosa, with some estimates of up to 20–30% of college women in the US, and often occur surreptitiously without telltale weight loss. Lifetime prevalence estimates range from 0.5% to 3% for anorexia nervosa and 1% to 19% for bulimia. So far, eating disorders are considered rare in developing countries, but prevalence often increases dramatically when Western influences such as television advertising are introduced, as was the experience in the South Pacific Islands.

The pathophysiology of anorexia nervosa is not well understood, and there is probably a combination of environmental and psychological factors with a biochemical imbalance of neurotransmitters, especially serotonin and its precursor 5-hydroxyindole acetic acid which tends to be reduced. There is a substantial biologic predisposition to run in families with heritability in twin studies of between 35% and 90%.

Eating disorders should be suspected in any adolescent below normal weight ranges or with recent weight loss, but other medical conditions such as intestinal malabsorption, inflammatory bowel disease, and malignancy should also be considered. It is important to realize that most height and weight charts represent cross-sectional population norms, which may not be as sensitive as longitudinal tracking or height velocity of individuals, because puberty occurs at different ages. For example, a 12-year-old who does not gain weight for 6 months may just be entering puberty, or might be severely affected by growth failure due to a malignancy or inflammatory bowel disease.

Physical signs and symptoms of inadequate caloric intake may include amenorrhea, cold hands and feet, dry skin and hair, constipation, headaches, fainting, dizziness, lethargy, hypothermia, bradycardia, orthostatic hypotension, and edema. There is no specific laboratory diagnosis, but there are often endocrine and electrolyte abnormalities especially hypokalemia, hypophosphatemia, and hypochloremic metabolic alkalosis from vomiting, which may require careful supplementation.

Treatment may be very difficult and prolonged, often involving family-based therapy and adolescent-focused behavior therapy. Outpatient treatment teams should include a medical provider, a mental health provider, and a registered dietitian. Occasionally, long inpatient stays in a locked unit are required to maintain weight. With severe anorexia and accompanying malnutrition, there is a high risk of refeeding syndrome with edema, possible arrhythmias and sudden death from electrolyte abnormalities, so protocols have been developed to provide a slow increase of calories, supplemented by adequate amounts of phosphorus and potassium. The anorexic patient's persistent distorted view of body image reality is very resistant to casual counseling.

The consequences of anorexia nervosa can be quite severe and include menstrual dysfunction, cardiovascular disease, arrhythmias, anemia, liver disease, swollen joints, endocrinopathies, cerebral atrophy, and even sudden death. There is a significant bone loss or osteopenia associated with amenorrhea and lack of estrogen stimulation, which is not completely reversed even with hormone replacement. Anorexia nervosa is also associated with other psychiatric diagnoses such as depression, anxiety, personality disorders, obsessive compulsive disorder, and substance abuse, and psychiatric problems often continue to remain an issue even when normal weight is

maintained. Prognosis is relatively poor compared to other adolescent medical illnesses, with 33% persistence at 5 years and 17% at 11 years. Six percent died within 5 years and 8.3% by 11 years.

Other Nutritional Diseases

In many countries of the world, HIV infection and acquired immunodeficiency syndrome (AIDS) has become one of the leading causes of undernutrition and cachexia, especially in younger patients. Indeed, many of the syndromes and consequences of protein-energy malnutrition are also seen in AIDS cachexia, such as frequent respiratory and other infections, diarrhea, malabsorption, and rashes. Weight loss is an AIDS-defining symptom, and weight loss of one-third of usual weight usually signifies terminal illness. Fortunately, new generations of antiretroviral and other medications have dramatically slowed the progression of HIV infection in many patients, as well as reducing the vertical transmission rate. Proper attention to nutrition, with early enteral energy and micronutrient supplementation, is an important part of care, which is best instituted long before weight loss becomes manifest.

Specific Nutrient Requirements

The Institute of Medicine periodically convenes several committees of nutrition scientists to review the scientific literature and recommend levels of daily dietary nutrients that would keep 95% of the population from developing deficiencies. Recent editions of Dietary Reference Intakes (DRIs) have also specified Estimated Average Requirements (EARs), Adequate Intakes (AIs), and upper limits (ULs).

Calcium

Calcium is a major component of bone, providing structural skeletal support to the human body. The approximately 2–3 kg of bone calcium in each person also provides a storage reservoir for the small percentage of ionized calcium that allows muscle to contract, nerves to communicate, enzymes to function, and cells to react. The body has developed several hormonal mechanisms, including vitamin D, parathyroid hormone, and calcitonin, to protect the small amount of ionized calcium in the blood from changing drastically. Tight control of blood calcium levels is needed because unduly low blood calcium might result in uncontrolled tetanic muscle contractions and seizures, whereas high blood calcium levels may cause kidney stones and muscle calcifications. To increase blood calcium levels, vitamin D and its metabolites increase calcium absorption from the intestinal tract, parathyroid hormone increases calcium reabsorption from the kidney, and both increase resorption of calcium from the bone.

During the early years of life, calcium is deposited in the bone as it grows, but after about the third decade, there is a steady decline in bone calcium. This is especially marked after menopause in women, when estrogen declines, and often

leads to bone loss (osteopenia) to below a threshold that predisposes women in particular to fractures (osteoporosis). Osteoporosis is not just a disease of the elderly, and may occur in much younger patients, especially athletic young women, those with anorexia nervosa, those on steroids and other medications, and in anyone on prolonged bed rest, including astronauts experiencing long periods of weightlessness.

Dietary calcium is often seen as the most limiting factor in the development of peak bone mass, and strategies to increase dietary calcium have been promoted. Other factors in the development of bone mineral include height, weight, racial background and inheritance, gender, activity, vitamin D deficiency, parathyroid hormone deficiency, vitamin A, vitamin K, growth hormone, phosphorus, and magnesium. Phosphorus, the other major component of bone mineral, is relatively common in the diet.

In the 2010 DRIs, it is recommended that 9- to 18-year-olds take in 1300 mg of calcium per day.

Only a small percentage of the population takes in the RDA for calcium. The estimated average calcium intake in American women is only approximately 500–600 mg a day, and is even much lower in developing world, as low as 200 mg a day. From calcium tracer studies performed since the 1950s, intestinal calcium absorption ranges from 10% to 40% of ingested calcium, with a higher percentage absorption with lower calcium intakes. A large percentage (usually 70–80%) of dietary calcium is from milk and dairy products, which provides approximately 250 mg calcium per 8 ounce glass of milk, and most studies show better absorption from dairy products than from vegetable sources. However, many people, especially non-Caucasians, develop relative lactose intolerance after childhood, and are reluctant to increase their dairy food intake.

Thus, attention has focused on whether supplementation or fortification of calcium especially during adolescence will ensure achievement of peak bone mass. Calcium supplementation in adolescent females has shown short-term increases in bone mineral density, but this may be because it increases mineralization in a limited amount of trabecular bone, and it remains to be seen whether this leads to long-term improvement or protection against future fractures. Further, most studies still assume that increased bone mineral density is synonymous with reduced fracture risk, although fractures may depend on many other factors such as optimal bone architecture and lack of falls. Although the majority of scientific opinion probably favors increased dietary calcium intake in adolescence, the factors that control bone mineralization are not yet completely understood, and long-term protection against eventual bone loss and fractures remains to be demonstrated by randomized clinical trials. Recent studies have raised the possibility that calcium supplementation (in the form of tablets) may be associated with an increased risk of heart disease, and raise the question of whether it is better to increase dietary calcium intake via foods rather than through the use of calcium supplements.

Iron

The 2004 DRIs recommend that males and females aged 9–13 years consume 8 mg of iron per day. For males aged 14–18,

the iron requirement is 11 mg of iron per day, and for females aged 14–18 who have reached menarche, the requirement is 15 mg day⁻¹. Iron deficiency is one of the most common vitamin or mineral deficiencies in the world, affecting 20% or more of women and children especially in developing countries. Adolescent women who have started menses or who are pregnant are particularly at risk for developing iron deficiency. Iron deficiency may be present before the appearance of typical hematological indicators of ferropenic anemia. Anemia (low hemoglobin or red cell volume) may lead to reduced school and work performance and may affect cognitive function, as well as leading to cardiovascular and growth problems. Diagnosis is made most simply by hemoglobin level or packed red cell volume (hematocrit) and red cell morphology, or alternatively by transferrin saturation, serum ferritin, or serum iron level. Microscopic examination of a red cell smear typically shows red cells that are small (microcytic) and pale (hypochromic).

Folate

Folate is a vitamin that is responsible for one-carbon methyl transfer in a variety of cellular reactions, including formation of purines and pyrimidines which make up DNA and RNA. Folate deficiency may result in megaloblastic anemia, as forming red cells fail to divide. As of 1998, all enriched grain products in the United States are fortified with folic acid, the synthetic form of folate. Therefore, although folate nutrition may be marginal in adolescents given that the best source is green leafy vegetables, folate deficiency is now rare as a result of fortification. Epidemiologic evidence suggests that folic acid supplementation early in pregnancy, at levels that are higher than usual dietary intake (200–400 mcg d⁻¹) reduced the incidence of neural tube defects (anencephaly and spina bifida) in newborns. Supplementation needs to be started early in pregnancy, within the first 8 weeks and before most pregnancies are apparent, so should involve most women of childbearing age. The decision to fortify grains and cereals with folic acid in the US will also reduce serum homocysteine levels, lowering the risk of cardiovascular disease.

Zinc and Other Minerals

Zinc is a component of many metalloenzymes including those needed for growth, pancreatic enzymes, and intestinal secretions. Overt zinc deficiency is uncommon in children and adolescents, but several studies over the past decade have

indicated that inadequate zinc intake may be widespread in children from low-income countries. These studies indicate that marginal zinc intake may result in increased susceptibility to respiratory and gastrointestinal infections, and delayed growth. Zinc deficiency can lead to impaired taste (hypogeusia) and appetite.

In spite of fortification programs, iodine deficiency continues to be a significant public health problem in many countries, particularly in the highlands of Southeast Asia. Iodine deficiency results in hypothyroidism and goiter. During gestation, iodine deficiency has devastating and irreversible effects on the central nervous system of the fetus.

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Requirements for Growth and Optimal Health

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Abbreviations

BMI Body mass index
EAR Estimated average requirement

NMES Nonmilk extrinsic sugars
NSP Nonstarch polysaccharide
RNI Reference nutrient intake

Glossary

Cross-sectional study A study that provides a one-off observation of a population.

Growth percentile A way of comparing individual children to a reference group using percentages.

Longitudinal study A study that provides a series of observations of a population, or follows a specific cohort of individuals for a defined period of time.

Osteoporosis A progressive disease which causes bone loss, leading to fractures.

Reference nutrient intake A nutrient recommendation which would be expected to meet the needs of 97.3% of the population.

Underreporting Self-reporting of food intake that deliberately or unconsciously underestimates food, energy or nutrient intakes.

Introduction

Adolescence is the period of transition between childhood and adulthood. This reflects not only the physical and emotional changes experienced by the adolescent, but also the development of dietary behaviors. Although younger children tend to resist new foods (i.e., neophobia), adolescents may use food to assert their independence, but not always in a beneficial way. This section covers development during adolescence and highlight nutrients that are important during this time. Information on adolescent energy and nutrient intakes from a broad range of countries is presented. The findings are put in context with dietary recommendations.

Physical Changes During Adolescence

Adolescence is generally assumed to be the period of human development from 10 to 18 years, a time during which rapid growth and physical maturity take place.

Growth

During prepubescent childhood, the growth of boys and girls follows a similar trajectory, although boys may be slightly taller and heavier than girls. The pubertal growth spurt begins around the ninth year in girls, lasting up to 3½ years, with boys starting their growth spurt approximately 2 years later. This means that girls can reach their full height 2 years before boys do. UK standards for height and weight during adolescence are presented in Table 1. International reference

growth standards are developed by the World Health Organization (WHO). Maximum height velocity is generally seen in the year preceding menarche for girls and at around 14 years of age for boys. On average, weight velocity peaks at 12.9 years of age for girls and 14.3 years of age for boys. Annual growth rates during adolescence can be as much as 9 cm/8.8 kg in girls and 10.3 cm/9.8 kg in boys. It is not fully known when growth ceases. Certainly, height gains of up to 2 cm can still occur between 17 and 28 years of age.

Energy and protein intakes per kilogram body weight have been observed to peak during maximal growth, suggesting increased requirements during adolescence. Undernutrition in this crucial window of development can result in a slow height increment, lower peak bone mass, and delayed puberty. Overnutrition is not without its risks. It is believed that obesity in young girls can bring about an early menarche, which then increases the risk of breast cancer in adulthood. Menarche is deemed precocious if it occurs before the age of 8 years. Rising childhood obesity levels in Western countries have resulted in more girls experiencing precocious menarche.

Important nutrients for growth include protein, iron, calcium, vitamin C, vitamin D, and zinc. Calcium, in particular, has a key role in bone development, and huge increments in bone density are seen during adolescence under the influence of sex hormones. Bone density peaks in the early twenties and a low bone density at this time is related to an increased risk of osteoporosis in later life, especially for women. Studies have suggested that body mass index (BMI) in adolescence is the best predictor of adult bone density, explaining why children who experience anorexia nervosa are likely to have a higher risk of osteoporosis.

Table 1 Percentiles for height, weight and body mass index

<i>(a) Boys</i>						
Age (years)	Height (cm)		Weight (kg)		Body mass index	
	2nd	50th	98th	2nd	50th	98th
11	130.1	143.4	156.7	25.8	34.6	52.6
16	158.2	173.4	188.6	44.5	60.6	85.7
18	163.1	177.1	191.1	51.7	68.8	90.3
<i>(b) Girls</i>						
Age (years)	Height (cm)		Weight (kg)	Body mass index		
	2nd	50th		2nd	50th	98th
11	130.3	144.1	157.9	14.02	17.48	23.88
16	151	163.2	175.4	16.37	20.44	27.76
18	151.5	163.6	175.6	16.99	21.19	28.62

For more information, contact the Child Growth Foundation: childgrowthfoundation.org.

Adipose Stores

There are few differences in body fat between boys and girls in the prepubertal stage. However, during puberty, girls develop adipose tissue at a greater rate than boys, laying down stores in the breast and hip regions. The pattern for boys is rather different and tends toward a more central deposition. Methods for estimating fatness in adolescents include weight for height, BMI (weight in kilograms/height in square metres), skinfold thickness measures, bioelectrical impedance analysis, densitometry, magnetic resonance imaging, dual-energy X-ray absorptiometry, and computed tomography. Waist circumference is gaining popularity as a useful proxy of fatness in the field. Many researchers argue that it is a better predictor than BMI of the central adipose stores that place the individual most at risk from later obesity, diabetes, and coronary heart disease.

UK standards for BMI and waist circumference are shown in [Table 1](#). As adolescents are still growing, it is important to use age- and sex-appropriate appropriate standards to assess over- and underweight. The 85th percentile of BMI is often used as the lower cutoff point for classification of overweight, whereas the 5th is taken as an upper cutoff for underweight. Some surveys have suggested that adult obesity risk can be tracked from childhood, citing BMI at adolescence as a strong predictor of adult obesity. However, this information should be used with caution in practice because adolescents have not yet reached their full height and may still revert to a normal BMI without dietary intervention.

Sexual Development

In girls, the onset of menarche at around 13 years is triggered by the attainment of a specific level of body fat, with taller, heavier girls more likely to experience an early menarche. Vigorous exercise, for example, gymnastics and endurance running, can delay the menarche, both due to the physiological effects of regular training and the depletion of body fat. Iron becomes more important for girls as menstrual periods become regular and heavier, and there is good evidence that the iron status of many teenage girls is inadequate. Low iron status is due to a combination of higher requirements (i.e., menstrual periods and growth) and poor nutritional practices, such as dieting, missing breakfast; and avoiding red meat.

Dietary Recommendations

In general, each country has its own nutritional recommendations for adolescents, which are developed by expert bodies using a combination of deficiency studies and extrapolations from adult studies. In the UK, US, and Canada, guidelines have evolved from a simple Recommended Dietary Intake to the more complex bell-shaped distribution with a mean representing the intake likely to satisfy the needs of 50% of the population. The upper extreme, at the 97.5th centile, represents the intake likely to meet the needs of the majority of the population, whereas the lower extreme, at the 2.5th centile, represents the lowest acceptable intake. Current UK Reference Nutrient Intakes (RNI), presented in [Table 2](#), cover a

Table 2 UK Dietary guidelines for adolescents

(a) Dietary Reference Values for macronutrients											
Age group	Sex	Energy (MJ)	Protein (g)	NSP (g)	Total fat% energy	Saturated fat% energy	Starch/intrinsic sugars% energy	NMES% energy			
11–14 yr	M	9.27	42.1	18	35	11	39	11			
	F	7.92	41.2	18	35	11	39	11			
15–18 yr	M	11.51	55.2	18	35	11	39	11			
	F	8.83	45.0	18	35	11	39	11			
(b) Reference Nutrient Intakes for vitamins and minerals											
Age group	Sex	Vitamin B ₂ (mg)	Vitamin B ₆ (mg)	Niacin (mg)	Vitamin B ₁₂ (mcg)	Folate (mcg)	Vitamin C (mg)	Vitamin A (mcg)	Calcium (mg)	Iron (mg)	Zinc (mg)
11–14 yr	M	0.9	1.2	15	1.2	200	35	600	1000	11.3	9.0
	F	0.7	1.1	12	1.0	200	35	600	800	14.8	9.0
15–18 yr	M	1.1	1.3	18	1.5	200	40	700	1000	11.3	9.5
	F	0.8	1.1	14	1.2	200	40	600	800	14.8	7.0

Key: y, years; MJ, megajoules; mg, milligrams; mcg, micrograms; %energy, percentage of food energy; NMES, nonmilk extrinsic sugars (similar to added sugars).

range of nutrients from fat and sugars to the main micro-nutrients. Dietary guidelines are an important reference point for nutrition scientists and dietitians, but it must also be borne in mind that they relate to the average needs of populations, rather than to the needs of individuals.

As well as numerical recommendations, many nations have adopted more descriptive or visual methods of promoting the ideal diet. This makes sense as recommended nutrient intakes are poorly understood by the public and need to be put into context by health professionals. Communication tools such as the plate model, pyramid system, food groups, and traffic light systems can help to get healthy eating messages across to adolescents.

Dietary Intakes

It is often assumed that most adolescents in Western countries have a nutritionally inadequate diet yet, despite reported low intakes of some micronutrients in surveys, there is little evidence of widespread clinical deficiencies, or indications that adolescents are failing to achieve appropriate heights and weights. Iron is the exception, where mean intakes are low and clinical markers suggest deficiency across several age groups, particularly in girls. There is also justifiable concern about the general healthiness of diets eaten by 'at risk' subgroups of adolescents such as dieters, smokers, vegans, and those who regularly consume alcohol.

Mean daily intakes of energy and selected micronutrients from a selection of major international surveys of adolescents are presented in Table 3. Caution should be taken when interpreting data from dietary surveys because underreporting can be a feature of these. Selective underreporting, often focused on energy-dense or high-fat foods, can partially explain low reported intakes of energy and certain micronutrients. It is also difficult to make comparisons between the data from different countries given the range of dietary assessment methods used. There is normally a trade-off between the sample size and methodology that sees the larger surveys favoring less precise methods such as 24 h recalls or food frequency questionnaires in order to make data collection more economical.

Energy, Protein, and Salt

Despite height and weight data that are consistent with expected results, mean energy intakes appear to be low when compared with dietary recommendations in many studies of adolescents, particularly in lower income groups. Although low energy intakes can be of concern in individuals, on a population level, this phenomenon may be due to a number of reasons. These include dieting, low physical activity levels, and underreporting, where subjects subconsciously or deliberately misreport dietary intakes. Popular sources of energy in the adolescent diet included cereal products (providing around one-third of energy), savory snacks, potatoes, meat/meat products, white bread, milk/dairy products, biscuits/cakes, spreading fats, and confectionery. Beverages (i.e., soft drinks, juices, and alcohol) provide a significant amount of

Table 3 Key international surveys of adolescent dietary intakes

Country	Date carried out	Sex (age, years)	Energy (MJ)	Energy (kcal)	Protein% en	CHO% en	Sugars (g)	Fat% en	Iron (mg)	Calcium (mg)	A (mcg)	B ₁ (mg)	B ₂ (mg)	B ₆ (mcg)	B ₁₂ (mcg)	Niacin (mg)	Folate (mcg)	Carbon (mg)
Australia	24 h DR 1995	M 12–15	11.59	2777	15.1	50.9	33.5	24.7	16.1	1093	1296	2.4	3.0	–	–	46.0	271	121
		M 16–18	13.53	3233	15.4	49.6	32.9	24.5	17.9	1280	1186	2.3	3.0	–	–	53.5	313	154
Austria	7DUR, 24 h DR 1991, 2002	F 12–15	8.53	2038	8.5	51.1	33.1	25.6	11.0	784	1130	1.5	2.0	–	–	33.4	206	124
		F 16–18	8.69	2076	8.7	50.1	32.1	24.0	11.1	801	877	1.5	1.8	–	–	35.3	217	126
		M 11–14	9.49	2268	13.2	48.2	–	35.2	13.0	903	–	1.4	1.6	1.5	5.7	–	229	113
		M 15–18	11.65	2784	12.9	50.0	–	37.2	15.4	1002	–	1.4	1.7	1.5	–	–	247	140
Belgium	3DUR, FFQ 1991, 1995	F 10–14	9.49	2268	12.6	49.4	–	35.8	10.2	834	–	1.1	1.4	1.3	5.0	–	217	132
		F 15–18	8.49	2029	12.7	49.5	–	33.5	13.4	784	–	1.0	1.2	1.2	4.0	–	201	99
		M 11–12	11.49	2746	11.6	–	–	–	–	–	–	–	–	–	–	–	–	–
		M 12–18	13.06	3122	13.0	48.6	149	37.2	13.4	913	–	1.5	1.7	1.6	–	–	–	83
Canada	24 h DR 2004	F 11–12	11.72	2802	11.6	–	–	–	–	–	–	1.0	–	–	–	–	–	–
		F 1–18	9.44	2256	14.9	48.8	112	36.7	8.2	805	–	1.2	1.3	1.2	–	–	–	78
		M 9–13	10.28	2467	14.6	54.5	–	31.0	16.5	1219	–	2.1	2.4	1.8	4.6	–	–	157
		M 14–18	12.08	2901	15.2	52.7	–	31.5	19.1	1300	–	2.4	2.7	2.2	5.5	–	–	163
Denmark	7DUR 1995	F 9–13	8.49	2037	14.0	55.5	–	30.5	13.5	993	–	1.7	2.0	1.5	3.5	–	–	146
		F 14–18	8.53	2048	14.4	54.3	–	30.9	13.1	917	–	1.6	1.9	1.5	3.3	–	–	147
		M 11–14	10.90	2605	–	51.0	–	35.0	–	1286	–	1.5	2.2	1.7	6.7	27.0	304	79
		M 15–18	12.15	2903	14.0	–	–	35.0	–	1362	–	1.5	2.3	–	7.1	30.0	295	80
Finland	48 h DR, 2007	F 11–14	8.70	2079	–	51.0	–	34.0	–	1061	–	1.1	1.7	1.4	5.1	23.0	238	72
		F 15–18	9.70	2318	14.0	–	–	34.0	–	1121	–	1.2	1.8	1.5	5.5	23.0	266	79
		M 13–14	8.3	1978	16.7	53.2	–	30.0	10	1273	524	1.3	2.2	2.1	5.3	28	203	87
		F 13–14	6.7	1602	16.0	54.1	–	29.8	8.9	1032	537	1.1	1.7	1.6	3.9	22	190	93
France	DH, 1DWR 1988, 1993–4	M 10–13	–	–	–	47.8	142.5	–	–	1250	–	1.2	2.1	1.7	11.0	–	–	88
		M 11–14	10.83	2587	15.4	–	–	36.5	12.6	835	–	1.4	1.8	1.8	5.6	–	253	91
		M 11–18	–	–	15.7	–	–	–	–	–	–	1.0	2.2	–	–	–	–	–
		M 13–18	12.10	2892	14.9	48.8	126.8	36.0	12.5	1300	–	1.4	–	2.0	7.0	–	–	127
Germany	DH, 3–d/7–d Recall, 1DWR 1985–95, 1998	F 10–13	–	–	–	47.7	113.3	–	–	1100	–	1.0	1.8	1.5	7.5	–	–	99
		F 11–14	8.84	2112	15.9	–	–	–	11.4	835	–	–	1.8	1.8	5.6	17.0	253	91
		F 11–18	–	–	16.1	–	–	–	–	–	–	1.3	–	–	–	–	–	–
		F 13–18	9.16	2188	16.1	45.7	98.2	–	10.4	1100	–	–	1.7	1.4	7.0	–	–	112
		M 10–12	9.08	2170	12.9	46.0	–	38.0	12.4	795	–	1.1	1.2	1.3	4.4	24.0	221	87
		M 13–14	10.41	2487	13.2	45.6	–	37.5	14.3	893	–	1.3	1.5	1.4	5.5	28.6	245	98
Germany	1985–95, 1998	M 15–18	11.14	2661	13.2	46.9	–	38.3	14.8	902	–	1.4	1.7	1.6	5.7	30.4	263	97
		F 10–12	7.78	1860	12.9	47.6	–	36.4	11.1	681	–	1.0	1.2	1.1	4.0	20.4	203	87

Greece 1DWR, 24 h DR 1993–4, 1999	F 13–14	8.49	2028	12.6	45.1	–	39.2	12.2	754	–	1.1	1.1	1.2	4.2	24.1	210	98
	F 15–18	8.59	2052	13.1	46.5	–	37.0	12.3	728	–	1.0	1.3	1.5	4.4	24.6	216	92
	M 10–11	–	–	15.7	44.0	–	–	11.0	963	–	–	–	–	–	–	–	119
	M 12–14	8.90	2126	15.6	45.0	97	40.0	13.5	1011	–	1.7	2.1	1.8	4.7	18.0	226	112
Ireland DH 1988	M 14–16	9.00	2151	15.0	47.2	–	–	13.8	871	–	2.5	2.0	1.9	4.3	19.2	251	123
	F 10–11	–	–	15.6	44.0	–	41.8	10.0	851	–	–	–	–	–	–	–	108
	F 12–14	9.70	2318	14.7	48.0	88	–	10.1	748	–	1.4	1.6	–	4.1	–	212	108
	F 14–16	7.08	1692	14.5	46.0	–	–	9.4	771	–	2.4	1.5	1.3	3.2	13.2	217	118
Japan UR n/a	M 11–14	11.3	2700	–	50.3	–	36.3	14.7	1208	–	1.8	2.5	2.2	4.9	40.2	246	76
	M 15–17	14.0	3346	14.2	49.3	–	36.0	19.3	1549	–	2.2	3.1	2.6	7.2	51.7	306	95
	F 11–14	9.10	2174	–	50.2	–	36.0	–	962	–	–	1.9	1.7	3.9	32.0	198	76
	F 12–15	–	–	–	–	–	–	12.4	–	–	1.4	–	–	–	–	–	–
Netherlands 2DUR 1997–8	F 15–17	8.90	2127	13.9	48.9	–	37.1	11.6	950	–	1.3	1.8	1.6	4.0	32.0	182	79
	M 15–19	10.6	2545	14.4	51.9	–	28.3	8.6	633	978	1.2	1.4	1.3	8.1	16.6	303	89
	F 15–19	8.0	1918	15.1	50.4	–	29.7	7.4	516	875	0.9	1.2	1.1	6.4	13.2	268	91
	M 13–16	10.9	2605	13.1	51.2	188	35.5	10.9	1045	778	1.2	1.6	1.6	3.9	–	–	79
New Zealand 24 h DR, FFQ 1997	M 16–19	11.6	2772	13.3	49.5	184	35.4	11.5	1095	972	1.3	1.6	1.8	4.4	–	–	71
	F 13–16	8.7	2079	13.7	50.3	146	35.9	9.0	904	724	1.0	1.4	1.3	3.4	–	–	81
	F 16–19	9.1	2175	13.4	50.3	152	35.5	9.9	908	754	1.2	1.4	1.4	3.4	–	–	81
	M 15–18	12.4	2963	15.0	49.0	82	35.0	15.2	957	505	1.8	2.1	1.8	4.9	43.0	280	155
Norway 1DWR, FFQ n/a	F 15–18	8.86	2117	14.0	51.0	69	34.0	10.4	783	342	1.3	1.5	1.1	3.2	28.0	203	120
	M 11–14	–	–	–	–	–	31.1	–	–	–	–	–	–	–	–	–	–
	M 13–14	15.0	3585	–	–	–	–	–	1625	–	2.1	2.8	–	–	–	–	110
	M 13–15	–	–	13.4	–	–	–	–	–	–	–	–	–	–	–	–	–
Portugal 24 h DR, 1995	F 11–14	10.9	2605	–	54.9	–	28.9	–	–	–	–	–	–	–	–	–	–
	F 13–14	–	–	–	–	–	–	–	1142	–	1.6	2.1	–	–	–	–	104
	F 13–15	–	–	13.7	–	–	–	–	–	–	–	–	–	–	–	–	–
	M 12–18	8.86	2117	–	49.1	–	–	–	890	–	–	–	–	–	–	–	–
Spain 24 h DR 1998–2000	M 13–17	9.41	2248	17.6	–	–	–	–	–	–	–	–	–	–	–	–	77
	F 12–18	9.40	2248	–	53.4	–	33.3	–	853	–	–	–	–	–	–	–	–
	F 13–17	8.14	1945	17.8	–	–	–	–	–	–	–	–	–	–	–	–	99
	M 10–13	9.63	2302	–	–	–	–	15.1	1010	521	1.5	1.9	1.9	8.3	24.8	162	70.0
Sweden 7DUR 1989–90, 1993–4	M 14–17	10.7	2565	–	–	–	–	16.6	1031	551	1.6	1.9	2.0	8.9	26.2	178	76.3
	F 10–13	8.15	1949	–	–	–	–	12.7	862	453	1.3	1.6	1.5	6.7	20.1	140	69.7
	F 14–17	8.28	1979	–	–	–	–	12.5	823	415	1.3	1.5	1.5	7.2	21.1	149	73.7
	M 13–14	–	–	–	–	–	–	17.4	1279	–	–	–	–	–	–	–	–
	M 14–16	8.90	2127	–	52.6	–	32.1	18.2	1406	–	1.8	2.4	2.0	6.6	33.5	178	68
	M 17–18	10.50	2509	14.7	–	–	–	–	1472	–	1.8	2.8	2.2	8.7	36	138	77

(Continued)

Table 3 Continued

Country	Date carried out	Sex (age, years)	Energy (MJ)	Energy (kcal)	Protein% en	CHO% en	Sugars (g)	Fat% en	Iron (mg)	Calcium (mg)	A (mcg)	B ₁ (mg)	B ₂ (mg)	B ₆ (mcg)	B ₁₂ (mcg)	Niacin (mg)	Folate (mcg)	Carbon (mg)
Switzerland	7DUR 1994–5	F 13–14	–	–	–	49.4	–	–	–	1061	–	–	–	–	–	–	–	–
		F 14–15	–	7.21	1722	–	–	–	13.4	1046	–	1.4	1.8	1.5	4.9	24.9	144	68
		F 17–18	–	7.88	1884	14.2	–	–	13.3	966	–	1.2	1.8	1.5	5.5	23.0	105	77
		M 11–12	–	–	–	13.3	–	–	–	–	–	–	–	–	–	–	–	–
		M 13–14	–	11.98	2863	–	–	–	16.0	1311	–	1.5	2.2	–	–	–	–	185
		M 15–18	–	12.56	3001	–	–	35.0	–	1157	–	1.3	1.8	–	–	–	–	163
Turkey	3DUR, 2004	F 11–12	–	–	–	49.4	–	–	–	–	–	–	–	–	–	–	–	–
		F 13–14	–	7.90	1887	–	–	37.4	9.3	819	–	–	1.3	–	–	–	–	110
		F 15–18	–	8.12	1939	–	–	35.8	–	832	–	1.5	1.3	–	–	–	–	146
		M 12–14	–	8.210	1961	16.8	–	34.3	11.2	715	428	0.8	1.4	1.2	–	–	121 ^a	86.3
		M 15–17	–	8.880	2122	17.6	–	33.3	12.2	732	488	0.8	1.5	1.2	–	–	130 ^a	76.1
		F 12–14	–	6.960	1664	15.2	–	34.4	9.6	632	430	0.7	1.1	1.2	–	–	218 ^a	85.0
USA	24 h DR 2007–8	F 15–17	–	6.530	1560	15.4	–	34.8	8.8	600	409	0.6	1.1	0.9	–	–	206 ^a	69.3
		M 12–19	–	60.6	2424	15	152	33.0	16.6	1173	680	1.9	2.6	2.3	6.7	28.9	198 ^b	86.6
		F 12–19	–	7.75	1861	14	54	33.0	13.8	878	528	1.5	1.8	1.6	4.1	20.8	154 ^b	73.8

^aFolic acid data only.^bFolate from food sources only.

Key: CHO, carbohydrate; DR, Dietary recall; DH, Diet history; FFQ, Food frequency questionnaire; UR, Unweighed record; WR, Weighed record.

Vitamin A = micrograms retinol equivalent.

Dates refer to when the surveys were carried out. For some countries, data refer to a combination of more than one survey.

energy due to their popularity with adolescents and this has led to concern about their impact on obesity risk. In UK dietary surveys, beverages not including milk provided 9% of total daily calories in 11–18 years old.

In Western countries, average protein intakes are considerably in excess of requirements for all ages and both sexes. The main sources in adolescent diets are meat and meat products (contributing around one-third of protein), cereals, bread, and dairy products. It is believed that protein requirements in adolescents are between 0.8 and 1.0 g kg⁻¹ body mass, although this does not take into account any additional needs that may relate to regular participation in sport and exercise. As a proportion of energy, protein intakes tend to be higher in Southern European countries, Australia, and New Zealand compared with intakes in the United States and Northern European countries.

High intakes of salt are a risk factor for abnormal blood pressure which, in turn, increases the risk of heart disease. Health experts believe that reductions in salt from childhood can help to maintain normal vascular health. UK dietary surveys of 11–18 years old report daily sodium intakes of approximately 2280 mg in girls and 2970 mg in boys. This equates to daily salt intakes of 5 and 6.5 g, respectively, with the target being less than 6 g day⁻¹. Meat products, such as burgers and sausages, provide around one-third of salt intakes with bread and processed foods also contributing significantly.

Fats

Average fat intakes as a proportion of energy vary considerably across Western countries. In the UK, intakes are close to the recommendation of 35% energy, suggesting increased public awareness as previous surveys reported intakes of 38–40% energy from fat. However, intakes of saturated fat, at 14% energy, still exceed the target of 11% food energy. It is worth noting that population averages hide subgroups with more extreme intakes. In the case of saturated fat, a considerable number of adolescents have intakes approximately 17% energy that may increase their risk of heart disease. Main sources of saturated fat in the adolescent diet include meat and meat products (approximately 20%), savory snacks and fried foods. Fat intakes in other European countries are 36–38% energy, with the highest fat intakes reported in Finland, Greece, Belgium, Germany, Switzerland, and Spain. In the United States, where the dietary target for fat is 30% of energy, intakes are approximately 32% energy from fat.

Not all dietary fats need to be reduced. There is increasing evidence that long-chain ω -3 fats from marine sources, specifically docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are beneficial for health. Rich sources of these are oily fish, such as salmon, trout, mackerel, and sardines, whereas shellfish also make an important contribution. Studies show that high intakes of oily fish, DHA, and EPA can help maintain heart health and cognitive function in adults. Emerging data suggest a role for DHA and EPA in brain development in infants, and maintenance of the normal cognitive function in school-age children. Adolescents often have poor intakes of oily fish and, unless offered alternative sources

such as marine oil supplements, may miss out on the benefits offered by DHA and EPA.

Carbohydrates and Fiber

Average carbohydrate intakes in Western populations are close to the recommendation of 50% energy. The main sources in the adolescent diet are breakfast cereals, bread, savory snacks, vegetables and potatoes. Fiber intakes, expressed as nonstarch polysaccharide (NSP), are 10–13 g per day in the UK, which is approximately 70% of the adult guideline. Vegetables, potatoes, and savory snacks together contribute 40% of NSP. Interestingly studies have not always found consistent relationships between fiber intakes and bowel movements in young people. Sugar intakes peak in adolescence before declining to adult levels and are higher in boys compared with girls. In UK surveys, mean intakes of added sugars are 16% of energy and the largest contributor is nondiet soft drinks, providing 40% of sugars, followed by confectionery and preserves. Children from lower income households tended to have lower intakes of protein and NSP but higher intakes of sugars and fats compared with their more affluent peers.

Recommendations to reduce fat are often accompanied by those urging a decrease in added sugars due to concerns about obesity, dental health, and micronutrient adequacy. However, inverse relationships between fat and sugars are often seen in observational surveys suggesting that reductions in dietary fat may occur, in part, due to increases in foods containing added sugars. Most cross-sectional surveys also report inverse relationships between sugar intakes and BMI, suggesting that leaner people have higher sugar intakes, but lower fat intakes. This may seem counterintuitive given concerns about the impact of sugar on obesity risk; however, an explanation could be that heavier people either restrict their sugar intake or underreport sugar-containing foods. Nevertheless, apart from one intervention study relating to soft drinks, the available evidence does not support a relationship between higher sugar intakes and an increased risk of obesity. This was confirmed by the European Food Safety Authority in 2010 following a review of the evidence.

Several reviews have also considered the evidence linking sugar consumption with diet adequacy, responding to concerns that higher sugar intakes result in diets, which are low or inadequate in vitamins and minerals. The findings, based on surveys of children and adolescents in the United States and Europe, suggest that a broad range of sugar consumption is consistent with adequate micronutrient intakes. This may be due to fortification of popular sugar-containing foods, for example breakfast cereals and dairy foods. Lower levels of vitamins and minerals tend to be seen only at the upper and lower extremes of sugar consumption, suggesting that these diets lack variety.

Micronutrients

The main contributors to sources of vitamins and minerals are breakfast cereals, milk, bread, chips/potatoes, and eggs. Surveys that report comparisons between intakes and recommendations have found satisfactory intakes for most

micronutrients when population averages are considered. Intakes of vitamins B₁, B₂, B₆, B₁₂, vitamin C, and niacin greatly exceeded recommendations in the UK, perhaps reflecting high-protein intakes and the fortification of breakfast cereals, bread, and beverages. Average intakes that fall below recommendations have been seen for iron, calcium, magnesium, and zinc. In girls, mean selenium and iodine intakes can also be lower than recommendations.

As mentioned earlier, averages hide subgroups of adolescents whose nutrient intakes fall below acceptable levels for health. UK data suggest that 3–47% of adolescents have intakes of vitamin A, iron, calcium, magnesium, potassium, zinc, selenium, and iodine that fall below the population minimum, suggesting that they may be at risk from deficiency. The nutrients of most concern in adolescence are iron and calcium, which are important for the normal growth and development. Intakes of folate are particularly important for girls, some of whom may fall pregnant in their teens. Certain practices, such as smoking and drinking alcohol, can increase requirements for micronutrients, suggesting that specific groups of adolescents may be more at risk from a poor nutritional status.

Mean iron intakes are particularly low in 11–18 year old girls, at 58–63% of the recommendation (see Table 4), reflecting avoidance of iron-rich foods, such as red meat and offal. It is important to address low iron intakes because these can lead to poor iron status and, in some case, anemia. The best source of iron is red meat which provides the easily absorbed form, called heme iron. However, most iron in the adolescent diet comes from foods, such as breakfast cereals, which supply the less-well-absorbed nonhaem iron. Iron absorption can be improved by consuming vitamin C rich foods or beverages at meal times.

An adequate calcium intake during childhood and adolescence is important for establishing an optimal peak bone mass that can be maintained throughout adulthood. Low intakes of calcium are a risk factor for osteoporosis in later life. Although average calcium intakes tend to be close to recommended levels, there are groups of adolescents with intakes below adequate levels. In UK 11–14 year olds, 12% of boys and 24% of girls had intakes below the lower reference nutrient intake, while in 15–18 year olds, the figures were 9% and 19%, respectively. This suggests a risk of deficiency. Good sources of calcium are milk, cheese, yogurt, soya products, tinned fish and, in many countries, fortified grain products.

Concern has been expressed that the rise in soft drink consumption has displaced milk from the diets of adolescents and this could be contributing to the low calcium intakes found in many surveys. Fluid milk consumption has fallen dramatically over the last decade in Western countries and this is due to a range of factors including preference for other beverages, concerns about weight management, and the perception that milk is for younger children. Although improving diet is important, it should not be forgotten that physical activity is also important for optimal bone health.

Impact of Lifestyle on Nutrition

Young people consume particular foods and diets for a variety of reasons, mostly unrelated to their nutritional content.

Table 4 Average daily intakes of UK adolescents from UK National Diet and Nutrition Surveys

Sex (age) Sample size	Energy MJ	Protein% en	CHO% en	NMES% en	Fat% en	NSP (g)	Iron (mg)	Calcium (mmg)	A (mcg)	B ₁ (mg)	B ₂ (mg)	B ₆ (mcg)	B ₁₂ (mcg)	Niacin (mg)	Folate (mcg)	Carbon (mg)
National Diet and Nutrition Survey (Bates, <i>et al.</i> , 2010) ^a																
M 11–18 yr N = 114	9.07	14.8	50.7	16.3	34.5	13.1	11.1 98% RNI	919 92% RNI	776 120% RNI	1.69 170% RNI	1.72 137% RNI	2.6 192% RNI	4.8 359% RNI	39.4 238% RNI	256 128% RNI	94.4 252% RNI
F 11–18 yr N = 110	7.02	14.4	49.4	14.8	35.4	10.8	8.5 58% RNI	702 88% RNI	619 103% RNI	1.26 169% RNI	1.28 117% RNI	2.1 191% RNI	3.9 289% RNI	30.7 237% RNI	193 97% RNI	72.4 194% RNI
Low Income National Diet and Nutrition Survey (Nelson, <i>et al.</i> , 2007) ^b																
M 11–18 yr N = 200	9.36 93% EAR	13.1	50.5	17.2	36.4	12.6	11.4 101% RNI	913 91% RNI	625 98% RNI	1.82 187% RNI	1.70 137% RNI	2.3 176% RNI	4.6 346% RNI	35.7 220% RNI	232 116% RNI	74.5 201% RNI
F 11–18 yr N = 215	7.85 97% EAR	13.3	50.4	16.3	36.3	11.5	9.3 63% RNI	723 90% RNI	568 95% RNI	1.47 201% RNI	1.30 118% RNI	1.9 181% RNI	3.7 286% RNI	29.1 229% RNI	201 101% RNI	78.0 213% RNI

^aBates B, Lennox A, and Swan G (2010) National Diet and Nutrition Survey Headline results from year 1 of the rolling program (2008–09). London: Food Standard Agency and the Department of Health.

^bNelson M, Erens B, Bates B, *et al.* (2007) Low Income Diet and Nutrition Survey. Three Volume Survey, Executive Summary. London: The Stationary Office.

Key: CHO, carbohydrate; EAR, Estimated Average Requirement; RNI, Reference Nutrient Intake; NMES, Non-milk extrinsic sugars (similar to added sugars); NSP, Non starch polysaccharide.

These include weight control (whether justified or not), peer group pressure, celebrity endorsement, convenience, personal ideologies (e.g., veganism), or enhancement of sporting prowess. As energy and nutrient intakes are influenced by eating patterns, it is important to consider lifestyle when interpreting dietary information or development health promotion messages.

Breakfast

Breakfast can be a nutrient-dense, low fat meal, yet is often omitted by adolescents. Around 10% of younger children miss breakfast, rising to 20% as adulthood is approached. Boys are more likely than girls to eat breakfast, and favor cereals rather than bread or cooked foods. Data on breakfast habits reveal higher intakes of sugars, fiber and micronutrients, such as folate, niacin, iron, calcium, and zinc, amongst regular consumers of breakfast cereals. Fat intakes, as a proportion of energy, are lower when breakfast cereals are consumed. Surveys of adolescents have found an inverse relationship between breakfast cereal consumption and body mass index, suggesting that eating breakfast is a useful strategy for weight control.

Food Choices at School

Although the popularity of school lunches has diminished over the last 10 years, they are still eaten regularly by many children, particularly those from lower socioeconomic groups. School lunches have been criticized in the past for containing a high proportion of fat and delivering low levels of vitamins, minerals, and ω -3 fatty acids. The introduction of school meal standards in a number of countries has significantly improved the nutritional content of school lunches. However, this has not always benefited adolescents who often prefer to assert their independence by buying food out with the school environment. Foods purchased from cafes and take-aways tend to be less healthy than the meals offered at school, and opportunities to choose fruits and vegetables are few.

Fruit and Vegetable Consumption

Data on intakes of fruit and vegetables show that adolescents, particularly girls, have lower intakes than adults and younger children. In the UK, where the recommendation is 400 g (expressed as five portions of fruit/vegetables per day), average intakes in 15–18 year olds are only 200 g. Indeed, only 22% of boys and 7% of girls meet the five portions-a-day target.

Snacking and Soft Drink Consumption

There has been a general shift over the last few decades toward more meals eaten outside the home and a greater proportion of daily energy consumed as snacks and soft drinks. Concerns about the possible impact of snacks on diet quality and the risk of obesity are not always borne out by the evidence, although dietary assessment is hindered by a lack of consensus on what constitutes a 'snack'. Observational studies have found that frequent snackers have similar nutrient intakes to

those who snack infrequently. With respect to body size, snacking is often associated with a lower, rather than a higher, BMI. The few intervention studies that have examined snacking behavior report full or partial compensation for the additional calories provided by snacks by a reduction in the energy from meals. This suggests that snacking itself is not harmful but that adolescents should choose their snacks wisely, focusing on those that make some contribution to micronutrient intakes. Intervention studies looking at the impact of sugar-sweetened soft drinks give a different picture and tend to find less compensation for the additional calories and a reduction in nutrient density of the diet. This suggests that high intakes of sweetened soft drinks, as are often found in adolescence, pose a risk for obesity and could be detrimental for diet adequacy.

Smoking

The proportion of adolescent smokers rises with age and is between 8 and 20% with an average exposure, in older children, of around 40 cigarettes per week. Since the 1980s, smoking has decreased in adolescent boys but not in girls. Smokers tend to have different dietary habits from non-smokers and this is reflected in their nutrient intakes. Studies have found that smokers consume fewer dairy foods, whole-meal bread, fruit, breakfast cereals, and more coffee, alcohol, and chips. Smokers' diets tend to be lower in fiber, vitamin B₁ and vitamin C compared with nonsmokers. In a study of 18 year olds, male smokers had a higher percentage energy from fat and lower intakes of sugars and iron. Contrary to beliefs, there is no evidence that smoking helps to control body weight in young people.

Alcohol Consumption

In the UK, alcohol is consumed by 10% of 11–14 years old, and 37–46% of 15–18 year olds with older boys most likely to drink alcohol. Other European surveys have found higher proportions, 60–90% in 14–18 year old males. Although US surveys have found similar proportions to the UK. The average contribution of alcohol to energy intakes in the NDNS is just over 1%, with higher contributions reported by Danish and Irish studies (around 2–5% energy). Excess alcohol intake can increase micronutrient requirements but few younger adolescents fall into this category. However, regular consumption of alcohol contributes to obesity because the energy provided by alcoholic drinks rarely displaces energy from other food sources.

Socioeconomic Status

Differences in diet are sometimes seen between children from different social classes or income groups. In the UK, children from a lower socioeconomic background consume fewer low fat dairy foods, fruit juice, salad vegetables, high fiber cereals, fruit juices, and fruit than children from a higher socioeconomic background. This impacts on mean daily nutrient intakes with poorer children consuming lower amounts of protein, sugars, carbohydrate, vitamin C and fiber. Some

Table 5 Average daily intakes of adolescents from the European Nutrition & Health Report (2009)^a

Sex (age)	Protein % en	CHO % en	Sucrose % en	Fat % en	Fiber (g)	Iron (mg)	Calcium (mg)	Vit D (mcg)	Folate (mcg)	Iodine (mcg)	Sodium (g)
M 1–15 yr	12–17	43–56	5–29	28–41	9–24	5.7–14.0	554–1104	1.4–5.3	116–304	48–299	1.6–6.3
F 1–15 yr	12–17	42–55	5–29	28–42	6–21	5.4–12.1	560–1049	1.2–6.3	109–278	51–299	1.5–5.4
M 14–24 yr	13–18	42–54	13–16	31–40	14–26	11.8–16.3	675–1362	1.3–5.4	175–312	93–133	2.4–4.1
F 14–24 yr	12–17	42–55	12–18	29–40	14–22	8.9–12.8	659–1212	1.5–3.4	161–266	78–106	2.2–3.2

^aData presented as ranges.

% en, as percentage of total daily energy.

CHO, carbohydrate.

surveys have also found higher fat intakes and a greater risk of obesity in children from lower socioeconomic backgrounds.

Physical Activity

Regular physical activity impacts on nutrition and health in a number of ways, for example, by maintaining normal energy balance that lowers the risk of obesity, by supporting the normal heart function, and by promoting bone density. However, adolescents, particularly girls, have become far less physically active in recent years. The European Youth Heart Study found that 82% of 15-year old boys, but only 62% of girls of that age, met physical activity targets. At present, WHO recommends that children and young people are physically active for at least 60 min per day⁻¹. This includes sports and exercise, but also walking. Reducing sedentary behaviors, for example, TV viewing, video games, is also important for obesity prevention.

Sleep

Although sufficient sleep is vital for normal growth and cognitive development in childhood and adolescence, short sleep duration can also impact on nutritional intake and obesity risk. Studies in adolescents and young adults have found associations between a lack of nighttime sleep and increases in BMI. Short sleep duration could influence body weight by disrupting appetite control, reducing physical activity levels (e.g., due to tiredness), or affecting thermoregulation.

Conclusions

Most adolescents in Western countries are consuming adequate energy and protein to support normal growth. However, intakes of micronutrients in subgroups of the population are lower than recommendations and may not be sufficient for optimal health. These include iron, calcium, zinc, folate, and vitamin A. For iron, there is good evidence of clinical deficiency in low iron consumers, particularly girls. As energy intake is the best predictor of micronutrient adequacy, care must be taken when advising adolescents about weight management because calorie restriction can reduce intakes of vitamins and minerals.

Longitudinal studies that attempt to link early diet with the incidence of later disease suggest that high intakes of fruit,

vegetables, wholegrains, and oily fish are markers of good health in later life. However, this beneficial dietary pattern has not been adopted by most adolescents. Indeed, despite the public health efforts of government and health professionals, adolescents remain a hard to reach group. (Table 5).

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See also: Adolescents: Nutritional Problems of Adolescents. Dietary Guidelines, International Perspectives. Growth and Development: Physiological Aspects

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Glossary

Aging A progressive sequence of detrimental age-related changes that occur in every individual of a given species, at varying rates. These changes lead to a breakdown in normal homeostatic mechanisms so that the functional capacity of the body and its ability to respond to a wide variety of extrinsic and intrinsic agents is often reduced. This causes degradation of structural elements within the cells, tissues, and organs of the body, leading eventually to the onset of age-related disorders and ultimately death.

Apoptosis Programed cell death which is important for changes that occur during development, and for cell

turnover. Approximately 50–70 billion cells per day die through apoptosis in the human adult. Apoptosis is a normal event and differs from necrosis, which is cell death due to injury. Insufficient atrophy results in excessive cell proliferation such as in cancer.

Hayflick phenomenon (or Hayflick limit) The number of times a cell population can divide before it dies.

Senescence The process of becoming old.

Somatic mutation Changes in DNA that can occur in any cell except sperm or eggs. They cannot be passed on to the next generation, but can cause cancer or other diseases.

Introduction

The aging processes, and interventions to ameliorate them, have fascinated humans since the dawn of civilization. Research into aging is now a vital area of human endeavor, as our species reaches the limits of its longevity and faces the prospect of an aging population.

An individual's life expectancy is contributed to by the interaction of intrinsic (genetic and epigenetic) factors with extrinsic (environmental and life style) factors (**Figure 1**). In the world's more developed countries (MDCs) life expectancy at birth in the 1900s was around 47 years. By the end of the twentieth century this rose to a mean of 78 and 76 years in Western Europe and North America, respectively, with many individuals living much longer. This dramatic increase in average life expectancy has been largely due to improvements in environmental conditions such as nutrition, housing, sanitation, and medical and social services, leading to a large increase in the number of older people around the world. This change in the age structure of society is compounded by the decreasing fertility levels in the world's populations leading to large gains in worldwide median population ages. Our aging populations have a growing number and proportion of older people and, importantly, a growing number and proportion of very elderly people.

Based on current rates and trends in population growth by the year 2025 the elderly population (aged 65 and above) in the world's MDCs will increase by more than 50%, and will more than double worldwide. The very elderly (aged 80 and above) is the fastest growing section of the elderly population. This changing demographic picture will result in a large increased prevalence worldwide of long-term illness, disability,

and the degenerative diseases associated with aging. These alterations in the proportions of the population of working age and those beyond working age will significantly impact the funding and costs of healthcare for all nations, making research into aging of critical international importance.

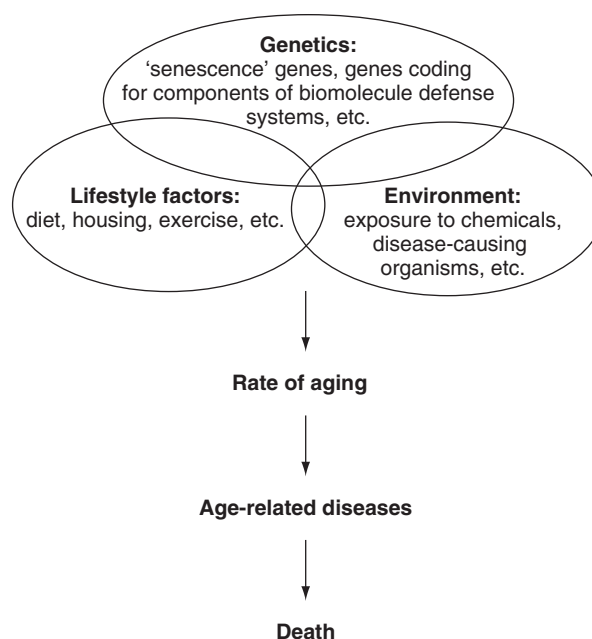


Figure 1 Interactive factors that contribute to the aging process. Reproduced from Barnett YA (1994) Nutrition and the ageing process. *British Journal of Biomedical Sciences* 51: 278–287.

Theories of Aging

The biological manifestations that occur with aging affect the entire hierarchical structure of living systems. Age-related effects are seen in the accumulation of damaged cellular biomolecules (e.g., advanced glycosylation end products, lipid peroxidation products, genetic damage, and mutation), damaged organelles (mitochondria), and loss of cellular function, which contributes to dysfunction of the body's tissues, organs, and systems. These hierarchical changes have paved the way for more than 300 theories in an attempt to explain how and why aging occurs. They have previously been broadly categorized into: (1) programed or genetic theories; and (2) damage accumulation (stochastic) theories. However, these categories are not proven to be entirely comprehensive or mutually exclusive and it is likely that there is a shifting range throughout the life span that reflects a decreasing influence of genetic factors and an increasing influence of stochastic events.

Programed and Genetic Theories

Programed and genetic theories propose that the process of aging follows a biological timetable, perhaps a continuation of the one that regulates childhood growth and development. There are a number of lines of evidence supporting these theories.

Longevity Genes

It is clear that aging is controlled to some extent by genetic mechanisms. The distinct differences in life span among species are a direct indication of genetic control, at least at the species level. A number of genes have been identified in yeast, nematode worms (*Caenorhabditis elegans*), and fruit flies (*Drosophila melanogaster*) that significantly increase the organism's potential maximum life span. The products of these genes are involved in stress response and resistance, development, signal transduction, transcriptional regulation, and metabolic activity. However, the genetics of longevity have not been as revealing in mammalian studies. In mouse systems genes involved with immune response have been implicated in longevity, as has the 'longevity gene' *p66^{shc}*, which is involved in signal transduction pathways that regulate the cellular response to oxidative stress. In humans, a number of mitochondrial DNA polymorphisms are associated with longevity. Linkage analysis in human systems has associated certain genes on chromosome 4 with exceptional longevity. Further support for human longevity genes may be provided by the observation that siblings and parents of centenarians live longer. The major histocompatibility complex (MHC), the master genetic control of the immune system, may also be a gene system controlling aging, because a number of genetic defects that cause immunodeficiency shorten the life span of humans. Certain MHC phenotypes have also been associated with malignancy, autoimmune disease, Alzheimer disease, and xeroderma pigmentosum in humans.

Accelerated Aging Syndromes

No distinct phenocopy exists for normal aging, but several genetic diseases/syndromes display some features of accelerated

aging, including Hutchinson-Gilford syndrome (classic early onset Progeria), Werner syndrome, and Down syndrome. Patients with these syndromes suffer from many signs of premature aging including hair loss, early graying, and skin atrophy, and premature age-related diseases such as atherosclerosis, osteoporosis, and glucose intolerance. The defined genetics involved in these syndromes provides strong evidence for the genetic basis of aging.

Neuroendocrine Theories

These theories propose that functional decrements in neurons and their associated hormones are pivotal to the aging process. An important version of this theory suggests that the hypothalamic-pituitary-adrenal (HPA) axis is the key regulator of mammalian aging. The neuroendocrine system regulates early development, growth, puberty, the reproductive system, metabolism, and many normal physiological functions. Functional changes to this system could exert effects of aging throughout an organism. However, the cells of the neuroendocrine system are subject to the normal cellular aging processes found in all cells, and changes in the neuroendocrine system may be secondary expressions of the aging phenotype.

Immunologic Theory and Immunosenesence

Deterioration of the immune system with aging ('immunosenesence') may contribute to morbidity and mortality due to decreased resistance to infection and, possibly, certain cancers in the aged. T-cell function decreases and autoimmune phenomena increase in elderly. Although the immune system obviously plays a central role in health status and survival, its cells are subject to the normal cellular aging processes found in all cells. Changes to the immune system may be secondary expressions of the aging phenotype.

Cellular Senescence

At the cellular level, most, if not all, somatic cell types have a limited replicative capacity *in vitro* before they senesce and die. The number of cell population doublings *in vitro* is inversely correlated with donor age. This is called the 'Hayflick phenomenon' after the scientist credited with its discovery. This limit in the capacity of a cell type or tissue to divide and replenish itself would have major repercussions *in vivo*. There is evidence that replicative senescence is related to *in vivo* aging, but definitive evidence that senescent cells accumulate *in vivo* is lacking to date. Many alterations to normal cellular physiology are exhibited with the senescent phenotype, indicating that senescent cells exist in a growth state that is quite distinct from that of young cells and are subject to a complex alteration to their cellular physiology. Several explanations for limiting the number of cell population doublings have been proposed, including a tumor suppressive mechanism. One is that the shortening of telomeres, the sequences of noncoding DNA located at the end of chromosomes, is a measure of the number of cell divisions that a cell has experienced. These telomeres may act as specialized regions of the genome, a sacrificial 'sentinel' zone, for the detection of DNA damage being noncoding, more prone to damage, and less prone to repair than the genome as a whole. Damage to telomeres

transposes to telomere shortening, and loss of telomere higher order structure may trigger senescence and/or apoptosis.

Studies involving fusion of normal cells (subject to senescence) with immortal cell lines *in vitro* have clearly demonstrated that the senescent phenotype is dominant, and that unlimited division potential results from changes in normal growth control mechanisms. These fusion studies have also revealed the existence of several dominant genes associated with the process of cellular senescence. These genes reside on a number of chromosomes, including 1, 4, and X.

Disposable Soma Theory

The disposable soma theory suggests that aging is due to stochastic background damage to the organism, i.e., damage that is not repaired efficiently because the energy resources of the somatic cells are limited. So, instead of wasting large amounts of energy in maintaining the whole body in good condition, it is far more economical to simply repair the heritable stem cell genetic material, in order to ensure the survival of the species. In this way the future of the species is secured at the expense of individual lives. When the somatic energy supply is exhausted, the body ages and dies, but the genetic material survives (in the next generation).

Damage Accumulation (Stochastic) Theories

The 'damage' or 'error' theories emphasize intrinsic and environmental insults to our cellular components that accumulate throughout life and gradually cause alterations in biological function and the physiological decline associated with aging.

Somatic Mutation and DNA Repair

Damage to DNA occurs throughout the lifetime of a cell. If this damage is not repaired or removed then mutations may result. Mutations may result in the synthesis of aberrant proteins with altered or absent biological function; alterations to the transcriptional and translational machinery of a cell; and deregulation of gene control. The accumulation of mutations on their own, or in combination with other age-related changes, may lead to alterations in cellular function and ultimately the onset of age-related disease.

Error Catastrophe

This theory suggests that damage to mechanisms that synthesize proteins results in faulty proteins, which accumulate to a level that causes catastrophic damage to cells, tissues, and organs. Altered protein structure has been clearly demonstrated to occur with age; however, most of these changes are posttranslational in nature, and hence do not support this theory of aging. Such changes to protein structure may result in progressive loss of 'self-recognition' by the cells of the immune system and thus increase the likelihood that the immune system would identify self-cells as foreign and launch an immune attack. Indeed, the incidence of autoimmune episodes is known to increase with age.

Cross-Linking

The cross-linking theory states that an accumulation of cross-linked biomolecules caused by covalent or hydrogen bonds damages cellular and tissue function through molecular aggregation and decreased mobility. The modified malfunctional biomolecules accumulate and become increasingly resistant to degradation processes and may represent a physical impairment to the functioning of organs. There is evidence *in vitro* for such cross-linking over time in collagen and in other proteins, and in DNA. Many agents exist within the body that have the potential to act as cross-linking agents, e.g., aldehydes, antibodies, free radicals, quinones, citric acid, and polyvalent metals, to name but a few.

Free Radicals

The most popular, widely tested and influential of the damage accumulation theories of aging is the 'free radical' theory, first proposed by Harman in 1956. Free radicals from intrinsic and extrinsic sources (Table 1) can lead to activation of cytoplasmic and/or nuclear signal transduction pathways, modulation of gene and protein expression, and also alterations to the structure and ultimately the function of biomolecules. Free radicals may thus induce alterations to normal cell, tissue, and organ functions, which may result in a breakdown of homeostatic mechanisms and lead to the onset of age-related disorders and ultimately death. It can be predicted from this theory that the life span of an organism may be increased by slowing down the rate of initiation of random free radical reactions or by decreasing their chain length. Studies have demonstrated that it is possible to increase the life span of

Table 1 Extrinsic and intrinsic sources of free radicals

Extrinsic sources	Intrinsic sources
Radiation: ionizing, ultraviolet	Plasma membrane: lipoxygenase, cyclooxygenase, NADPH oxidase
Drug oxidation: paracetamol, carbon tetrachloride, cocaine	Mitochondria: electron transport, ubiquinone, NADH dehydrogenase
Oxidizing gases: oxygen, ozone, nitrogen dioxide	Microsomes: electron transport, cytochrome p450, cytochrome b ₅
Xenobiotic elements: arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd)	Peroxisomes: oxidases, flavoproteins
Redox cycling substances: paraquat, diquat, alloxan, doxorubicin	Phagocytic cells: neutrophils, macrophages, eosinophils, endothelial cells
Heat shock	Auto-oxidation reactions: Metal catalyzed reactions
Cigarette smoke and combustion products	Other: hemoglobin, flavins, xanthine oxidase, monoamine oxidase, galactose oxidase, indolamine dioxygenase, tryptophan dioxygenase
	Ischemia – reperfusion

cells *in vitro* by culturing them with various antioxidants or free radical scavengers. Antioxidant supplementation with a spin-trapping agent has been demonstrated to increase the life span of the senescence accelerated mouse, although as yet there is little evidence for increasing the life span of a normal mammalian species by such strategies.

Mitochondrial DNA Damage

This hypothesis combines elements of several theories, covering both the stochastic and genetic classes of aging theories. It is proposed that free radical reactive oxygen species generated in the mitochondria contribute significantly to the somatic accumulation of mitochondrial DNA mutations. This leads to a downward spiral wherein mitochondrial DNA damage results in defective mitochondrial respiration that further enhances oxygen free radical production, mitochondrial DNA damage, and mutation. This leads to the loss of vital bioenergetic capacity eventually resulting in aging and cell death.

The absence of evidence that exclusively supports any one theory leaves no doubt that aging is due to many processes, interactive and interdependent, that determine life span and death.

Age-Related Diseases

Regardless of the molecular mechanisms that underlie the aging process, a number of well-characterized changes to the structure and therefore the function of the major cellular biomolecules (lipids, proteins, carbohydrates, and nucleic acids) are known to occur with age (Table 2). The age-related alterations to the structure and therefore the function of

cellular biomolecules have physiological consequences and may directly cause or lead to an increased susceptibility to the development of a number of diseases (Figure 2).

Cellular biomolecules are constantly exposed to a variety of extrinsic and intrinsic agents that have the potential to cause damage. A number of defense systems exist, e.g., antioxidant enzymes and DNA repair systems, which aim to reduce, remove, or repair damaged biomolecules. These defense systems are not perfect, however, and biomolecular damage may still occur. Such damage can result in the degradation of structural elements within the cells, tissues, and organs of the body, leading to a decline in biological function and eventually to disease and death.

The physiological alterations with age proceed at different rates in different individuals. Some of the common changes seen in humans are: the function of the immune system decreases by the age of 30 years, reducing defenses against infection or tumor establishment and increasing the likelihood of autoimmune disorders; metabolism starts to slow down at around 25 years of age; kidney and liver function decline; blood vessels lose their elasticity; bone mass peaks at the age of 30 years and drops approximately 1% per year thereafter; the senses fade; the epidermis becomes dry and the dermis thins; the quality of and need for sleep diminish; and the brain loses 20% of its weight, slowing recall and mental performance. A number of age-related diseases may develop due to the tissue, organ, and system deterioration (Table 3).

Modification of the Aging Process

Can the adverse consequences of aging be prevented? Through the ages many have pursued the elixir of life. Attempts to

Table 2 Major age-related alterations in biomolecule structure and the resultant physiological consequences of such structural changes

Biomolecule	Alteration	Physiological consequence
Lipids	Lipid peroxidation	Oxidized membranes become rigid, lose selective permeability and integrity. Cell death may occur Peroxidation products can act as cross-linking agents and may play a role in protein aggregation, the generation of DNA damage and mutations, and the age-related pigment lipofuscin
Proteins	Racemization, deamination, oxidation, and carbamylation	Alterations to long-lived proteins may contribute to aging and/or pathologies. For example, modified crystallins may aggregate in the lens of the eye thus leading to the formation of cataracts Cross-linking and formation of advance glycosylation end-products (AGEs), which can severely affect protein structure and function Effects on the maintenance of cellular homeostasis
Carbohydrates	Fragmentation, depolymerization glucose auto-oxidation	Alters physical properties of connective tissue. Such alteration may be involved in the etiology and pathogenesis of osteoarthritis and other age-related joint disorders Glycosylation of proteins <i>in vivo</i> with subsequent alteration of biological function; for example, glycosylation of insulin in patients with diabetes may result in altered biological function of insulin and contribute to the pathogenesis of the disease
Nucleic acids	Strand breaks base adducts loss of 5-methyl cytosine from DNA	Damage might interfere with transcription, translation, and DNA replication, reducing a cell's capacity to synthesize vital polypeptides/proteins. In such circumstances cell death may occur. The accumulation of hits in critical cellular genes associated with the control of cell growth and division results in the process of carcinogenesis Dedifferentiation of cells (5-methylcytosine plays an important role in switching off genes as part of gene regulation) If viable, such dedifferentiated cells may have altered physiology and may contribute to altered tissue/organ function

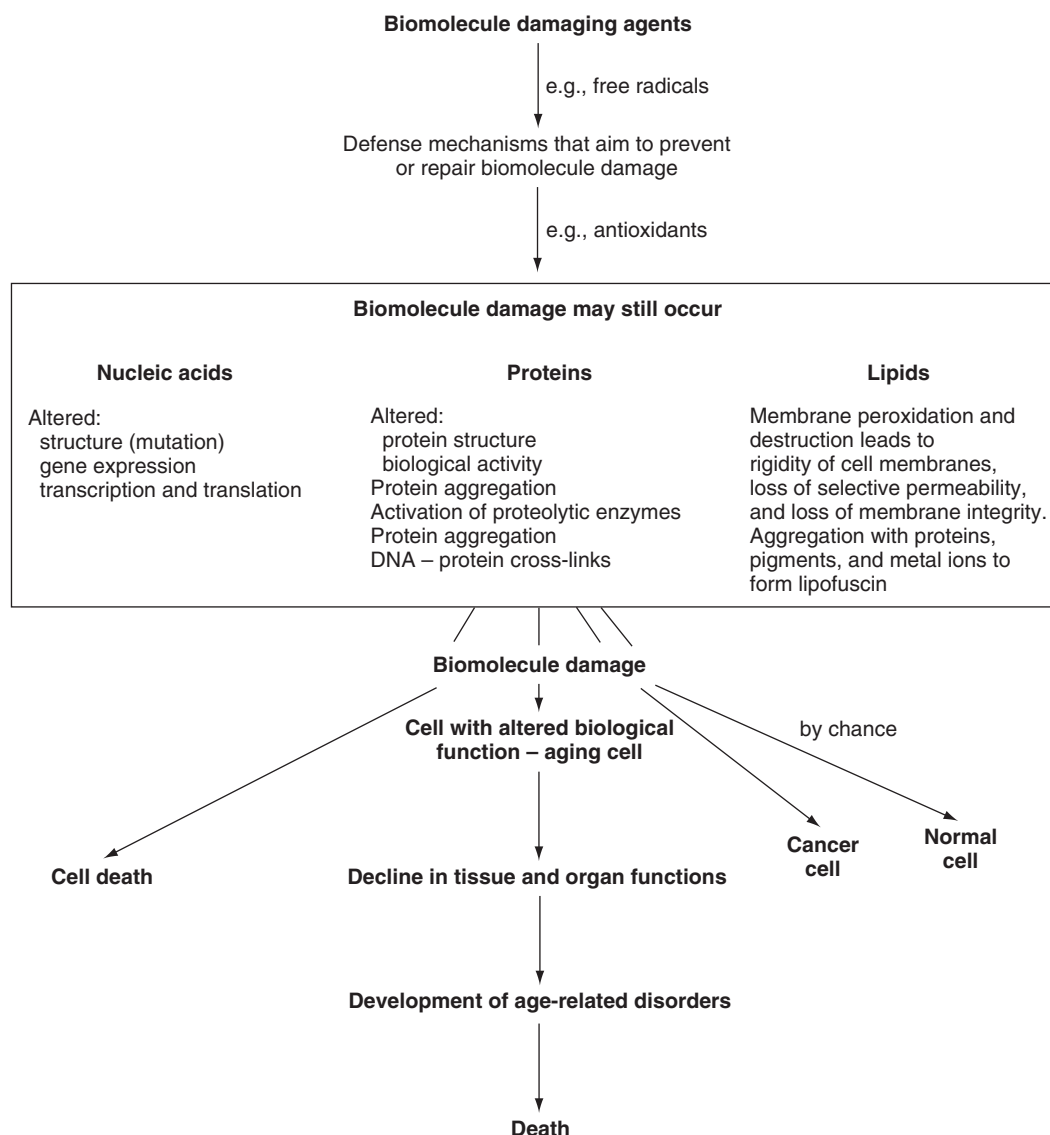


Figure 2 Biomolecule damage and the aging process. Reproduced from Barnett YA (1994) Nutrition and the aging process. *British Journal of Biomedical Sciences* 51: 278–287.

Table 3 Major age-related alterations *in vivo* and the resultant pathological conditions

Body system	Pathological changes
Cardiovascular	Atherosclerosis, coronary heart disease, hypertension
Central nervous system	Reduction of cognitive function, development of various dementias (e.g., Alzheimer's disease and Parkinson's disease)
Endocrine	Noninsulin-dependent diabetes, hypercortisolemia
Hematopoietic	Anemia, myelofibrosis
Immune	General decline in immune system function, particularly in T cells
Musculoskeletal	Osteoporosis, osteoarthritis, skeletal muscle atrophy
Renal	Glomerulosclerosis, interstitial fibrosis
Reproductive	Decreased spermatogenesis, hyalinization of semeniferous tubules
Respiratory	Interstitial fibrosis, decreased vital capacity, chronic obstructive pulmonary disease
Sense organs	Cataracts, senile macular degeneration, diabetic retinopathy
All systems	Cancer

Table 4 Effects of vitamins and micronutrients on age-related disorders

<i>Vitamin or micronutrient</i>	<i>Possible effect on age-related disorder</i>
Vitamins B ₆ , E copper, zinc, and selenium	Impairment of immune function in older humans if inadequate amounts
Vitamins C, E, and carotenoids	Increased amount in the diet is associated with delayed development of various forms of cataract
Carotenoids and zinc	Dietary supplementation associated with a decreased risk of age-related macular degeneration
Selenium	Absolute or relative deficiency associated with development of a number of cancers (not breast cancer)
Vitamin C, β -carotene, α -tocopherol, and zinc	Dietary supplementation may decrease the rate of development of atherosclerosis
Selenium, copper, zinc, lithium, vanadium, chromium, and magnesium	Dietary deficits are associated with an increased risk of cardiovascular disease
Vitamins B ₁₂ , B ₆ , and folate	Adequate levels throughout a lifetime may prevent some of the age-related decrease in cognitive function
Chromium	Deficiency is associated with an increased risk of Type 2 diabetes mellitus

increase the average life expectancy and quality of life in the elderly can only succeed by slowing the aging process itself. In humans, the rate of functional decline associated with aging may be reduced through good nutrition, exercise, timely health care, and avoidance of risk factors for age-related disease.

Nutritional Modification

It is clear that diet contributes in substantial ways to the development of age-related diseases and that modification of the diet can contribute to their prevention and thus help to improve the quality of life in old age. Macronutrient intake levels can play a significant role in the progression of age-related diseases and affect the quality of life. For example, the total and proportional intakes of polyunsaturated fatty acids and saturated fatty acids in the Western diet may have an effect on the incidence of atherosclerosis and cardiovascular diseases.

Our dietary requirements also change as we age and if such changes are not properly addressed this could lead to sub-optimal nutritional status. This challenge is compounded by a decrease in the body's ability to monitor food and nutrient intakes. Dietary intake and requirements are complex issues, intertwined with many health and life style issues. However, most research points toward the need for a varied diet as we age, with an increased emphasis on micronutrient intake levels.

An exemplary diet for healthy aging can be found in the traditional diet of Okinawa, Japan. Okinawans are the longest-living population in the world according to the World Health Organization, with low disability rates and the lowest frequencies of coronary heart disease, stroke, and cancer in the world. This has been attributed to healthy life style factors such as regular physical activity, minimal tobacco use, and developed social support networks as antistress mechanisms, all of which are underpinned by a varied diet low in salt and fat (with monosaturates as the principal fat) and high levels of micronutrient and antioxidant consumption.

Vitamins and Micronutrients

The mechanisms by which certain vitamins and micronutrients mediate their protective effect on age-related disorders is based on their abilities to prevent the formation of free radicals or scavenging them as they are formed, either

directly (e.g., vitamins C, E, and β -carotene) or indirectly (e.g., copper/zinc superoxide dismutase, manganese-dependent superoxide dismutase, selenium-dependent glutathione peroxidase) (Table 4). Only by exploring more fully the underlying molecular mechanisms of aging and the major classes of antioxidants will it be possible to establish their role, and develop strategies for using various classes of antioxidants to reduce the effects of aging. Other dietary components may also have a beneficial effect in preventing or delaying the onset of age-related disease. For example, as a deterrent against the onset of osteoporosis, adults should ensure adequate calcium and vitamin D intakes.

Dietary Energy Restriction

The effect of caloric restriction on life span has only been demonstrated convincingly in rodents. Feeding mice and rats diets deficient in energy (approximately 35% of that of animals fed ad libitum, after the initial period of growth) retards the aging of body tissues, inhibits the development of disease and tumors, and prolongs life span significantly. The exact mechanism of action of dietary energy restriction remains to be elucidated, but may involve modulation of free radical metabolism, or the reduced hormone excretion that occurs in dietary restricted animals may lower whole body metabolism resulting in less 'wear and tear' to body organs and tissues.

Current investigations into the effects of dietary energy restriction (by approximately 30%) on the life spans of primates, squirrels, and rhesus monkeys continue. Caloric restriction in rhesus monkeys leads to reductions in body temperature and energy expenditure consistent with the rodent studies. These investigations should have direct implications for a dietary energy restriction intervention aimed at slowing down the aging process in humans, should any humans wish to extend their life span at such a cost. Once the mechanisms of effects of caloric restriction on longevity are understood it may be possible to develop drugs that act through these mechanisms directly, mitigating the need for diets that interfere with the quality of life.

Molecular Biological Interventions and the Aging Process

Accelerated aging syndromes show degenerative characteristics similar to those appearing during normal aging. The mutations

leading to these disorders are being identified and their roles in the aging process are being elucidated. Examining differences in the genetic material from normal elderly people and those with progeria should provide a better understanding of the genetic mechanisms of aging. Identification of a control gene or genes that inhibit the action of the genes producing the progeroid phenotype might make it possible to slow down aberrant protein production in normal people as well.

As an example, the genetic defect that predisposes individuals to the development of Werner syndrome has been elucidated. Individuals with this disease carry two copies of a mutant gene that codes for a helicase enzyme (helicases split apart or unwind the two strands of the DNA double helix). DNA helicases play a role in DNA replication and repair. In light of the biological function of these enzymes it has been proposed that premature aging in Werner syndrome is caused by defective helicase preventing DNA repair enzymes from removing background DNA damage, which thus becomes fixed as mutations, with consequent deleterious effects on cellular function. It remains to be determined whether increasing the fidelity or activity of helicases in cells will extend their life span.

Because loss of telomeric DNA sequences may lead to replicative senescence in dividing cells, in theory by preventing such telomere loss the life span of the cell could be extended. A naturally occurring enzyme, telomerase, exists to restore telomeric DNA sequences lost by replication. Telomerase is normally only functional in germ cells. Manipulating certain cell types (e.g., cells of the immune system) to regulate the expression of telomerase may extend their functional life span. Drugs that enhance telomerase activity in somatic cells are being developed. However, cellular senescence has been implicated as a tumor suppressor mechanism and it has been found that cancer cells express telomerase. An uncontrolled expression of this enzyme in somatic cells may lead to the onset of malignancy through uncontrolled cell proliferation. Thus, any intervention aiming to increase life span based on the cellular expression of telomerase must strike a balance between maintaining controlled cell division and uncontrolled proliferation.

A number of single gene mutations have been identified that affect metabolic function, hormonal signaling, and gene

silencing pathways. In future it may be possible to develop drugs to mimic the antiaging effects that these genes exert.

See also: Antioxidants. Cancer: Epidemiology and Associations Between Diet and Cancer. Coronary Heart Disease: Lipid Theory; Prevention. Fats and Oils. Growth and Development: Physiological Aspects. Nutrient Requirements: International Harmonization. Older People: Nutritional Management of Geriatric Patients; Nutritional Requirements. Protein Digestion and Bioavailability. Protein: Requirements and Role in Diet; Synthesis and Turnover

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Contents

Absorption, Metabolism, and Physiological Effects Effects of Consumption on Diet and Nutritional Status

Absorption, Metabolism, and Physiological Effects

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Glossary

Alcohol dehydrogenase Alcohol dehydrogenase (ADH) is an enzyme that couples oxidation of ethanol to reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH.

ADH has a wide range of substrates and functions, including dehydrogenation of steroids and oxidation of fatty acids.

Aldehyde dehydrogenase Aldehyde dehydrogenase (ALDH) is an enzyme that couples oxidation of acetaldehyde to reduction of NAD^+ . The presence of ALDH in tissues may reduce the toxic effects of acetaldehyde.

Blood ethanol concentration Blood ethanol concentration is commonly used as a measure of intoxication. It is commonly expressed as mg l^{-1} , g dl^{-1} , or mmol l^{-1} .

Catalase Catalase is a common enzyme found in nearly all living organisms. The main action is the decomposition of hydrogen peroxide (H_2O_2) to water. It can also oxidize ethanol in a reaction that requires H_2O_2 .

First-pass metabolism of ethanol First-pass metabolism of ethanol reduces the concentration of ethanol before it reaches the systemic circulation.

Microsomal ethanol oxidizing system The microsomal ethanol oxidizing system (MEOS) is another pathway of ethanol metabolism. The key enzyme of the MEOS is cytochrome P4502E1 (CYP2E1). This pathway requires oxidation of NADPH to NADP^+ .

After caffeine, ethanol is the most commonly used recreational drug worldwide. Alcohol is synonymous with ethanol, and drinking often describes the consumption of beverages containing ethanol. In the United Kingdom (UK), a unit of alcohol (standard alcoholic drink; [Table 1](#)) contains 8 g of ethanol (ethyl alcohol). The Department of Health, the National Institute for Clinical Excellence (NICE), and several of the medical Royal Colleges in the UK have recommended sensible limits for alcohol intake based on these units of alcohol. However, as the amount of ethanol in one unit or a standard alcoholic drink varies throughout the world ([Table 2](#) and [Table 3](#)), the unit system does not allow international comparisons. Recommendations for sensible limits for alcohol intake also vary worldwide.

Despite these guidelines, the quantity of alcohol consumed varies widely. Many enjoy the pleasant psychopharmacological effects of alcohol. However, some experience adverse reactions due to genetic variation of enzymes that metabolize alcohol. Misuse of alcohol undoubtedly induces pathological changes

in most organs of the body. Some data have suggested that alcohol may be beneficial in the reduction of ischemic heart disease.

Many of the effects of alcohol correlate with the peak concentration of ethanol in the blood during a drinking session. It is therefore important to understand the factors that influence the blood ethanol concentration (BEC) achieved from a dose of ethanol.

Physical Properties of Ethanol

Ethanol is produced from the fermentation of glucose by yeast. Ethanol ([Figure 1](#)) is highly soluble in water due to its polar hydroxyl (OH) group. The nonpolar (C_2H_5) group enables ethanol to dissolve lipids and thereby disrupt biological membranes. As a relatively uncharged molecule, ethanol crosses cell membranes by passive diffusion.

Absorption and Distribution of Alcohol

The basic principles of alcohol absorption from the gastrointestinal (GI) tract and subsequent distribution are well understood. Beverages containing ethanol pass down the esophagus into the stomach. The endogenous flora of the GI tract can also transform food into a 'cocktail' containing several alcohols including ethanol. This is particularly important if there are anatomical variations in the upper GI tract (e.g., diverticulae).

Table 1 Unit system of ethanol content of alcoholic beverages^a

Beverage containing ethanol	Units of ethanol
Half pint of low-strength beer (284 ml)	1
Pint of beer (568 ml)	2
500 ml of high-strength beer	6
Pint of cider	2
One glass of wine (125 ml)	1
Bottle of wine (750 ml)	6
One measure of spirits (e.g., whisky, gin, vodka)	1
Bottle of spirits (e.g., vodka 750 ml)	36

^aThe unit system is a convenient way of quantifying consumption of ethanol and offers a suitable means to give practical guidance. However, there are several problems with the unit system. The ethanol content of various brands of alcoholic beverages varies considerably (for example, the ethanol content of beers/ales is in the range 0.5–9.0% so a pint may contain 2–5 units) and the amounts of alcohol consumed in homes bear little in common with standard measures. Similarly, variations in the strength (9–14%) and serving size (125–250 ml or more) of wine makes quantization of self reported intakes rather difficult.

Table 2 Geographical variation in the amount of ethanol in one unit

Country	Amount of ethanol (g)
Sweden	20
Japan	19.75
United States	14
Australia and New Zealand	10
United Kingdom	8

The unit system does not permit international comparisons.

Table 3 UK guidelines for the consumption of alcohol^a

	Men (units)		Women (units)	
	Weekly ^b	Daily ^c	Weekly ^b	Daily ^c
Low risk	0–21	3–4	0–14	2–3
Hazardous	22–50	≥4	15–35	≥3
Harmful	>50		>35	≥1–2 ^d

^aGuidelines regarding the consumption of alcohol are designed to reduce harm. The Royal Colleges' (1995) guidelines are for weekly consumption rates, and the Department of Health's (1995) guidelines are for daily consumption.

^bRecommendations of the Working Group of the Royal Colleges of Physicians, Psychiatrists and General Practitioners (UK).

^cRecommendations of the Department of Health (UK).

^dConsumption of alcohol may increase the risk of miscarriage so NICE recommends that when pregnant or trying to conceive, women should be advised to abstain from alcohol completely for at least the first 3 months of pregnancy. Women who wish to drink while pregnant, should be advised not to get drunk or binge drink (drink over 7.5 UK units during a single session). They should be advised to limit their intake to no more than 1 or 2 UK units of alcohol once or twice a week. How much alcohol is safe during pregnancy is uncertain, but there is no evidence that this low level of alcohol intake harms the fetus. However, many other countries recommend that women should abstain from alcohol while pregnant, trying to conceive or breastfeeding.

Alcohol continues down the GI tract until absorbed. The ethanol concentration therefore decreases down the GI tract. There is also a concentration gradient of ethanol from the lumen to the blood. The concentration of ethanol is much higher in the lumen of the upper small intestine than in plasma (Table 4). Alcohol diffuses passively across the cell membranes of the mucosal surface into the submucosal space and then the submucosal capillaries.

Absorption occurs across all of the GI mucosa but is fastest in the duodenum and jejunum. The rate of gastric emptying is the main determinant of absorption because most ethanol is absorbed after leaving the stomach through the pylorus.

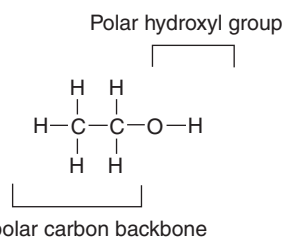


Figure 1 Chemical Structure of Ethanol (Ethyl Alcohol).

Table 4 Approximate ethanol concentrations in the gastrointestinal tract and in the blood of a 70 kg male after consumption of 7 units of alcohol^a

Site	Ethanol concentration	
	g dl ⁻¹	mmol l ⁻¹
Stomach	8	1740
Jejunum	4	870
Ileum	0.1–0.2	22–43
Blood (15–120 minutes after dosage)	0.1–0.2	22–43

^aEthanol appears in the blood as quickly as 5 minutes after ingestion and is rapidly distributed around the body. A dose of 0.8 g ethanol per kg body weight (56 g ethanol (7 units) consumed by a 70 kg male) should result in a blood ethanol concentration of 100–200 mg dl⁻¹ (22–43 mmol l⁻¹) between 15 and 120 minutes after dosage. Highest concentrations occur after 30–90 minutes.

Alcohol diffuses from the blood into tissues across capillary walls. Ethanol concentration equilibrates between blood and the extracellular fluid within a single pass. However, equilibration between blood water and total tissue water may take several hours, depending on the cross-sectional area of the capillary bed and tissue blood flow.

Ethanol enters most tissues but its solubility in bone and fat is negligible. Therefore, in the postabsorption phase, the volume of distribution of ethanol reflects total body water. Thus, for a given dose, BEC will reflect lean body mass.

Metabolism of Alcohol

The rate at which alcohol is eliminated from the blood by oxidation varies from 6 to 10 g h⁻¹. This is reflected by the BEC, which falls by 9–20 mg dl⁻¹ h⁻¹ after consumption of ethanol. After a dose of 0.6–0.9 g per kg body weight without food, elimination of ethanol is approximately 15 mg dl blood⁻¹ h⁻¹. However, many factors influence this rate and there is considerable individual variation.

Absorbed ethanol is initially oxidized to acetaldehyde (Figure 2) by one of three pathways (Figure 3):

1. Alcohol dehydrogenase (ADH)–cytosol
2. Microsomal ethanol oxidizing system (MEOS)–endoplasmic reticulum
3. Catalase–peroxisomes

Alcohol Dehydrogenase

ADH couples oxidation of ethanol to reduction of nicotinamide adenine dinucleotide (NAD⁺) to NADH. ADH has a

wide range of substrates and functions, including dehydrogenation of steroids and oxidation of fatty acids.

Alcohol Dehydrogenase Isoenzymes

ADH is a zinc metalloprotein with five classes of isoenzymes that arise from the association of eight different subunits into dimers (Table 5). These five classes of ADH are the products of five gene loci (ADH1–5). Class 1 isoenzymes generally require a low concentration of ethanol to achieve 'half-maximal activity' (low K_m), whereas class 2 isoenzymes have a relatively high K_m . Class 3 ADH has a low affinity for ethanol and does not participate in the oxidation of ethanol in the liver. Class 4 ADH is found in the human stomach and class 5 has been reported in liver and stomach. Whereas the majority of ethanol metabolism occurs in the liver, gastric ADH is responsible for a small portion of ethanol oxidation.

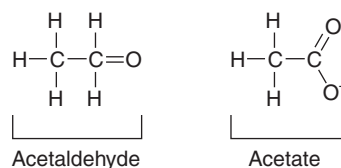


Figure 2 Chemical structures of acetaldehyde and acetate, the products of ethanol metabolism. Acetaldehyde and acetic acid/acetate are the current preferred or common names for these chemicals. However, some texts may use their systematic names, i.e., ethanal and ethanoic acid, respectively.

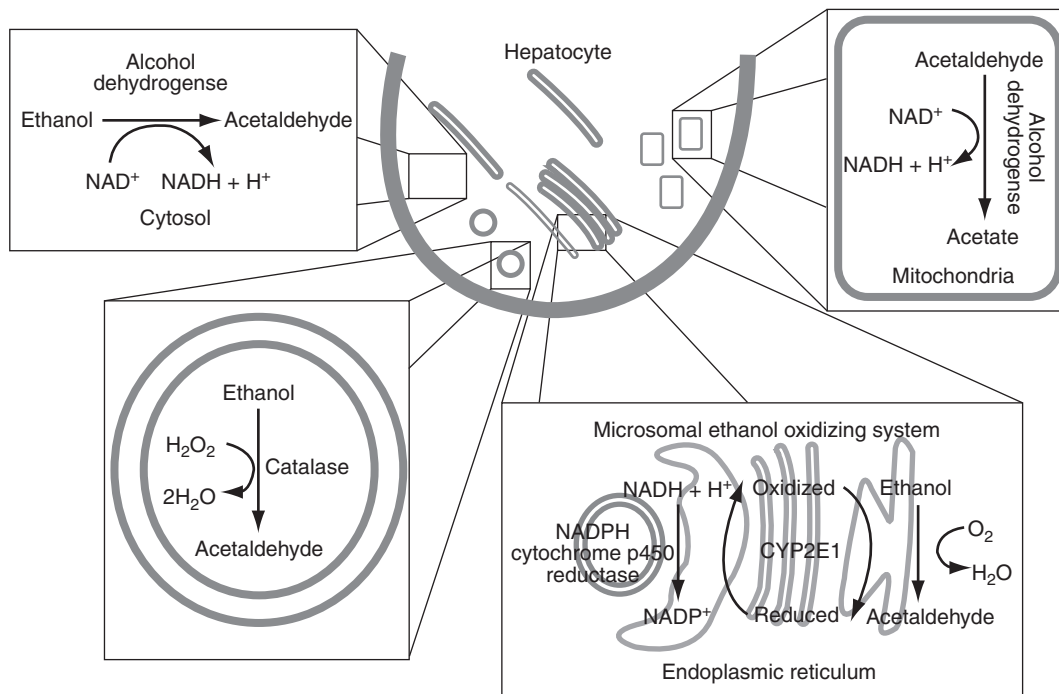


Figure 3 Pathways of ethanol metabolism.

Table 5 Classes of alcohol dehydrogenase isoenzymes

Class	Subunit	Location	K_m (mmol l ⁻¹) ^a	V_{max}
1				
ADH1	α	Liver	4	54
ADH2	β	Liver, lung	0.05–34	*
ADH3	γ	Liver, stomach	0.6–1.0	*
2				
ADH4	π	Liver, cornea	34	40
3				
ADH5	χ	Most tissues	1000	*
4				
ADH7	σ, μ	Stomach, esophagus, other mucosae	20	1510
5				
ADH6	—	Liver, stomach	30	*

^a K_m supplied is for ethanol; ADH also oxidizes other substrates.

*Reliable data not available.

Source: Reproduced with permission from Kwo PY and Crabb DW (2002) Genetics of ethanol metabolism and alcoholic liver disease. In: Sherman DIN, Preedy VR, and Watson RR (eds.) *Ethanol and the Liver. Mechanisms and Management*, pp. 95–129. London: Taylor & Francis.

Table 6 Classes of aldehyde dehydrogenase isoenzymes

Class	Structure	Location	K_m (μ mol l ⁻¹) ^a
1			
ALDH1	$\alpha 4$	Cytosolic Many tissues: highest in liver	30
2			
ALDH2	$\alpha 4$	Mitochondrial Present in all tissues except red blood cells Liver > kidney > muscle > heart	1

^a K_m supplied is for acetaldehyde; ALDH also oxidizes other substrates.

Source: Reproduced with permission from Kwo PY and Crabb DW (2002) Genetics of ethanol metabolism and alcoholic liver disease. In: Sherman DIN, Preedy VR, and Watson RR (eds.) *Ethanol and the Liver. Mechanisms and Management*, pp. 95–129. London: Taylor & Francis.

Catalase

Peroxisomal catalase, which requires the presence of hydrogen peroxide (H₂O₂), is usually of little significance in the metabolism of ethanol. Metabolism of ethanol by ADH inhibits catalase activity because H₂O₂ production is inhibited by the reducing equivalents (NADH) produced by ADH. However, metabolism of ethanol by catalase may be more significant if the other pathways for ethanol metabolism are inhibited, for example, by mitochondrial damage in a chronic alcoholic.

Microsomal Ethanol Oxidizing System

Chronic administration of ethanol with nutritionally adequate diets increases clearance of ethanol from the blood. In 1968, the MEOS was identified. The MEOS has a higher K_m for ethanol (8–10 mmol l⁻¹) than ADH (0.2–2.0 mmol l⁻¹) so at low BEC, ADH is more important. However, unlike the other pathways, MEOS is highly inducible by chronic alcohol consumption. The key enzyme of the MEOS is cytochrome

P4502E1 (CYP2E1). Chronic alcohol use is associated with a 4- to 10-fold increase of CYP2E1 due to increases in mRNA levels and rate of translation.

Acetaldehyde Metabolism

Acetaldehyde is highly toxic but is rapidly converted to acetate. This conversion is catalyzed by aldehyde dehydrogenase (ALDH) and is accompanied by reduction of NAD⁺ (Figure 3). There are several isoenzymes of ALDH (Table 6). The most important are ALDH1 (cytosolic) and ALDH2 (mitochondrial). The presence of ALDH in most tissues may reduce the toxic effects of acetaldehyde.

In alcoholics, the oxidation of ethanol is increased by induction of MEOS. However, the capacity of mitochondria to oxidize acetaldehyde is reduced. Hepatic acetaldehyde therefore increases with chronic ethanol consumption. A significant increase of acetaldehyde in hepatic venous blood reflects the high tissue level of acetaldehyde.

Metabolism of Acetate

The final metabolism of acetate derived from ethanol remains unclear. However, some important principles have been elucidated:

1. The majority of absorbed ethanol is metabolized in the liver and released as acetate. Acetate release from the liver increases $2\frac{1}{2}$ times after ethanol consumption.
2. Acetyl-CoA synthetase catalyzes the conversion of acetate to acetyl-CoA *via* a reaction requiring adenosine triphosphate. The adenosine monophosphate produced is converted to adenosine in a reaction catalyzed by 5'-nucleosidase.
3. Acetyl-CoA may be converted to glycerol, glycogen, and lipid, particularly in the fed state. However, this only accounts for a small fraction of absorbed ethanol.
4. The acetyl-CoA generated from acetate may be used to generate adenosine triphosphate *via* the Krebs's cycle.
5. Acetate readily crosses the blood-brain barrier and is actively metabolized in the brain. The neurotransmitter acetylcholine is produced from acetyl-CoA in cholinergic neurons.
6. Both cardiac and skeletal muscle are very important in the metabolism of acetate.

Based on these observations, future studies on the effects of ethanol metabolism should focus on skeletal and cardiac muscle, adipose tissue, and the brain.

Nonoxidative Metabolism of Alcohol

Nonoxidative metabolism of alcohol, which results in formation of ethyl esters from fatty acids occurs in several organs which lack an oxidative system to metabolize alcohol (e.g., pancreas, heart, and adipose tissue). These organs often develop alcohol-induced disease so fatty acid ethyl esters may play a role in the pathogenesis of the lesions induced by alcohol consumption. The nonoxidative metabolism of ethanol may be more significant if the other pathways for ethanol metabolism are inhibited.

Blood Ethanol Concentration

The relationship between BEC and the effects of alcohol is complex and varies between individuals and with patterns of drinking. Many of the effects correlate with the peak concentration of ethanol in the blood and organs during a drinking session. Other effects are due to products of metabolism and the total dose of ethanol ingested over a period of time. These two considerations are not entirely separable because the ethanol concentration during a session may determine which pathways of ethanol metabolism predominate.

It is of considerable clinical interest to understand what factors increase the probability of higher maximum ethanol concentrations for any given level of consumption.

Factors Affecting Blood Ethanol Concentration

Gender Differences in Blood Ethanol Concentration

Women achieve higher peak BEC than men given the same dose of ethanol per kilogram of body weight. The volume of

distribution of ethanol reflects total body water. Because the bodies of women contain a greater proportion of fat, it is not surprising that the BEC is higher in women. However, gender differences in the gastric metabolism of ethanol may also be relevant.

Period Over which the Alcohol is Consumed

Rapid intake of alcohol increases the concentration of ethanol in the stomach and small intestine. The greater the concentration gradient, the faster the absorption of ethanol and therefore peak BEC. If alcohol is consumed and absorbed faster than the rate of oxidation, then BEC increases.

Effects of Food on Blood Ethanol Concentration

The peak BEC is reduced when alcohol is consumed with or after food. Food delays gastric emptying into the duodenum. This attenuates the sharp early rise in BEC seen when alcohol is taken on an empty stomach. Food also increases elimination of ethanol from the blood. The area under the BEC/time curve (AUC) is reduced (**Figure 4**). The contributions of various nutrients to these effects have been studied, but small, often conflicting, differences have been found. It appears that the caloric value of the meal is more important than the precise balance of nutrients.

In animal studies ethanol is often administered with other nutrients in liquid diets. The AUC is less when alcohol is given in a liquid diet than with the same dose of ethanol in water. The different blood ethanol profile in these models may affect the expression of pathology.

However, food increases splanchnic blood flow, which maintains the ethanol diffusion gradient in the small intestine. Food-induced impairment of gastric emptying may be partially offset by faster absorption of ethanol in the duodenum.

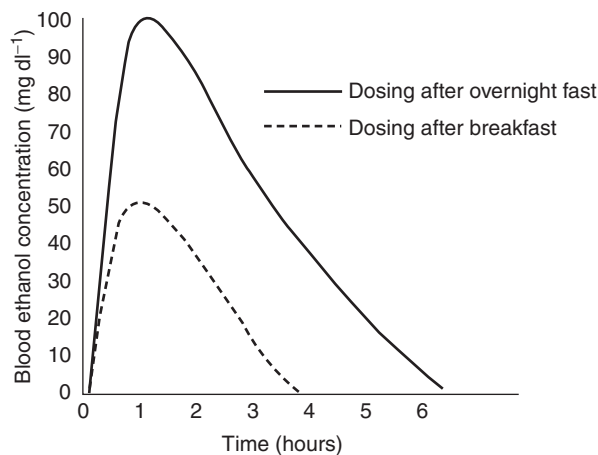


Figure 4 Blood ethanol concentration curve after oral dosing of ethanol. A subject ingested 0.8 g kg^{-1} ethanol over 30 minutes either after an overnight fast or after breakfast. The peak blood ethanol concentration and the area under the curve are reduced if ethanol is consumed with food.

Beverage Alcohol Content and Blood Ethanol Concentration

The ethanol concentration of the beverage consumed (Table 7) affects ethanol absorption and can affect BEC. Absorption is fastest when the concentration is 10–30%. Below 10%, the low ethanol concentration in the GI tract reduces diffusion and the greater volume of liquid slows gastric emptying. Concentrations above 30% irritate the GI mucosa and the pyloric sphincter, increasing secretion of mucus and delaying gastric emptying. Some evidence has shown that even low concentrations of ethanol (e.g., 4%, as found in beer) may cause minor lesions in the gastric mucosa though they may be insignificant pathologically.

First-Pass Metabolism of Ethanol

The AUC is significantly lower after oral dosing of ethanol than after intravenous or intraperitoneal administration. The total dose of intravenously administered ethanol is available to the systemic circulation. The difference between AUC_{oral} and AUC_{iv} represents the fraction of the oral dose that was either not absorbed or metabolized before entering the systemic circulation (first-pass metabolism (FPM)). The ratio of AUC_{oral} to AUC_{iv} reflects the oral bioavailability of ethanol.

The investigation of ethanol metabolism has primarily focused on the liver and its relationship to liver pathology. However, gastric metabolism accounts for approximately 5% of ethanol oxidation and 2–10% is excreted in the breath, sweat, or urine. The rest is metabolized by the liver.

After absorption, ethanol is transported to the liver in the portal vein. Some is metabolized by the liver before reaching the systemic circulation. However, hepatic ADH is saturated at a BEC that may be achieved in an average-size adult after consumption of one or two units. If ADH is saturated by ethanol entering the liver from the systemic circulation *via* the hepatic artery, ethanol in the portal blood must compete for binding to ADH. Although hepatic oxidation of ethanol cannot increase once ADH is saturated, gastric ADH can significantly metabolize ethanol at the high concentrations in the stomach after initial ingestion. If gastric emptying of ethanol is delayed, prolonged contact with gastric ADH increases FPM. Conversely, fasting, which greatly increases the speed of gastric emptying, virtually eliminates gastric FPM.

Physiological Effects of Alcohol

Ethanol or the products of its metabolism affect nearly all cellular structures and functions.

Effects of Alcohol on the Central Nervous System

Ethanol generally decreases the activity of the central nervous system. In relation to alcohol, the most important neurotransmitters in the brain are glutamate, gamma-aminobutyric acid (GABA), dopamine, and serotonin.

Glutamate is the major excitatory neurotransmitter in the brain. Ethanol inhibits the *N*-methyl-D-aspartate (NMDA) subset of glutamate receptors. Ethanol thereby reduces the excitatory effects of glutamate. GABA is the major inhibitory neurotransmitter in the brain. Alcohol facilitates the action of the GABA-a receptor, increasing inhibition. Changes to these receptors seem to be important in the development of tolerance and dependence on alcohol.

Dopamine is involved in the rewarding aspects of alcohol consumption. Enjoyable activities such as eating or use of other recreational drugs also release dopamine in the nucleus accumbens of the brain. Serotonin is also involved in the reward processes and may be important in encouraging alcohol use.

The most obvious effects of ethanol intoxication on the central nervous system begin with behavior modification (e.g., cheerfulness, impaired judgment, and loss of inhibitions). These excitatory effects result from the disinhibition described previously (inhibition of cells in the brain that are usually inhibitory).

As a result of these effects, it is well recognized that operating vehicles such as cars or heavy machinery under the influence of ethanol is unsafe. However, the BEC after consumption of a specific amount of ethanol and the impairment caused by a specific BEC vary significantly between individuals. Despite this variation, BEC is used to define intoxication and provide a rough measure of impairment for legal purposes because it is an objective measurement that is difficult to contest.

Most countries have set maximum legally permissible BEC levels for drivers to reduce harm from 'drink driving.' Governments define these level after reviewing the available evidence. However, the definition of what is safe or acceptable varies between countries (Table 8). These BEC

Table 7 Alcohol content of selected beverages

Beverage	Alcohol content		
	^a g dl ⁻¹ (%)	^b mmol l ⁻¹	^b mol l ⁻¹
Low-strength beers	3–4	650–870	0.65–0.87
High-strength beers	8–9	1740–1960	1.74–1.96
Wine	7–14	1520–3040	1.52–3.04
Brandy	35–45	7610–9780	7.61–9.78
Vodka	35–50	7610–10870	7.61–10.87
Gin	35–50	7610–10870	7.61–10.87
Whisky	35–75	7610–16300	7.61–16.30

^aData obtained by reviewing the alcohol content of various beverages as stated on the product label.

^bData calculated from the information obtained from reviewing the alcoholic content of various beverages as stated on the product label.

Table 8 Legal limits of blood ethanol concentrations for driving^a

Legal limit ^b	Blood ethanol concentration	
	mg dl ⁻¹	mmol l ⁻¹
The Czech Republic, and Hungary	0	0
Norway and Sweden	20	4.3
Japan, Russian Federation, and Uruguay	30	6.45
France, Germany, Italy, and Australia	50	11
United Kingdom, ^c United States, and Canada	80	17

^aEthanol impairs judgment and coordination. It is well recognized that driving under the influence of ethanol is unsafe. However, the definition of what is safe or acceptable varies between countries and can change as a result of social, political, or scientific influences.

^bLegislation regarding legal limits of blood ethanol for driving may change.

^cIn the UK, the legal limit is currently 80 mg dl⁻¹ but the recently published North Review of Drink and Drug Driving Law strongly recommended that this limit be reduced to 50 mg dl⁻¹.

thresholds range from zero tolerance (0.0 mg ml⁻¹) to 0.8 mg ml⁻¹.

Some countries are considering the potential social benefits of lowering BEC limits. However, opponents cite factors such as the drinking culture, convenience, the unpalatability of tighter legislation and the impact on the alcohol industry.

The effects of ethanol are dose dependent (Table 9) and further intake causes agitation, slurred speech, memory loss, double vision, and loss of coordination. This may progress to depression of consciousness and loss of airway protective reflexes, with danger of aspiration, suffocation, and death.

This sequence of events is particularly relevant in the hospital setting, where patients may present intoxicated with a reduced level of consciousness. It is difficult to determine whether there is coexisting pathology such as an extradural hematoma or overdose of other drugs in addition to ethanol. Although measurement of BEC is helpful (Table 9), it is safest to assume that alcohol is not responsible for any disturbance in consciousness and to search for another cause.

Neuroendocrine Effects of Alcohol

Alcohol activates the sympathetic nervous system, increasing circulating catecholamines from the adrenal medulla. Hypothalamic–pituitary stimulation results in increased circulating cortisol from the adrenal cortex and can, rarely, cause a pseudo-Cushing's syndrome with typical moon-shaped face, truncal obesity, and muscle weakness. Alcoholics with pseudo-Cushing's show many of the biochemical features of Cushing's syndrome, including failure to suppress cortisol with a 48-h low-dose dexamethasone suppression test. However, they may be distinguished by an insulin stress test. In pseudo-Cushing's, the cortisol rises in response to insulin-induced hypoglycemia, but in true Cushing's there is no response to hypoglycemia.

Ethanol affects hypothalamic osmoreceptors, reducing vasopressin release. This increases salt and water excretion from the kidney, causing polyuria. Significant dehydration may result particularly with consumption of spirits containing high concentrations of ethanol and little water. Loss of

hypothalamic neurons (which secrete vasopressin) has also been described in chronic alcoholics, suggesting long-term consequences for fluid balance. Plasma atrial natriuretic peptide, increased by alcohol consumption, may also increase diuresis and resultant dehydration.

Alcoholism also affects the hypothalamic–pituitary–gonadal axis. These effects are further exacerbated by alcoholic liver disease. There are conflicting data regarding the changes observed. Testosterone is either normal or decreased in men, but it may increase in women. Estradiol is increased in men and women, and it increases as hepatic dysfunction deteriorates. Production of sex hormone-binding globulin is also perturbed by alcohol.

The development of female secondary sexual characteristics in men (e.g., gynecomastia and testicular atrophy) generally only occurs after the development of cirrhosis. In women, the hormonal changes may reduce libido, disrupt menstruation, or even induce premature menopause. Sexual dysfunction is also common in men with reduced libido and impotence. Fertility may also be reduced, with decreased sperm counts and motility.

Effects of Alcohol on Muscle

Myopathy is common, affecting up to two-thirds of all alcoholics. It is characterized by wasting, weakness, and myalgia and improves with abstinence. Histology correlates with symptoms and shows selective atrophy of type II muscle fibers. Ethanol causes a reduction in muscle protein and ribonucleic acid content. The underlying mechanism is unclear, but rates of muscle protein synthesis are reduced, whereas protein degradation is either unaffected or inhibited. Attention has focused on the role of acetaldehyde adducts and free radicals in the pathogenesis of alcoholic myopathy.

Alcohol and Nutrition

The nutritional status of alcoholics is often impaired. Some of the pathophysiological changes seen in alcoholics are direct consequences of malnutrition. However, in the 1960s, Charles Lieber demonstrated that many alcohol-induced pathologies, including alcoholic hepatitis, cirrhosis, and myopathy, are reproducible in animals fed a nutritionally adequate diet. Consequently, the concept that all alcohol-induced pathologies are due to nutritional deficiencies is outdated and incorrect.

Myopathy is a direct consequence of alcohol or acetaldehyde on muscle and is not necessarily associated with malnutrition. Assessment of nutritional status in chronic alcoholics using anthropometric measures (e.g., limb circumference and muscle mass) may be misleading in the presence of myopathy.

Acute or chronic ethanol administration impairs the absorption of several nutrients, including glucose, amino acids, biotin, folate, and ascorbic acid. There is no strong evidence that alcohol impairs absorption of magnesium, riboflavin, or pyridoxine, so these deficiencies are probably due to poor intakes. Hepatogastrointestinal damage (e.g., villous injury, bacterial overgrowth of the intestine, pancreatic damage, or

Table 9 Relationship between amount of ethanol consumed, blood ethanol concentration (BEC) and effect of ethanol on the central nervous system

<i>Alcohol consumed^a</i> (UK units)	<i>Possible BEC</i>	<i>Effect</i>
1–5	10–50 mg dl ⁻¹ 2–11 mmol l ⁻¹	No obvious change in behavior
2–7	30–100 mg dl ⁻¹ 7–22 mmol l ⁻¹ Euphoria Sociability	Increased self-confidence; loss of inhibitions Impaired judgment, attention, and control Mild sensorimotor impairment, delayed reaction times Legal limits for driving generally fall within this range (see Table 8)
8–15	90–250 mg dl ⁻¹ 20–54 mmol l ⁻¹	Loss of critical judgment Impairment of perception, memory, and comprehension Reduced visual acuity Reduced coordination, impaired balance Drowsiness
11–20	180–300 mg dl ⁻¹ 39–65 mmol l ⁻¹ Confusion	Disorientation Exaggerated emotional states Disturbances of vision and perception of color, form, motion, and depth Increased pain threshold Further reduction of coordination, staggering gait, slurred speech
15–25	250–400 mg dl ⁻¹ 54–87 mmol l ⁻¹ Stupor	Loss of motor functions Markedly reduced response to stimuli Marked loss of coordination, inability to stand/walk Incontinence Impaired consciousness
22–30	350–500 mg dl ⁻¹ 76–108 mmol l ⁻¹ Coma	Unconsciousness Reduced or abolished reflexes Incontinence Cardiovascular and respiratory depression (death possible)
38	> 600 mg dl ⁻¹ > 130 mmol l ⁻¹ Death	Respiratory arrest

^aApproximate amounts of alcohol required by a 70 kg male to produce the corresponding blood ethanol concentration (BEC) and intoxicating effects of ethanol. One UK unit of alcohol contains 8 g of ethanol. It should be noted that these theoretical data are provided for illustrative purposes only. The BEC and the effects after consumption of ethanol are dependent on several factors and varies significantly between individuals.

Source: Adapted with permission from Morgan MY and Ritson B (2003) *Alcohol and Health: A Handbook for Students and Medical Practitioners*, 4th edn. London: Medical Council on Alcohol.

cholestasis) may impair the absorption of some nutrients such as the fat-soluble vitamins (A, D, E, and K). In contrast, iron stores may be adequate as absorption is increased.

Effects of Alcohol on the Cardiovascular System

Alcohol affects both the heart and the peripheral vasculature. Acutely, alcohol causes peripheral vasodilatation, giving a false sensation of warmth that can be dangerous. Heat loss is rapid in cold weather or when swimming, but reduced awareness leaves people vulnerable to hypothermia. The main adverse effect of acute alcohol on the cardiovascular system is the induction of arrhythmias i.e., 'Holiday Heart'. These are often harmless and experienced as palpitations but can rarely be fatal. Chronic ethanol consumption can cause systemic hypertension and congestive cardiomyopathy. Alcoholic cardiomyopathy accounts for up to one-third of dilated cardiomyopathies but may improve with abstinence or progress to death.

The beneficial, cardioprotective effects of alcohol consumption have been broadcast widely. This observation is based on population studies of mortality due to ischemic heart disease, case-control studies, and animal experiments. However, there is no evidence from randomized controlled trials. The apparent protective effect of alcohol may therefore result from confounding factors. For example, the diets are different to those of nondrinkers. Even the diets of beer drinkers are different from those of wine drinkers. Furthermore, on the population level, the burden of alcohol-induced morbidity and mortality far outweighs any possible cardiovascular benefit.

Effects of Alcohol on Liver Function

Fundamental to the effects of ethanol is the liver, in which 60–90% of ethanol metabolism occurs. Ethanol displaces many of the substrates usually metabolized in the liver. Metabolism of ethanol by ADH in the liver generates reducing

equivalents. ALDH also generates NADH with conversion of acetaldehyde to acetate. The NADH:NAD⁺ ratio is increased, with a corresponding increase in the lactate:pyruvate ratio. If lactic acidosis combines with a β -hydroxybutyrate predominant ketoacidosis, the blood pH can fall to 7.1 and hypoglycemia may occur. Severe ketoacidosis and hypoglycemia can cause permanent brain damage. However, in general the prognosis of alcohol-induced acidosis is good. Lactic acid also reduces the renal capacity for urate excretion. Hyperuricemia is further exacerbated by alcohol-induced ketosis and acetate-mediated purine generation. Hyperuricemia explains, at least in part, the clinical observation that alcohol misuse can precipitate gout.

The excess NADH promotes fatty acid synthesis and inhibits lipid oxidation in the mitochondria, resulting in fat accumulation. Fatty changes within the liver are usually asymptomatic but can be seen on ultrasound or computed tomography scanning, and they are associated with abnormal liver toxicity tests (e.g., raised activities of serum γ -glutamyl transferase, aspartate aminotransferase, and alanine transaminases). The supposition that most of the hepatic damage in alcoholism is due to increases in the NADH:NAD ratio *per se* is somewhat outdated. Now, molecular and cellular processes and acetaldehyde toxicity have been shown to be major contributors to the disease process.

Progression to alcoholic hepatitis involves invasion of the liver by neutrophils with hepatocyte necrosis. Giant mitochondria are visible and dense cytoplasmic lesions (Mallory bodies) are seen. Alcoholic hepatitis can be asymptomatic but usually presents with abdominal pain, fever, and jaundice, or, depending on the severity of disease, patients may have encephalopathy, ascites, and ankle edema.

Continued alcohol consumption may lead to cirrhosis. However, not all alcoholics progress to cirrhosis. The reason for this is unclear. It has been suggested that genetic factors and differences in immune response may play a role.

In alcoholic cirrhosis there is fibrocollagenous deposition, with scarring and disruption of surrounding hepatic architecture. There is ongoing necrosis with concurrent regeneration. Alcoholic cirrhosis is classically said to be micronodular, but often a mixed pattern is present. The underlying pathological mechanisms are complex and are the subject of debate. Induction of the MEOS and oxidation of ethanol by catalase result in free radical production. Glutathione (a free radical scavenger) is reduced in alcoholics, impairing the ability to dispose of free radicals. Mitochondrial damage occurs, limiting their capacity to oxidize fatty acids. Peroxisomal oxidation of fatty acids further increases free radical production. These changes eventually result in hepatocyte necrosis, and inflammation and fibrosis ensue. Acetaldehyde also contributes by promoting collagen synthesis and fibrosis.

Alcohol and Facial Flushing

Genetic variations in ADH and ALDH may explain why particular individuals develop some of the pathologies of alcoholism and others do not. For example, up to 50% of Orientals have a genetically determined reduction in ALDH2 activity ('flushing' phenotype). As a result, acetaldehyde

accumulates after ethanol administration, with plasma levels up to 20 times higher in people with ALDH2 deficiency. Even small amounts of alcohol produce a rapid facial flush, tachycardia, headache, and nausea. Acetaldehyde partly acts through catecholamines, although other mediators have been implicated, including histamine, bradykinin, prostaglandin, and endogenous opioids.

This is similar to the disulfiram reaction due to the rise of acetaldehyde after inhibition of ALDH. Disulfiram is used therapeutically to encourage abstinence in alcohol rehabilitation programs. The aversive effects of acetaldehyde may reduce the development of alcoholism and the incidence of cirrhosis in 'flushers.' However, some alcoholics with ALDH2 deficiency and, presumably, higher hepatic acetaldehyde levels develop alcoholic liver disease at a lower intake of ethanol than controls.

Effects of Acetaldehyde

Acetaldehyde is highly toxic and can bind cellular constituents (e.g., proteins including CYP2E1, lipids, and nucleic acids) to produce harmful acetaldehyde adducts. Adduct formation changes the structure and the biochemical properties of the affected molecules. The new structures may be recognized as foreign antigens by the immune system and initiate a damaging response.

Adduct formation leads to retention of protein within hepatocytes, contributing to the hepatomegaly, and several toxic manifestations, including impairment of antioxidant mechanisms (e.g., decreased glutathione (GSH)). Acetaldehyde thereby promotes free radical-mediated toxicity and lipid peroxidation. Binding of acetaldehyde with cysteine (one of the three amino acids that comprise GSH) or GSH also reduces liver GSH content. Chronic ethanol administration significantly increases rates of GSH turnover in rats. Acute ethanol administration inhibits GSH synthesis and increases losses from the liver. Furthermore, mitochondrial GSH is selectively depleted and this may contribute to the marked disruption of mitochondria in alcoholic cirrhosis.

Effects of Acetate

The role of acetate in alcohol-induced pathology is not well understood. The uptake and utilization of acetate by tissues depend on the activity of acetyl-CoA synthetase. Acetyl-CoA and adenosine are produced from the metabolism of acetate. Acetate crosses the blood-brain barrier easily and is actively metabolized in the brain. Many of the central nervous system depressant effects of ethanol may be blocked by adenosine receptor blockers. Thus, acetate and adenosine may be important in the intoxicating effects of ethanol.

Ethanol increases portal blood flow, mainly by increasing GI tract blood flow. This effect is reproduced by acetate. Acetate also increases coronary blood flow, myocardial contractility, and cardiac output. Acetate inhibits lipolysis in adipose tissue and promotes steatosis in the liver. The reduced circulating free fatty acids (a source of energy for many tissues) may have significant metabolic consequences. Thus, some of

the effects of alcohol may be due to acetate, though this area is under explored.

See also: Alcohol: Effects of Consumption on Diet and Nutritional Status. Liver Disorders: Nutritional Management

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Effects of Consumption on Diet and Nutritional Status

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Glossary

Alcoholic liver disease Occurs in chronic alcoholic subjects and is characterized by steatosis (increased fat in the liver), inflammation, necrosis (death of liver cells), and fibrosis (scarring that leads to cirrhosis).

Chronic alcoholism Long-standing alcohol abuse.

Drink Unit of alcohol consumption, usually 12–15 g, as found in 12 oz of beer, 1.2 oz of spirits, and 4 oz of wine.

Moderate drinking Up to 2 drinks per day for men but 1 drink per day for women.

Nutritional consequences of alcoholism Weight loss, protein malnutrition, deficiencies of folate, pyridoxine, thiamine, vitamin A, vitamin D, zinc, and iron.

Risks of chronic alcoholism Liver disease, chronic pancreatitis, heart disease, anemia, and cognitive disorders.

Introduction

Alcohol is a component of the diet that provides 7.1 kcal g^{-1} and 5.6% of total dietary energy intake in the United States. When consumed in moderation, alcoholic beverages protect against cardiovascular disease, but when alcohol is consumed in excess it can become an addictive drug with potential for displacement of beneficial components of the diet, damage to several organ systems including the liver, brain, and heart, and increased risk of several cancers. The consumption of excessive amounts of alcohol contributes to generalized malnutrition, with particular effects on the availability and metabolism of both water- and fat-soluble vitamins including folate, thiamine, pyridoxine, niacin, and vitamins A and D. All the effects of alcoholism on nutritional status are magnified in the presence of alcoholic liver disease. This article addresses the benefits and risks of alcohol consumption and the effects of alcohol drinking on human nutritional status.

Effects of Alcohol Consumption on Dietary Intake

Alcohol is consumed by approximately two-thirds of adult Americans, and the estimated per capita annual consumption of alcohol exceeds 2 gal for each US citizen over age 14. In the United States, young adults between 18 and 25 years of age consume more alcohol than any other age group, and the preferred beverages are beer, wine, and spirits in that order. However, older men and women are more likely to develop liver, pancreatic, or neurological disease as a result of their chronic alcoholism. Adult men consume approximately three times more alcohol than adult women do. Among alcohol consumers, most are moderate drinkers, whereas approximately 7% are heavy drinkers at risk for addiction and organ damage. Moderate drinking can be defined as consuming no more than 2 drinks per day for men or 1 drink per day for women, where 1 drink contains 12–15 g of alcohol. Heavy drinking is defined as consuming more than 5 drinks on any given day per week for men or 4 drinks on any given

day per week for women. Chronic alcoholics are addicts who typically consume excessive amounts of alcohol on a daily basis. Binge drinkers are chronic alcoholics who escalate their alcohol intake over weeks or months, typically to the exclusion of the essential components of their regular diets. Alcoholic beverages differ in their alcohol content, such that spirits contain approximately $40 \text{ g } 100 \text{ mL}^{-1}$, wine approximately $12 \text{ g } 100 \text{ mL}^{-1}$, and beer approximately $4.5 \text{ g } 100 \text{ mL}^{-1}$. Thus, the amount of alcohol found in 12 oz of beer is roughly equivalent to the amount found in 4 oz of wine or 1.2 oz of spirits.

The effects of alcohol on dietary intake depend on the amount consumed each day and changes in overall eating behavior. Although alcohol contains 7.1 kcal g^{-1} , it is rapidly metabolized to acetaldehyde in the liver at rates up to 50 g h^{-1} , and none is stored as energy equivalents in the body. Furthermore, the metabolism of alcohol influences the metabolism of dietary fat and carbohydrate. There are three metabolic routes for the disposal of alcohol by the body: two in the liver and one in the stomach. Alcohol dehydrogenase (ADH) is present in the cytosol of hepatocytes and metabolizes relatively low levels of alcohol that would be expected after moderate drinking. The metabolism of alcohol by ADH causes a redox change that contributes to lipid synthesis in the liver, reduces gluconeogenesis, and increases lactate production. Thus, even moderate drinking can cause fatty liver with elevated serum triglyceride levels and, in the absence of dietary carbohydrate, may result in low blood glucose levels that impair concentration and even consciousness. The second liver enzyme, CYP2E1, is part of the cytochrome P450 family and metabolizes alcohol at levels to be expected after heavy drinking. During metabolism of high levels of alcohol, CYP2E1 utilizes adenosine triphosphate (ATP) energy units and thus 'wastes' stored calories, with resultant potential for weight loss. Another form of this enzyme, gastric CYP2E1, exists in the stomach and, as the first of the three alcohol-metabolizing enzymes to encounter alcohol, accounts for approximately 30% of all alcohol metabolism in men but only 10% in women. This gender difference may explain why

women's tolerance to alcohol is much less than men's, hence the recognized lower 'safe' level for moderate drinking in women.

The Potential Benefits of Moderate Alcohol Consumption

In 1992, French scientists published a report indicating that cardiovascular mortality was much less among wine-drinking residents of the Mediterranean southern provinces of France than their counterparts in northern provinces where wine is less frequently preferred, in spite of similar overall dietary components and rates of consumption of alcoholic beverages (Table 1). This report on the 'French Paradox' attributed specific cardioprotective benefit to wine but was soon tempered by *in vitro* studies that showed that the protective effect of wine on the oxidation of low-density lipoprotein could be mimicked by constitutive antioxidant flavonoids present not only in grapes but also in many other fruits and vegetables. Another epidemiological study concluded that the lower mortality risk among wine drinkers compared to nonwine

drinkers could be attributed in large part to a better lifestyle, including less smoking, more exercise, and better diet. Subsequent population studies defined J-shaped curves for alcohol-related mortality, where mortality is increased in abstainers and progressively in women who consume more than 1 drink per day and in men who consume more than 2 drinks per day. The benefits of moderate drinking are confined to reductions in incidences of coronary vessel occlusions and ischemic strokes but not of hemorrhagic strokes. Although red and white wine each contain protective antioxidant flavonoids, moderate amounts of alcohol also improve the circulating lipid profile by increasing levels of high-density lipoprotein and tissue plasminogen activator while reducing platelet adhesiveness.

The Risks of Excessive Alcohol Consumption

Unlike other drug abuses, chronic alcohol abuse affects many different organ systems, which include the liver, pancreas, heart, and brain, and also increases the risk of certain cancers (Table 1). Although these risks are apparent among the 7% of

Table 1 Benefits and risks of alcohol consumption

	<i>Minimal amount or duration of drinks per day</i>	<i>Mechanism</i>
Benefits		
Coronary disease protection	1–2 (women), 2–4 (men)	Flavonoid antioxidants
Cerebrovascular disease (nonhemorrhagic) protection		Elevated HDL lipoprotein Reduced platelet adhesiveness
Risks		
Cancer		
Oropharynx and esophagus	> 2 (women), > 4 (men)	Unknown; higher risk in smoking alcoholics
Breast (women)	> 2	Increases estrogen production
Colon	> 2 (women), > 4 (men)	Initiation risk increases with low folate, proliferation risk increases with excessive folate
Alcoholic liver disease		
Fatty liver	> 2	Increased liver fat synthesis, decreased oxidation and export
Alcoholic hepatitis	> 3 (women) × 10 years > 6 (men) × 15 years	Toxicity of alcohol metabolism
Alcoholic cirrhosis	> 3 (women) × 15 years > 6 (men) × 20 years	Increased collagen synthesis
Pancreas		
Pancreatitis	~ 10 years	Acute inflammation of pancreas
Pancreatic insufficiency	~ 10–15 years	Loss of exocrine and endocrine pancreatic cells
Cardiomyopathy	Binge drinking	Mitochondrial damage of muscle cells or thiamine deficiency
Neurological effects		
Acute trauma, e.g., motor vehicle accidents	1–2 in social setting	Legal intoxication
Coma and death	10–20 in rapid succession	Severe toxicity
Withdrawal syndrome	Follows binge drinking	Neuronal hyperexcitability
Wernicke–Korsakoff syndrome	Unknown	Thiamine deficiency
Anemia	Unknown	Combination of iron, folate, and pyridoxal deficiencies

Source: Reproduced from Halsted CH (2006) Alcohol: effects of consumption on diet and nutritional status. In: Caballero B, Allen L, and Prentice AM (eds.) *Encyclopedia of Human Nutrition*, 2nd edn., pp. 62–69. Amsterdam: Elsevier.

US citizens over age 14 who abuse alcohol, their prevalence is generally no less in countries such as France, Italy, and Spain where drinking wine with meals is considered part of the culture. The organ damage from chronic alcoholism may impact on processes of nutrient assimilation and metabolism, as is the case with chronic liver and pancreatic disease, or may be modulated in large part by nutrient deficiencies, for example, the moderating effects of thiamine on brain function. This section considers specific effects of alcohol abuse on certain organs as a background for consideration of specific effects on nutritional status.

Alcoholic Liver Disease

Alcoholic liver disease is among the top 10 causes of mortality in the United States, with somewhat higher mortality rates in western European countries where wine is considered a dietary staple and is a leading cause of death in Russia. Alcoholic liver disease accounts for approximately half of liver disease mortality in the United States. Several mechanisms are implicated in the pathogenesis of liver disease during alcohol drinking. Alcohol-induced translocation of bacterial lipopolysaccharide (LPS) from the intestinal lumen through the portal vein initiates an inflammatory process in the liver by activating tumor necrosis factor alpha (TNF- α), a cytokine that promotes oxidative injury with necrosis of liver cells and also has systemic effects including fever and anorexia with weight loss. Steatosis, or increased lipid in the liver, is initiated by several factors that include the effects of alcohol on liver methionine and adipokine metabolism that promotes lipid synthesis in liver cells, whereas other mechanisms reduce fatty acid oxidation and the export of lipid from the liver. Altered methionine metabolism in the liver also contributes to apoptosis, or cell death, and reduction of antioxidant glutathione. Fibrosis results from collagen synthesis by hepatic stellate cells and is, in part, initiated by their incorporation of apoptotic liver cells as well as a switch in vitamin A storage to production of collagen. Among the three stages of alcoholic liver disease, fatty liver is related to the acute effects of alcohol on hepatic lipid metabolism and is completely reversible with alcohol sobriety. By contrast, alcoholic hepatitis usually occurs after a decade or more of chronic alcoholism, is associated with steatosis and inflammation of the liver with necrosis of liver cells, and carries approximately 40% mortality risk within 6 months. Alcoholic cirrhosis represents irreversible scarring of the liver secondary to fibrosis with loss of liver cells and function and is usually preceded by alcoholic hepatitis. The scarring process greatly alters the circulation of blood through the liver in the sinusoids and is associated with increased blood pressure in the portal (visceral) circulation and shunting of blood flow away from the liver and through other organs such as the esophagus. The potentially lethal complications of portal hypertension include hemorrhage due to rupture of esophageal varices, ascites or accumulation of fluid in the abdominal cavity with potential for peritonitis, and hepatic encephalopathy due to inadequate hepatic detoxification of ammonia in the visceral blood that is shunted around the scarred liver. The risk of developing alcoholic cirrhosis is dependent on the amount of alcohol exposure regardless of the presence or

absence of malnutrition. For example, a study of well-nourished German male executives found that the incidence of alcoholic cirrhosis was directly related to the amount and duration of alcohol consumption such that daily ingestion of 160 g alcohol, equivalent to that found in somewhat less than a pint of whisky, predicted a 50% risk of cirrhosis on liver biopsy over a 15-year period. Other worldwide demographic data indicate that mortality rates from cirrhosis of the liver can be related to national per capita alcohol intake. These studies have defined the threshold risk for eventual development of alcoholic cirrhosis as 6 drinks per day for men and approximately half that for women. Of note, only approximately 20% of heavy drinkers develop clinically significant alcoholic liver disease, indicating that genetic susceptibility is a likely additional factor in the pathogenesis of this condition.

Pancreatitis and Pancreatic Insufficiency

Pancreatitis occurs less frequently than liver disease in chronic alcoholics and is characterized by severe attacks of abdominal pain due to inflammation of the pancreas. Pancreatic insufficiency is a feature of chronic pancreatitis, which occurs after multiple bouts of acute pancreatitis and leads to the eventual destruction of exocrine pancreatic cells that secrete digestive enzymes and of endocrine cells that secrete insulin. This destructive process is associated with progressive scarring of the pancreas together with distortion and partial blockage of the pancreatic ducts, which limit secretion and promote recurrent episodes of acute inflammatory pancreatitis. Because the pancreas is the site of production of proteases and lipases for protein and lipid digestion, destruction of more than 90% of the pancreas results in significant malabsorption of these major dietary constituents with consequent steatorrhea and weight loss, as well as diabetes secondary to reduced insulin secretion. Patients with pancreatic insufficiency exhibit severe loss of body fat and muscle protein. Because the absorption of fat-soluble vitamins is dependent on pancreatic lipase for solubilization of dietary fat, these patients are also at risk for deficiencies of vitamins A, D, and E.

Heart

Although coronary heart disease risk may be decreased by alcohol consumption, excessive alcohol use also impairs cardiac muscle function. Episodic heavy drinking bouts can lead to arrhythmias with potential for sudden death in the 'holiday heart' syndrome. Chronic alcoholics are prone to left-sided heart failure secondary to decreased mitochondrial function of cardiac muscle cells, possibly mediated by abnormal fatty acid metabolism. A specific form of high-output heart failure, or 'wet beriberi,' occurs in association with thiamine deficiency as described in more detail in the Section on Micronutrient Deficiencies in Chronic Alcoholism.

Neurological Effects

Approximately half of the 20 million chronic alcoholics in the US are affected by neuropsychological difficulties. The many neurological effects of acute and chronic alcohol abuse can be

categorized as those related directly to alcohol, those secondary to chronic liver diseases, and those mediated by thiamine deficiency. The variable effects of alcohol on the brain are related to several factors including the duration and amount of drinking, the age when drinking was started, malnutrition, genetic background, and family history of alcoholism. The stages of acute alcohol toxicity progress upward from legal intoxication with reduced reaction time and judgment, as occurs with blood alcohol levels greater than 0.08 g dl^{-1} that usually define legal intoxication, to coma and death with levels greater than 0.35 g dl^{-1} . Although mild intoxication is common with social drinking, coma and death have been described among college-aged males who consume excessive amounts of alcohol in a very short period of time. Automobile accidents, which account for a large portion of alcohol-related deaths, are more common in drunken pedestrians than drivers. Intoxication also leads to frequent falls and head trauma, and subdural hematoma presents with delayed but progressive loss of cognition, headaches, and eventual death. Chronic alcoholics are prone to episodes of alcohol withdrawal, which can be characterized by stages of tremulousness, seizures, and delirium tremens with hyperexcitability and hallucinations at any time up to 5 days after the last drink. This state of altered consciousness is distinct from hepatic encephalopathy in chronic alcoholic liver disease resulting from diversion of toxic nitrogenous substances around the scarred cirrhotic liver and is associated with progressive slowing of cerebral functions with stages of confusion, loss of cognition, and eventual coma and death. Progressive altered cognition and judgment can also result from cerebral atrophy following years of heavy drinking and may also be mediated by thiamine deficiency as described in greater detail in the Section on Micronutrient Deficiencies in Chronic Alcoholism.

Cancers

Chronic alcoholics are at increased risk for cancers of the oropharynx and esophagus, colon, and breast. The risk of oropharyngeal cancer is greatest when heavy smoking is combined with excessive daily alcohol consumption. Increased risk of squamous cell cancer of the esophagus is also compounded by smoking and may be associated with deficiencies of vitamin A and zinc. Breast cancer in women may be mediated through increased estrogen production during heavy alcohol intake. Colon cancer risk is increased among alcoholics with marginal folate deficiency, but excessive folate supplementation may cause benign adenomas to proliferate to malignancy.

Anemia

Chronic alcoholics who substitute large amounts of alcohol for other dietary constituents are at risk for developing anemia. The causes of anemia in chronic alcoholics are multifactorial, including iron deficiency secondary to occult bleeding from episodic gastritis or other gastrointestinal sites, folate deficiency from inadequate diet, malabsorption, and

increased renal excretion of folic acid, and deficiency of pyridoxine (vitamin B₆) due to abnormal effects on its metabolism. Consequently, the bone marrow may demonstrate absent iron and mixtures of megaloblastosis from folate deficiency and sideroblastosis from pyridoxine deficiency.

The Effects of Chronic Alcohol Consumption on Nutritional Status

Body Weight and Energy Balance

The effects of alcoholism on body weight are dependent on the timing and amount of alcohol consumption in relation to meals and on the presence or absence of organ damage, in particular, alcoholic liver disease (Table 2). Although body weight is usually unaffected by moderate alcohol consumption, chronic alcoholics who drink daily while substituting alcohol for other dietary constituents lose weight due to the energy-neutral effect of alcohol in the diet. Moderate drinkers on weight loss regimens are less likely to lose weight while consuming alcohol with their meals because one effect of alcohol is to decrease restraint over eating. At the same time, those who consume alcohol with high-fat meals are more likely to gain weight due to an acute effect of alcohol on reducing the metabolism of fat while it promotes its storage.

The presence of alcoholic liver disease results in significant changes in body composition and energy balance. According to large multicenter studies, alcoholic hepatitis patients demonstrate universal evidence for protein-calorie malnutrition, based on the physical findings of muscle wasting and edema, low levels of serum albumin and other visceral proteins, and decreased cell-mediated immunity, whereas their 6-month mortality is related in part to the severity of malnutrition. Anorexia is a major cause of weight loss in alcoholic liver disease. Furthermore, active alcoholic hepatitis contributes to increased resting energy expenditure. However, resting energy expenditure is normal in stable alcoholic cirrhotic patients who are also typically underweight or malnourished in part due to preferential metabolism of endogenous fat stores. At the same time, the digestion of dietary fat and the absorption

Table 2 Effects of alcohol on body weight

<i>Drinking behavior</i>	<i>Explanation</i>
Moderate drinking	
Reduce weight	Substitution of carbohydrate by alcohol; more likely in women
Increase weight	Decreased dietary restraint
Heavy drinking	
Reduce weight	Substitution of nonalcohol calories by alcohol calories, which are 'wasted' during metabolism
Increase weight	Alcohol metabolism decreases lipid metabolism in the liver and promotes fat storage

Source: Reproduced from Halsted CH (2006) Alcohol: effects of consumption on diet and nutritional status. In: Caballero B, Allen L, and Prentice AM (eds.) *Encyclopedia of Human Nutrition*, 2nd edn., pp. 62–69. Amsterdam: Elsevier.

of fat-soluble vitamins A, D, E, and K are decreased in cirrhotic patients due to diminished secretion of bile salts and pancreatic enzymes.

Micronutrient Deficiencies in Chronic Alcoholism

Chronic exposure to excessive amounts of ethanol is associated with deficiencies of multiple nutrients, in particular thiamine, folate, pyridoxine, vitamin A, vitamin D, zinc, and iron. The frequency of these deficiencies is increased in the presence of alcoholic liver disease, which results in decreased numbers of hepatocytes for vitamin storage and metabolism. Many of the clinical signs of alcoholic liver disease are related to vitamin deficiencies.

Thiamine Deficiency

Low-circulating levels of thiamine have been described in 80% of patients with alcoholic cirrhosis. Thiamine pyrophosphate is a coenzyme in the intermediary metabolism of carbohydrates, in particular for transketolases that play a role in cardiac and neurological functions. Although alcoholic beverages are essentially devoid of thiamine, acute exposure to alcohol decreases the activity of intestinal transporters required for thiamine absorption. The major neurological signs and symptoms of thiamine deficiency in alcoholics include peripheral neuropathy, partial paresis of ocular muscles, wide-based gait secondary to cerebellar lesions, cognitive defects, and severe memory loss. The presence of peripheral neuropathy is sometimes referred to as 'dry beriberi,' whereas the other symptoms constitute the Wernicke–Korsakoff syndrome. Although abnormal eye movements can be treated acutely by thiamine injections, the other signs are often permanent and contribute to the dementia that often afflicts alcoholics after years of drinking. 'Wet beriberi' refers to high-output cardiac

failure that can also occur in thiamine-deficient alcoholics and is responsive to thiamine therapy in addition to conventional treatment. Because endogenous thiamine is used during carbohydrate metabolism, acute cardiac failure can be precipitated by the administration of intravenous glucose to malnourished and marginally thiamine-deficient patients by depletion of remaining thiamine stores. This process can be prevented by the intravenous addition of water-soluble vitamins including thiamine to malnourished chronic alcoholic patients who are undergoing treatment for medical emergencies (Table 3).

Folate Deficiency

Folates, a family of vitamins with folic acid at its core, function in DNA synthesis and cell turnover and play a central role in methionine metabolism in the liver. Although originally recognized as a cause of megaloblastic anemia, the expanding known consequences of folate deficiency are related to elevated circulating homocysteine and include increased risk for neural tube defects and other congenital abnormalities in newborns as well as altered cognition in the elderly people. Before folate fortification of grains in the United States in 1998, the incidence of low serum folate levels in chronic alcoholics was at approximately 80%, but there are no data on the incidence of postfortification folate deficiency in chronic alcoholics. Megaloblastic anemia, due to the negative effects of folate deficiency on DNA synthesis, has been described in approximately one-third of chronic alcoholics. Furthermore, folate deficiency may play a role in the pathogenesis of alcoholic liver disease by reducing hepatic levels of S-adenosylmethionine with consequent reduction in antioxidant glutathione and DNA methylation with resultant increased activation of genes relevant to alcoholic liver injury.

Table 3 Common micronutrient deficiencies in chronic alcoholic patients

Deficiency	Cause	Effect
Thiamine	Poor diet Intestinal malabsorption	Peripheral neuropathy Wernicke–Korsakoff syndrome High-output heart failure
Folate	Poor diet Intestinal malabsorption Decreased liver storage Increased urine excretion	Megaloblastic anemia Hyperhomocysteinemia and liver disease Neural tube defect Altered cognition
Vitamin B ₆	Poor diet Displacement from circulating albumin Promotes urine excretion	Peripheral neuropathy Sideroblastic anemia
Niacin	Poor diet	Pellagra with dermatitis, diarrhea, and dementia
Pantothenic acid	Poor diet	Paresthesias 'burning feet' syndrome
Vitamin A	Malabsorption Increased biliary secretion	Night blindness May promote development of fibrosis in alcoholic liver disease
Vitamin D	Malabsorption Decreased sun exposure	Calcium deficiency Metabolic bone disease
Zinc	Poor diet Increased urine excretion	Night blindness Decreased taste Decreased immune function
Iron	Gastrointestinal bleeding	Anemia

Source: Reproduced from Halsted CH (2006) Alcohol: effects of consumption on diet and nutritional status. In: Caballero B, Allen L, and Prentice AM (eds.) *Encyclopedia of Human Nutrition*, 2nd edn., pp. 62–69. Amsterdam: Elsevier.

There are multiple causes of folate deficiency in chronic alcoholism. With the exception of beer, all alcoholic beverages are devoid of folate, and the typical diet of the binge-drinking chronic alcoholic does not include fresh vegetable sources and fortified grains. Chronic alcoholism causes intestinal folate malabsorption, decreased liver folate uptake, and accelerated folate excretion in the urine. In addition, alcoholic liver disease results in decreased liver stores of folate, so the duration of time for development of folate deficiency with marginal diet is shortened.

Pyridoxine Deficiency

Pyridoxine (vitamin B₆) is required for transamination reactions, including the elimination of homocysteine. Pyridoxine deficiency in chronic alcoholism is caused by poor diet, whereas displacement of pyridoxal phosphate from plasma albumin by the alcohol metabolite acetaldehyde increases its urinary excretion. Low serum levels of pyridoxal phosphate are common in chronic alcoholics, and pyridoxine deficiency is manifest by peripheral neuropathy and sideroblastic anemia. In alcoholic hepatitis, the serum level of alanine transaminase (ALT) is disproportionately low compared with aspartate transaminase (AST), due to the requirement of pyridoxine for ALT activity.

Vitamin B₁₂ Deficiency

The incidence of vitamin B₁₂ deficiency in chronic alcoholism is undefined because serum levels are often normal or increased due to the increased presence of B₁₂ analogs in the presence of alcoholic liver disease. Nevertheless, the intestinal absorption of vitamin B₁₂ is decreased in chronic alcoholics due to defective uptake at the ileum. Presumed low levels of vitamin B₁₂ in the liver may contribute to abnormal hepatic methionine metabolism with elevated serum homocysteine, because this vitamin is a cofactor for methionine synthase.

Other Less Common Water-Soluble Vitamin Deficiencies in Chronic Alcoholism

Niacin deficiency is typically found in less developed countries in association with decreased intake of the animal protein, in particular the amino acid tryptophan, which is a precursor of nicotinic acid and nicotinamide. Clinical symptoms of niacin deficiency constitute the syndrome known as pellagra, which can occasionally be found in chronic alcoholics as a component of severe malnutrition due to inadequate diet. These signs include the 'three D's' of chronic diarrhea, dermatitis including a scaly rash over sun-exposed areas such as the neck, forearms, and hands, and dementia with features of disorientation, confusion, memory loss, and psychosis. In addition to this typical triad, laboratory features include low urinary *N*-methylnicotinamide excretion. Recovery from pellagra in chronic alcoholism follows treatment for protein malnutrition with supplemental niacin and abstinence from alcohol.

Pantothenic acid is a component of coenzyme A, which is involved in many reactions related to lipid and carbohydrate metabolism, and is found in animal protein, dairy products, and whole grains. Pantothenic acid deficiency is rare and may sometimes be found in malnourished chronic alcoholics with symptoms of paresthesias that include the 'burning feet'

syndrome. Its causation in chronic alcoholism is most likely related to inadequate diet and generalized malnutrition. There is no specific diagnostic test, and the deficiency symptoms usually respond to restoration of nutrition and supplemental pantothenic acid.

Vitamin A Deficiency

Although serum levels of vitamin A are usually normal in chronic alcoholics, liver retinoids are progressively lowered through the stages of alcoholic liver disease.

The causes of vitamin A deficiency in alcoholic liver disease include malabsorption, which is due to decreased secretion of bile and pancreatic enzymes necessary for the digestion of dietary retinyl esters and their incorporation into water-soluble micelles before intestinal transport. In addition, the transport of retinol is impaired due to decreased hepatic production of retinol binding protein. Finally, the metabolism of alcohol induces microsomal enzymes that promote the production of polar retinol metabolites that are more easily excreted in the bile. The signs of vitamin A deficiency include night blindness with increased risk of automobile accidents and increased risk of esophageal cancer due to abnormal squamous cell cycling. Conversely, patients with alcoholic liver disease are more susceptible to vitamin A hepatotoxicity so that supplemental doses of vitamin A should be used with caution.

Vitamin D and Calcium Deficiencies

Chronic alcoholic patients are at increased risk for metabolic bone disease due to low vitamin D levels and hence decreased absorption of calcium. Alcoholic liver disease increases the likelihood of low circulating levels of 25-hydroxy vitamin D because of decreased excretion of bile required for absorption of this fat-soluble vitamin, poor diet, and often decreased sun exposure. Calcium deficiency results from low levels of vitamin D that is required to regulate its absorption, and also because the fat malabsorption that often accompanies alcoholic liver disease results in increased binding of calcium to unabsorbed intestinal fatty acids.

Zinc Deficiency

Zinc is a cofactor for many enzymatic reactions including retinol dehydrogenase, is stored in the pancreas, and circulates in the blood bound mainly to albumin. Chronic alcoholic patients are frequently zinc deficient due to poor diet, pancreatic deficiency, and increased urine excretion because of low zinc-binding albumin in the circulation. The consequences of zinc deficiency include night blindness from decreased production of retinal, decreased taste, and hypogonadism that may result in lowered testosterone levels and increased risk of osteoporosis in men. Because zinc is required for cellular immunity, its deficiency may contribute to increased infection risk in alcoholic patients.

Iron Deficiency

Chronic alcoholic patients are often iron deficient because of increased frequency of gastrointestinal bleeding, typically due to alcoholic gastritis or esophageal tears from frequent retching and vomiting, or from rupture of esophageal varices in patients with cirrhosis and portal hypertension. The major consequence of iron deficiency is anemia, which may be

compounded by the concurrent effects of folate and pyridoxine deficiencies. Conversely, increased exposure to iron, e.g., from cooking in iron pots, increases the likelihood and severity of alcoholic liver disease, because the presence of iron in the liver promotes oxidative liver damage during the metabolism of alcohol.

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ALUMINUM

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Glossary

Dysarthria Speech disturbance.

Dyspraxia Impaired or painful organ function.

Encephalopathy Disorder of the brain.

Hypochromic Erythrocyte hemoglobin percentage less than normal.

Microcytic Small erythrocyte.

Properties and Natural Occurrence

Aluminum (Al) is a soft (2.5–3 on the Mohs scale), light (2.7 g cc⁻³, therefore not a heavy metal), ductile, malleable metal. It is the most common metal and the third most common element in the earth's crust (comprising ~8%, primarily with silica), and is ubiquitously distributed throughout the environment. Its atomic number is 13. It has only one stable isotope, ²⁷Al. It is too reactive to occur in nature as the free metal. It exists as the trivalent ion (Al³⁺), therefore has no oxidation–reduction chemistry. Its preferred coordination number is 6, forming octahedral complexes in nature, including mammalian systems. Exposure to water, oxygen, and other oxidants leads to surface formation of Al oxide that provides a few nanometer thick film that has high resistance to corrosion and is virtually insoluble from pH 4.5 to 8.5. In aqueous solution, in the absence of cationic ligands that bind to it, its chemical form (species) is pH-dependent. The Al³⁺ ion with 6 waters of hydration predominates below pH 5.5, as Al(H₂O)₆³⁺. As the pH increases Al(OH)²⁺, Al(OH)₂⁺, Al(OH)₃, and Al(OH)₄⁻ predominate at pH 5.5, 6, 6.2, and above 6.2, respectively, resulting in its lowest solubility as Al(OH)₃ (gel) at pH 6.2. As a Lewis acid (electron pair acceptor, electrophile) it strongly complexes with multidentate carboxylate- and hydroxyl-/keto-containing ligands, for example citrate, through noncovalent interactions, generally involving ionic or electrostatic bonds. Aluminum is one of the slower ions kinetically. The mean residence time of water in Al(H₂O)₆³⁺ is ~1 s. Replacement of water by citrate has a complexation half-life of ~1–2 min. The chemical species of aluminum has a great impact on its kinetics and effects. The effective ionic radius of Al³⁺ is 54 pm, sufficiently similar to that of Fe³⁺ (65 pm) to engage in some of the same chemical and metabolic processes. Aluminum binds particularly strongly to phosphates, giving it the potential to bind with deoxyribonucleic acid, adenosine triphosphate, and many other biomolecules.

In contrast to its abundance in the earth's crust, most natural waters contain very little dissolved aluminum. Its average concentration in lakes, rivers, ground water, coastal sea water,

and open ocean water is ~150, 400, 100, 1–7, and 1 µg l⁻¹, respectively, reflecting the low solubility of some aluminum salts and its deposition in sediments as the hydroxide. Acidification by acid rain, mine runoff, or other means solubilizes aluminum, and can raise its concentrations in acidified lakes and rivers to 100–800 mg l⁻¹ or more, producing toxicity to aquatic organisms. The atmospheric aluminum concentration in nonindustrial rural and urban areas is typically 0.050–0.5 and 0.1–5 µg Al m⁻³, respectively. The World Health Organization (WHO) has a 'practicable level' of ≤0.1 and ≤0.2 mg l⁻¹ for large and small water treatment facilities. The European Union (EU) 'Indicator Parameter' directive for aluminum in drinking water is 0.2 mg l⁻¹. The US Environmental Protection Agency has a secondary, nonenforceable standard of 50–200 µg l⁻¹, based on esthetic (taste, sight, and smell) properties. Canada recommended an operational guidance level of <0.1 mg l⁻¹ for water treatment plants using aluminum-based coagulants and <0.2 mg l⁻¹ for other types of treatment systems. The Joint Food and Agriculture Organization (FAO) of the United Nations/WHO Expert Committee on Food Additives in 2006 and the Panel on Food Additives, Flavorings, Processing Aids and Food Contact Materials of the European Commission in 2007 established a tolerable weekly intake for aluminum of 1 mg per kg body weight per week. At the 74th meeting of the JECFA (June 14–23, 2011, Rome) a PTWI of 2 mg/kg body weight was established (ftp://ftp.fao.org/ag/agn/jecfa/JECFA_74_Summary_Report_4July2011.pdf). Similarly, the Agency for Toxic Substances and Disease Registry Minimal Risk Level for intermediate (15–364 days) oral aluminum intake was set at 1 mg kg⁻¹ day⁻¹.

Nonfood Uses

Aluminum and its compounds are widely utilized. They are used in building and construction, transportation, packaging, containers, water purification, sugar refining, brewing, paper production, glass, ceramics, rubber, abrasives, furnace linings, as wood preservatives, to waterproof textiles, and as a flame retardant. In personal care and medical applications it is in

antacids (that are also used as phosphate binders); buffered aspirin products; as aluminum chloride, aluminum zirconium glycine complex, and aluminum chlorohydrate in antiperspirants; in acne cleaning preparations; as an abrasive in dentifrices; in dental rinses and toothpastes to reduce dental hypersensitivity; as an astringent and antibacterial in products for dermatitis such as athlete's foot; in first aid antibiotic and antiseptic, diaper rash and prickly heat, insect sting and bite, sunscreen and suntan, and dry skin products; in antidiarrheal products; in vaginal douches; as a keratolytic in anorectal preparations; and as an adjuvant in vaccines to increase their antigenic properties.

Food Uses of Aluminum Compounds

United States Food and Drug Administration (FDA)-approved aluminum compounds that may be employed as food additives are listed in **Table 1**. These contribute to the high concentrations of aluminum in some of the foods in **Tables 2** and **3**. Canada, the EU, Norway, Iceland, and the United Kingdom also allow the use of aluminum as a food additive.

Aluminum in Beverages and Foods

Tables 2 and **3** show average values from studies published since 1985 for which there were multiple reported values for each entry in the table. The values shown are medians, which are much less sensitive to outliers than the mean. This former measure of central tendency was selected because there is considerable variability of reported results from some beverages and foods. This variation may result from poor analytical techniques, inadequate removal of soil and/or contamination, and differences due to climate and soil growing conditions.

Plants that accumulate aluminum deposit it in their leaves. The tea plant accumulates and concentrates aluminum, accounting for the higher aluminum concentration in this beverage than others. Addition of the anticaking agent sodium aluminosilicate to nondairy creamer and salt, particularly single-use packets, accounts for their high aluminum concentration. The higher aluminum content of soy-based infant formula derives from the higher aluminum content of soybean (**Table 2**) than cow milk.

Even though soil contains 3–10% aluminum, most plants and plant-eating animals contain little aluminum, due to its very low oral bioavailability (below) and limited transfer to eggs and milk. Most unprocessed foods contain $<5 \text{ mg kg}^{-1}$. The addition of aluminum as a food additive can greatly increase its food content, illustrated by baking powder, cake and pancake mixes, cakes and pancakes, and cheese containing sodium aluminum phosphate (SALP). Residual dirt may account for the higher aluminum concentration in lettuce and spinach. Some spices contain considerable aluminum, illustrated by paprika and pepper. Spice aluminum values are expressed based on their dry weight, whereas most other food aluminum concentrations are based on wet weight.

Table 2 Aluminum concentrations of beverages and nondairy creamer

<i>Beverage</i>	<i>Aluminum concentration (median; as mg l^{-1}, except for nondairy creamer)</i>
Beer	0.16
Coffee	0.24
Nondairy creamer – multiple serving container	38 mg kg^{-1}
Nondairy creamer – single serving packet	170 mg kg^{-1}
Apple juice	0.44
Orange juice	0.18
Pineapple juice	0.35
Tomato juice	0.67
Wine	0.90
Distilled spirit	0.42
Cola	0.25
Noncola soft drink	0.41
Black tea	3.0
Green tea	2.5
Instant tea	1.2
Herbal tea	0.31
Tap water	0.040
Mineral water	0.016
Cow milk	0.070
Human milk	0.043
Cow-based infant formula	0.019
Soy-based infant formula	3.9

Table 1 Some FDA-approved food additives containing aluminum, or aluminum agents that come in contact with foods, and their uses

Aluminum ammonium sulfate – Buffer and neutralizing agent
Aluminum calcium silicate – Anticaking agent, $<2\%$ by wt in table salt
Aluminum potassium sulfate – Buffer and neutralizing agent
Aluminum sodium sulfate – Buffer and neutralizing agent
Fatty acids, salts of aluminum – In foods as binder, emulsifier, and anticaking agent, coatings on fresh citrus fruit
1-Octenyl succinic anhydride and $<2\%$ aluminum sulfate – Modifier for food starch
Sodium aluminosilicate (also known as: sodium silicoaluminat) – Anticaking agent, $<2\%$ in dried whole eggs and egg yolks, and grated cheeses
Sodium aluminum phosphate (SALP) – acidic SALP as a leavening agent in self-rising flours and meals, basic SALP as an emulsifying agent in cheeses
Sodium aluminum sulfate – Cereal flours
Sodium calcium aluminosilicate hydrated (sodium calcium silicoaluminat) – Anticaking agent, $<2\%$
In lakes (Al salts of water-soluble artificial colors adsorbed onto alumina)
Aluminum borate as an antistatic and/or antifogging agent for olefin polymers intended as food-packaging materials
Aluminum-containing clarifying agent for polypropylene and polypropylene copolymers intended for use in contact with food

Table 3 Concentrations of aluminum in foods

<i>Food</i>	<i>Aluminum concentration (median; as mg kg⁻¹)</i>
Beef meat	1.2
Pork meat	2.2
Chicken meat	1.2
Luncheon meats	3.2
Bacon	2.4
Ham	0.85
Sausage	6.2
Fish	0.15
Eggs	0.27
Nuts (Almond, cashew, chestnut, walnut)	2.3–4.6
Peanuts and peanut butter	2.0
Pine nuts	38
Fruits (Apple, banana, grape, orange, peach, pear, plum, strawberry)	0.55–2.6
Watermelon	0.28
Raisin	10.5
Vegetables (Broccoli, cabbage, carrot, cauliflower, celery, corn, pepper, onion, parsnip, tomato)	0.30–1.5
Beans	4.0
Cucumber	2.2
Lettuce	5.5
Mushrooms	2.9
Peas	2.1
Potato	2.5
Soybean	7.8
Spinach	24
Corn Flour	5.3
Oats	4.0
Rice	3.3
Wheat flour	5.6
Baking Powder	70
Biscuits	22
Cake mix	445
Cakes (not stated to contain SALP)	6.3
Cakes (that contain SALP)	190
Cookies	6.9
White bread	3.6
Wheat bread	4.5
Cereal	1.0
Pancake mix	100
Pancakes	85
Pasta	5.5
Cheese	3.8
Goat cheese	15
Processed cheese	15
Frozen pizza cheese	415
Restaurant pizza cheese	2.9
Yoghurt	0.28
Goat yoghurt	2.8
Soup	1.2
Butter	1.4
Margarine	1.7
Olive oil	0.043
Sugar	1.7
Honey	0.5
Chocolate	9.4
Jellies and jams	4.1
Paprika	92
Pepper	31

(Continued)

Table 3 Continued

<i>Food</i>	<i>Aluminum concentration (median; as mg kg⁻¹)</i>
Pickles	7.4
Vinegar	0.21
Salt	2.4
Salt – Single serving packet	180
Infant foods	10
Infant strained foods	0.41

Aluminum in Foods from Processing, Packaging, and Storage

Food preparation and storage in contact with aluminum can increase the food's aluminum content more than non-aluminum containing cookware and storage containers. Many examples follow. Coffee brewed in an aluminum pot had an average of two times as much aluminum as when brewed in stainless steel. Water and milk boiled/cooked in aluminum cookware had more aluminum than when prepared in porcelain, stainless steel, teflon, or enamel cookware. In one study multiple vegetables cooked in old or new aluminum vessels had 40% and 80% more aluminum than when cooked in stainless steel. However, studies have not consistently shown higher aluminum in foods prepared in new versus old aluminum vessels, perhaps due to differences in vessel manufacture and surface coating, extent of use or cleaning before their study, and the food under study. Cottage cheese and yoghurt prepared in aluminum containers had greater aluminum concentrations than when prepared in boron glass or steel containers. Rhubarb boiled for 12 min in a steel pot had 0.8 and in an aluminum pot 13–20 mg aluminum l⁻¹. Blackcurrant juice boiled for 90 min in an aluminum vessel increased from 0.05 to 24 mg aluminum l⁻¹ when sugar was added before boiling and to 56 mg l⁻¹ when the sugar was added after it was brought to a boil.

Household aluminum foil is 0.18 mm thick. Beef, mutton, pork, water buffalo, chicken, and turkey baked in aluminum foil had on average a 2.5-fold increase in the aluminum content. Higher temperature (250 vs. 200 vs. 150 °C) contributed more than longer baking time (20 vs. 40 vs. 60 min) for beef, mutton, and pork whereas the longer baking time, not temperature, increased aluminum in chicken and turkey. Baking and grilling fish in aluminum foil increased its aluminum concentration by five-fold. Potatoes roasted in aluminum foil had 2.5-fold more aluminum than when roasted in stainless steel. Tomato cooked for 10 min in an aluminum pan had ~60% more aluminum when compared to a steel pan. After cooking the tomato was stored for 72 h at 4 °C during which the aluminum concentration increased ~140% in food stored in both aluminum and steel pans.

Storage of tap water in an aluminum bottle or siphon increased its aluminum concentration. Milk stored in an aluminum pan increased in aluminum concentration ~14-fold. Milk stored in an aluminum-lined container had more aluminum than when stored in plastic or glass. The

aluminum concentration in beer stored in aluminum cans was consistently higher (average 60%) than in steel cans or glass bottles, did not increase over time when stored at 5 °C, but did when stored at room temperature (increasing by 35–70% of the original aluminum concentration per month in the first 5 months and 10% per month over 6 years). Wine stored for 2 years in an aluminum can had more aluminum than when stored in a glass bottle or steel can. The aluminum concentration in apple, lemon, and orange (but not tropical fruit) juice stored in aluminum cans increased over time and the aluminum concentration in grapefruit juice stored in aluminum cans increased with increased storage temperature. Soft drinks (colas, Fanta orange, and soda water, but not Pocari Sweat or orange squash) stored in aluminum cans had a higher aluminum content than when stored in plastic or glass. The aluminum concentration of lemon, orange and cola drinks stored in aluminum cans for 12 months increased ~one-, two- and threefold monthly above the original aluminum concentration, respectively. Tomato paste stored in steel and aluminum cans increased ~25 and 60% over 2.5 years, respectively. Storage of tomato puree and tamarind in an aluminum pan for 72 h increased its aluminum content 31- and 17-fold, respectively. Addition of salt to the tomato and sugar to the tamarind increased their aluminum content an additional ~15 and 9%, respectively. Aluminum mobilization during food preparation and storage in contact with aluminum is greatest for acidic foods (e.g., tomato pH ~4.5 and rhubarb ~3.2), consistent with the chemical species of aluminum as a function of pH; solubility increases below pH 6.2.

Exposure and Dietary Intake

Dietary intake provides the greatest percentage of aluminum intake for the typical human. Daily average drinking water consumption is estimated to be 1.4 l. Intake of the water with the median aluminum concentration of 0.04 mg l⁻¹ (Table 2) would provide ~0.055 mg Al. Fifty studies conducted since the mid-1980s in Australia, Brazil, Canada, China, France, Germany, Hungary, India, Italy, Japan, the Netherlands, Portugal, Slovenia, Spain, Sweden, Taiwan, Turkey, the UK, and the US have reported daily dietary aluminum intakes. The intakes were based on total diet studies, market basket surveys, dietary records, calculations based on food aluminum levels, and duplicate diets and portions. Intake by males generally exceeded that of females. Median daily aluminum intake of adults in these studies was ~5.7, adolescents 7.4, and infants/young children 2 mg. Reported intakes were highest in China and Taiwan. Intake in the US and Canada was higher than Europe. Japan was the lowest. The greater daily aluminum intake in the US and Canada than Europe and Japan may be due to a higher utilization of SALP and other approved aluminum food additives in processed foods. Aluminum intake in food is ~100-fold greater than from drinking water. As tea contains more aluminum than other beverages, its consumption can contribute 50% of the daily aluminum intake in those who consume considerable amounts of this beverage when other sources do not have large amounts of aluminum.

Bioavailability and Biotransformation

Aluminum bioavailability is low. The main routes of uptake are by inhalation and through the gastrointestinal tract, and medical routes such as injections and during dialysis. Absorption from the gastrointestinal tract, lungs and after underarm application (presumably transdermal) is ~0.1–0.4%, 1.5–2%, and up to 0.012%, respectively. Aluminum injected in vaccines is slowly absorbed as it dissolves, and may ultimately reach 100%. Aluminum absorption from the gastrointestinal tract appears to be primarily in the distal intestine. There is evidence supporting several mechanisms of intestinal aluminum absorption, including paracellular diffusion, an interaction with calcium uptake, and sodium transport processes. Owing to the ubiquitous presence of aluminum, which creates measurable levels in all tissues and fluids and potential contamination problems, and its low bioavailability, determination of oral bioavailability at relevant exposures is very difficult using ²⁷Al. More precise measurements of absorption have been made using ²⁶Al. Owing to its high cost and very long half-life (~700 000 years), its decay rate is too low for practical application as a radioisotopic tracer. Its quantification by accelerator mass spectrometry is exquisitely sensitive. The limit of detection is ~1 000 000 atoms (~4 × 10⁻¹⁷ g). This enables administration to human subjects of doses that present no significant radiation risk and the conduct of exposure dose-relevant pharmacokinetic studies. Studies conducted in rats have shown oral bioavailability from water, tea, acidic SALP in a food (biscuit), and basic SALP in cheese to be ~0.3%, 0.4%, 0.1%, and 0.2%, respectively. Studies in humans showed uptake from drinking water and aluminum hydroxide to be ~0.2% and 0.1% respectively. Oral aluminum uptake is increased by citrate, and other carboxylic acids to a lesser extent. Oral aluminum bioavailability has also been shown to be increased by the solubility of the aluminum species; fluoride; low iron, calcium or sodium status; and uremia. In contrast, silicon-containing compounds appear to reduce its absorption and/or enhance its urinary elimination as hydroxylaluminosilicate species. Some results suggest oral aluminum absorption is increased in Alzheimer's disease (AD) and Down's subjects.

Biokinetics of Aluminum in Blood

The blood concentration of aluminum in normal humans is believed to be <2 µg l⁻¹. As this is very low and aluminum is ubiquitously present, much attention must be paid to collection, storage, and handling of blood samples to avoid contamination. At equilibrium serum and red blood cell aluminum concentrations are approximately equal. The volume of distribution of aluminum is initially equal to the blood volume, consistent with its equal concentration in serum and the blood cells. It then increases over time as it distributes and accumulates in tissues. Within blood plasma, ~91% is bound to transferrin, an iron-transport protein, ~7–8% is bound to citrate, and the remainder to phosphate and hydroxide. Formation of the aluminum transferrin- and aluminum citrate-complexes occurs within minutes. Generally

the strength of the binding (stability constant) for Al^{3+} with ligands is 3 to 4 orders of magnitude lower than for Fe^{3+} . Consequently, aluminum will not displace iron from transferrin or most other ligands. Transferrin-receptor-mediated endocytosis may mediate aluminum uptake into the brain and other organs. Citrate enhances aluminum distribution out of the blood and into tissues and its renal clearance (by increasing the filterable fraction of aluminum), suggesting a mechanism for uptake of this aluminum species that is different from that mediating aluminum transferrin cell uptake.

Aluminum Tissue Deposition and Body Retention

Aluminum distributes unequally throughout the body in normal and aluminum-intoxicated humans. Because of its very low bioavailability by most routes of exposure and the effective clearance of aluminum from blood by the kidney, aluminum concentrations in the human are low compared to most exposure sources. The normal human body burden of aluminum is ~60 mg. Steady state tissue aluminum concentrations in normal adults are (in mg kg^{-1} wet weight unless stated otherwise): lung: 20, bone: 1–3 mg per kg dry weight, liver and spleen: 1, kidney: 0.5, heart: 0.45, muscle: 0.4, brain: 0.35, and blood: 0.002. Approximately 60%, 25%, 10%, 3%, 1%, 0.3%, 0.25, and 0.2% of the aluminum body burden is in the bone, lung, muscle, liver, brain, heart, kidney, and spleen, respectively. Aluminum localizes at the mineralization front and in osteoid of bone. Lung aluminum may be from particles from the environment, occupational exposure, and distribution from the blood. Insoluble particles trapped in the lung are very slowly cleared from the lung. Blood and tissue concentrations are higher in uremia and higher still in dialysis encephalopathy. Approximately 80% of an intravenous dose of aluminum citrate was excreted within a week, suggesting the remainder was retained within the body, some of which was excreted over a longer time. This finding indicates that under conditions of continuous intake, aluminum accumulates in the body, even in subjects with normal renal function. Brain, bone and serum aluminum concentration increase with age. Hair aluminum concentration as an indicator of aluminum body burden has not been validated. Problems include the lack of standardized hair collection procedures.

Aluminum clearance is characterized by multiple half-lives that are estimated in hours, days, and years, suggesting multiple compartments. The initial half-life (distribution from the vascular compartment) is a few hours. The brain, bone, liver, and kidney aluminum half-life in rats was estimated to be 150–1635, 520, 430 and 400 days, respectively. Allometric scaling from this animal species with a lifespan of 2 years to the human with an 80 year life span suggests the aluminum half-life in human brain might be decades or even greater than the lifespan. Estimates of the terminal half-life in the human suggest it may be as great as 50 years, attributed to the aluminum in bone. The slow release of aluminum from its depot in bone, due to the low rate of bone turnover (~10% per year), may account for the prolonged half-life seen in most organs, including the brain, and prolonged elevated urinary aluminum excretion after significant occupational aluminum exposure. Increased bone turnover rates compared to the

healthy adult, as seen in children who exhibit high rates of bone growth and turnover or geriatrics, may accelerate aluminum release from bone.

Biological monitoring of aluminum exposure can be conducted with urine, which is thought to indicate recent exposure, and plasma, which is thought to better reflect the aluminum body burden and long-term exposure. However, neither is a good predictor of the aluminum body burden, which is better estimated by bone aluminum, the desferrioxamine challenge test, or combined measurement of serum parathyroid hormone and the desferrioxamine test. Desferrioxamine is a chelator that binds aluminum to form aluminioxamine, iron and many other trivalent metals, and increases their urinary elimination. Bone biopsy is needed for definitive diagnosis.

Aluminum Excretion

The primary route of aluminum elimination is via the kidneys, accounting for >95%, presumably by glomerular filtration of aluminum citrate. Humans who consume the average daily aluminum intake of 6 mg would be expected to excrete 4–12 μg of aluminum daily, with a typical urinary aluminum concentration of 2–10 $\mu\text{g l}^{-1}$, although several industrial studies reported higher values in controls (often 20 $\mu\text{g l}^{-1}$) and even higher in exposed workers. Reduced or lack of renal function creates the risk of aluminum accumulation and toxicity. Bile (feces) accounts for most of the remaining excreted aluminum, although it is present in saliva, sweat, and semen.

Toxicity of Systemic Aluminum

There is no good evidence that aluminum is essential for the human. The toxicity of aluminum has been extensively reviewed by the WHO, for the US Department of Health and Human Services, and most extensively by a multinational group led by Daniel Krewski. Occupational exposure to high levels of aluminum has been associated with lung fibrosis from stamped aluminum powder, asthma from aluminum salts and welding fumes, and increased oxidative stress. Contact dermatitis has been reported. Neurobehavioral toxicity has been seen in aluminum welders with urine aluminum > 100 $\mu\text{g l}^{-1}$. When hemodialysis was initially extensively used, some patients developed a progressive encephalopathy that was fatal within 6 months (see the Section Aluminum-Induced Encephalopathy). This was due to aluminum contamination of the dialysis fluids and administration of aluminum-based phosphate binders to form an insoluble aluminum phosphate in the intestine, facilitating phosphate elimination, a goal not well achieved by dialysis. The renal dialysis patient is highly susceptible to aluminum accumulation and toxicity from aluminum in dialysis fluids because the aluminum can diffuse across the dialysis membrane, it quite rapidly and very strongly binds to transferrin in the blood, and these patients lack the primary route of aluminum elimination, renal function. Aluminum as a phosphate binder presents a problem simply due to the very large

oral dose of aluminum administered, paired with some oral aluminum absorption. Exposure to lower levels of aluminum than those that caused aluminum-induced encephalopathy can produce a low turnover bone disease (see the Section Aluminum-Induced Bone Disease) and anemia (see the Section Aluminum-Induced Microcytic Anemia).

Another high risk group for aluminum toxicity are premature infants who are fed intravenously, because they do not tolerate oral feeding. The total parenteral nutrition feeding solutions given intravenously can contain significant aluminum, which is primarily derived from the calcium gluconate and phosphates used as components of the solution. As this feeding solution is given intravenously, and therefore 100% bioavailable, to premature infants whose kidneys are not fully matured and therefore less able to excrete the aluminum, they are at risk of sufficient aluminum accumulation to develop metabolic bone disease, cholestatic hepatitis, and reduction of mental development. To address this concern the US FDA adopted a labeling requirement for aluminum in large and small volume parenterals used to prepare total parenteral nutrition solutions.

Studies in mice, rats, rabbits, and dogs have shown aluminum has the potential to produce neurobehavioral toxicity, affect the male reproductive system, produce embryotoxicity, and affect development. Based on these concerns, the Joint FAO/WHO Expert Committee and the European Commission established a tolerable weekly intake for aluminum of 1 mg per kg body weight per week, as noted earlier in the Section Properties and Natural Occurrence. At the 74th meeting of the JECFA (June 14 to 23, 2011, Rome) a PTWI of 2 mg/kg body weight was established (ftp://ftp.fao.org/ag/agn/jecfa/JECFA_74_Summary_Report_4July2011.pdf).

Aluminum-Induced Encephalopathy

Encephalopathy has been associated with the use of hemodialysis and peritoneal-dialysis fluids that contain significant amounts of aluminum, the administration of massive amounts of aluminum salts orally to bind phosphate in the gastrointestinal tract to renal-impaired humans, aluminum instillation into the urinary bladder to stop hemorrhaging, and neurosurgical implants of aluminum-containing biomaterials. Dialysis (associated) encephalopathy (also known as: dialysis dementia) is characterized by dyspraxia, dysarthria, emotional changes, trembling, ataxia, myoclonus, and fatal convulsions. It is associated with elevated levels of aluminum in the brain, and serum aluminum $> 80 \mu\text{g l}^{-1}$. With recognition of the role of aluminum in dialysis-related toxicity, reduction of aluminum exposure guided by a recommendation of the US Association for the Advancement of Medical Instrumentation that water used in the preparation of dialysate solution contain $< 10 \mu\text{g l}^{-1}$, and use of alternatives to aluminum as an oral phosphate binder, occurrences of aluminum-induced encephalopathy have become unusual.

Aluminum-Induced Bone Disease

Aluminum-induced low-turnover bone disease is manifest as osteomalacia and an adynamic bone disease. Aluminum-

induced bone disease in dialysis patients is seen when serum aluminum is $> 30 \mu\text{g l}^{-1}$ and when stains show aluminum at 30% of the trabecular bone surface. Aluminum interferes with parathyroid hormone synthesis and function. In osteomalacia, aluminum is localized at the mineralization front, where it disrupts bone mineralization by inhibiting calcium accretion and osteoblast activity, increases bone resorption, and results in an increase of the nonmineralized bone, leading to painful fractures. In adynamic bone disease, aluminum decreases osteoid formation and bone mineralization.

Aluminum-Induced Microcytic Anemia

A microcytic, hypochromic anemia is associated with elevated plasma aluminum in chronic renal failure patients. Aluminum interferes with the development of hematopoietic progenitor cells. It may produce anemia by disrupting iron metabolism, disorganizing the erythrocyte membrane, and/or inducing oxidative stress to the erythrocyte membrane.

Up to 5 mg kg^{-1} of the chelator desferrioxamine once or twice a week has been shown to be safe and effective for long-term treatment of aluminum overload, and reduction of aluminum-induced encephalopathy, bone disease, and anemia.

Evidence for a Role in AD

Aluminum has been implicated in the etiology of AD. Hallmark neuropathological signs include neurofibrillary tangles (NFTs), senile plaques (SP), and cerebrovascular amyloid. Early onset AD usually has a familial link, due to gene mutations which result in increased secretion of neurotoxic amyloid β ($A\beta$) protein. No specific gene mutations have been associated with late-onset/sporadic forms of AD which account for 85–95% of AD cases. The lack of identified hereditary links for the majority of AD cases suggests environmental factors are likely to interact with other factors to cause this disease. Aluminum is one of the suggested environmental contributors. The genesis of the hypothesis that aluminum plays a role in the etiology of AD was an observation reported in 1965 of neurofibrillary degeneration in rabbit brain after intracerebral aluminum injection, which resembled, but was not identical to, the NFTs of AD. Similarly, the neuropathology in dialysis encephalopathy is different from that seen in AD. The observation of elevated aluminum in post-mortem brain samples (that typically weighed scores of milligrams) of humans with AD reported in 1973 was interpreted as suggesting a role for aluminum in AD. This was followed by many studies, some of which found a few-fold or less higher concentration of aluminum in AD victim brains than in controls, and some which did not. Studies investigating an elevated level of aluminum in AD brain using microprobe techniques, such as energy dispersive (electron probe) X-ray microanalysis, secondary ion mass spectrometry, and laser microprobe mass spectroscopy which can quantify aluminum within a cell, NFT or SP, as well as aluminum-selective stains have also produced mixed results. If aluminum is elevated in AD brain, it is not reflected in cerebrospinal fluid aluminum, which has generally not been found to be elevated. Even if aluminum is elevated in AD brain, it would not prove

cause and effect. The neuronal degeneration of AD may result in accumulation of metals, such as aluminum.

Another approach to address the potential role of aluminum in AD is the epidemiological study of the association between the concentration of aluminum in drinking water and AD incidence, comparing geographic regions where drinking water aluminum concentrations differ. Again, the results of many such studies are not consistent. The majority reported an increased risk of AD associated with higher drinking water aluminum concentration. Some of the differences were statistically significant. A major review published in 2007 conducted a risk characterization of the route of aluminum intake, the exposure level of concern, and aluminum exposure in the general population and calculated a margin of exposure (ratio of the exposure level of concern to the exposure level). The exposure level of concern was based on an epidemiological study that showed a relative risk of AD of 2.14 associated with a drinking water aluminum concentration $> 100 \mu\text{g l}^{-1}$. However, drinking water is not the major source of aluminum for the general population. The only epidemiological study of AD and aluminum consumption from food was too small to draw firm conclusions, although it found a significant odds ratio for higher association of AD with a food category typically high in aluminum (pancakes, waffles, biscuits, muffins cornbread, and corn tortillas).

Aluminum has been shown to produce many effects in the brain that have been suggested as contributors to its neurotoxicant effects, and contributing to AD. These have included promotion of the formation and accumulation of A β and aggregation of hyperphosphorylated tau protein, deficits of cortical cholinergic neurotransmission, increased oxidative injury, and increased inflammation and oxidative injury.

The controversy of a contributory role of aluminum in AD has not been resolved. As there are many contributing factors to AD, it will be extremely difficult to demonstrate the lack of contribution of any one factor, such as aluminum, to this disease.

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See also: Aging. Food Safety: Heavy Metals

AMINO ACIDS

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Metabolism

Specific Functions

Chemistry and Classification

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Introduction

Amino acids are a series of small organic molecules whose prime importance lies in the fact that they are the monomers from which proteins are made. The form and functions of proteins depend on the sequence in which the amino acids are joined together, because each amino acid has specific chemical and physical properties. In this article the structures and chemical properties of each amino acid are outlined, with an indication of how this affects the metabolic role of the free amino acid and how it affects the behavior of the amino acid residue within a protein. These chemical properties also form the basis for methods of analysis of amino acids. Some amino acids can be synthesized within the body from other molecules whereas others cannot, so the final section explains the basis of the classification into essential and nonessential amino acids.

Chemical Structures and Nomenclature

Amino acids have the general formula shown in **Figure 1**.

The central carbon atom in this structure is called the α -carbon, and the amino and carboxyl groups attached to it are known as the α -amino group and the α -carboxyl group. The R groups of the twenty amino acids, which can be incorporated into proteins are shown in **Table 1**; these R groups give the different amino acids their specific chemical and physical properties.

It should be noted that the α -amino group acts as a weak base and is always protonated at physiological pH; similarly the α -carboxyl group acts as weak acid and at physiological pH

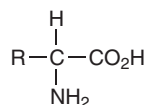


Figure 1 Amino acid structure.

is always ionized. Thus free amino acids in biological material exist as zwitterions, as shown in **Figure 2**.

The α -carbon atom is asymmetric, so that amino acids show stereoisomerism; the exception to this is glycine, where the R group is a second hydrogen atom. Most of the amino acids found in nature are in the L-form, and only L-amino acids can be used for protein synthesis in higher organisms. However, D-amino acids may be ingested from bacterial sources, and if high concentrations accumulate they could be toxic. The human body has a D-amino acid oxidase enzyme, found in the liver and the kidney, which disposes of these molecules by oxidative deamination.

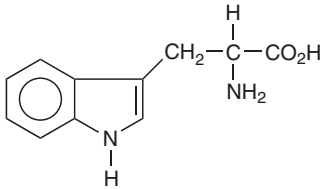
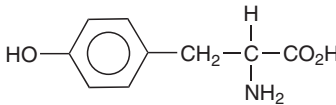
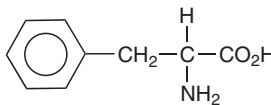
The most important common chemical property of the amino acids is their ability to form peptide bonds with one another. The α -amino group of one amino acid reacts with the α -carboxyl group of another to form a peptide bond with the elimination of water (see **Figure 3**). The results of this process are conventionally known as peptides or oligopeptides if they contain from two to 20 amino acid residues, or polypeptides, which may contain from 21 to several thousand amino acid residues. The polypeptides may undergo further processing, including chemical modification, before taking up their final conformation as proteins.

Each amino acid also has specific chemical properties, which depend on the nature of the R group. This affects the behavior of the free amino acids and the corresponding amino acid residues in peptides and polypeptides. For convenience the amino acids may be considered in groups according to some common properties.

Small Neutral Amino Acids – Glycine and Alanine

The small side chains, a hydrogen atom and a methyl group, have little effect on the shape of a peptide chain. The free amino acids tend to be heavily involved as metabolic intermediates. Glycine is a precursor of purines, porphyrins, bile acids, and creatine; it acts as a neurotransmitter and as a conjugating substance, which aids the excretion of xenobiotics by making them more water-soluble. Alanine is the

Table 1 Amino acid characteristics

Name (3 letter code; 1 letter code)	Structure	Molecular weight	pKa
<i>Small neutral amino acids</i>			
Glycine (Gly; G)	$\begin{array}{c} \text{H} \\ \\ \text{H}-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	75	2.35 9.78
Alanine (Ala; A)	$\begin{array}{c} \text{H} \\ \\ \text{CH}_3-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	89	2.35 9.87
<i>Branched-chain amino acids</i>			
Valine (Val; V)	$\begin{array}{c} \text{CH}_3 \quad \text{H} \\ \diagdown \quad \\ \text{CH}-\text{C}-\text{CO}_2\text{H} \\ / \quad \\ \text{CH}_3 \quad \text{NH}_2 \end{array}$	117	2.29 9.74
Isoleucine (Ile; I)	$\begin{array}{c} \quad \quad \text{CH}_3 \quad \text{H} \\ \quad \quad \quad \\ \text{CH}_3-\text{CH}_2-\text{CH}-\text{C}-\text{CO}_2\text{H} \\ \quad \quad \quad \\ \quad \quad \quad \text{NH}_2 \end{array}$	131	2.32 9.76
Leucine (Leu; L)	$\begin{array}{c} \quad \quad \text{CH}_3 \quad \quad \text{H} \\ \quad \quad \quad \quad \\ \text{CH}_3-\text{CH}-\text{CH}_2-\text{C}-\text{CO}_2\text{H} \\ \quad \quad \quad \\ \quad \quad \quad \text{NH}_2 \end{array}$	131	2.33 9.74
<i>Aromatic amino acids</i>			
Tryptophan (Trp; W)		204	2.43 9.44
Tyrosine (Tyr; Y)		181	2.20 9.11 10.13
Phenylalanine (Phe; F)		165	2.16 9.18
<i>Hydroxyl-containing amino acids</i>			
Serine (Ser; S)	$\begin{array}{c} \text{H} \\ \\ \text{HO}-\text{CH}_2-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	105	2.19 9.21
Threonine (Thr; T)	$\begin{array}{c} \text{CH}_3 \quad \text{H} \\ \quad \\ \text{HO}-\text{CH}-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	119	2.09 9.10

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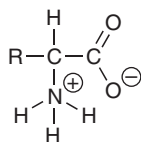
Table 1 Continued

Name (3 letter code; 1 letter code)	Structure	Molecular weight	pKa
<i>Sulfur-containing amino acids</i>			
Cysteine (Cys; C)	$\text{HS}-\text{CH}_2-\underset{\text{NH}_2}{\overset{\text{H}}{\text{C}}}-\text{CO}_2\text{H}$	121	1.92 8.35 10.46
Methionine (Met; M)	$\text{CH}_3-\text{S}-\text{CH}_2-\text{CH}_2-\underset{\text{NH}_2}{\overset{\text{H}}{\text{C}}}-\text{CO}_2\text{H}$	149	2.13 9.28
<i>Imino acid</i>			
Proline (Pro; P)	$\begin{array}{c} \text{CH}_2-\text{CH}_2 \\ \quad \\ \text{CH}_2-\text{N}-\text{C}-\text{H} \\ \quad \\ \text{H} \quad \text{CO}_2\text{H} \end{array}$	115	1.95 10.64
<i>Acidic side chains</i>			
Aspartic acid (Asp; D)	$\begin{array}{c} \text{CO}_2\text{H} \quad \text{H} \\ \quad \\ \text{CH}_2-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	133	1.99 3.90 9.90
Glutamic acid (Glu; E)	$\begin{array}{c} \text{CO}_2\text{H} \quad \text{H} \\ \quad \\ \text{CH}_2-\text{CH}_2-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	147	2.10 4.07 9.47
<i>Amides</i>			
Asparagine (Asn; N)	$\begin{array}{c} \text{CONH}_2 \quad \text{H} \\ \quad \\ \text{CH}_2-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	132	2.10 8.84
Glutamine (Gln; Q)	$\begin{array}{c} \text{CONH}_2 \quad \text{H} \\ \quad \\ \text{CH}_2-\text{CH}_2-\text{C}-\text{CO}_2\text{H} \\ \\ \text{NH}_2 \end{array}$	146	2.17 9.13
<i>Basic side chains</i>			
Histidine (His; H)	$\begin{array}{c} \text{Imidazole ring} \\ \\ \text{CH}_2-\underset{\text{NH}_2}{\overset{\text{H}}{\text{C}}}-\text{CO}_2\text{H} \end{array}$	155	1.80 6.04 9.76
Lysine (Lys; K)	$\text{NH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\text{CH}_2-\underset{\text{NH}_2}{\overset{\text{H}}{\text{C}}}-\text{CO}_2\text{H}$	146	2.16 9.18 10.79
Arginine (Arg; R)	$\begin{array}{c} \text{NH}_2 \\ \\ \text{C}-\text{NH}-\text{CH}_2-\text{CH}_2-\text{CH}_2-\underset{\text{NH}_2}{\overset{\text{H}}{\text{C}}}-\text{CO}_2\text{H} \\ \\ \text{HN} \end{array}$	174	1.83 8.99 12.48

(Continued)

Table 1 Continued

Name (3 letter code; 1 letter code)	Structure	Molecular weight	pKa
<i>Nonprotein amino acids</i>			
Ornithine	$\text{NH}_2\text{—CH}_2\text{—CH}_2\text{—CH}_2\text{—}\overset{\text{H}}{\underset{\text{NH}_2}{\text{C}}}\text{—CO}_2\text{H}$	132	1.71 8.69 10.76
Citrulline	$\overset{\text{CONH}_2}{\text{NH}}\text{—CH}_2\text{—CH}_2\text{—CH}_2\text{—}\overset{\text{H}}{\underset{\text{NH}_2}{\text{C}}}\text{—CO}_2\text{H}$	175	Not determined
γ -Aminobutyric acid (GABA)	$\overset{\text{CH}_2\text{—CO}_2\text{H}}{\text{CH}_2\text{—CH}_2\text{—NH}_2}$	103	4.03 10.56
Homocysteine	$\text{HS—CH}_2\text{—CH}_2\text{—}\overset{\text{H}}{\underset{\text{NH}_2}{\text{C}}}\text{—CO}_2\text{H}$	117	2.22 8.87 10.86

**Figure 2** Zwitterionic structure of an amino acid.

transamination product of pyruvic acid, and is thus closely associated with the metabolism of carbohydrates, acting as a major precursor for gluconeogenesis.

Branched-Chain Amino Acids – Valine, Leucine, and Isoleucine

These have bulky, nonpolar side chains, so these residues are often found within the hydrophobic core of proteins. Isoleucine has an extra chiral center, so four optical isomers are theoretically possible, but only L-isoleucine (and not L-allo-isoleucine) is found in proteins. Branched-chain amino acids are metabolized initially in muscle and adipose tissue rather than liver, where most of the other amino acids are metabolized.

Aromatic Amino Acids – Tryptophan, Tyrosine, and Phenylalanine

These are also bulky and nonpolar, and may interact with other hydrophobic molecules. The phenolic hydrogen of tyrosine is weakly acidic and can form hydrogen bonds to create cross-links, or can be donated during catalysis. Tyrosine residues on certain membrane-bound receptors become

phosphorylated by tyrosine kinase domains, thereby initiating a signal transduction cascade. Tryptophan is important as a precursor of the neurotransmitter 5-hydroxytryptamine (serotonin), and of the nicotinamide-containing coenzymes NAD and NADP. Phenylalanine can be converted to tyrosine in the body, but not vice versa. Tyrosine is a precursor of the catecholamines, the thyroid hormones and the pigment melanin.

Hydroxyl-Containing Amino Acids – Serine and Threonine

These are polar, weakly acidic molecules but uncharged at neutral pH. They can form hydrogen bonds and are thus quite soluble. Threonine has an additional chiral center, but again only L-threonine is found in proteins. Serine is found at the active center of some enzymes. It is also the usual site of attachment for the carbohydrate residues in glycoproteins and for the phosphoryl groups in phosphoproteins.

Sulfur Containing Amino Acids – Cysteine and Methionine

Methionine is nonpolar but cysteine is polar. It can form weak hydrogen bonds with oxygen and nitrogen, it is also weakly acidic and is sometimes found at the active site of enzymes. Cysteine also acts as a reducing agent within the cell, both as the free amino acid and in the form of the antioxidant tripeptide glutathione. The sulfhydryl groups of two cysteine residues can be oxidized to form the double amino acid cystine, and this is the predominant form of the amino acid in extracellular fluid. When the same reaction occurs between cysteine residues in adjacent polypeptide chains a strong, covalent disulfide bond is formed, which gives the protein a rigid structure. This appears to be particularly important in

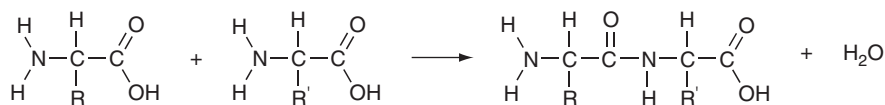


Figure 3 Peptide bond formation.

stabilizing extracellular or secreted proteins. Methionine can be converted to S-adenosyl-methionine, the donor of methyl groups in transmethylation reactions. Methionine can be converted to cysteine in the body, but not vice versa.

Selenium can replace sulfur in some cysteine and methionine residues, particularly when selenium intake is high. Certain proteins such as the antioxidant protein glutathione peroxidase require a selenocysteine residue at their active site. Incorporation of a selenocysteine residue during protein synthesis is specified by a UGA codon. This is normally a stop codon, but if there is a so-called SelenoCysteine Insertion Sequence element (SECIS) in the mRNA it is read as encoding a selenocysteine residue.

Imino Acid – Proline

Since its structure contains a secondary amine rather than a primary amine, proline is actually an imino acid rather than an amino acid, but it forms peptide bonds and is incorporated into proteins just like an amino acid. It causes an abrupt and rigid change of direction in the polypeptide chain, and this has a major effect on the final conformation of the protein. The carbon at the 4 position can be hydroxylated to form hydroxyproline. Every third residue of the structural protein collagen is a hydroxyproline residue.

Acidic Side Chains – Aspartic Acid and Glutamic Acid

These are dicarboxylic acids, though at physiological pH they exist almost entirely in the anionic form and so should be referred to as aspartate and glutamate. They are mainly found on the surfaces of proteins. The free amino acids play a central role in transamination reactions, equilibrating rapidly with their corresponding keto acids oxaloacetate and 2-oxoglutarate. Glutamate is a precursor for the inhibitory neurotransmitter γ -aminobutyric acid (GABA). The monosodium salt of glutamate is used in the food industry as a savoury flavor enhancer.

Amides – Asparagine and Glutamine

Although they are uncharged these molecules are strongly polar. They are often found on the surface of proteins, where they can form hydrogen bonds with water or with other polar molecules. The conversion of glutamate to glutamine is central to the disposal of ammonia, and to the maintenance of acid–base balance. Glutamine is a precursor for the synthesis of purines and pyrimidines. It is also a precursor for gluconeogenesis and is the main source of energy for enterocytes and leucocytes. There is some evidence that glutamine may play a role in the control of protein metabolism and that it may be beneficial in augmenting the immune response in critically ill patients.

Basic Side Chains – Histidine, Lysine, and Arginine

These are hydrophilic amino acids that are positively charged at neutral pH. The imidazole group of histidine has a pK_a just below 7, so it is weakly ionized at physiological pH, giving it some buffering capacity and making it useful at the active site of many enzymes. Histidine is also a precursor for the physiologically active amine histamine. Arginine is an intermediate in the urea cycle and a precursor for polyamine synthesis. It is also the precursor for nitric oxide, which appears to have many physiologically important properties, including that of an endothelial derived relaxing factor (EDRF) and a cell signalling molecule in the coordination of the inflammatory response. Although arginine, like glutamine, is a nonessential amino acid there is some evidence that increasing the dietary supply of arginine can improve clinical outcome in critically ill patients. Lysine is the limiting amino acid in cereals and cereal-based diets.

Post-Translational Modification

Some amino acid residues may become chemically modified after they have been incorporated into polypeptide chains. They will thus be present when the protein is degraded, but cannot be reutilized for protein synthesis.

Hydroxylation of proline to hydroxyproline is mainly associated with collagen (see the Section, Imino Acid – Proline, above). Hydroxylysine is also found in collagen.

The side-chain nitrogen atoms of the dibasic amino acids (histidine, arginine, and lysine) can all be methylated. For example, N^{ϵ} -methylhistidine (3-methylhistidine) is found mainly in the contractile proteins actin and myosin, so that detection of N^{ϵ} -methylhistidine in a food sample usually indicates the presence of meat. It has also been suggested that measurement of the urinary excretion of N^{ϵ} -methylhistidine could provide an index of the rate of breakdown of myofibrillar proteins in skeletal muscle, though interpretation is complicated by the presence of N^{ϵ} -methylhistidine derived from other tissues.

The hydroxyl groups of serine, threonine, and tyrosine can all be phosphorylated. Phosphoserine residues bind calcium and are found in proteins such as casein. Another calcium binding residue is γ -carboxyglutamic acid, which is found in prothrombin.

The hydroxyl groups of serine can also be glycosylated to form glycoproteins and proteoglycans. The amide group of asparagine can also be glycosylated.

The ϵ -N of certain lysine residues can be oxidized by the copper containing enzyme lysyl oxidase to form allysine. Four allysine residues in adjacent polypeptide chains may then condense to form desmosine (see Figure 4). This covalent link gives considerable strength and elasticity to the connective tissue protein elastin.

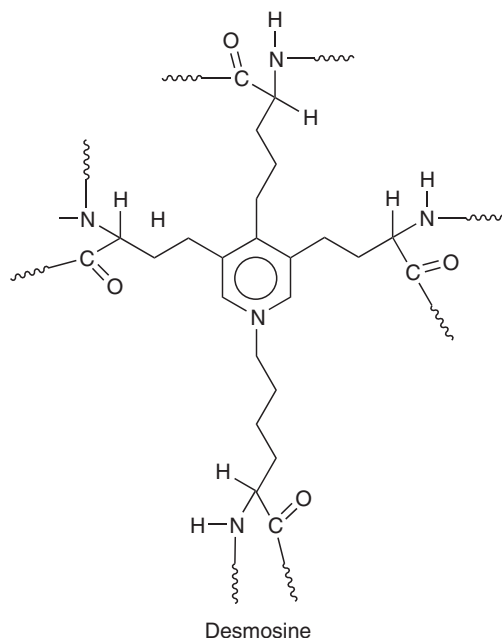
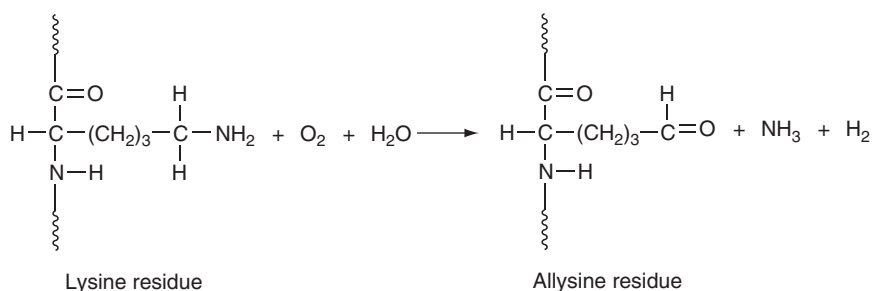


Figure 4 Formation of allysine and structure of desmosine.

The ϵ -N of lysine residues is also susceptible to chemical reactions within food systems. It undergoes the Maillard reaction with carbonyl groups of carbohydrates to form a series of brown and slightly bitter products. This is an integral part of the baking process when producing bread, cakes, and biscuits, although there is some evidence that large quantities of some Maillard products may be toxic or carcinogenic. On the other hand, because the lysine in Maillard products is not biologically available when the food is ingested this can seriously reduce the protein quality of heat-treated animal feedstuffs.

Proteins within living systems can also be damaged by covalent binding to other molecules (usually reactive biochemicals) to form adducts, thereby rendering the protein inoperative or immunogenic. Adducts can be formed by the reaction of an aldehyde function with a receptive nucleophilic center in the protein, particularly the ϵ -amino groups on lysine residues but also the α -amino terminus, the thiol groups on cysteine residues, the imidazole groups on histidine residues and the phenolic groups on tyrosine residues. The aldehydes that may be involved in adduct formation include malondialdehyde and 4-hydroxy-2-nonenal, which are produced by free radical damage to polyunsaturated fatty acids in cell membranes, and acetaldehyde, which is produced when

alcohol is metabolized. Adduct formation may play a role in the pathological processes leading to diseases such as alcoholic cirrhosis and coronary heart disease.

Nonprotein Amino Acids

There are several amino acids found in biological systems, which are not incorporated into proteins. Ornithine and citrulline, for example, are intermediates of the urea cycle; GABA is a neurotransmitter.

Homocysteine is an intermediate in the transsulfuration pathway for the conversion of methionine to cysteine. Homocystinuria is an inborn error of metabolism, which is characterized by the accumulation of high concentrations of homocysteine, and this leads to severe cardiovascular disease at an early age. However, there is a much more common mutation in the enzyme 5,10-methylenetetrahydrofolate reductase that causes a moderate increase in plasma homocysteine concentration in more than 10% of the population. A high plasma homocysteine concentration appears to be an independent risk factor for cardiovascular disease in the population as a whole, although the mechanism is not yet

known. Supplementing the diet with folic acid is often effective in reducing plasma homocysteine concentration because methyltetrahydrofolate is a substrate for the remethylation of homocysteine by the vitamin B₁₂-dependent enzyme methionine synthase. An inverse relationship has been observed between plasma homocysteine concentration and folate status in many studies, which has led to the proposal that plasma homocysteine concentration could be used as a biomarker of folate intake.

Taurine, or 2-aminoethanesulfonic acid, is sometimes classified as an amino acid although in fact it is an amino sulfonic acid rather than a conventional amino acid as it does not have a carboxylic acid group. It is found in high concentrations within the cells of many tissues. Numerous physiological functions have been ascribed to taurine, though its only definite role is in the conjugation of bile acids as taurocholic acid.

Peptides

As well as free amino acids and proteins, significant amounts of amino acids are present in physiological systems as small peptides. One of the most important is the tripeptide glutathione (γ -glutamylcysteinylglycine), which acts as an intracellular antioxidant.

Dipeptides found within the cell include carnosine (β -alanylhistidine) and its methylated derivatives anserine and balenine. These may act as buffers; no other physiological role has yet been identified.

Peptides are also used in food systems. For example, cysteine containing peptides, or cysteine itself, are used as improvers in bread making, to speed up the cross linking, which is required to give the bread its texture.

Another peptide that is used in the food industry is aspartame, which is composed of aspartic acid and phenylalanine. It is a very powerful sweetener, which does not have the bitter aftertaste of some other intense sweeteners.

Analysis

The analysis of amino acids is based on chromatographic techniques. Traditional amino acid analyzers involve separation of the amino acids mixture on a column of ion exchange resin using a series of sodium or lithium citrate buffers of increasing pH. The column effluent is then reacted with ninhydrin and passed through a spectrophotometer, which can detect and quantify a series of peaks, the areas of which are proportional to the concentration of the relevant amino acid. This method is still used, though high-performance liquid chromatography (HPLC) hardware is usually employed. Other postcolumn detection systems can be used, replacing the ninhydrin reagent with orthophthalaldehyde (OPA) or fluorescamine and detecting the product fluorimetrically, thereby increasing the sensitivity.

Amino acids can also be separated by HPLC on a reversed phase column. The mobile phase is usually based on an aqueous buffer with a gradient of increasing concentration of acetonitrile. In this case the amino acids are usually converted

to a fluorimetrically detectable (or ultraviolet absorbing) form before being injected onto the column. A wide variety of derivatizing agents can be used for this, including OPA, 1-fluoro-2,4-dinitrobenzene (FDNB), dansyl chloride, phenylisothiocyanate (PITC), and 9-fluorenylmethyl chloroformate (FMOC).

It is also possible to measure amino acids using gas-liquid chromatography, but this has never been popular, perhaps because the sample clean-up and derivatization steps are more laborious. The amino acids have to be converted to volatile derivatives before analysis, commonly either *N*-trifluoroacetyl-*n*-butyl or *N*-heptafluorobutyl-isobutyl esters. Gas-liquid chromatography is potentially a very sensitive method. It can also be coupled directly with mass spectrometry, for identification of unknown compounds or for measurement of tracer enrichment when carrying out metabolic studies with stable isotopes.

These analytical methods can be applied equally to the measurement of amino acids in proteins, after hydrolysis, or free amino acids in physiological fluids such as plasma, urine or tissue extracts. For physiological fluids the protein must first be removed, and this is usually accomplished by precipitating with an acid such as sulfosalicylic acid or an organic molecule such as acetonitrile. The chromatographic requirements for physiological fluids are more demanding than for protein hydrolysates because there will be many more contaminating substances producing extra peaks from which the amino acid peaks must be resolved, so the run time is generally rather longer.

Proteins have to be hydrolyzed before their amino acid composition can be measured. This is done by heating to 110 °C with an excess of 6 M HCl, either under nitrogen or in a vacuum. Proteins are usually hydrolyzed for 24 h, but this actually represents a compromise because some amino acids, including valine and isoleucine, may take longer than 24 h to liberate completely whereas others, including tyrosine, threonine, and serine, are progressively destroyed. Thus for complete accuracy a protein should be hydrolyzed for different lengths of time (usually between 16 and 72 h) and appropriate extrapolations made to the analytical values for each amino acid.

Acid hydrolysis destroys tryptophan, so a separate alkaline hydrolysis is needed to measure this amino acid. The sulfur containing amino acids are also partially oxidized during acid hydrolysis, so the protein may be oxidized with performic acid before hydrolysis and the oxidation products of cysteine and methionine measured. Finally, acid hydrolysis converts the amides glutamine and asparagine to their parent dicarboxylic acids, so values are often reported as total [glutamic acid plus glutamine] and [aspartic acid plus asparagine]. If separate values are required for the amides the protein must be subjected to enzymatic hydrolysis.

Classification

From a nutritional point of view the most important classification of amino acids is the division between those which are essential (or indispensable) and those which are non-essential (or dispensable). Essential amino acids may be

Table 2 Essential amino acids for the rat

Valine
Isoleucine
Leucine
Tryptophan
Phenylalanine
Threonine
Methionine
Histidine
Lysine
(Arginine)

defined as those that the body cannot synthesize in sufficient quantities.

This classification is based on work carried out by Rose in the 1930s. Young, rapidly growing rats were fed on purified diets from which one amino acid was removed. For some of the amino acids this made no difference to the rats' growth rate – these are the nonessential amino acids shown in **Table 2**. For the essential amino acids removal from the diet resulted in immediate cessation of growth, followed by loss of weight, decline in food intake and eventual death of the rats. The response to the removal of arginine was less dramatic, as the rats continued to grow, but at a reduced rate. Thus it appeared that the rat can synthesize arginine, but not at a high enough rate to support maximal growth.

It has subsequently been shown that the reason why certain amino acids are essential is that their carbon skeletons cannot be synthesized in mammalian cells. So long as the carbon skeletons are present, all amino acids except threonine and lysine can be formed by transamination. It should be noted, however, that tyrosine can only be synthesized from phenylalanine and cysteine can only be synthesized from methionine.

Rose also determined which amino acids are essential for man, by carrying out nitrogen balance experiments on healthy young adult volunteers. He showed that nitrogen balance could be maintained on a diet in which the only source of nitrogen was a mixture of the 10 amino acids that are essential for the rat. He then found that histidine and arginine could also be removed without affecting nitrogen balance. Thus the eight amino acids, which are essential for adult man are shown in **Table 3**.

Table 3 Essential amino acids for man

Valine
Isoleucine
Leucine
Phenylalanine
Tryptophan
Threonine
Methionine
Lysine

More recent work has identified certain circumstances, usually associated with disease or recovery from malnutrition, in which the addition of particular nonessential amino acids to an otherwise adequate diet appears to cause an unexpected improvement in either nitrogen balance or growth rate. It is hypothesized that the rate at which the body can synthesize these particular amino acids is limited, and that under extreme circumstances the requirement for them becomes greater than the rate at which they can be synthesized. These amino acids are thus sometimes called conditionally essential amino acids, and the list of them would include glycine, arginine, histidine, and glutamine.

See also: Amino Acids: Metabolism; Specific Functions. Protein Digestion and Bioavailability. Protein: Quality and Sources; Requirements and Role in Diet; Synthesis and Turnover

Further Reading

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Metabolism

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Introduction

Amino acids are generated within the body from three different sources. They enter the body from protein in the diet and nonessential (dispensable) amino acids are synthesized from other metabolic intermediates, but by far the largest quantities of free amino acids arise from the breakdown of tissue proteins. Similarly there are three metabolic fates for amino acids. Amino acid disposal is dominated by protein synthesis, but amino acids are also oxidized to carbon dioxide, water, and urea or they may be metabolized to other small molecules. The pathways involved in each of these processes will be considered and this will be followed by a discussion of the movement of amino acids between different compartments within the body (Figure 1).

Amino Acid Supply

Dietary Intake

Protein is digested in the stomach by pepsins and in the small intestine by proteolytic enzymes from the pancreas. The products of digestion are mainly small peptides, which are then taken up by the intestinal epithelium and hydrolyzed to free amino acids. The portal circulation transports these amino acids to the liver, where approximately 75% of the amino acids are metabolized. The remaining 25% then enter the systemic circulation for transport to other tissues.

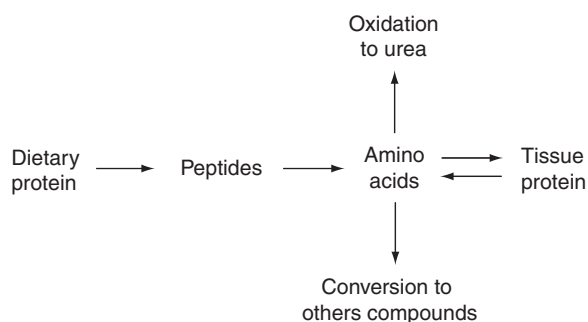


Figure 1 Overview of amino acid metabolism.

Amino Acid Biosynthesis

The essential (indispensable) amino acids must be supplied in the diet because their carbon skeletons cannot be synthesized in the human body, whereas the nonessential amino acids can be synthesized from common intermediates of the central metabolic pathways within the cell, i.e., glycolysis, the pentose phosphate pathway and the tricarboxylic acid cycle (TCA cycle). So long as the keto-analogues are present almost all amino acids can be generated by the process of transamination. The exceptions are threonine and lysine. Threonine is a poor substrate for mammalian transaminase enzymes, whereas the keto-analogue of lysine, α -oxo- ϵ -aminocaproate, is unstable and cyclizes spontaneously to pipercolic acid.

Glutamic Acid, Glutamine, Proline, and Arginine

Glutamic acid is synthesized by transamination of 2-oxoglutarate, a TCA cycle intermediate. This reaction represents the first stage in the catabolism of many other amino acids, particularly the branched chain amino acids. Vitamin B₆ is a cofactor for all transamination (aminotransferase) reactions.

Glutamine is made from glutamic acid and ammonium in an energy-requiring reaction catalyzed by glutamine synthetase. The synthesis of glutamine plays an important role in the removal of the ammonium formed in peripheral tissues by deamination of amino acids, as it is transported to the liver and used for urea synthesis.

Glutamic acid can be phosphorylated to γ -glutamyl phosphate by ATP, and this can then be dephosphorylated to glutamic- γ -semialdehyde. This undergoes nonenzymatic cyclization to Δ^1 -pyrroline-5-carboxylate, which can then be reduced to proline (see Figure 2).

Arginine is made from ornithine via the reactions of the urea cycle (see Figure 10). Ornithine can theoretically be made by transamination of glutamic- γ -semialdehyde, but as mentioned in the previous paragraph this cyclizes spontaneously to pyrroline-5-carboxylate. Thus in practice glutamate is first acetylated by acetyl CoA to *N*-acetyl glutamate, so that when this is converted to *N*-acetyl glutamic- γ -semialdehyde the amino group is blocked and cannot cyclize. The *N*-acetyl glutamic- γ -semialdehyde is then transaminated to *N*-acetyl ornithine and this is deacetylated to ornithine (see Figure 2).

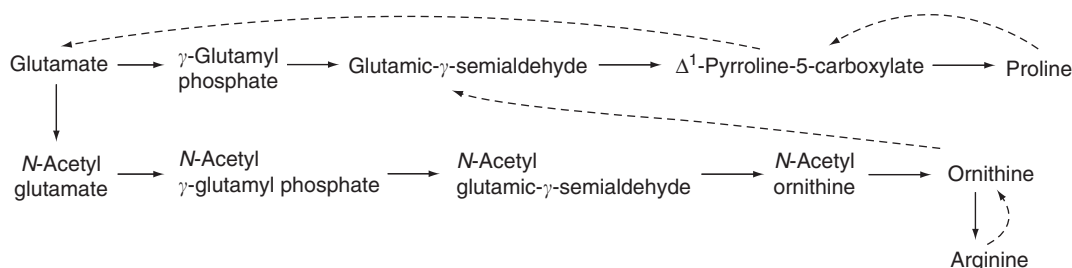


Figure 2 Synthesis and catabolism of proline and arginine. Solid lines indicate biosynthetic pathways; broken lines indicate catabolic pathways.

Aspartic Acid and Asparagine

Aspartic acid is derived from transamination of oxaloacetic acid, a TCA cycle intermediate. As with glutamic acid synthesis, this represents a common mechanism for removing amino groups from many other amino acids. Asparagine is made from aspartic acid by transfer of the amide group from glutamine.

Alanine

Alanine is made by transamination of pyruvic acid, which is generated by glycolysis.

Serine and Glycine

Serine and glycine are readily interconvertible via methylene tetrahydrofolate, which either condenses with a glycine molecule to yield serine or is cleaved to yield glycine and tetrahydrofolate (see Figure 3). However, there are also separate biosynthetic pathways for both molecules. Glycine can be synthesized by transamination of glyoxylate, which arises from the pentose phosphate pathway. Serine can be made by dephosphorylation of 3-phosphoserine, which is made by sequential dehydrogenation and transamination of 3-phosphoglycerate, a glycolytic intermediate (see Figure 3).

Histidine

Histidine is synthesized by a relatively long pathway, which has no branch points and does not lead to the formation of any other important intermediates. The main precursors are phosphoribosyl pyrophosphate and ATP, with the α -amino group arising by transamination from glutamate (see Figure 4).

Cysteine

In man and other animals cysteine can only be synthesized from the essential amino acid methionine. Methionine reacts with ATP to form S-adenosylmethionine, an important methylating agent within the cell. Transfer of the methyl group results in the formation of S-adenosylhomocysteine, which is then converted to homocysteine. Homocysteine can condense with serine to form cystathionine, which is then cleaved by cystathionase to yield cysteine (see Figure 5).

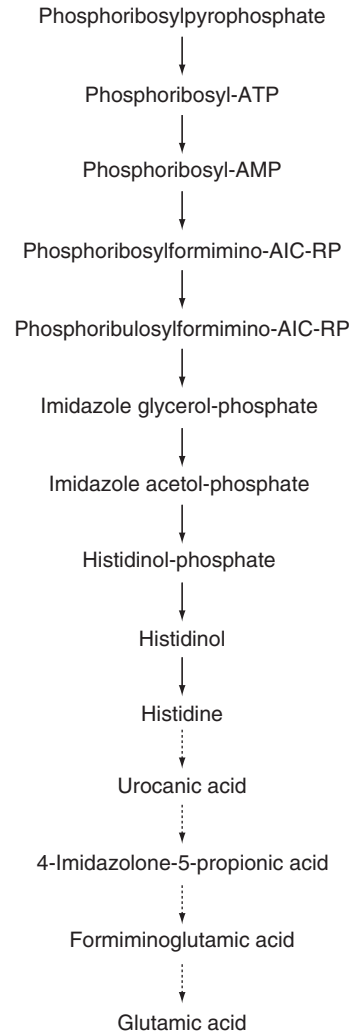


Figure 4 Synthesis and catabolism of histidine. Solid lines indicate biosynthetic pathways; broken lines indicate catabolic pathways.

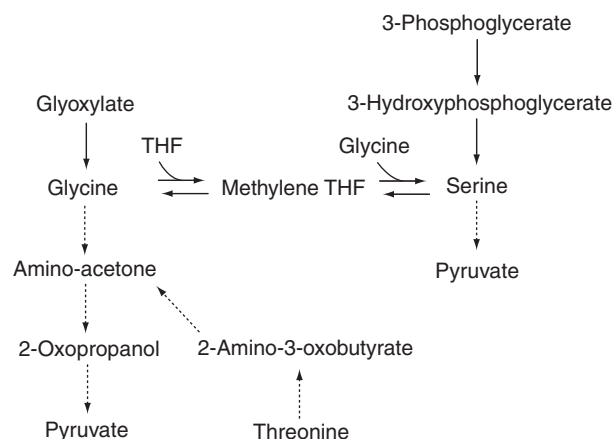


Figure 3 Synthesis and catabolism of glycine, serine, and threonine. Solid lines indicate biosynthetic pathways; broken lines indicate catabolic pathways. THF, tetrahydrofolate.

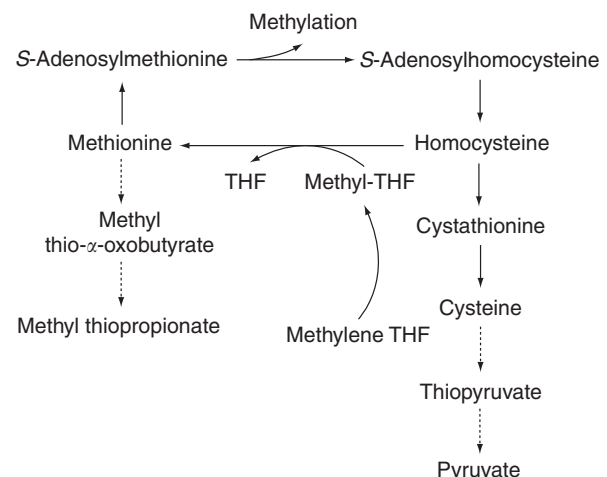


Figure 5 Synthesis and catabolism of methionine and cysteine. Solid lines indicate biosynthetic pathways; broken lines indicate catabolic pathways. THF, tetrahydrofolate.

An alternative fate for homocysteine is remethylation to methionine. The methyl donor for this reaction can be either methyltetrahydrofolate, in a reaction for which vitamin B₁₂ is a cofactor, or betaine. Remethylation seems to be quite sensitive to folate status, and plasma homocysteine is becoming accepted as a biomarker of nutritional status with respect to folate. Homocystinuria is an important inborn error of metabolism that is caused by impaired activity of cystathionine synthetase, the enzyme that catalyzes the condensation of homocysteine with serine. One of the consequences of homocystinuria is premature cardiovascular disease. There is now considerable evidence that milder elevations of plasma homocysteine, caused by poorly active variants of the methylenetetrahydrofolate reductase enzyme (which is required to make the methyl donor methyltetrahydrofolate) or by low folic acid status may be an important risk factor for cardiovascular disease throughout the population.

Tyrosine

In mammals, including man, tyrosine can only be formed by hydroxylation of the essential amino acid phenylalanine in a reaction that utilizes tetrahydrobiopterin as a cofactor. The inborn error of metabolism phenylketonuria is caused by a failure of the enzyme phenylalanine hydroxylase.

Protein Breakdown

Amino acids are continuously released by the hydrolysis of proteins. This occurs by several different mechanisms. Much intracellular proteolysis occurs within lysosomes, which provide the acidic environment within which enzymes such as cathepsins operate. However, there are also cytosolic proteolytic enzymes, which operate at neutral or alkaline pH. These include the enzymes, which hydrolyze proteins bound to ubiquitin. There are also extracellular proteinases which degrade extracellular proteins such as collagen.

Disposal of Amino Acids

Protein Synthesis

Protein synthesis represents the major route of disposal of amino acids. Amino acids are activated by binding to specific molecules of transfer RNA and assembled by ribosomes into a sequence that has been specified by messenger RNA, which in turn has been transcribed from the DNA template. Peptide bonds are then formed between adjacent amino acids. Once the polypeptide chain has been completed the subsequent folding, post-translational amino acid modifications and protein packaging are all determined by the primary sequence of amino acids. The rate of protein synthesis is controlled by the rate of transcription of specific genes, by the number and state of aggregation of ribosomes and by modulation of the rate of initiation of peptide synthesis.

Amino Acid Catabolism

Many amino acids can be converted to other useful molecules within the cell and the same pathways may also lead to

oxidation of the amino acid. It is therefore convenient to consider these metabolic fates together.

Glycine, Serine, and Threonine

The interconversion of glycine and serine has already been mentioned (see Figure 3), and this can act as a mechanism for disposal of either amino acid. In quantitative terms, however, the main tendency is for both to be converted to the common intermediate methylene tetrahydrofolate, which acts as a methyl donor in many important biosynthetic reactions including the conversion of dUMP to dTMP for DNA synthesis.

An alternative pathway for serine catabolism is deamination to pyruvate. However, the K_m of this enzyme is relatively high, so the pathway would only operate at high serine concentrations (see Figure 3).

Another pathway of glycine catabolism is by condensation with acetyl CoA to form amino-acetone. This is then transaminated and dehydrogenated to yield carbon dioxide and pyruvate. Amino-acetone is also formed by the NAD-linked dehydrogenation of threonine, followed by the spontaneous decarboxylation of the unstable intermediate 2-amino-3-oxobutyrate, and this appears to be the main pathway of catabolism of threonine in mammals (see Figure 3).

Glycine is also an important precursor for several important larger molecules. Purines are synthesized by a pathway which begins with the condensation of glycine and phosphoribosylamine. Porphyrins, including heme, are synthesized from glycine and succinyl CoA via δ -aminolaevulinic acid. Creatine synthesis involves the addition of the guanidino nitrogen from arginine to glycine. Glycine is also used to conjugate many foreign compounds, allowing them to be excreted in the urine. Glycine also conjugates with cholic acid to form the major bile acid glycocholic acid.

Glutamic Acid, Glutamine, Proline, and Arginine

Glutamic acid can be transaminated to 2-oxoglutarate, which can enter the TCA cycle. The amino group would be transferred to aspartate, which would then enter the urea cycle. Alternatively, glutamate can be deaminated by glutamate dehydrogenase, with the resulting ammonium entering the urea cycle as carbamoyl phosphate. Decarboxylation of glutamate yields γ -aminobutyric acid, an important inhibitory neurotransmitter.

Glutamine is deamidated to glutamic acid in the kidney, this process being central to the maintenance of acid-base balance and the control of urine pH. Glutamine also acts as a nitrogen donor in the synthesis of purines and pyrimidines.

Proline is metabolized by oxidation to glutamic acid, although the enzymes involved are not the same as those which are responsible for the synthesis of proline from glutamic acid (see Figure 2).

Arginine is an intermediate of the urea cycle, and is metabolized by hydrolysis to ornithine. Ornithine can transfer its δ -amino group to 2-oxoglutarate, forming glutamic- γ -semialdehyde, which can then be metabolized to glutamate (see Figure 2). Ornithine can also be decarboxylated to putrescine, which in turn can be converted to other polyamines such as spermidine and spermine.

Arginine can also be oxidized to nitric oxide and citrulline. Nitric oxide appears to be an important cellular signalling

molecule which has been implicated in numerous functions including relaxation of the vascular endothelium and cell killing by macrophages. In the vascular endothelium nitric oxide is made by two different nitric oxide synthase isozymes, one of which is inducible whereas the other acts constitutively. Both require tetrahydrobiopterin as a cofactor.

Aspartic Acid and Asparagine

Aspartic acid can be transaminated to oxaloacetic acid, a TCA cycle intermediate. Alternatively, when aspartic acid feeds its amino group directly into the urea cycle the resulting keto acid is fumarate, another TCA cycle intermediate. Aspartic acid is also the starting point for pyrimidine synthesis. Asparagine is metabolized by deamidation to aspartic acid.

Lysine

In mammals lysine is catabolized by condensing with 2-oxoglutarate to form saccharopine, which is then converted to α -aminoadipic acid and glutamate. The α -aminoadipic acid is ultimately converted to acetyl CoA. In the brain some lysine is metabolized via a different pathway to pipercolic acid (see Figure 6). Lysine is also the precursor for the synthesis of carnitine, which carries long chain fatty acids into the mitochondrion for oxidation. In mammals this process starts with three successive methylations of a lysine residue in a protein. The trimethyllysine is then released by proteolysis before undergoing further reactions to form carnitine.

Methionine and Cysteine

The conversion of methionine to cysteine via the so-called trans-sulphuration pathway has already been mentioned

(see Figure 5). This pathway appears to act mainly as a biosynthetic pathway for the synthesis of cysteine. There is an alternative pathway for methionine catabolism that involves transamination to methyl thio- α -oxobutyrate and thence to methyl thiopropionate.

Cysteine can be transaminated to thiopyruvate, which then undergoes desulphuration to pyruvate and hydrogen sulphide (see Figure 5). Cysteine can also be oxidized to cysteine sulphonic acid, which can then be decarboxylated to hypotaurine, which is then oxidized to taurine. High concentrations of taurine are found within most cells of the body, although its role is far from clear. In the liver the main fate of taurine is the production of taurocholic acid, which acts as an emulsifier in the bile.

Another key role for cysteine is in the synthesis of the tripeptide glutathione, which is an important intracellular antioxidant.

Leucine, Isoleucine, and Valine

The branched chain amino acids are unusual in that the first step in their metabolism occurs in muscle rather than liver. This step is transamination, producing α -oxoisocaproic acid, α -oxo- β -methyl valeric acid and α -oxoisovaleric acid. These ketoacids are then transported to the liver for decarboxylation and dehydrogenation. Subsequent catabolism yields acetyl CoA and acetoacetate in the case of leucine, acetyl CoA, and propionyl CoA from isoleucine and succinyl CoA from valine (see Figure 7).

Histidine

The first step in histidine metabolism is deamination to urocanic acid. Subsequent metabolism of this compound can follow several different pathways, but the major pathway is the one which involves formiminoglutamic acid (FIGLU), which is demethylated by a tetrahydrofolic acid dependent reaction to glutamic acid (see Figure 4). This forms the basis of the FIGLU test for folate status. Another physiologically important pathway of histidine metabolism is decarboxylation to histamine, for which vitamin B₆ is a cofactor.

Phenylalanine and Tyrosine

Because mammalian enzymes cannot break open the benzene ring of phenylalanine, the only important pathway for catabolism of this amino acid is through hydroxylation to tyrosine. If the phenylalanine hydroxylase enzyme is lacking, as in phenylketonuria, a high concentration of phenylalanine accumulates and it is converted to phenylpyruvate, phenyllactate, and phenylacetate, which are toxic.

Tyrosine is transaminated to *p*-hydroxyphenylpyruvate, which is then decarboxylated to homogentisic acid. This is subsequently metabolized to acetoacetic acid and fumaric acid (see Figure 8). Small amounts of tyrosine are hydroxylated to 3,4 dihydroxyphenylalanine (DOPA), which is then decarboxylated to the catecholamines dopamine, noradrenaline and adrenaline. DOPA can also be converted to the pigment melanin. In the thyroid gland protein bound tyrosine is iodinated to the thyroid hormones tri-iodothyronine and thyroxine.

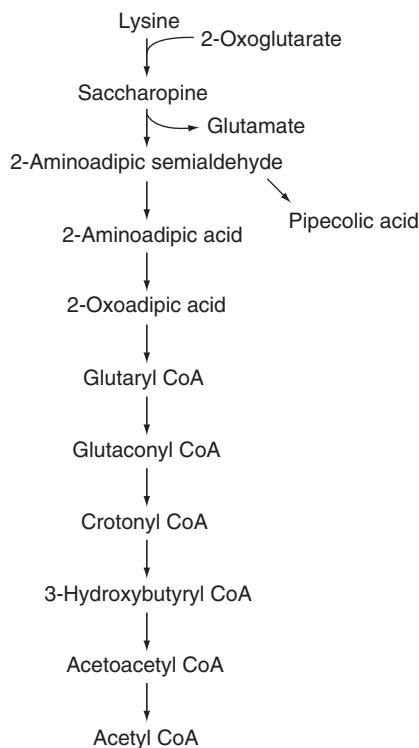


Figure 6 Metabolism of lysine.

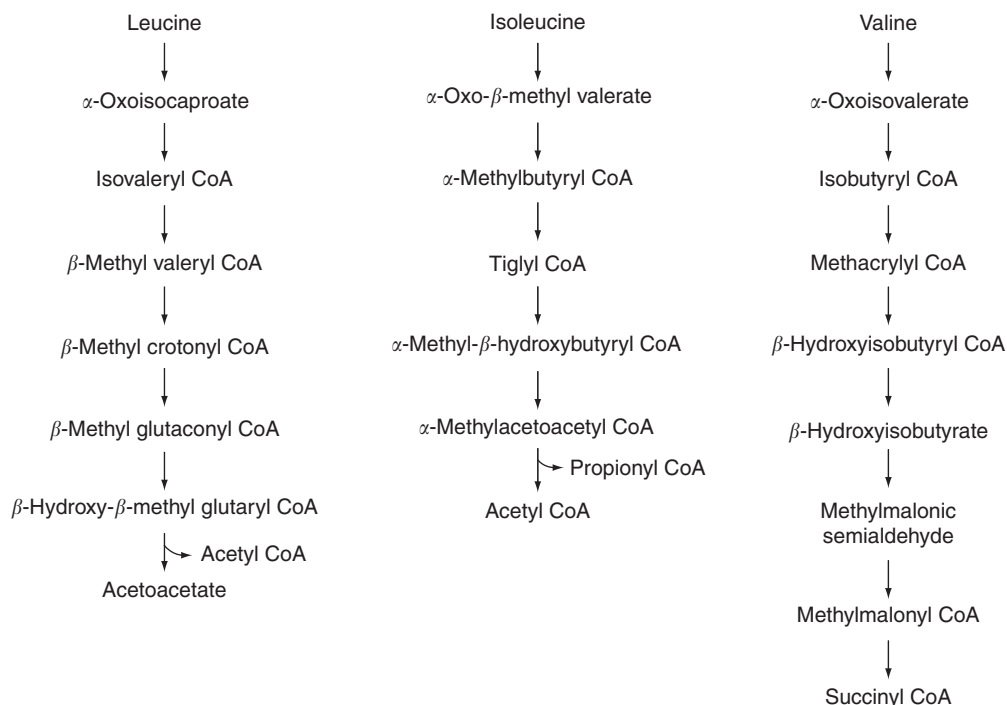


Figure 7 Metabolism of the branched-chain amino acids.

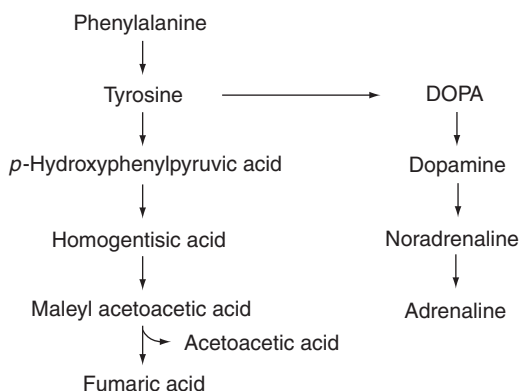


Figure 8 Metabolism of phenylalanine and tyrosine.

Tryptophan

Tryptophan is oxidized by the hormone-sensitive enzyme tryptophan oxygenase to *N*-formyl kynurenine, which then follows a series of steps to yield amino-carboxymuconic semialdehyde. Most of this undergoes enzymic decarboxylation, leading ultimately to acetyl CoA. However, a small proportion undergoes nonenzymatic cyclization to quinolic acid, which leads to the formation of NAD. This is why excess dietary tryptophan can meet the requirement for the vitamin niacin (see [Figure 9](#)).

One of the steps in the catabolism of tryptophan is catalyzed by the vitamin B₆-dependent enzyme kynureninase. If vitamin B₆ status is inadequate and a large dose of tryptophan is administered much of the tryptophan will be metabolized by an alternative pathway to kynurenic and xanthurenic acids, which will be excreted in the urine.

This is the basis of the tryptophan load test for vitamin B₆ status.

A small amount of tryptophan undergoes hydroxylation to 5-hydroxytryptophan, which is then decarboxylated to the physiologically active amine 5-hydroxytryptamine (serotonin).

Alanine

Alanine is metabolized by transamination to pyruvate.

Urea Cycle

From the above it can be seen that the metabolism of most amino acids involves removal of the amino groups by transamination. 2-Oxoglutarate is the main acceptor of these amino groups, being converted to glutamate, which can then be deaminated to release ammonium. However, ammonium is highly toxic and cannot be allowed to accumulate, so it is converted to urea, which is the form in which most of the nitrogen derived from protein is excreted from the body. Urea is formed in the liver by the cyclic series of reactions shown in [Figure 10](#). It can be seen that only one of the nitrogen atoms in the urea molecule is actually derived from ammonium, via carbamyl phosphate. The other nitrogen atom comes from aspartic acid, which is formed by transamination of oxaloacetic acid.

The rate of production of urea by the liver is normally greater than the rate of urea excretion in the urine. This is because some of the urea diffuses into the colon, where it is hydrolyzed to ammonia by bacteria. The ammonia can be absorbed and taken up by the liver where it can be re-incorporated into amino acids, thereby augmenting the net

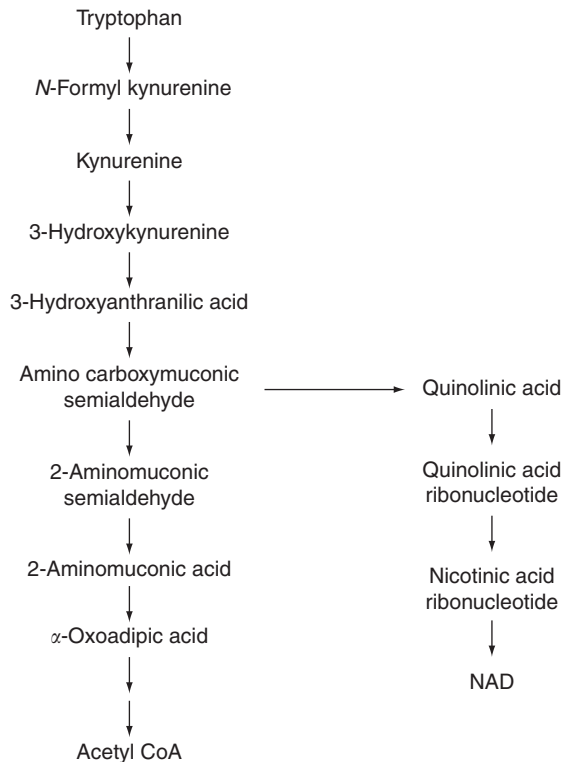


Figure 9 Metabolism of tryptophan.

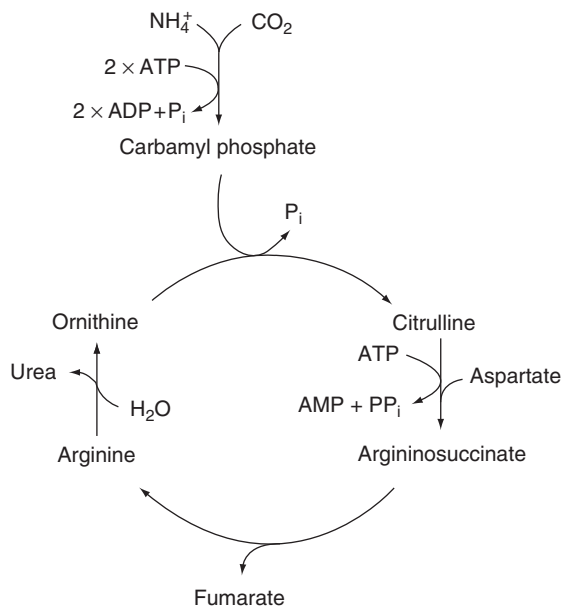


Figure 10 The urea cycle.

supply of nonessential amino acids. The colonic bacteria can also use ammonia to synthesize essential amino acids. There is evidence that some of these essential amino acids can also be absorbed and utilized by the human body. However, the rate at which this happens is clearly not sufficient to meet the body's requirements for essential amino acids.

Table 1 Amino acid transport systems

System	Sodium dependence	Preferred amino acids
A	Yes	Alanine, serine, glycine, methionine, proline
L	No	Leucine, isoleucine, valine, methionine, phenylalanine, tyrosine, tryptophan, histidine
ASCP	Yes	Alanine, serine, cysteine, proline
Ly	Yes	Lysine, histidine, arginine, ornithine
X_A^-	Yes	Aspartate
X_G^-	Yes	Glutamate
X_C^-	No	Aspartate, glutamate, cystine
y^+	Yes	Lysine, arginine, histidine
β	Yes	β -Alanine, taurine
$\text{b}^{0,+}$	No	Lysine, leucine
Gly	Yes	Glycine, sarcosine
N	Yes	Histidine, glutamine, asparagine
Imino	Yes	Proline

Glucogenic and Ketogenic Amino Acids

The carbon skeletons that are left after the amino acids have been transaminated are converted to common intermediates of the central metabolic pathways of the cell, so are ultimately used to provide energy. Clearly, for an adult in energy and nitrogen balance, energy will be derived from amino acids in the same proportion as protein is present in the diet. For most human diets this is in the range 10–15% of energy.

Under certain circumstances, such as starvation, diabetes, or a high fat diet, the body may need to synthesize glucose from amino acids rather than oxidizing them directly. Experiments with diabetic dogs fed on single amino acids have shown that most of the amino acids can be converted to glucose. They are therefore classified as glucogenic. However, leucine and lysine cannot be converted to glucose, so under these circumstances they give rise to acetoacetic acid and are thus classified as ketogenic. This classification can be related to the catabolic pathways outlined above. The ketogenic amino acids are those which are metabolized only to acetyl CoA, whereas those that are metabolized to pyruvate or TCA cycle intermediates are glucogenic. Tryptophan, phenylalanine, tyrosine, isoleucine, methionine, and cysteine are both glucogenic and ketogenic.

Interorgan Exchange of Amino Acids

Amino Acid Pools

Free amino acids make up only approximately 2% of the total amino acid content of the body, the rest being present as protein. The concentrations of free amino acids are regulated largely by modulation of their catabolic pathways, though in the case of nonessential amino acids there is also some regulation of the rate at which they are synthesized. There is some evidence that the rates of protein synthesis and degradation are regulated by amino acid supply and that this is another homeostatic mechanism acting to maintain free amino acid

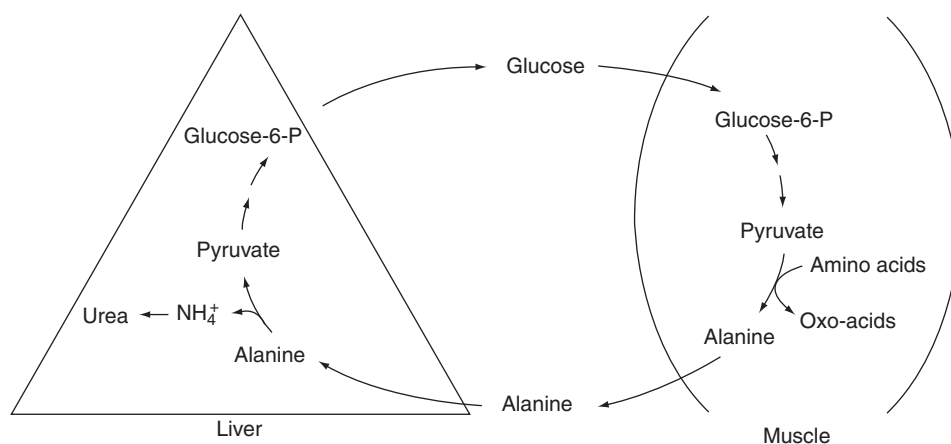


Figure 11 The glucose–alanine cycle.

concentrations within safe limits. Protein degradation is suppressed following a meal containing protein, and the rate of protein synthesis may be increased, so that there is net storage of amino acids as protein. Subsequently, in the postabsorptive state these changes in the rates of protein synthesis and breakdown are reversed, so that there is net release of amino acids from protein. In nongrowing adults these changes balance out over a 24-h period, so that there is no net change in body protein content. The amplitude of these diurnal changes in the rates of protein synthesis and degradation appears to vary in direct proportion to the amount of protein which an individual habitually consumes.

Free amino acids are found in all cells of the body and in extracellular fluid. They are transported between tissues in the plasma and transported into cells by a variety of transport mechanisms which are relatively specific for particular groups of amino acids (see Table 1). Amino acids are also present in red blood cells, but their role in interorgan transport appears to differ from that of plasma. For example, the plasma amino acid concentration increases as blood traverses the gastrointestinal tract after a meal whereas the amino acid content of blood cells actually decreases.

Metabolism in Different Organs

The liver is responsible for most of the deamination of amino acids, except for the branched chain amino acids, which are transaminated in muscle. Oxidation of amino acids is one of the main sources of energy for the liver. The liver is also the main site of gluconeogenesis, extracting large amounts of glutamine and alanine from the plasma for this purpose. The liver is the only site of urea synthesis.

Skeletal and cardiac muscle and adipose tissue are the main sites for transamination of the branched chain amino acids, and the resulting ketoacids are transported to the liver for oxidation. However, in fasting and diabetes the capacity of muscle to oxidize branched chain ketoacids increases markedly. In the postabsorptive state there is a net loss of amino acids from muscle, whereas in the fed state there is net uptake, reflecting the changes in net protein deposition and loss. However, at all times there is net output of alanine and glutamine from muscle, representing the disposal of the

amino groups from the branched chain amino acids. Muscle also takes up glucose, which is metabolized to supply the carbon skeletons for alanine and glutamine. Thus there is a well-recognized glucose–alanine cycle between muscle and liver (see Figure 11).

The kidney is a prime site of glutamine deamidation, producing ammonium to maintain acid–base balance and regulate the pH of the urine. Glutamine also serves as a substrate for gluconeogenesis in the kidney.

Glutamine is the major energy source for the small intestine, and at least part of the glutamine is derived from the lumen of the gut. Much of the glutamine is metabolized to pyruvate, which is then transaminated and exported to the liver as alanine. Some glutamine is also converted by the gut to citrulline, which then circulates to the kidney to be converted to arginine. Glutamine is also a major energy source for lymphocytes and monocytes when the immune system is activated.

See also: Amino Acid: Chemistry and Classification. Folic Acid. Inborn Errors of Metabolism: Classification and Biochemical Aspects; Nutritional Management of Phenylketonuria. Niacin and Pellagra. Protein Digestion and Bioavailability. Protein: Synthesis and Turnover. Vitamin B₆: Physiology

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Specific Functions

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Glossary

Deficiency In clinical practice the term amino acid deficiency is commonly used to indicate low plasma levels of specific amino acids. However a more proper definition would be an insufficient flux to maintain full functionality of the concerning amino acid. Flux may be unrelated to pool size (plasma or intracellular concentration) and therefore in many instances amino acid deficiency cannot be simply counteracted by direct supplementation.

Essential amino acid Strictly an essential amino acid is an amino acid that cannot be made by the organism itself. The term is also applied to amino acids of which the endogenous synthesis rate is insufficient to meet

physiological demands. These meets may depend on varying clinical conditions such as critical illness or trauma. Nonessential amino acids that may become deficient during such conditions are commonly referred to as conditionally essential amino acids.

Flux (also: turnover) Rate at which an amino acid is added to (rate of appearance) and removed from (rate of disappearance) a specific pool. In metabolic steady-state conditions these rates are equal. Different pools that can be distinguished are the extracellular (plasma) pool, intracellular pools, and subcellular pathways. The fluxes within these three pools can vary greatly and are often unrelated to each other.

Introduction

Apart from being the building blocks of proteins, many amino acids are indispensable for certain vital functions or have specific functions of their own. They can function as neurotransmitters, as precursors for neurotransmitters and other important metabolites, including crucial oligo- and polypeptides, as a stimulus for hormonal release, and in inter-organ nitrogen transport and nitrogen excretion. Consequently, manipulation of free amino acid levels by dietary or topical supplementation may support and modulate these specific functions.

Amino Acid Flux, Concentration, and Function

Many amino acids have or support specific functions by serving as precursors or substrates for reactions in which vital end products are produced. The availability of amino acids to serve these purposes is determined by the rate at which they are released into the plasma and other pools in which these reactions take place, as well as by the rate of disappearance through excretion, protein synthesis, or conversion to other amino acids. The rate of this release, referred to as amino acid flux, is determined by the breakdown of (dietary) proteins or the conversion from other amino acids. In fact, 80–90% of amino acid flux is derived from the whole body protein breakdown and only 10–20% from the diet. Increased demand for one or more amino acids generally leads to an increased flux of the required amino acids across specific organs. Because it is the flux of an amino acid that determines its availability for metabolic processes, flux is far more important for maintenance of specific functions than the plasma concentration. In fact it is striking that fluxes of some amino acids can double without significantly affecting plasma levels despite the fact that the plasma pool may be quantitatively negligible compared to the flux per hour. Plasma amino acid

concentrations must therefore be subjected to strong regulatory mechanisms. Increased demand and utilization of a specific amino acid may lead to decreased plasma and tissue concentrations, which may act as a signal to increase flux. Thus, a low plasma concentration in itself does not necessarily imply that supply of the amino acid in question is inadequate, but it may indicate that there is increased turnover of the amino acid and that deficiencies may result when dietary or endogenous supply is inadequate. Other factors determining the amino acid concentration are induction of enzymes and stimulation or blocking of specific amino acid transporters affecting the exchange and distribution of amino acids between different compartments. The regulation of plasma and tissue concentrations of specific amino acids may also be executed by the fact that release of the amino acid by an organ (e.g., muscle) and the uptake of that amino acid by another organ (e.g., liver) are subjected to a highly integrated network including the action of cytokines and other hormones.

Structurally each amino acid consists of an amino group, a carboxyl group, and carbon skeleton consisting of at least one carbon atom. Each amino acid is thus characterized by specific side groups attached to the carbon skeleton. When this specific side group is metabolized but the amino and carboxyl groups remain intact, one amino acid is metabolized to another. Such conversions can occur repeatedly, giving rise to metabolic pathways by which (part of) the carbon backbone of a single amino acid (with its amino and carboxyl groups) can pass through a succession of different amino acids. These pathways can be found both intracellularly (e.g., urea cycle), intercellularly (e.g., glutamine synthesis and breakdown in the liver), or on an inter-organ basis (e.g., the synthesis of arginine in the kidney from citrulline, which is formed from glutamine in the intestines). Because of this interconvertibility, groups of amino acids rather than one specific amino acid contribute to specific functions. Apart from the rate at which these amino acids interconvert, the rate at which they gain access to the tissue where the specific end

products exert their functions is also an important determinant of deficiencies of amino acids.

Amino Acid Deficiencies and Supplementation

In many diseases and during undernutrition, diminished turnover of amino acids can occur. These deficiencies may concern specific amino acids in certain diseases or a more generalized amino acid deficiency. The resulting functional deficits can contribute to the symptoms, severity, and progress of the disease. In some instances these deficits can be counteracted by simple supplementation of the deficient amino acids. Amino acid supplementation is also applied to enhance turnover and improve amino acid function in nondeficient patients.

However, amino acid supplementation in nondeficient states does not necessarily lead to an increased function because the organism utilizes what is programmed by regulating hormones and cytokines. In addition, the exchange between intra- and extracellular amino acid pools is largely dependent on amino acid transporters. Most of these transporters have a low K_m . Consequently cellular amino acid uptake mostly cannot be increased by a simple increment of plasma amino acid levels. An additional factor to consider is that metabolic processes can be subjected to counter regulatory feedback mechanisms. Some important metabolic processes served by a specific amino acid require only a marginal part of the total flux of that amino acid. The question may be raised whether true shortages may arise in such pathways, and supplemented amino acids may be disposed off in pathways other than those serving to improve a specific function.

Assessment of Amino Acid Function

The effectiveness of amino acid supplementation, particularly with respect to clinical effectiveness, can be assessed at four levels. First, the intervention should lead to an increased local or systemic concentration of the amino acid in question. The conversion of amino acids in (inter-organ) metabolic pathways can lead to an increase in the levels of amino acids other than the one supplemented, increasing, or mediating its functionality. Alternatively, supplementation of one amino acid may decrease the uptake of other amino acids because they compete for a common transporter. Second, the metabolic process for which the supplemented amino acid forms the substrate should be stimulated or upregulated by this increased amino acid availability. Third, this enhanced metabolic activity must lead to physiological changes. Fourth, these changes must be clinically effective in a desirable fashion (Table 1).

Alanine

Alanine and glutamine are the principal amino acid substrates for hepatic gluconeogenesis and ureagenesis. Alanine is produced in peripheral tissues in transamination processes with glutamate, branched chain amino acids, and other amino acids; following its release into the systemic circulation,

alanine is predominantly taken up by the liver and to a lesser extent by the kidney. Here, alanine can be deaminated to yield pyruvate and an amino group, which can be used for transamination processes, ureagenesis, or its derived amino group can be excreted in the urine as ammonia. Thus, the alanine released from peripheral tissues may be converted to glucose in the liver or kidney and eventually become a substrate for peripheral glycolysis. This so-called glucose-alanine cycle may be especially relevant during metabolic stress and critical illness when the endogenous alanine release from peripheral tissues is increased. Alanine is often used as the second amino acid in glutamine dipeptides that are applied to increase the solubility and stability of glutamine in nutritional solutions.

Supplementation

No clinical benefits have been ascribed to supplementation with alanine, although it has never been considered whether the beneficial effects of the dipeptide alanine-glutamine, which are generally ascribed to glutamine, may also be due to alanine. In this context, it should be realized, however, that alanine itself constitutes the strongest drive for hepatic ureagenesis (leading to breakdown of alanine).

Arginine, Citrulline, Ornithine, and Proline

Arginine is best known as the precursor for nitric oxide (NO). The conversion to NO is catalyzed by the enzyme nitric oxide synthase (NOS), and results in coproduction of the amino acid citrulline. Depending on its site of release, NO exerts several functions including stimulation of the pituitary gland, vasodilatation, neurotransmission, and immune modulation. Arginine is also a precursor for urea synthesis in the urea cycle, which has an important function in the excretion of waste nitrogen from the body. A full urea cycle is only present in the liver, but the arginase enzyme that converts arginine to urea and ornithine is, to a limited extent, also found in other tissues and cells, such as brain, kidney, small intestine, and red blood cells. Ornithine is utilized for the formation of proline, polyamines (putrescine, spermine, and spermidine), glutamic acid, and glutamine. Arginine is involved in collagen formation, tissue repair, and wound healing *via* proline, which is hydroxylated to form hydroxyproline. This role in wound healing may additionally be mediated by stimulation of collagen synthesis by NO, although this claim is still under investigation. It is currently thought that arginine availability is regulated by the balance between NOS and arginase enzyme activity, which subsequently determines substrate availability for NO and ornithine production. Proline also stimulates hepatocyte DNA and protein synthesis. Polyamines are potent inducers of cell differentiation (Figure 1).

In addition to synthesis of NO, urea, and ornithine, arginine is used for synthesis of creatine, which is an important constituent of skeletal muscle and neurons and acts as an energy source for these tissues. Furthermore, arginine may be catabolyzed to agmatine, which acts as a cell-signaling molecule. Arginine not only acts as an intermediate in the synthesis of functional products, but also is a potent stimulus for

Table 1 Specific functions of amino acids and their intermediate products

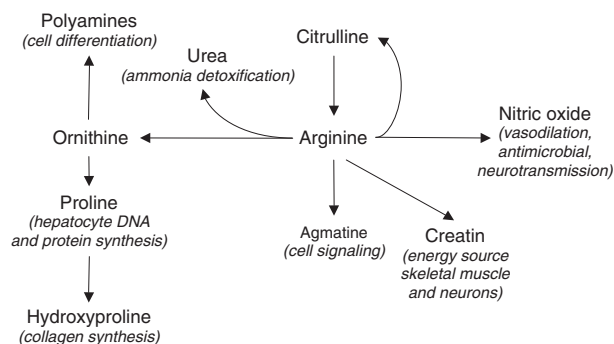
<i>Amino acid</i>	<i>Intermediate products</i>	<i>Function</i>	<i>Supplementation efficacy</i>
Alanine	Pyruvate	Gluconeogenesis Nitrogen transport	Data too limited
Arginine	Nitric oxide	Vasodilatation Immunomodulation Neurotransmission	Positive effects of arginine-containing immunonutrition on morbidity in surgical and trauma patients suggested; further research required
	Urea Creatine Agmatine	Ammonia detoxification Muscle constituent/fuel Cell signaling Ornithine precursor	
Citrulline	Arginine production		
Ornithine	Polyamines	Cell differentiation Proline precursor	Improves healing of burns (ornithine- α -ketoglutarate)
Proline	Hydroxyproline	Hepatocyte DNA, protein synthesis Collagen synthesis	
Asparagine		Aspartic acid precursor	(Asparaginase-induced asparagine depletion is therapeutic in leukemia)
Aspartic acid	Oxaloacetate, fumarate	Gluconeogenesis	
Methionine		Cysteine precursor (<i>see arginine</i>)	
Cysteine	Glutathione	Antioxidant	Improves antioxidant status in undernutrition, inflammatory diseases
	Taurine	Bile acid conjugation, neuronal cell development, regulation of membrane potential, calcium transport, antioxidant	Reduces contrast-induced nephropathy in renal failure Mucolysis, symptom reduction in COPD Hepatoprotective in acetaminophen intoxication
Glutamic acid	Glutamine α -Ketoglutarate Glutathione γ -Aminobutyric acid	Ammonia disposal Gluconeogenesis Antioxidant Inhibition CNS Excitation CNS (NMDA receptor)	
Glutamine	Ammonia	Inter-organ nitrogen transport	Intravenous administration reduces infectious morbidity in trauma patients, burn patients, and surgical patients
	Purines, pyrimidines	Renal HCO_3^- production RNA synthesis, DNA synthesis Glutamic acid precursor	
Glycine		Inhibition CNS (glycine receptor), Excitation CNS (NMDA receptor)	Adjuvant to antipsychotics, may reduce negative symptoms of schizophrenia
	Glutathione Creatine	Antioxidant (<i>see arginine</i>) Serine precursor	
Serine	D-Serine	Excitation CNS (NMDA receptor) Glycine precursor Cysteine precursor	Adjuvant to antipsychotics, may reduce negative symptoms of schizophrenia
Threonine	Glycine Serine	Brain development	
Histidine	Histamine	Immunomodulation Gastric acid secretion	
Lysine	Carnitine	Mitochondrial oxidation of long-chain fatty acids	Reduces chronic stress-induced anxiety
	Glutamate		

(Continued)

Table 1 Continued

Amino acid	Intermediate products	Function	Supplementation efficacy
<i>Branched chain amino acids</i>			
Isoleucine	α -Keto- β -methylvaleric acid		Upper gastrointestinal hemorrhage
Leucine	α -Ketoisocaproic acid	Important in regulation of energy and protein metabolism Substrate for glutamine synthesis	Improve protein malnutrition and restore amino acid and neurotransmitter balance in hepatic failure and hepatic encephalopathy (supplemented BCAA)
Valine	α -Ketoisovaleric acid		
Phenylalanine		Tyrosine precursor	
Tyrosine	L-dopa	Dopamine synthesis	Possible slight improvement of cognitive functions after physical or mental exhaustion. Metabolites are powerful pharmacotherapeutic drugs
	Dopamine	Movement, affect on pleasure, motivation	
	Noradrenaline, adrenaline	Activation of sympathetic nervous system (fight-or-flight response)	
	Tri-iodothyronine, thyroxine	Regulation of basal metabolic rate	
Tryptophan	Kynureninic acid	CNS inhibition	No scientific evidence for beneficial effects of supplementation
	Quinolinic acid	CNS excitation	
	Serotonin	Mood regulation Sleep regulation Intestinal motility	
	Melatonin	Regulation of circadian rhythms	

Different fonts indicate: nonessential amino acids, **essential amino acids**, and *conditionally essential amino acids*.

**Figure 1** Specific functions of arginine metabolism.

the release of several hormones, such as insulin, glucagon, somatostatin, and growth hormone, illustrating its pharmacological characteristics.

Arginine can be synthesized by the body from citrulline. However, because virtually all arginine produced in the liver is trapped within the urea cycle, kidney is the only arginine-synthesizing organ that significantly contributes to the total body pool of free arginine. Diminished renal arginine synthesis has been found in patients with renal failure and in highly catabolic conditions, like sepsis, burn injury, or trauma (which may be related to concomitant renal failure). In these situations, arginine may be considered a conditionally

essential amino acid and it has been suggested that arginine supplementation can become useful in these situations.

Citrulline is formed from glutamine, glutamic acid, and proline in the intestine. Plasma citrulline concentration reflects intestinal metabolic function and has recently been introduced as a potential marker for (reduced) enterocyte mass.

Supplementation

Based on its pluripotent functions, arginine has been widely used in supplemental nutrition for surgical patients, patients with burns, and patients with sepsis and cancer in order to modify the inflammatory response, to enhance organ perfusion, and to stimulate wound healing. However, despite these promising theoretical benefits, clinical results of arginine supplementation remain rather disappointing. The use of NO donors that have vasodilatory actions is an established therapeutic modality in coronary artery disease and for erectile dysfunction. Poor accessibility of plasma arginine to intracellular NOS may be an explanation for the limited clinical effects of arginine supplementation.

Using citrulline as an arginine-delivering substrate has been suggested, but has not been applied clinically. Ornithine is supplied as part of the ornithine- α -ketoglutarate molecule (see glutamine in the Section Glutamine, Glutamic acid, and Ornithine- α -Ketoglutarate). Creatine is widely used by

professional and recreational athletes as a nutritional supplement, although the ascribed performance-enhancing effects have not been proven.

Asparagine and Aspartic Acid

Asparagine can be converted by asparaginase to ammonia and aspartic acid, which is the precursor of the citrate cycle intermediates oxaloacetate and fumarate; this reaction is reversible. In fasting humans, asparagine and aspartic acid are utilized as precursors for *de novo* synthesis of glutamine and alanine in muscle.

Supplementation

The claim that asparagine or aspartic acid supplementation improves endurance has not been confirmed in human studies. Asparaginase, which degrades asparagine, is widely used in the treatment of pediatric leukemia because the resulting asparagine depletion leads to apoptosis of leukemic cells.

Cysteine, Cystine, Methionine, and Taurine

Methionine is converted to cysteine and its dipeptide cystine. In addition, methionine is a precursor for creatine (see the Section Arginine, Citrulline, Ornithine, and Proline). The potential for formation of disulfide bonds between its thiol (-SH) groups makes protein-bound cysteine important in the folding and structural assembly of proteins. Reduced cysteine thiol groups are found in protein (albumin), free cysteine, and in the principle intracellular antioxidant tripeptide glutathione (see glycine, glutamic acid in the Section Glycine, Serine, and Threonine and Glutamine, Glutamic acid, and Ornithine- α -Ketoglutarate) for which free cysteine is the synthesis rate-limiting constituent. Through the formation of disulfides (e.g., cystine, cysteinyl-glutathione, glutathione disulfide, mercaptalbumin) thiol-containing molecules can scavenge the oxygen-derived free radicals. The ratio between oxidized and reduced thiol groups reflects the cellular redox state. Owing to its small pool size, cysteine deficiencies rapidly occur during malnutrition (Figure 2).

Cysteine is also the precursor for taurine, which is abundant in all mammalian cells, particularly in neuronal cells and

lymphocytes, but it is not a true amino acid and is not incorporated in proteins. Taurine is involved in the conjugation of bile acids and may act as an antioxidant. Moreover, taurine is an osmolyte by virtue of the fact that through its transporter its intracellular concentrations are between 50- and 100-fold higher than in the extracellular compartment. This gradient contributes to the maintenance of the cellular hydration state. Similarly, it has been proposed that taurine is involved in stabilization of cell membrane potential and regulation of Ca^{2+} transport through several calcium ion channels. Based on these characteristics it has been suggested that taurine is involved in the control of cardiac muscle cell contraction, which has led to the addition of taurine to commercially available energy drinks. Its high level in lymphocytes suggests an important role in immunological resistance to infections. Taurine plays an important part in the development and maintenance of neuronal and especially retinal cells.

Supplementation

Although methionine is the only sulfur-containing essential amino acid, it has not been considered as part of the supplementation regimes. Because cysteine easily oxidizes to cystine, which has a poor solubility, it is generally supplemented in the form of *n*-acetylcysteine (NAC). Both directly and indirectly, as a precursor for glutathione, NAC has attracted attention as a potentially protective agent against oxidative injury in numerous conditions including endurance exercise, ischemia reperfusion injury, adult respiratory distress syndrome (ARDS), and cystic fibrosis. In addition, NAC has mucolytic properties in chronic obstructive pulmonary disease (COPD) patients by reducing disulfide bonds of polymers in mucus, blocking their reactivity. Currently, more or less established indications for NAC supplementation are the protection against nephropathy, induced by the administration of iodine containing contrast agents for radiological imaging in patients with chronic renal failure, in the reduction of the number of exacerbations and disability in COPD patients, and in the treatment of liver injury induced by acetaminophen intoxication. However, it has been suggested that glutathione depletion by buthionine sulfoximine administration potentiates the effect of radiotherapy by increasing the susceptibility of tumor cells to radiation-induced oxidative injury.

In a few studies it has been demonstrated that taurine supplementation improves retinal development in premature babies receiving parenteral nutrition. Human data on the efficacy of taurine supplementation in so-called energy drinks are very limited. In the absence of taurine supplementation in children, taurine concentrations drop, suggesting its conditional indispensability also in the postneonatal period. This has led to the addition of taurine to standard feeding formulas for infants and growing children.

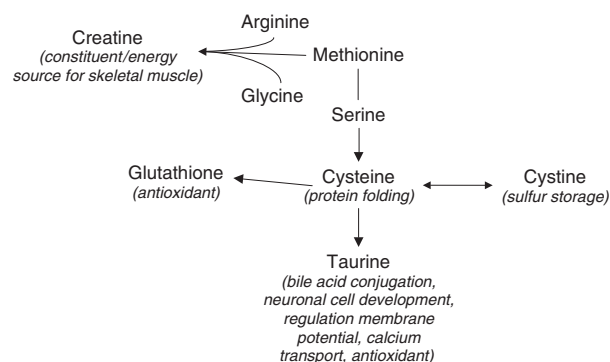


Figure 2 Specific functions of sulfur-containing amino acids.

Glutamine, Glutamic acid, and Ornithine- α -Ketoglutarate

Glutamine is the most abundant amino acid in plasma and tissue. In glutamine-consuming cells it is readily converted by

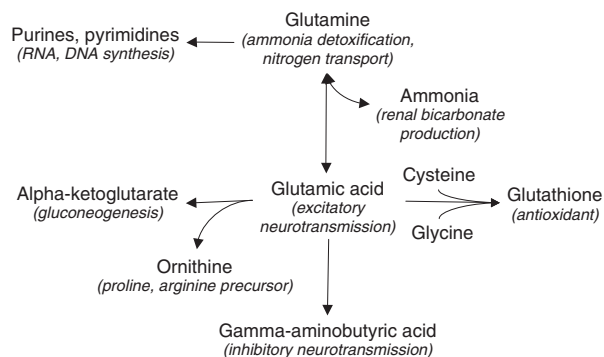


Figure 3 Specific functions of glutamine and glutamine degradation products.

the enzyme glutaminase to form ammonia and glutamic acid, which is the primary intermediate in almost all routes of glutamine degradation. In the presence of ammonia this process can occur in reverse, catalyzed by the enzyme glutamine synthetase. In contrast to glutamic acid, glutamine can easily pass through the cellular membrane, thus exporting waste nitrogen out of the cell and serving as an inter-organ nitrogen carrier. In the kidney, glutamine donates NH_3 , which is the acceptor for protons released from carbonic acid, to form NH_4^+ and thus facilitates the formation of HCO_3^- , which is essential in plasma pH regulation (Figure 3).

Following conversion to glutamic acid and subsequently α -ketoglutarate, glutamine may supplement intermediates of the citrate cycle. In this manner glutamine serves as the preferred fuel for rapidly dividing cells of, for example, the immune system cells and intestinal mucosa. In the brain, glutamic acid is the most abundant excitatory neurotransmitter and the precursor for gamma-aminobutyric acid, which is an important inhibitory neurotransmitter. Glutamine is a direct precursor for purine and pyrimidine and therefore is involved in RNA and DNA synthesis and cell proliferation. In addition it is a constituent of the tripeptide glutathione, which is the principal intracellular antioxidant in eukaryotes (see cysteine and glycine in the Sections Cysteine, Cystine, Methionine, and Taurine and Glycine, Serine, and Threonine).

Supplementation

Of all the compounds discussed above glutamine is the most extensively applied in clinical and experimental amino acid supplementation, often in the form of the more soluble and stable dipeptides alanyl- and glycyl-glutamine. Glutamic acid and α -ketoglutarate are less ideally suited for the use in feeding formulas because of poor inward transport of glutamic acid and poor solubility and stability of α -ketoglutarate. Moreover, glutamic acid has been related to the 'Chinese restaurant syndrome', characterized by light-headedness and nausea after consumption of Chinese food containing glutamic acid for flavor improvement. However, scientific evidence is weak. Numerous experimental and clinical studies have suggested that glutamine supplementation has positive effects on immune function, intestinal mucosal integrity, nitrogen balance, and glutathione concentration in a wide variety of conditions.

Nevertheless, the true benefit of glutamine supplementation is difficult to quantify in clinical practice. Its benefit has especially been claimed in the critically ill and surgical patients in whom clinical outcome is multifactorial. Recent meta-analyses support the view that glutamine supplementation is safe and may reduce infectious morbidity and hospital stay in surgical patients. A positive effect of glutamine supplementation on morbidity and mortality in critical illness, trauma patients, and burn patients has been demonstrated in a few well-designed clinical trials. However, due to the paucity of such trials reliable meta-analyses are not possible in these latter patient categories. Moreover due to high intestinal glutaminase activity, parenteral glutamine supplementation should be considered distinctively from enteral glutamine supplementation, which leads to much lower systemic glutamine levels. It has been demonstrated in some small clinical series that supplementation with ornithine- α -ketoglutarate may improve wound healing in burnt patients, benefiting from the combined actions of both α -ketoglutarate and ornithine (see arginine and ornithine in the Section Arginine, Citrulline, Ornithine, and Proline).

Glycine, Serine, and Threonine

Threonine is an essential amino acid, which can be converted to glycine in the liver and subsequently to serine. Glycine is a constituent of glutathione (see cysteine and glutamic acid in the Sections Glutamine, Glutamic acid, and Ornithine- α -Ketoglutarate and Cysteine, Cystine, Methionine, and Taurine) and is a versatile neurotransmitter in the central nervous system. Through the glycine receptor it has a direct inhibitory neurotransmitter function but it is also a ligand for the glycine site at the N-methyl-D-aspartate (NMDA) glutamic acid receptor. Activation of this glycine site is needed for NMDA activation, which makes glycine a mediator in the excitatory neurotransmitter effects of glutamic acid. Besides a role in the central nervous system, glycine is also thought to possess antiinflammatory properties, but to date these properties have only been demonstrated in the test tube. Furthermore, glycine can react with arginine and methionine to form creatine (see arginine in the Section Arginine, Citrulline, Ornithine, and Proline). Finally, glycine, like taurine, is a conjugate for bile acids.

Glycine is convertible to serine in a reversible reaction, which can be converted to its stereoisomeric form D-serine; this is also a ligand for the glycine site at the NMDA receptor. Furthermore, serine is an intermediate in the pathway from methionine to cysteine and a precursor for pyrimidines and purines and as such is involved in cell proliferation. It is also a precursor for gluconeogenesis, albeit of lesser importance than glutamine and alanine.

Supplementation

Based on their excitatory effects on the central nervous system both glycine and D-serine have been implicated in the treatment of schizophrenia. As adjuvant therapy to standard psychopharmacological treatment they may reduce the negative symptoms of the disease. High doses of threonine in adults

have been used as tentative therapy for spastic syndromes, a therapy that probably acts through increased glycine formation. A negative effect of excessive threonine, which is abundant in bovine infant formula nutrition, has been considered in experimental studies on brain development, and it has been suggested that this happens through its conversion to glycine and serine or the competition of amino acid transport across the blood–brain barrier.

Histidine

Histidine is the precursor for histamine, which is important for the immune system by mediating growth and functionality of immune cells. Excessive release of histamine from mast cells induces the clinical signs of allergy (dilation of capillaries and larger blood vessels, increased capillary permeability and swelling, itching, and anaphylactic shock). These phenomena are affected *via* the H1 receptor, which is found in smooth muscle cells of the vascular wall and bronchi, among others. Furthermore, histamine acts as a neurotransmitter and mediates gastric acid production. The latter occurs *via* the H2 receptor found in gastric mucosa. There is no literature available on the potential relationship between histidine availability and histamine production and action.

Supplementation

H1 receptor antagonists are applied in the treatment of allergy and H2 receptor antagonists have been shown to be very effective in the inhibition of gastric acid secretion and have greatly improved the treatment of individuals with peptic ulcer disease and acid reflux esophagitis. Histamine is present in abundance in many dietary sources; no beneficial effects of supplementation of either histidine or histamine are known.

Branched Chain Amino Acids (Isoleucine, Leucine, Valine)

Branched chain amino acids (BCAAs) are essential amino acids, which together compose approximately one third of the daily amino acid requirement in humans. BCAAs, and especially leucine, play an important role in the regulation of energy and protein metabolism. BCAAs are primarily oxidized in skeletal muscle and not in the liver. BCAAs donate their amino groups to furnish glutamic acid in muscle in transamination reactions yielding the α -ketoacids α -ketoisocaproic acid, α -keto- β -methylvaleric acid, and α -ketoisovaleric acid. These transamination products of BCAAs can give rise to succinate, which enters the citrate cycle and contribute to the ATP production by aerobic substrate oxidation. This is important during the change from rest to exercise. After consumption of protein-containing meals, a larger part of the BCAA passes through the liver and is taken up by the muscle where it primarily contributes to protein synthesis and synthesis of glutamine, which accounts for approximately 70% of the amino acid release from muscle. The importance of the essential branched chain amino acids for protein synthesis is

strikingly exemplified by the negative nitrogen balance and catabolism that follows upper gastrointestinal bleeding caused by ingestion of large amounts of hemoglobin (which lacks isoleucine). Leucine has been suggested to regulate the turnover of protein in muscle cells by inhibiting protein degradation and enhancing protein synthesis. This has led to a worldwide interest in the possible use of BCAAs in general, and leucine in particular, for metabolic support.

In liver failure the plasma concentrations of the aromatic amino acids (AAAs) tyrosine, phenylalanine, and tryptophan increase, probably because they are predominantly broken down in the liver, whereas the plasma levels of BCAAs decrease while they are degraded in excess in muscle as a consequence of hepatic failure-induced catabolism. As AAAs and BCAAs are all neutral amino acids and share a common transporter across the blood–brain barrier (system L carrier), changes in their plasma ratio are reflected in the brain, subsequently disrupting the neurotransmitter profile of the catecholamines and indoleamines (see tyrosine and tryptophan in the Sections Tryptophan and Phenylalanine and Tyrosine). It has been hypothesized that this disturbance contributes to the multifactorial pathogenesis of hepatic encephalopathy. In line with this hypothesis it has been suggested that normalization of the amino acid pattern by supplementing extra BCAAs counteracts hepatic encephalopathy.

Supplementation

Specialized formulas that are widely used for hepatic failure and hepatic encephalopathy are based on a high content of BCAAs to improve protein malnutrition and restore the amino acid and neurotransmitter balance. Although BCAA-enriched formulas have been proven to improve neurological status in comatose liver patients it is not certain that this is achieved by the addition of BCAAs specifically, because of a lack of adequate control groups.

Because BCAAs compete with tryptophan for uptake by the brain, they have (in line with the ascribed benefits in hepatic encephalopathy) been applied as competitive antagonists for tryptophan transport, reducing tryptophan-induced cognitive impairment (see tryptophan in the Section Tryptophan).

Isoleucine, which is absent in the hemoglobin molecule, can be supplemented to patients with upper gastrointestinal bleeding to restore the balance of amino acids that are taken up by the splanchnic organs. This has been demonstrated to improve mainly the protein synthesis in liver and muscle in small observational studies. Prospective randomized clinical trials are, however, still lacking.

Lysine

Lysine is an essential amino acid that is mainly provided by meat products and is therefore limited in diets where wheat is the primary protein source. Lysine is also the first rate-limiting amino acid in milk-fed newborns for growth and protein synthesis. Lysine is catabolyzed to glutamate and acetyl-CoA and is also the precursor for the synthesis of carnitine, which is needed for mitochondrial oxidation of long-chain fatty acids.

Supplementation

Lysine supplementation in patients with renal failure is contraindicated, as the amino acid shows some degree of nephrotoxicity.

Phenylalanine and Tyrosine

Phenylalanine is hydroxylated to tyrosine by the enzyme phenylalanine hydroxylase. The inborn error of metabolism that leads to phenylketonuria is characterized by a deficiency of this enzyme.

Tyrosine is the precursor for dihydroxyphenylalanine (dopa), which can successively be converted to the catecholamines dopamine, noradrenaline (norepinephrine) and adrenaline (epinephrine). Although only a small proportion of tyrosine is used in this pathway, this metabolic route is extremely relevant. Dopamine is an important neurotransmitter in different parts of the brain and is involved in movement and affects pleasure and motivation. Disruption of dopamine neurons in the basal ganglia is the cause of Parkinson's disease. Noradrenaline and adrenaline are the most important neurotransmitters in the sympathetic nervous system. The sympathetic nervous system becomes activated during different forms of emotional and physical arousal, and results in the induction of phenomena such as increased blood pressure and heart rate, increased alertness, and decreased intestinal motility (fight-or-flight response). Besides acting as a precursor for catecholamines, tyrosine can be iodinated and as such is the precursor for the thyroid hormones tri-iodothyronine and thyroxine. These hormones are important regulators of general whole body rate of metabolic activity.

Supplementation

The processes described in the paragraph above quantitatively contribute only marginally to total tyrosine turnover and the limited data on tyrosine supplementation in phenylketonuria suggest that tyrosine deficiency is not causal in the development of cognitive dysfunction in the disease, although tyrosine becomes an essential amino acid in phenylketonuria. In two studies tyrosine supplementation has been found to modestly increase mental status and cognitive performance following exhausting efforts such as prolonged wakefulness and intensive military training. In contrast, tyrosine derivatives (L-dopa, noradrenaline, adrenaline) have strong pharmacological properties. L-dopa is the direct precursor of dopamine synthesis and has been found to have strong beneficial effects in Parkinson's disease. The fact that administration of tyrosine as the physiological precursor of catecholamines has no or minor effects on catecholamine-induced sympathetic activity, whereas the effects of the catecholamines or more direct precursors is very strong, suggests that tyrosine hydroxylation to L-dopa is not limited by substrate availability.

Tryptophan

Functional end products of the essential amino acid tryptophan arise mainly through two distinctive pathways. The

major pathway is degradation of tryptophan by oxidation, which fuels the kynurenine pathway. The second and quantitatively minor pathway is hydroxylation of tryptophan and its subsequent decarboxylation to the indoleamine 5-hydroxytryptamine (serotonin) and subsequently melatonin. The metabolites of the kynurenine pathway, indicated as kynurenes, include quinolinic acid and kynurenic acid. Quinolinic acid is an agonist of the NMDA receptor (see glutamic acid in the Section Glutamine, Glutamic acid, and Ornithine- α -Ketoglutarate), whereas kynurenic acid is a nonselective NMDA receptor antagonist with a high affinity for the glycine site of the NMDA receptor (see glycine in the Section Glycine, Serine, and Threonine), and as such is a blocker of amino acid modulated excitation of the central nervous system. Imbalance between kynurenic acid and quinolinic acid can lead to an excitotoxic neuronal cell death and is believed to play a role in the development of several neurological diseases such as Huntington's chorea and epilepsy. In addition, an immunomodulatory role is suggested for several metabolites of the kynurenine pathway.

Serotonin is synthesized in the central nervous system and is involved in the regulation of mood and sleep. In addition, it is found in high quantities in neurons in the gastrointestinal tract where it is involved in the regulation of gut motility. Tryptophan competes with BCAAs for transport across the blood-brain barrier and the ratio between tryptophan and BCAAs therefore determines the uptake of both (groups of) amino acids by the brain (see BCAAs in the Section Branched Chain Amino Acids (Isoleucine, Leucine, Valine)). Because albumin has a strong tryptophan binding capacity, the plasma albumin concentration is inversely related to the plasma concentration of free tryptophan and as such influences the BCAA to tryptophan ratio and hence the brain uptake of both BCAAs and tryptophan. It has been suggested that increased plasma AAAs (tyrosine, phenylalanine, and tryptophan) levels in patients with liver failure are caused by the inability of the liver to degrade these amino acids. The resulting change in the ratio between AAA and BCAA plasma levels has been implied in the pathogenesis of hepatic encephalopathy because this may cause marked disturbances in transport of both AAAs and BCAAs across the blood-brain barrier, leading to disturbed release of indoleamines and catecholamines in the brain (see BCAAs in the Section Branched Chain Amino Acids (Isoleucine, Leucine, Valine)). High tryptophan concentrations have been associated with chronic fatigue disorders and hepatic encephalopathy whereas low tryptophan plasma concentrations have been implicated in the etiology of mood disorders, cognitive impairment, and functional bowel disorders. Melatonin, which is produced in the degradation pathway of serotonin during the dark period of the light-dark cycle, is an important mediator of circadian rhythms.

Supplementation

Inhibition of serotonin reuptake from the neuronal synapse and the subsequent increase in its functionality is one of the mainstays of the pharmacological treatment of depression. Like many amino acids, tryptophan is commercially available as a

nutritional supplement or a so-called smart drug, claiming to reduce symptoms of depression, anxiety, obsessive-compulsive disorders, insomnia, fibromyalgia, alcohol withdrawal, and migraine. However, no convincing clinical data are available to support these claims. In contrast tryptophan depletion induced by ingestion of a tryptophan-deficient amino acid mixture, is widely used in experimental psychiatry to study the biological background of various psychiatric disorders.

See also: Amino Acids: Chemistry and Classification. Brain and Nervous System: Biology, Metabolism, and Nutritional Requirements. Carbohydrates: Regulation of Metabolism. Cytokines: Nutritional Aspects. Electrolytes: Acid-Base Balance. Glucose: Metabolism and Maintenance of Blood Glucose Level. Inborn Errors of Metabolism: Classification and Biochemical Aspects; Nutritional Management of Phenylketonuria. Protein: Quality and Sources; Requirements and Role in Diet; Synthesis and Turnover

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Introduction

Free radicals, including reactive oxygen species (ROS), are formed in the human body as a result of oxidative metabolism, i.e., as a result of the many chemical reactions and metabolic processes that occur in the body. Free radicals are capable of modifying important molecules such as DNA, lipids, and proteins, and therefore affecting their ability to function or causing them to function abnormally. These processes are often referred to as oxidative damage. Antioxidants have the ability to scavenge and neutralize free radicals, or are necessary to enable other molecules to perform such a function. The body's antioxidant system is complex and consists of various intracellular and extracellular, endogenous and exogenous, and aqueous and lipid-soluble components that act in concert to prevent formation of ROS and terminate chains of ROS-initiated peroxidation of biological substrates. Antioxidants present in the diet include vitamin C, vitamin E, and carotenoids. Metals and minerals that are key components of antioxidant enzymes, such as zinc or selenium, are also referred to as antioxidants. Many antioxidants can also act as pro-oxidants in certain conditions, such as the presence of transition metals or at high concentrations. Intervention studies demonstrating higher risk of cancer in smokers with β -carotene supplementation have, for example, attributed this finding to its pro-oxidant effect (see section on β -Carotene).

Oxidant or oxidative stress is a pro-oxidant shift in the oxidant-antioxidant balance caused by a relative or absolute deficiency of antioxidants. A pro-oxidant shift promotes damaging oxidative changes to important cellular constituents, and this may in turn lead to cellular dysfunction and, ultimately, to aging, disability, and disease. Long-term oxidative stress has therefore been linked with a number of chronic diseases including coronary heart disease (CHD), cancer, cataract, dementia, and stroke.

Other plant components, including polyphenols, have also been shown to possess antioxidant potential. To date, most of the evidence on antioxidant potential of polyphenols comes from *in vitro* studies, many possessing higher antioxidant potential *in vitro* than vitamins and carotenoids. However, although polyphenols show strong antioxidant properties *in vitro*, there has been much discussion about whether they are present in sufficient quantities *in vivo* to influence antioxidant activities. Studies have shown that polyphenols do not appear to be circulating in the blood at high enough concentrations to contribute significantly to the body's total

antioxidant capacity. Further, it has been estimated that, after ingestion, around 90–95% of polyphenols undergo molecular changes. These changes to the structure of polyphenols can moderately or even radically change the 'biological activities' of polyphenols found in *in vitro* studies. However, it has been suggested that polyphenols may exert an indirect antioxidant effect, by protecting endogenous antioxidant enzymes in the human body. Owing to a lack of concise evidence of the potential antioxidant properties of polyphenols in the human body, these will not be discussed in this article.

The Antioxidant Hypothesis

A predominantly plant-based diet, high in fruit and vegetables, reduces the risk of developing several chronic diseases, including cancer and cardiovascular disease (CVD). It is often assumed that antioxidants, including vitamin C, vitamin E, and carotenoids, contribute to this protection by interfering passively with oxidative damage to DNA, lipids, and proteins. This hypothesis is supported by numerous *in vitro* studies in animals and humans. A large number of descriptive, case-control, and cohort studies have also demonstrated an inverse association between high intakes or plasma levels of antioxidants and risk of CVD and cancer at numerous sites, as well as other conditions associated with oxidative damage, such as age-related macular degeneration, cataracts, and chronic obstructive pulmonary disease (COPD).

These findings provided a strong incentive for the initiation of intervention studies to investigate whether a lack of dietary antioxidants is causally related to chronic disease risk and if providing antioxidant supplements confers benefits for the prevention and treatment of these conditions. This article summarizes the findings of the largest primary and secondary trials published to date and considers their implications for future research and current dietary advice.

Cardiovascular Disease

Of all the diseases in which excess oxidative stress has been implicated, CVD has the strongest supporting evidence. Oxidation of low-density lipoprotein (LDL) cholesterol appears to be a key step in the development of atherosclerosis, a known risk factor in the development of CVD. Small studies have demonstrated reductions in LDL oxidation (mostly *in vitro*) following supplementation with dietary antioxidants

(particularly vitamin E, which is primarily carried in LDL-cholesterol), suggesting that they may provide protection against the development of heart disease. A number of large intervention trials using disease outcomes (rather than biomarkers such as LDL oxidation) have also been conducted to try to demonstrate a protective effect of vitamin E, β -carotene, and, to a lesser extent, vitamin C supplements on CVD. Most have been carried out in high-risk groups (e.g., smokers) or those with established heart disease (i.e., people with angina or who have already suffered a heart attack).

Primary Prevention

The results of most primary prevention trials have not been encouraging (Table 1). For example, in the Finnish Alpha-Tocopherol Beta-Carotene Cancer prevention (ATBC) study, approximately 30 000 male smokers received vitamin E (50 mg d⁻¹ of α -tocopherol), β -carotene (20 mg d⁻¹), both, or an inactive substance (placebo) for approximately 6 years. There was no reduction in risk of major coronary events with any of the treatments despite a 50% increase in blood vitamin E concentrations and a 17-fold increase in β -carotene levels. Moreover, with vitamin E supplementation, there was an unexpected increase in risk of death from hemorrhagic stroke and a small but significant increase in mortality from all causes with β -carotene supplementation (relative risk (RR), 1.08; 95% confidence interval (CI), 1.01–1.16). In the Physicians' Health Study (PHS) II, looking at the effect of 400 IU α -tocopherol supplementation on alternate days in approximately 14 000 male physicians, the risk of hemorrhagic stroke almost doubled with vitamin E supplementation during 8 years of follow-up (hazard ratio (HR) 1.99, 95% CI, 1.13–3.52). An increase in CVD deaths was also observed in the Beta-Carotene and Retinol Efficacy Trial (CARET), which tested the effects of combined treatment with β -carotene (30 mg d⁻¹) and retinyl palmitate (25 000 IU d⁻¹) in 18 000 men and women with a history of cigarette smoking or occupational exposure to asbestos compared to the placebo group (RR, 1.26; 95% CI, 0.99–1.61). However, during 6-year follow-up after stopping supplements no effect of supplementation was found. In contrast, in the Women's Health Study (WHS), which looked at the effect of 600 IU α -tocopherol (alternate days) in approximately 40 000 female nurses over a period of 10 years, a significant reduction in cardiovascular deaths (RR, 0.76; 95% CI, 0.59–0.98), largely attributable to fewer sudden deaths, was observed.

Secondary Prevention

The most positive results from secondary prevention trials came from the Cambridge Heart Antioxidant Study (CHAOS), a controlled trial on 2002 heart disease patients with angiographically proven coronary atherosclerosis randomly assigned to receive a high dose of vitamin E (400 or 800 IU d⁻¹) or placebo (Table 2). Those receiving the supplements were 77% less likely to suffer from nonfatal heart disease over the 1½-year trial period than those who did not receive vitamin E (RR, 0.23; 95% CI, 0.11–0.47), although there was no reduction in CVD deaths. However, other large

secondary prevention trials with longer follow-up have been less encouraging. For example, in a further analysis of the ATBC study, the β -carotene supplementation was associated with an increased risk of CHD deaths among men who had a previous heart attack and were thus at high risk of subsequent coronary events. There were significantly more deaths from fatal CHD in the β -carotene group (RR, 1.75; 95% CI, 1.16–2.64) and in the combined β -carotene and vitamin E group (RR, 1.58; 95% CI, 1.05–2.40) compared to the placebo group. The Heart Outcomes Prevention Evaluation (HOPE) study observed no benefit from vitamin E supplementation (400 IU d⁻¹) on CVD or all-cause mortality. The Heart Protection Study (HPS) in the UK examined the effect of 5 years of supplementation with a cocktail of antioxidant vitamins (600 mg vitamin E, 250 mg vitamin C, and 20 mg β -carotene) alone or in combination with the lipid-lowering drug Simvastatin or placebo in more than 20 000 adults with CHD, other occlusive arterial disease, or diabetes mellitus. Although blood levels of antioxidant vitamins were substantially increased, no significant reduction in the 5-year mortality from vascular disease or any other major outcome was noted. In the Italian GISSI-Prevenzione Trial dietary fish oils reduced the risk of fatal or nonfatal CVD in men and women who had recently suffered from a heart attack but vitamin E supplementation (300 mg daily for 3½ years) did not provide any benefit. In these three trials, no significant adverse effects of vitamin E were observed. The PSH II study was designed as a primary prevention study including more than 14 000 male physicians, 754 of which had prevalent CVD. Analysis of this sup-sample showed that there was a nonsignificant decrease of total CVD (HR 0.82, 95% CI, 0.63–1.09), myocardial infarction (MI) (HR 0.88, 95% CI, 0.50–1.55), stroke (HR 0.74, 95% CI, 0.47–1.16) and cardiovascular mortality risk (HR 0.91, 95% CI, 0.70–1.17) with vitamin E supplementation, and of MI (HR 0.57, 0.32–1.02) with vitamin C supplementation. In the Women's Antioxidant Cardiovascular Study (WACS) in over 8000 postmenopausal women with a history of CVD or at least 3 cardiac risk factors, supplementation with vitamin E, β -carotene nor vitamin C showed any effect on total cardiovascular events, MI, stroke, or deaths.

Systematic reviews and meta-analyses of the clinical trials to date have therefore concluded that supplementation with any single antioxidant nutrient or combination of nutrients has not demonstrated any consistent benefit for the treatment or prevention of CVD.

Cancer

The oxidative hypothesis of carcinogenesis asserts that carcinogens generate ROS that damage RNA and DNA in cells, predisposing these cells to malignant changes and enhanced cancer risk. Most, but not all, damage is corrected by internal surveillance and repair systems involving dietary antioxidants, as well as endogenous antioxidant mechanisms. Antioxidants are therefore proposed to prevent cell damage by neutralizing free radicals and oxidants, thus preventing subsequent development of cancer.

Table 1 Summary of large intervention trials (> 1000 subjects) investigating the role of antioxidants and CVD in primary prevention

<i>Trial</i>	<i>Characteristics of subjects</i>	<i>Sex</i>	<i>Length of follow-up (years)</i>	<i>Treatment</i>	<i>Effect of antioxidant supplementation</i>
ATBC	29 133 smokers, Finland	Male	6	50 mg α -tocopherol or 20 mg β -carotene	No significant effect on fatal or nonfatal CHD or total strokes with either supplement Increase in deaths from hemorrhagic stroke in vitamin E group Increase in hemorrhagic stroke (+ 62%) and total mortality (+ 8%) in β -carotene group
CARET	14 254 smokers, 4060 asbestos workers, USA	Male and female	4	30 mg β -carotene and 25 000 IU retinol	Increase in deaths from CVD (+ 26%) (terminated early)
LCPS	29 584 poorly nourished, China	Male and female	5	15 mg β -carotene, 30 mg α -tocopherol, and 50 μ g selenium	Small decline in total mortality (− 9%)
NPCT	1004 subjects with nonmelanoma skin cancer but without CVD, USA	Male and female	7.6	200 μ g selenium daily	No effect on total CVD events, MI, stroke, or CVD mortality Reduction in deaths from stroke in men (− 55%) but not women
PHS	22 071 physicians, USA	Male	12	50 mg β -carotene or aspirin (alternate days)	No effect on fatal or nonfatal MI or stroke
PHS II	13 887 physicians, USA	Male	8	400 IU α -tocopherol (alternate days) or 500 mg ascorbic acid (daily) or placebo	No effect of vitamin E or vitamin C on CV events, MI, stroke, or CV mortality. Significantly increased risk of hemorrhagic stroke (+ 99%) with vitamin E

POPADAD	1276 adults with type 1 or type 2 diabetes and asymptomatic peripheral arterial disease, UK	Male and female	6.7	Antioxidant capsule (200 mg α -tocopherol, 100 mg ascorbic acid, 25 mg pyridoxine hydrochloride, 10 mg zinc sulphate, 10 mg nicotinamide, 0.4 mg lecithin, 0.8 mg sodium selenite) or 100 mg aspirin or placebo daily	No effect of antioxidant on CVD deaths or events
PPP	4495 with one or more CVD risk factors, Italy	Male and female	3 $\frac{1}{2}$	Low-dose aspirin or 300 mg α -tocopherol	No effect on CVD deaths or events (but inadequate power due to premature interruption of trial)
SCPS	1720 with recent nonmelanoma skin cancer, Australia	Male and female	8	50 mg β -carotene	No effect on CVD mortality
SUVIMAX	13 017, France	Male and female	7.5	Combination of 120 mg ascorbic acid, 30 mg vitamin E, 6 mg of β -carotene, 100 μ g selenium, 20 mg zinc, daily	No effect on incidence of ischaemic CVD
VACP II	1204 former asbestos workers, Australia	Male and female	5	30 mg β -carotene or 25 000 IU retinol (no placebo group)	No effect of β -carotene on CHD deaths
WHS	39 876, United States	Female	2 10	50 mg β -carotene (alternate days) 600 IU α -tocopherol (alternate days)	No effect on fatal or nonfatal CVD No effect on total CV events, MI or stroke Significant 24% reduction in CV deaths (largely attributable to fewer sudden deaths)

ATBC, Alpha-Tocopherol Beta-Carotene Prevention Study; CARET, Beta Carotene and Retinol Efficacy Trial; LCPS, Linxian Cancer Prevention Study; NPCT, Nutritional Prevention of Cancer Trial; PHS, Physicians' Health Study; POPADAD, Prevention of Progression of Arterial Disease and Diabetes; PPP, Primary Prevention Project; SCPS, Skin Cancer Prevention Study; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants (Vitamin and Mineral Antioxidant Supplementation Study); VACP, Vitamin A and Cancer Prevention; WHS, Women's Health Study; CHD, coronary heart disease; MI, myocardial infarction; CV, cardiovascular.

Table 2 Summary of large intervention trials (>1000 subjects) investigating the role of antioxidants and CVD in secondary prevention^a

<i>Trial</i>	<i>Characteristics of subjects</i>	<i>Sex</i>	<i>Length of follow-up (years)</i>	<i>Treatment</i>	<i>Effect of antioxidant supplementation</i>
ATBC	1862 smokers with previous MI, Finland	Male	5½	50 mg α -tocopherol or 20 mg β -carotene	No effect on total coronary events (fatal and nonfatal) Increase in deaths from fatal CHD in β -carotene (+75%) and combined β -carotene/vitamin E group (+58%) versus placebo
	1795 heavy smokers with previous angina, Finland				No effect on symptoms or progression of angina or on total coronary events
CHAOS	2002 patients with coronary atherosclerosis, UK	Male and female	1½	300 or 800 IU α -tocopherol	Reduction in nonfatal MI (−77%) but no effect on CVD mortality
GISSI	11 324 patients with recent MI, Italy			300 mg α -tocopherol or 1 g <i>n</i> -3 PUFA	No benefit from vitamin E
HOPE	9541 known CVD or diabetes, Canada	Male and female	4–6	400 IU α -tocopherol or ACE inhibitor	No effect on MI, stroke, or CVD death
HPS	20 536 with known vascular disease or at high risk, UK	Male and female	≥5	20 mg β -carotene, 600 mg α -tocopherol, and 250 mg vitamin C	No effect on fatal or nonfatal MI or stroke
WACS	8171 postmenopausal women with history of CVD or at least 3 cardiac risk factors, USA	Female	9.4	600 IU α -tocopherol or 50 g β -carotene (both every other day) or 500 mg vitamin C (daily)	No effect of vitamin C, tocopherol or β -carotene on total CV events, MI, stroke or deaths

^aSecondary prevention is defined as including patients with known or documented vascular disease.

GISSI, GISSI Prevenzione Trial; HOPE, Heart Outcomes Prevention Evaluation Study; HPS, Heart Protection Study; WACS, Women's Antioxidant Cardiovascular Study; MI, myocardial infarction; PUFA, polyunsaturated fatty acids.

β -Carotene

Many of the randomized controlled trials (RCTs) investigating a protective role for antioxidant nutrients in cancer prevention (Table 3) have focused on β -carotene. A study in Linxian, China, of a rural population with poor nutritional status found that supplementation with a combination of β -carotene, selenium, and vitamin E for 5 years provided a 21% reduction in stomach cancer mortality and a 13% reduction in all cancer deaths. Although interesting, the population studied was likely to have very low intakes of a number of micro-nutrients and this study does not contribute to knowledge about the effects of individual antioxidants or offer any insight into their effects on populations with good nutritional status.

The findings of a number of large double-blind RCTs in well-fed subjects using high-dose β -carotene supplements (either alone or in combination with other agents) have generally been unsupportive of any protective effect, although most have only focused on high-risk groups (e.g., smokers, asbestos workers, and older age groups), although newer studies provide data from the general population or subjects with a health issue not related to cancer risk (e.g., established

CVD or CVD risk factors). In the ATBC Cancer Prevention Trial, in which 29 000 male smokers were randomly assigned to receive β -carotene, α -tocopherol or placebo each day, β -carotene showed no protective effect on the incidence of any type of cancer after approximately 6 years. In fact, concern was raised following the publication of the findings of this trial because those randomized to receive β -carotene had an 18% higher risk of lung cancer (RR, 1.18; 95% CI, 3–36) as well as an 8% higher total mortality than nonrecipients. Subgroup analyses suggested that the adverse effect of β -carotene on lung cancer risk was restricted to heavy smokers and that the risk appeared to be transient, being lost at follow-up 4–6 years after cessation of supplementation.

The CARET was also terminated early because of similar findings; subjects receiving a combination of supplements (30 mg β -carotene and vitamin A daily) experienced a 28% increased risk of lung cancer incidence compared with the placebo group (RR, 1.28; 95% CI, 1.04–1.57). Subgroup analyses also suggested that the effect was found in current, but not former, smokers. In contrast, in the PHS, supplementation of male physicians with 50 mg β -carotene on alternate days had no effect on cancer incidence (men who

Table 3 Summary of large intervention trials (> 1000 subjects) investigating the role of antioxidants and cancer in primary prevention

<i>Trial</i>	<i>Characteristics of subjects</i>	<i>Sex</i>	<i>Length of follow-up (years)</i>	<i>Treatment</i>	<i>Effect of antioxidant supplementation</i>
ATBC	29 133 smokers, Finland	Male	5–8	50 mg α -tocopherol or 20 mg β -carotene	18% increase in lung cancer in β -carotene group (no effect in vitamin E group) 34% reduction in incidence of prostate cancer in vitamin E group No effect of either vitamin on colorectal, pancreatic, or urinary tract cancer, or cancer of the oral cavity/pharynx, esophagus and larynx
CARET	14 254 smokers, 4060 asbestos workers, USA	Male and female	4	30 mg β -carotene and 25 000 IU retinol	Lung cancer increased by 28%
HPS	20 536 at high CVD risk, UK	Male and female	≥ 5	20 mg β -carotene, 600 mg α -tocopherol, and 250 mg vitamin C	No effect on cancer incidence or mortality
LCPS	29 584 poorly nourished, China	Male and female	5	15 mg β -carotene, 30 mg α -tocopherol, and 50 μ g selenium	Cancer deaths declined by 13% Stomach cancer declined by 21%
NSCPT	1621 (73% without skin cancer at baseline), Australia	Male and female	4 $\frac{1}{2}$	30 mg β -carotene with or without sunscreen application	No effect on basal cell or squamous cell carcinoma
PHS	22 071 physicians, United States	Male	12	50 mg β -carotene or aspirin (alternate days)	No effect on incidence of malignant neoplasms or nonmelanoma skin cancer
SELECT	35 533, USA, Canada, and Puerto Rico	Male	5.5	200 μ g selenium or 400 IU vitamin E or both	No effect of either treatment on prostate cancer incidence No effect on lung, colorectal and all other cancers, and cancer deaths
SUVIMAX	13 017, France	Male and female	7.5	Combination of 120 mg ascorbic acid, 30 mg vitamin E, 6 mg of β -carotene, 100 μ g selenium, 20 mg zinc, daily	No effect on overall cancer incidence in whole cohort, but significant reduction (– 31%) of overall cancer incidence in men No effect on overall skin cancer incidence in total sample; but increased incidence in women taking antioxidants, not in men No effect on nonmelanoma skin cancer
	5141 men, France	Male	8.5–9	See above	No effect on overall prostate cancer incidence Significant reduction (– 48%) of prostate cancer in those with baseline PSA levels $< 3 \mu\text{g l}^{-1}$
VACP II	1204 former asbestos workers, Australia	Male and female	5	30 mg β -carotene or 25 000 IU retinol (no placebo group)	No effect of β -carotene on cancer mortality

(Continued)

Table 3 Continued

<i>Trial</i>	<i>Characteristics of subjects</i>	<i>Sex</i>	<i>Length of follow-up (years)</i>	<i>Treatment</i>	<i>Effect of antioxidant supplementation</i>
WHS	39 876, USA	Female	2	50 mg β -carotene (alternate days)	No effect on cancer incidence
			10	600 IU α -tocopherol (alternate days)	No effect on overall cancer incidence and death, no effect on site-specific cancers (breast, lung, colon, rectum, and stomach)
WACS	7627 postmenopausal women with history of CVD or at least 3 cardiac risk factors, USA	Female	9.4	600 IU α -tocopherol or 50 g β -carotene (both every other day) or 500 g vitamin C (daily)	No effect of any antioxidant on total cancer incidence and mortality Higher lung cancer rates in vitamin C group (+84%)

LCPS, Linxian Cancer Prevention Study; NSCPT, Nambour Skin Cancer Prevention Trial; SELECT, Selenium and Vitamin E Cancer Prevention Trial; SUVIMAX, Supplémentation en Vitamines et Minéraux Antioxydants (Vitamin and Mineral Antioxidant Supplementation Study); VACP, Vitamin A and Cancer Prevention; PSA, prostate-specific antigen.

were smokers did not experience any benefit or harm). The HPS also demonstrated no effect on 5-year cancer incidence or mortality from supplementation with 20 mg β -carotene in combination with vitamins E and C in individuals at high risk of CVD, despite increases in blood concentrations of these nutrients (plasma β -carotene concentrations increased 4-fold). They did not, however, find any harmful effects from these vitamins. The WACS in approximately 7600 postmenopausal women having established CVD or CVD risk factors showed no effect of supplementation with β -carotene (in combination with vitamin E or vitamin C or placebo) on total cancer incidence and mortality. Interestingly, the Vitamin and Mineral Antioxidant Supplementation Study (SUVIMAX) found a significant 31% reduction of total cancer incidence in men (RR 0.69, 95% CI, 0.53–0.91) taking a low-dose supplement containing β -carotene along with vitamin C, vitamin E, selenium, and zinc, but found no effect in women (RR 1.04, 95% CI, 0.85–1.29). The authors suggested that the difference in men and women may be explained by differences in nutrient status at baseline, but that lower baseline status alone could not entirely explain the observed differences. In men, supplementation with this mixture of low-dose micronutrients showed no effect on overall prostate cancer incidence, but showed a significant reduction of prostate cancer (HR 0.52, 95% CI, 0.29–0.92) in those with baseline prostate-specific antigen (PSA) levels $<3 \mu\text{g l}^{-1}$ (high PSA levels can be an indicator for higher risk of prostate cancer or existing prostate cancer).

A number of trials have attempted to investigate the effect of β -carotene supplementation on nonmelanoma skin cancer, the most common forms of which are basal cell and squamous cell carcinomas (these types of cells are both found in the top layer of the skin). However, none have shown any significant effect on skin cancer prevention. For example, the PHS found no effect after 12 years of β -carotene supplementation on the development of a first nonmelanoma skin cancer. The Nambour Skin Cancer Prevention Trial (NSCPT) of 1621 men and women followed for nearly 5 years (most of

whom had no history of skin cancer at baseline) showed that those supplemented with 30 mg β -carotene did not experience any reduction in risk of basal cell or squamous cell carcinoma or the occurrence of solar keratoses (precancerous skin growths that are a strong determinant of squamous cell carcinoma). The SUVIMAX found no effect of low-dose β -carotene along with vitamin C, vitamin E, selenium, and zinc on risk of skin cancer in the total sample, but found an increased frequency of skin cancer in the female group (1.3% vs. 0.7%, $p = .02$). A 5-year trial of 1805 men and women with recent nonmelanoma skin cancer (the Skin Cancer Prevention Study) also found that supplementation with 50 mg of β -carotene gave no protection against either type of skin cancer, although this may have been because these cancers have a long latency period of approximately 12 years (Table 4).

Together, these trials suggest that β -carotene supplements offer no protection against cancer prevention in healthy individuals and, among smokers, may actually increase the risk of lung cancer. Investigators have sought to explain these findings by proposing that components of cigarette smoke may promote oxidation of β -carotene in the lungs, causing it to exert a pro-oxidant (rather than antioxidant) effect and act as a tumor promoter.

Vitamin C

Only one RCT has investigated the effect of vitamin C alone in primary prevention of cancer. In the WACS trial 500 g of vitamin C per day for an average of 9.5 years had no effect on total cancer incidence and mortality in postmenopausal women with history of CVD or cardiac risk factors, but was associated with higher lung cancer rates in vitamin C group (RR 1.84, 95% CI 1.14–2.97). Data from a small number of trials of vitamin C in combination with other nutrients have not provided any support for a role for high-dose vitamin C supplementation in cancer prevention (Table 3). The Linxian trial found no significant effect of supplementing Chinese

Table 4 Summary of large intervention trials (> 1000 subjects) investigating the role of antioxidants and cancer in secondary prevention^a

<i>Trial</i>	<i>Characteristics of subjects</i>	<i>Sex</i>	<i>Length of follow-up (years)</i>	<i>Treatment</i>	<i>Effect of antioxidant supplementation</i>
NPCT	1312 with history of basal or squamous cell carcinoma, United States	Male and female	4½	200 µg selenium	No effect on incidence of skin cancer overall Increased risk of squamous cell carcinoma (+25%) and of total nonmelanoma skin cancer (+17%) Reduced cancer mortality (−50%), cancer incidence (−37%), prostate cancer (−63%), colorectal cancer (−58%), and lung cancer (−46%)
SCPS	1805 with recent nonmelanoma skin cancer, USA	Male and female	5	50 mg β-carotene	No effect on occurrence of new nonmelanoma skin cancer

^aSecondary prevention defined as subjects with documented cancer including nonmelanoma skin cancer (although some of the primary prevention trials did not exclude those with nonmelanoma skin cancer at baseline).

NPCT, Nutritional Prevention of Cancer Trial; SCPS: Skin Cancer Prevention Study.

men and women with 120 mg vitamin C and 30 µg molybdenum daily for 5 years on the risk of cancers of the esophagus or stomach. The polyp prevention study, a trial of 864 patients with previous adenoma, found no effect of either β-carotene or a combination of vitamins E and C (1000 mg) on the incidence of subsequent colorectal adenomas. The HPS also found no beneficial effects of supplementation with these three vitamins on cancer mortality. However, trials have generally been carried out on those with diets containing sufficient amounts of vitamin C and there is a need for further studies in people with low intakes. As mentioned above, in the SUVIMAX study a low-dose supplement containing vitamin C along with β-carotene, vitamin E, selenium, and zinc found a significant reduction in total cancer incidence in men (RR 0.69, 95% CI, 0.53–0.91) but not in women.

Vitamin E

The ATBC trial showed no significant effect of α-tocopherol supplementation (50 mg d^{−1}) on risk of lung, pancreatic, colorectal, or urinary tract cancers among heavy smokers (Table 3). However, in a post hoc subgroup analysis a 34% reduction in the risk of prostate cancer was seen in men who received this supplement. Although interesting, prostate cancer was not a primary endpoint of this study. In the Selenium and Vitamin E Cancer Prevention Trial (SELECT) study, where prostate cancer was the primary outcome, no effect of vitamin E supplementation over a period of 5.5 years in around 35 000 men was found. In this study, no effect on any other cancer site was observed, including lung, colorectal and all other cancer sites, and there was no effect on cancer death rates. The WHS and WACS trials, both carried out in women, also found no effect of vitamin E supplementation on overall cancer incidence or deaths. The HPS found no effect of vitamin E in combination with vitamin C and β-carotene on cancer incidence or mortality, and the SUVIMAX study found a reduction of total cancer incidence in men but not women with low dose vitamin E along with vitamin C, β-carotene,

selenium, and zinc. Two smaller, short-term intervention studies found no effect of α-tocopherol supplementation on mammary dysplasia or benign breast disease. Several trials have also been unable to demonstrate a protective effect of vitamin E supplementation on the risk or recurrence of colorectal adenomatous polyps.

Selenium

A few trials have suggested that selenium supplementation may have a protective effect on liver cancer in high-risk groups living in low-selenium areas. For example, the provision of selenium-fortified salt to a town in Qidong, China, with high rates of primary liver cancer, reduced the incidence of this cancer by 35% compared with towns that did not receive this intervention. Some trials have also demonstrated the incidence of liver cancer to be significantly reduced in subjects with hepatitis B and among members of families with a history of liver cancer receiving a daily supplement of 200 µg of selenium for 4 and 2 years, respectively. The Nutritional Prevention of Cancer Trial (NPCT) in the US also supported a possible protective role of selenium in 1312 patients (mostly men) with a previous history of skin cancer who were supplemented with 200 µg selenium per day for 4½ years (Table 4). Those receiving selenium demonstrated significant reductions in the risk of total cancer incidence (37%) and mortality (50%) compared to those receiving placebo. The selenium-treated group also had substantial reductions in the incidence of lung, colorectal, and prostate cancers of 46, 58, and 63%, respectively. However, recurrent squamous cell carcinoma was increased by 25% and total nonmelanoma skin cancer by 17%. Further analysis showed the protective effect on prostate cancer to be confined to those with lower baseline PSA and plasma selenium levels. However, the SELECT study, one of the largest human cancer prevention trials ever undertaken in 45 533 healthy males from the US, Puerto Rico, and Canada, found no effect of 200 µg of selenium on prostate cancer, lung cancer, colorectal cancer and all other

cancers, or on cancer deaths. Comparison of this study to other clinical trials involving selenium and to animal studies suggests that the source of the selenium supplement, L-selenomethionine, and the relatively high initial levels of selenium in the enrolled men may have contributed to this outcome.

Other Diseases Associated with Oxidative Damage

Type 2 Diabetes

Type 2 diabetes is associated with elevated oxidative stress (especially lipid peroxidation) and declines in antioxidant defense. This is thought to be due in part to elevated blood glucose levels (hyperglycemia), but severe oxidative stress may also precede and accelerate the development of type 2 diabetes and then of diabetic complications (CVD and microvascular complications such as retinopathy, neuropathy, and nephropathy).

Some of the trials looking at antioxidants and CVD and cancer have also reported on the association of the examined antioxidants and the risk of diabetes. In the WACS women with a history of CVD or at least 3 CVD risk factors but free of diabetes at baseline ($n = 6574$) received 500 mg vitamin C daily, or 600 IU α -tocopherol every other day, or 50 mg β -carotene every other day. During a median follow-up of 9.2 years no significant effect of any of the treatments could be found: RR in vitamin C group was 0.89 (95% CI, 0.78–1.02), RR in vitamin E group 1.13 (95% CI, 0.99–1.29) and RR in β -carotene group 0.97 (95% CI, 0.85–1.11). Analysis of data from the ATBC study in 27 379 smokers free of diabetes at baseline showed that neither supplementation with α -tocopherol (50 mg d^{-1}) nor with β -carotene (20 mg d^{-1}) for 5–8 years had an effect on diabetes risk over a median follow-up of 12.5 years. Data from the WHS from almost 39 000 healthy women free of diabetes, cancer, and CVD at baseline and receiving either 600 IU of α -tocopherol or placebo on alternate days showed no effect of study treatment on diabetes risk over a median follow-up period of 10 years. In a smaller study, the NPCT, in 1202 participants with a history of non-melanoma skin cancer but no baseline diabetes treatment with 200 $\mu g d^{-1}$ selenium was associated with an increased risk of developing diabetes (HR 1.55, 95% CI, 1.03–2.33) after an average follow-up of 7.7 years.

Small-scale human trials have shown administration of high doses of vitamin E to reduce oxidative stress and improve some CVD risk factors, such as blood glycated hemoglobin, insulin, and triglyceride levels, in people with diabetes. Such trials have also indicated benefit from vitamin E in improving endothelial function, retinal blood flow, and renal dysfunction. However, the findings of large clinical trials investigating the role of individual or a combination of antioxidant nutrients in reducing the risk of CVD and microvascular complications in people with diabetes have generally been disappointing. For example, the HOPE trial investigated the effects of vitamin E and the drug Ramipril in patients at high risk for CVD events and included a large number of middle-aged and elderly people with diabetes (more than 3600). An average of $4\frac{1}{2}$ years of supplementation with 400 IU of vitamin E per day was found to exert no beneficial or harmful effect on

CVD outcomes or on nephropathy. The Primary Prevention Project (PPP) trial found no effect of vitamin E (300 mg d^{-1}) supplementation for 3 or 4 years in diabetic subjects, and the HPS, which included a number of people with diabetes, also reported no benefit of a combination of antioxidant vitamins on mortality or incidence of vascular disease. In the ATBC study, no effect of supplementation with α -tocopherol (50 mg d^{-1}) nor β -carotene (20 mg d^{-1}) was found during the intervention period (median 6.1 years) on the risk of macrovascular complication or total mortality in 1700 men with type 2 diabetes at baseline. No essential changes were found in these effects when the follow-up was extended up to 19 years. In another study, the Prevention of Progression of Arterial Disease and Diabetes (POPADAD) study, no effect of an antioxidant capsule (containing 200 mg α -tocopherol, 100 mg vitamin C, 25 mg pyridoxine hydrochloride, 10 mg zinc sulphate, 10 mg nicotinamide, 9.4 mg lecithin, and 0.8 mg sodium selenite) was found on CVD risk in 1276 adults with type 1 or type 2 diabetes and an ankle brachial pressure index of 0.99 or less but no symptomatic CVD at baseline over a median follow-period of 6.7 years. However, a meta-analysis of two studies reporting by diabetes subtype, the Haptoglobin (Hp) 2-2 genotype which is characterised by a markedly increased risk of CVD compared to other types, found that patients with Hp 2-2 diabetes taking vitamin E supplements reduced the risk of cardiovascular events (combined odds ratio (OR) 0.58, 95% CI 0.40–0.86).

Asthma and Chronic Obstructive Pulmonary Disease (COPD)

Asthma is a chronic inflammatory disease resulting in reversible airways bronchoconstriction which affects a large number of children and adults. Epidemiological studies suggest that intake and status of antioxidant nutrients are inversely associated with the risk of asthma and wheezing. A number of studies have also demonstrated a beneficial effect of fruit and vegetable intake on lung function. For example, regular consumption of fresh fruit rich in vitamin C (citrus fruits and kiwi) has been found to have a beneficial effect on reducing wheezing and coughs in children.

In COPD patients, many of the pathophysiological changes associated with the disease are produced through the generation of oxygen free radicals by activated inflammatory cells. Antioxidant nutrients have therefore been suggested to play a role in the prevention and treatment of these conditions. Common examples of COPD are emphysema and chronic bronchitis. COPD mainly affects smokers or people with a smoking history.

Vitamin C is the major antioxidant present in extracellular fluid lining of the lung, and intake in the general population has been inversely correlated with the incidence of asthma, bronchitis, and wheezing and with pulmonary problems. Although some trials have shown high-dose supplementation (1–2 g d^{-1}) to improve symptoms of asthma in adults and protect against airway responsiveness to viral infections, allergens, and irritants, this effect has been attributed to the antihistaminic action of the vitamin rather than to any antioxidant effect. The results of these trials have also been inconsistent, and a Cochrane review of nine RCTs concluded that there is insufficient evidence to recommend a specific role

for vitamin C in the treatment of asthma. However, a need for further trials to address the question of the effectiveness of vitamin C in asthmatic children was highlighted.

Other dietary antioxidants have been positively associated with lung function in cohort studies but the findings of clinical trials have been mixed. In a study of 158 children with moderate to severe asthma, supplementation with vitamin E (50 mg d^{-1}) and vitamin C (250 mg d^{-1}) led to some improvement in lung function following ozone exposure. A study in 72 adults with asthma receiving 500 mg vitamin E daily for 6 weeks did not show any effect on bronchial reactivity to methacholine, lung function, morning peak flow, or any other outcome measures. A Cochrane review looking at selenium and asthma only identified one study meeting their pre-defined criteria. The study in 24 patients suffering from chronic asthma found that $100 \mu \text{ d}^{-1}$ of selenium for 14 days was associated with significant improvements in subjective asthma symptoms but this improvement could not be validated by significant changes in the separate clinical parameters of lung function and airway hyperresponsiveness. A subsequent RCT in 197 participants found no effect of $100 \mu \text{g}$ daily for 24 weeks on asthma-related quality of life, lung function, asthma symptom scores, or any other outcome measures. The ATBC trial found no benefit from supplementation with α -tocopherol (50 mg d^{-1}) and β -carotene (20 mg d^{-1}) on symptoms of COPD, despite the fact that those with high dietary intakes and blood levels of these vitamins at baseline had a lower prevalence of chronic bronchitis and dyspnoea.

Macular Degeneration and Cataracts

The eye is at particular risk of oxidative damage due to high oxygen concentrations, large amounts of oxidizable fatty acids in the retina, and exposure to ultraviolet rays. In Western countries, age-related macular degeneration (AMD) is the leading cause of blindness among older people. Cataracts are also widespread among the elderly and occur when the lens is unable to function properly due to the formation of opacities within the lens. These develop when proteins in the eye are damaged by photooxidation; these damaged proteins build up, clump, and precipitate. It has been proposed that antioxidants may prevent cellular damage in the eye by reacting with free radicals produced during the process of light absorption.

The results of intervention trials in this area have also been mixed. The age-related eye disease study in the US investigating the effects of combined antioxidant vitamins C (500 mg), E (400 IU), and β -carotene (15 mg) with and without 80 mg zinc daily for 6 years showed some protective effect (a reduction in risk of approximately 25%) on the progression of moderately advanced AMD but no benefit on the incidence or progression or early AMD or cataracts. Further analysis of the data showed that an individual's response could be related to a specific genotype. The lutein antioxidant supplementation trial, a 12-month study of 90 patients with AMD, found significant improvements in visual function with 10 mg d^{-1} lutein (one of the major carotenoids found in the pigment of a normal retina) alone or in combination with a number of other antioxidant nutrients. The Roche European Cataract Trial, providing a combined daily supplement of

β -carotene, vitamin C, and vitamin E among adults with early signs of age-related cataract, showed a small deceleration in the progression of cataract after 3 years.

However, the Linxian trial found no influence of vitamin supplementation on risk of cataract; the ATBC trial found no reduction in the prevalence of cataracts with vitamin E, β -carotene, or both among male smokers; and the PHS of more than 22 000 men showed no benefit from 12 years of supplementation with β -carotene (50 mg on alternate days) on age-related maculopathy or cataract incidence. In fact, current smokers at the beginning of this trial who received the supplement experienced an increased risk of cataract (by approximately 25%) compared to the placebo group. The Vitamin E, Cataract, and Age-Related Maculopathy Trial also reported no effect of supplementation with vitamin E for 4 years (500 IU d^{-1}) on the incidence or progression of cataracts or AMD.

Immune Function – Common Cold

Antioxidants, vitamins, and minerals have also been linked with immune function. Deficiency of these nutrients, including vitamin C, vitamin E, vitamin A, selenium, and zinc, has been associated with compromised immune function. A Cochrane systematic review looked at the effects of the use of vitamin C supplements on the incidence of colds in the normal population. Based on 30 trials including almost 12 000 study participants on the use of vitamin C supplements (doses from 200 mg or higher), the reviewers concluded that supplements did not reduce the incidence of colds in the normal population. Vitamin C supplementation taken for prophylaxis reduced the duration of common cold symptoms by 8% in adults and by 13.6% in children, but no effect was found when vitamin C was taken therapeutically (i.e., after the onset of the common cold). Despite the lack of effectiveness in reducing the risk of a common cold infection in the normal population, vitamin C supplementation could reduce the risk of a common cold by half in study participants exposed to short periods of extreme physical or cold stress, or both (including marathon runners and skiers). In the ATBC study long-term vitamin E and β -carotene supplementation had no overall effect on common cold incidence. Among subjects aged 65 years or older, the incidence of colds was slightly lower in the vitamin E group compared with the control (unsupplemented) group ($\text{RR} = 0.95$; 95% $\text{CI} = 0.90\text{--}1.00$); this reduction was greatest among older city dwellers who smoked fewer than 15 cigarettes per day ($\text{RR} = 0.72$; 95% $\text{CI} = 0.62\text{--}0.83$). A Cochrane review on the use of zinc for common cold treatment based on seven studies including around 800 study participants concluded that, overall, treatment with zinc lozenges did not reduce the duration of cold symptoms.

Possible Explanations for the Disagreement between the Findings of Observational Studies and Clinical Trials

Various explanations have been given for the different findings of observational studies and intervention trials. Clearly, non-randomized studies are unable to exclude the possibility that

antioxidants are simply acting as a surrogate measure of a healthy diet or lifestyle and that the protective effect of certain dietary patterns, which has been presumed to be associated with dietary antioxidants, may in fact be due to other compounds in plant foods, substitution of these foods for others, or a reflection of other health behaviors common to people who have a high fruit and vegetable intake. However, although intervention studies provide a more rigorous source of evidence than observational studies, they are not without weaknesses from a nutritional perspective. Many of the trials to date have been criticised for their use of high risk populations and high doses of single supplements, insufficient duration of treatment, and follow-up and lack of consideration of the impact of genetic variability. More recent trials that have attempted to answer some of these criticisms. For example, SUVIMAX was designed to assess the efficacy of supplementation among more than 12 000 healthy men and women over a 7.5-year period with a cocktail of antioxidant vitamins and minerals at doses achievable by diet (approximately one to three times the daily recommended dietary allowances) on premature death from CVD and cancer. This low-dose antioxidant supplementation had no effect on vascular disease incidence but lowered total cancer incidence in men, but not in women. This suggests that the contradictory results of observational and interventional studies may be due to differences in the effects of antioxidants in relation to supplement doses (nutritional vs. pharmacological), baseline antioxidant status (different between gender or nutritional status), and health status of subjects (healthy vs. cancer high-risk subjects). This study concluded that antioxidant supplementation may have a beneficial effect on cancer incidence only in healthy subjects who are not exposed to cancer risk, and with a particularly low baseline status. In contrast, high doses of antioxidant supplements may be deleterious in high-risk subjects without any clinical symptoms in whom the initial phase of cancer development has already started. Studies are also now attempting to look at genetic variation by studying the effects of antioxidants in individuals with specific genotypes. For example, in a meta-analysis of two trials (Hope and ICARE), vitamin E supplementation was associated with reduced risk of CVD in individuals with diabetes mellitus and the hap-toglobin 2-2 genotype, both of which are associated with increased risk of the disease.

Conclusion

Although there is a substantial body of evidence that diets rich in plant foods (particularly fruit and vegetables) convey health benefits, as do high plasma levels of several antioxidant nutrients found in these foods, a causal link between lack of antioxidants and disease occurrence or between antioxidant administration and disease prevention remains to be established. There is a lack of understanding of the mechanisms underpinning the apparent protective effect of plant foods and, as yet, no clear picture of which components are effective and hence no way of predicting whether all or just some plant foods are important in this respect.

If future trials do demonstrate a reduction in chronic disease risk with antioxidant supplementation, this cannot be

definitively attributed to the antioxidant effect of these nutrients because other biological functions may also play a role. For example, in addition to retarding LDL oxidation, vitamin E may help to protect against CVD *via* its action on platelet aggregation and adhesion or by inhibition of the proliferation of smooth muscle cells. Vitamin E and other nutrients that are classified as antioxidants have also been shown to modulate pathways of cell signalling and gene expression. Furthermore, although vitamin C, vitamin E, and selenium have been shown to decrease the concentration of some of the biomarkers associated with oxidative stress, the relationship between many of these biomarkers and chronic disease remains to be elucidated.

The intervention studies highlight the lack of information on the safety of sustained intakes of moderate to high doses of micronutrient supplements and long-term harm cannot be ruled out, particularly in smokers. Further evidence is required regarding the efficacy, safety, and appropriate dosage of antioxidants in relation to chronic disease. Currently, the most prudent public health advice continues to be to consume a variety of plant foods.

See also: Ascorbic Acid: Deficiency States. Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Cancer: Epidemiology and Associations Between Diet and Cancer. Carotenoids: Chemistry, Sources and Physiology; Health Effects. Coronary Heart Disease: Lipid Theory. Diabetes Mellitus: Classification and Chemical Pathology; Dietary Management; Etiology and Epidemiology. Lipoproteins. Lung Diseases. Selenium. Stroke Nutritional Management. Vitamin E: Metabolism and Requirements; Physiology and Health Effects

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APPETITE

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Physiological and Neurobiological Aspects
Psychobiological and Behavioral Aspects

Physiological and Neurobiological Aspects

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Glossary

Episodic factors Peripheral signals generated by the recent consumption of food and which can influence intake in the short term, for example, ghrelin and CCK.

Hedonic control Reward based pathways that can override homeostatic regulation. They stimulate energy intake by increasing the desire to consume highly palatable foods.

Homeostatic control The control of energy balance achieved by increasing the motivation to eat following depletion of the body's energy stores.

Hunger The motivation to seek and consume food. It is often the initiator of a feeding event.

Hyperphagia Increased appetite for and excessive intake of food.

Orexigenic A substance that increases or stimulates the appetite.

Satiation Begins within a meal and generates negative feedback signals designed to terminate food intake. In doing so satiation determines meal size.

Satiety The end point of the process of satiation. It begins at the end of one meal and continues until the start of the next, thus determining the intermeal interval. It is characterized by a lack of drive to consume as well as feelings of fullness.

Tonic factors Peripheral signals generated by the metabolism and storage of energy which influence intake over the long term, for example, leptin and insulin.

Appetite Expression

Appetite can be defined as the tendency to seek and consume food. Typically, this consummatory behavior is characterized by discrete eating events (meals and snacks) which are under the combined control of a wealth of factors including peripheral physiological signals, central neural processes, and psychological experiences.

Peripherally generated factors typically arise as a consequence of food ingestion. Both episodic and tonic factors contribute to the control of feeding behavior but they differ in terms of their duration of effect. Episodic factors are generated by recent consumption and influence intake in the short term. Tonic factors provide influence over the long term and are generated by metabolism and the storage of energy. In turn, both classes of peripheral factors provide input to hypothalamic central nervous system (CNS) neurons which are key to the long-term control of body weight.

The regulatory components so far discussed (peripheral factors and hypothalamic CNS neurons) underpin the homeostatic expression of appetite. In combination with the psychological experiences of hunger and satiety, their collective action maintains the body's energy stores. Eating is driven by need (hunger) as the energy stores are depleted. Once this need is met negative feedback signals are generated to bring the period of eating to an end (satiation). These signals ultimately lead to a state of satiety in which the hunger drive and eating behavior are inhibited. The satiety cascade (*see* Psychological Experiences: Hunger, Satiety and the Satiety Cascade) depicts the events which stimulate eating along with the processes triggered by the ingestion of food which terminate intake.

When food is freely available this homeostatic appetite system defends well against energy deficit. However, as demonstrated by the current obesity pandemic, defense against energy excess is less potent resulting in an asymmetrical

regulatory system more sensitive to under- than over-consumption. In turn hedonic neuronal systems in the brain can stimulate overconsumption by signaling the sensory pleasure derived from food. Pairing the regulation of food intake with pleasure and reward in this way can lead to the less potent homeostatic energy surplus defense mechanisms being over-ridden, promoting hyperphagia and obesity.

Interrelated Levels of the System

Before considering the individual systems (psychological events, peripheral physiological signals, and central neural processes) responsible for the expression of appetite in detail, it will be beneficial to indicate how they interact during

the course of a meal. Understanding is best achieved by conceptualizing the appetite-regulating system in terms of three distinct but coordinated domains (Figure 1):

1. Psychological events (e.g., sensations of hunger, satiety, and hedonics) accompany behavioral actions (food intake; meals and snacking behavior) and their associated measurable consequences (energy intake and food choice).
2. Peripheral physiological signals arise as a consequence of nutrient absorption and subsequent utilization or storage.
3. Neural processing concerns how neurochemical (classic neurotransmitters, neuropeptides, and hormones) and metabolic signals of the body's energy status are detected in the CNS.

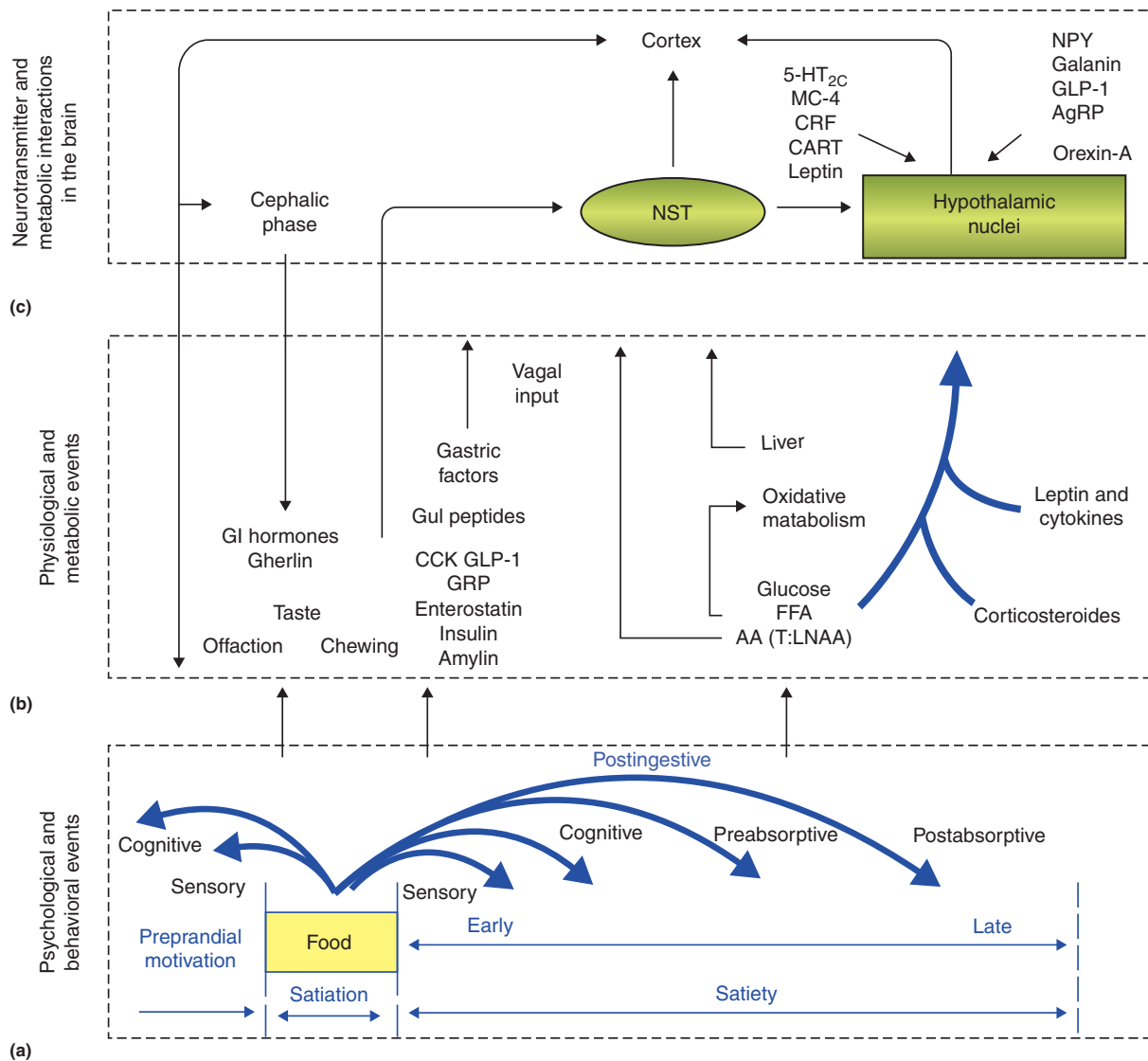


Figure 1 The psychobiological expression of appetite and the three levels of operation: (a) psychobiological and behavioral events; (b) physiological and metabolic operations; and (c) neurochemical and metabolic interactions within the CNS. Abbreviations: 5-HT, serotonin; AA, amino acids; AgRP, agouti-related peptide; CART, cocaine and amphetamine-regulated transcript; CCK, cholecystokinin; CRF, corticotrophin releasing factor; FFA, free fatty acids; GI, gastrointestinal; GLP-1, glucagon-like peptide-1; GRP, gastric releasing peptide; MC, melanocortin; NPY, neuropeptide Y; NST, nucleus tractus solitarius; T:LNAA, tryptophan large neutral amino acid ratio.

The expression of appetite reflects the synchronous operation of events and processes in all three domains.

Psychological Experiences: Hunger, Satiety, and the Satiety Cascade

To understand the nature of appetite we must consider its flux before (preprandial), during (prandial), and after (postprandial) a meal. The self-reported psychological experiences of hunger and satiety, and the transition between the two (pre to post consumption or across the full day) underpin this flux. Hunger is the motivation to seek and consume food and it is often the initiator of a feeding episode. Hunger can be triggered by changes in levels of blood glucose or the increase in certain stimulatory factors, such as ghrelin (*see* Episodic Signals: Hunger). Processes that feedback to bring the resulting feeding episode to an end are termed satiation. Satiation processes ultimately lead to the state of satiety in which the hunger drive and eating behavior are inhibited. Although satiation determines meal size, satiety determines the length of the postmeal interval (**Figure 2**).

Preconsumption physiological signals are generated by the sight and smell of the food preparing the body for ingestion.

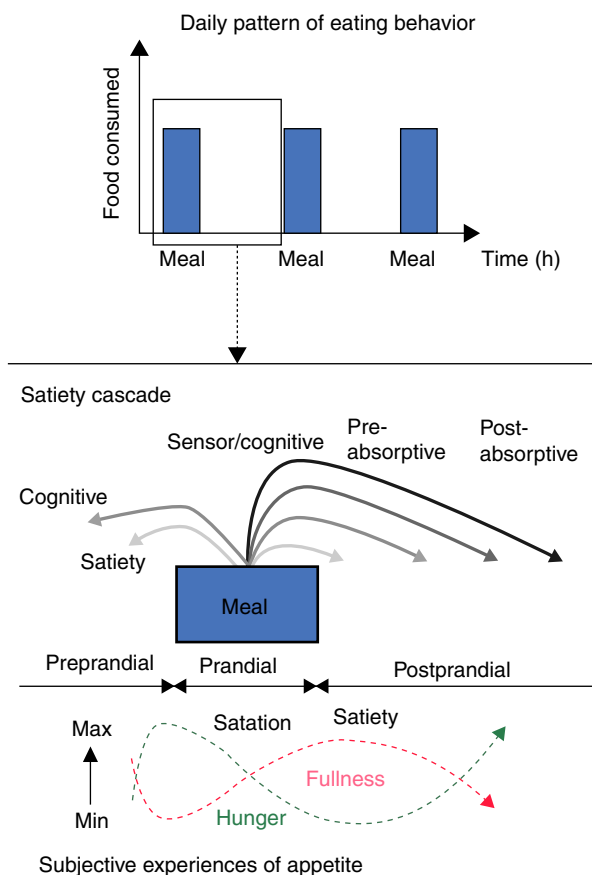


Figure 2 The satiety cascade. The signals generated prior to (preprandial), during (prandial) and after (postprandial) the consumption of a meal critical to short-term (episodic) meal-by-meal appetite regulation throughout the day.

Such afferent sensory information, carried to the brainstem *via* cranial nerves, stimulates hunger before eating and during consumption (prandial phase). During the prandial phase the CNS receives postingestive sensory signals from the gut which reflect the amount and the nutritional content of the food eaten. Mechanoreceptors in the gut signal the distension of the gut lining caused by the presence of food and as such aid in the estimation of the amount of food consumed. Gut chemoreceptors detect the presence of various nutrients, thus providing information on the nutritional composition of the food consumed. Prandial and postprandial signals are generated by the detection of nutrients absorbed from the gastrointestinal tract into the peripheral circulation (postabsorptive satiety signals). Circulating nutrients are either metabolized in the periphery (e.g., liver) and activate CNS receptors (e.g., in the brainstem) or they enter the CNS directly and act as postabsorptive satiety signals.

Although acceptance or rejection of foods can depend on the psychological states, for example, hunger and satiety discussed above, it is also important to recognize the impact of learned experiences on appetite. Learning has been shown to be a fundamental part of the processes controlling food intake and choice with ingestive behavior being modified through experience. Early research relating to learned experiences of appetite focused on conditioned taste aversion (the association of a tastant with experiences of illness) in animals. However, cognitive, social, cultural, ecological, and environmental factors are now recognized as contributors to mechanisms of learning relevant to appetite control in humans.

Central Neural Processes: Structure of the Appetite System

The control of energy balance depends critically on the CNS. The brain integrates multiple biological signals in order to determine the energy requirement of the body and to modify the experience of hunger and initiate the relevant behavioral actions in response to this. The CNS regions that control energy homeostasis are accessible to numerous circulating hormones and other factors including information generated by the sensory experience of eating and from the periphery indicating the ingestion, absorption, and metabolism of energy. Information reaches the CNS *via* three main routes:

1. The central control of appetite is guided by the peripheral signals (discussed in *Peripheral Physiological Signals*). These include signals derived from receptors in the gut (mechanoreceptors and chemoreceptors) and from metabolic changes in the liver. They are sent *via* afferent vagal signals to the Nucleus of the Solitary Tract/Area Postrema (NTS/AP) complex in the brainstem and from there upwards to the hypothalamus and other forebrain regions.
2. Regulatory signals are also derived from receptors within the CNS, particularly the brainstem. These detect circulating levels of nutrients, metabolites, and other regulatory factors.
3. Specific substances, for example, glucose and neurotransmitter precursors, have the ability to cross the blood-brain barrier and enter the brain. Here they alter neurochemical activity in key appetite-regulating sites.

Within the CNS itself are specific neuronal populations that recognize these signals and act in a network to integrate the multiple inputs, and help determine energy intake and expenditure. Primary locations involved in the regulation of food intake in mammals include the hypothalamus, and the amygdala and nucleus accumbens (Nac) of the cortico-limbic system. Structures in the brainstem also play an important role.

The NTS/AP are adjacent areas in the medulla of the brainstem which are key sites for integration of various afferent signals. Vagally transmitted gustatory signals (including gastric distension and portal-vein glucose levels) are relayed from the periphery. There is evidence to indicate that the levels of circulating nutrients can also be detected by receptors located in these brainstem areas. Finally, afferent sensory information from the mouth (carried by the cranial nerves), including taste, also converges on this region.

Hypothalamic nuclei play key roles in the control of hunger and satiety. For example, injection of various substances into the paraventricular nucleus (PVN) influences intake (either increases or decreases it), whereas lesions of this region result in hyperphagia (increased intake), reduced energy expenditure, and obesity. Other key hypothalamic sites include the Arcuate Nucleus (ARC), Dorsomedial Hypothalamic Nucleus (DMH), Lateral Hypothalamic Area (LHA), and the Ventromedial Hypothalamic Nucleus (VMH). The ARC is readily accessible to peripherally generated signals (both episodic and tonic) indicating nutritional status. It contains functionally discrete populations of neurons including the orexigenic (stimulatory) neuropeptide Y (NPY) and agouti-related peptide (NPY-AgRP) containing neurons and the anorexigenic (inhibitory) pro-opiomelanocortin and cocaine and amphetamine related transcript (POMC-CART) containing neurons. The ARC also has extensive reciprocal connections with the other hypothalamic appetite-regulating regions. In addition they receive afferent information *via* the NTS/AP.

Other key limbic sites identified as playing critical roles in appetite regulation include the Nac and amygdala of the forebrain. These sites contain an extensive neural system that processes appetitive and rewarding aspects of food intake; palatability and pleasure are arguably the most powerful motivators of food intake. The Nac contains both dopaminergic and opiodergic neuronal pathways and thus acts as an interface between motivation and actual feeding behavior. Reciprocal connections have also been identified between the Nac and the LHA. These connections offer a means of interaction between homeostatic and nonhomeostatic regulatory pathways. The amygdala is also an important component of reward circuitry, with several subnuclei of the amygdala influencing reward-related food intake. As food reward is a key contributor to learned experiences of appetite, a central role for the basolateral amygdala in the acquisition of conditioned taste aversions, a robust mechanism *via* which learning can impact on appetite in animals, is not surprising.

Peripheral Physiological Signals

Episodic Signals: Satiety

Episodic signals are predominantly generated by the gastrointestinal tract in response to the physical or chemical

presence of food. As a consequence their levels tend to rise and fall in coordination with periods of eating. Humans are periodic feeders and usually meals are separated by periods of 3–5 h during which little food is consumed. This periodicity of meal eating is compatible with the time taken by the gastrointestinal tract to process a meal. On this basis it seems obvious that the stomach must play some role in meal termination (satiety). In fact, stomach distension is considered an important satiety signal with experimental evidence indicating that gastric distension can arrest eating behavior, although this effect is typically acute in nature. However, gastric distension alone does not appear to be capable of signaling satiety and as such cannot be the only factor contributing to the control of meal size.

Over the last four decades rapid advances have been made in the identification and function of numerous peripheral hormones responsible for signaling satiety. Those that have been recognized as having a significant role include Cholecystokinin (CCK), Glucagon-Like Peptide-1 (GLP-1), Oxyntomodulin and Peptide YY (PYY). Considerable research has confirmed the status of CCK as a hormone mediating meal termination (satiety) and possibly early phase satiety. Endogenous CCK is released into the blood from the I-cells in the proximal intestinal tract following the detection of fat and protein in the gut. CCK has two known receptors, CCK-1 and CCK-2. CCK-1 is primarily responsible for the behavioral correlates of appetite regulation. The distribution of CCK-1 receptors indicates a dual pathway for CCK to communicate with the brain. The primary route of communication between these regions is believed to be *via* the vagal nerve and the brainstem with signals being relayed from here to the hypothalamus; however, CCK-1 receptors are also found in the hypothalamus itself suggesting that CCK can also directly communicate with this region without vagal mediation. Direct infusions of CCK dose-dependently reduce food intake in mice, rats, and monkeys. Infusions of the octapeptide CCK-8 in humans reduce both meal intake and meal length, indicating an involvement in satiety. Peripheral CCK-8 administration has also been shown to increase the release of serotonin in the hypothalamus. This neurotransmitter has been implicated in the integration of episodic satiety signals (*see* Integration of Episodic and Tonic Signals Within the CNS).

Glucagon-Like Peptide (GLP)-1 is synthesized in L cells (cells that line the GI tract containing regulatory peptide hormones) located in the distal small intestine and released predominantly in response to carbohydrate but also responsive to fat digestion. Receptors for GLP-1 are found throughout the CNS and peripheral tissues. Central anatomical regions of the NTS (brainstem), AP (brainstem), and ARC (hypothalamus) involved in the regulation of appetite contain GLP-1 receptors. Although there is evidence to suggest that GLP-1 can cross the blood–brain barrier to act directly on receptors located in the brain, similar to CCK the primary route of communication is believed to be *via* the vagal nerve. The principle role of GLP-1 in appetite regulation is to release insulin and inhibit glucagon; however, it also delays gastric emptying, inhibits food intake, and aids in the metabolism and absorption of specific macronutrients. A series of infusion studies employing GLP-1 or exendin-4 (a naturally occurring

GLP-1 analog), have shown that GLP-1 significantly decreases *ad libitum* food intake through reductions in hunger in lean and obese humans. Like GLP-1 Oxyntomodulin is also released from intestinal L cells after meals. Acute administration of the peptide to humans delays gastric emptying, reduces gastric and pancreatic hormone release, and ultimately decreases food intake. These anorectic effects of oxyntomodulin are mediated largely *via* the GLP-1 receptor.

Peptide YY (PYY)₃₋₃₆ is released from distal intestinal endocrine cells following detection of fatty acids, fiber, and bile acid in the gut. The release of PYY₃₋₃₆ causes a decrease in gastric emptying and consequently a reduction in food intake. Moreover, when coinfused with GLP-1 an additive effect on energy intake is observed. Differences between lean and obese individuals have been reported in terms of natural circulating PYY₃₋₃₆ concentrations. There appears to be a ubiquitous suppression of PYY₃₋₃₆ levels in obesity compared to their lean counterparts. The peptide also does not seem to have the same potency for restricting food intake in the obese. PYY₃₋₃₆ selectively binds to Neuropeptide Y₂ receptors which are heavily involved in restricting food intake. The distribution of Y₂ receptors is widespread throughout the CNS. Such a widespread distribution suggests that, as with other anorexic peripheral hormones, PYY₃₋₃₆ can act both directly and indirectly (*via* the brainstem) on the hypothalamus to control intermeal satiety dependent on the caloric load of the previous meal. Recent work employing functional magnetic imaging (fMRI) has demonstrated that PYY not only modulates neural activity in brain areas associated with homeostatic appetite control, but also within higher-cortical areas associated with reward and hedonic control. When PYY was present in high concentrations (mimicking the prandial state) food intake control was found to switch from homeostatic to hedonic brain areas. Moreover, in the presence of PYY, neural activity in these higher brain areas was reported to predict feeding behavior, independently of meal-related sensory experiences or neural activity in homeostatic brain areas.

Satiety is also controlled by signals generated through postabsorptive fuel metabolism. The products of food digestion may be metabolized in peripheral tissues or organs or alternatively may enter the brain directly. Most research has focused on glucose and fat metabolism in the hepatportal area and indicates that changes in the metabolism of both nutrients influences feeding by a common mechanism. Research suggests that satiety is associated with an increase in fuel oxidation. Indirect evidence is provided by the use of metabolic inhibitors to block oxidation pathways or impair fuel availability, leading to increases in food intake. As combined blockade of glucose and fat metabolism produces a greater response than inhibiting the two pathways separately a synergism is implied. Evidence suggests that changes in hepatic ATP concentrations provide the common satiety signal, with this being relayed to the brain *via* vagal afferent neurons. Pathways in the CNS that are sensitive to this metabolic signaling have begun to be mapped out. However, direct entry of metabolites into, and action on receptors in the CNS, may also contribute to their satiety actions. One central factor clearly associated with episodic satiety rather than tonic energy status is serotonin (*see* Integration of Episodic and Tonic Signals Within the CNS).

Episodic Signals: Hunger

The intimate contact of mainly chemical, but also physical stimuli with receptors in the mucosa of the nose and mouth set up orosensory effects of food stimuli. These signals are in turn transmitted to the brain by afferent fibers of primary olfactory, gustatory, and somatosensory neurons of cranial nerves 1, 5, 7, 9, and 10. These peripheral inputs appear to make contact with dopamine and opioid neurotransmitters in the brain. The cephalic phase of appetite control refers to the physiological responses initiated by the sight or smell of food. These are anticipatory and prepare the digestive system for the imminent ingestion of food. Cephalic phase responses occur in the mouth (anticipatory secretion of saliva), stomach, and small intestine and represent preprandial changes that are precursors for the onset of a meal.

Additionally, it has been proposed that changes in blood glucose levels may provide a signal for meal initiation. Evidence provides some support for a role for transient declines in blood glucose in humans leading to increased expression of hunger and the initiation of eating. Glucose sensing also occurs centrally at different levels from the hindbrain to the hypothalamus. These brain areas, together with peripheral glucose sensors, are anatomically and functionally related, representing a network that monitors glucose availability and is involved in appetite control.

Potent feeding responses can also be obtained by micro-injection of peptides peripherally or centrally in animals. Ghrelin is unlike other peripheral hormones as it stimulates rather than inhibits eating behavior. Ghrelin is responsive to nutritional status with concentrations peaking just before meal initiation and fall following intake dependent on the energy value of the food consumed. These data suggest that ghrelin is a potent episodic meal initiator supported by the observation that both peripheral and central infusions of ghrelin have been shown to stimulate food intake in rats and mice, an effect in part mediated by central NPY (*see* Central Hunger Signals). Infusions of ghrelin in lean participants also lead to increases in food intake through concurrent increases in premeal hunger and prospective consumption. Ghrelin is also responsive to weight status. Obese individuals appear to have lower levels of circulating ghrelin levels which do not respond to food intake in the same way as in lean participants. This indicates that excessive weight may cause dysregulation in this hormone's circulatory profiles. When administered during fMRI studies ghrelin has been shown to increase neural activity in hedonic brain areas in response to food-related images. This would suggest that one way in which ghrelin increases food intake is by enhancing hedonic and incentive responses to food-related cues.

Central Hunger Signals

A number of peptides including β -endorphin, dynorphin, NPY, orexins (OX-A and OX-B), agouti-related peptide (AgRP), and melanin-concentrating hormone (MCH) have all been reported to increase food intake when injected into the brains of animals.

NPY is one of the most potent stimulators of food intake identified to date. It is widely distributed throughout the CNS with high concentrations in the hypothalamic

appetite-regulating nuclei. NPY injection into the CNS or directly into the PVN or LHA promotes meal initiation and marked hyperphagia, delaying the onset of satiety. The hyperphagic effects of NPY are mediated by specific NPY receptor subtypes. To date, six receptor subtypes (Y_1 – Y_6) have been cloned and characterized. Present understanding suggests that Y_1 receptors mediate the effects of NPY on meal size, although much attention has recently been placed on the Y_5 receptor, which is assumed to serve the role of the NPY 'feeding receptor'. Endogenous NPY is sensitive to a variety of peripherally generated signals, being stimulated by the gut peptide ghrelin but inhibited by amylin, the adiposity signal leptin, and the satiety neurotransmitter serotonin.

Melanin-concentrating hormone (MCH) is expressed in the zona incerta and LHA of the hypothalamus. MCH-containing neurons project widely throughout the CNS suggesting an involvement in numerous physiological functions. However, the most widely investigated role for MCH is in the regulation of energy homeostasis. Repeated central injections to satiated rats produce a rapid and dose-dependent increase in food intake, whereas chronic central infusion of MCH to rodents results in persistent hyperphagia and enhanced body weight and adiposity. Two MCH receptors have been identified and of these MCHR1 is found in high levels in the Nac, amygdala, and hypothalamus indicating it is likely to mediate the orexigenic effects of MCH in conjunction with other feeding-related functions such as taste, reward, and olfaction.

The endogenous orexin system consists of two peptides termed Orexin A and B along with two receptors, orexin-1 and orexin-2. The strongest and most reliable effect on food intake is produced by orexin-A. Orexin neurons are stimulated by falls in plasma glucose and by fasting but are promptly inhibited by prandial satiety signals such as the presence of food in the gut. These central responses along with evidence that peripherally located orexin neurons may sense nutritional status in order to prepare the digestive tract for the ingestion of food suggests that the orexin system may constitute a mechanism for initiating feeding episodes. More recently, the identification of a prominent role for the orexins in regulating arousal has led to the suggestion that the orexins function to coordinate energy homeostasis and sleep/wake states.

Tonic Signals

Appetite not only relates to daily food intake and eating behavior but must also respond to the long-term (tonic) energy status of the individual. Tonic signals arise from tissue stores, the metabolic status of which provides a signal to indicate the status of the stores and drive intake if energy reserves are low. Organs implicated in energy storage include the liver, pancreas, and adipose tissue. They secrete various circulatory factors known to act as potent determinants of food intake, for example, leptin and insulin. The number of potentially active metabolites and by-products produced by metabolism of different nutrients is vast, providing a wide range of potential signaling factors.

One of the classical theories of appetite control, the lipostatic hypothesis, is based around the principal that tonic appetite control is achieved *via* signals that inform the brain of

the status of adipose tissue stores. In addition to the identified lipostatic signals leptin (*see* Leptin) and insulin, increasing evidence indicates that lipids also act as cellular messengers in the CNS being detected by fuel-sensing hypothalamic neurons to indicate the body's nutritional status. Levels of substances such as adiponectin and cytokine signals such as the interleukins and tumor-necrosis factors may also be influenced by adipose tissue. Levels of other circulating hormones such as gonadal steroids also reflect the body's fat mass.

Leptin

Leptin is one of the most important 'adiposity' signals identified to date, acting to indicate the size of adipose tissue stores to the CNS and counteract any change in fat mass in order to maintain a constant body weight. It was discovered in 1994 as a consequence of a long series of studies which demonstrated that the dramatic obesity of *ob/ob* and *db/db* mice was caused by single-gene mutations of an unknown hormone and its receptor, respectively. Molecular genetic methods identified the likely culprit as the adipocyte hormone leptin, named from the Greek word 'leptos' meaning thin. Consistent with this, acute central or peripheral administration of leptin to rodents reduces food intake, whereas chronic administration also decreases fat mass without altering lean tissue content.

In general, leptin circulates at levels proportional to body fat, with levels increasing with adiposity thus demonstrating its ability to respond to weight gain and energy status. Leptin also enters the CNS in proportion to its plasma concentration. Here leptin's hypophagic effects are mediated by various neuropeptide systems located in the hypothalamus, medulla, and other sites which express the extended functional variant of the leptin receptor (OB-Rb). Within the hypothalamus leptin's targets include appetite-stimulating neurons (e.g., NPY) that are inhibited by leptin, and appetite-inhibiting neurons (e.g., those expressing components of the melanocortin system) that are stimulated by leptin. In addition to its interaction with central regulatory mechanisms there is evidence of a synergy between leptin and the episodic satiety factor CCK. Co-injection of subthreshold doses of leptin and CCK dose-dependently decreases food intake, an effect that is lost in the presence of a CCK-1 receptor antagonist indicating the importance of a functioning CCK pathway to the synergistic relationship.

The proportional relationship between leptin levels and body fat is lost during short-term severe energy restriction when leptin levels fall to a larger extent than would be expected from changes in adiposity alone. Falling leptin levels induce a starvation response, powerfully stimulating appetite and food intake to restore the negative energy balance.

Integration of Episodic and Tonic Signals Within the CNS

As stated previously, the control of energy balance depends critically on the CNS. The various CNS regions that control energy homeostasis (brainstem, hypothalamus, and other limbic structures) are accessible to the numerous circulating hormones and other factors discussed above. Within these central locations are specific neuronal populations that

recognize these signals and act in a network to integrate the multiple inputs, and help determine energy intake and expenditure. Serotonin (5-HT) has been identified as a key CNS satiety player in the short-term regulation of food intake. It acts to mediate the effects of episodic meal generated satiety signals such as those produced by CCK and macronutrient ingestion. In turn 5-HT has been shown to suppress CNS NPY release and inhibit hunger. The melanocortins have been identified as another critical CNS neuropeptide system. They are integral in the action of leptin on intake and in contrast to 5-HT they appear to mediate the effect of tonic energy status on appetite. However, both CNS satiety factors have a common downstream target in NPY, the functioning of which is also inhibited by melanocortin agonists.

Serotonin (5-HT) activation has been associated with the within-meal processes of satiation and the postmeal state of satiety for over 30 years. As mentioned previously, activation of the 5-HT system is linked to peripheral signals triggered by fat ingestion, for example, CCK. In addition, CNS levels of the 5-HT precursor tryptophan are influenced by dietary carbohydrate. Increased CNS 5-HT functioning as a consequence of these signals reduces food intake and body weight in both

rodents and humans; effects that are also demonstrated by serotonergic drugs. The 5-HT receptors currently thought to be directly involved in mediating the satiety effect of 5-HT are 5-HT_{1B} and the 5-HT_{2C} receptors. More recently, attention has been focused on 5-HT₆ receptors antagonism of which reduces food intake and body weight gain. However it remains to be determined whether these effects are consistent with satiety. The PVN and other hypothalamic appetite-regulating brain areas appear central to 5-HT induced hypophagia. Levels of NPY within the PVN are suppressed on 5-HT activation. Conversely inhibition of 5-HT receptors enhances NPY functioning.

Like leptin, the role of the melanocortin system was revealed through the study of a genetic mouse model of obesity (the agouti mouse). Such animals are obese and demonstrate marked hyperphagia as a consequence of excessive levels of agouti protein, an endogenous agonist of melanocortin receptors. Two melanocortin receptor subtypes (MC3-R and MC4-R) have been located within the hypothalamus. Both of these probably act to mediate the hypophagic effects of the melanocortin peptides. However, a number of studies have placed MC4-R in a central role. As mentioned previously, the

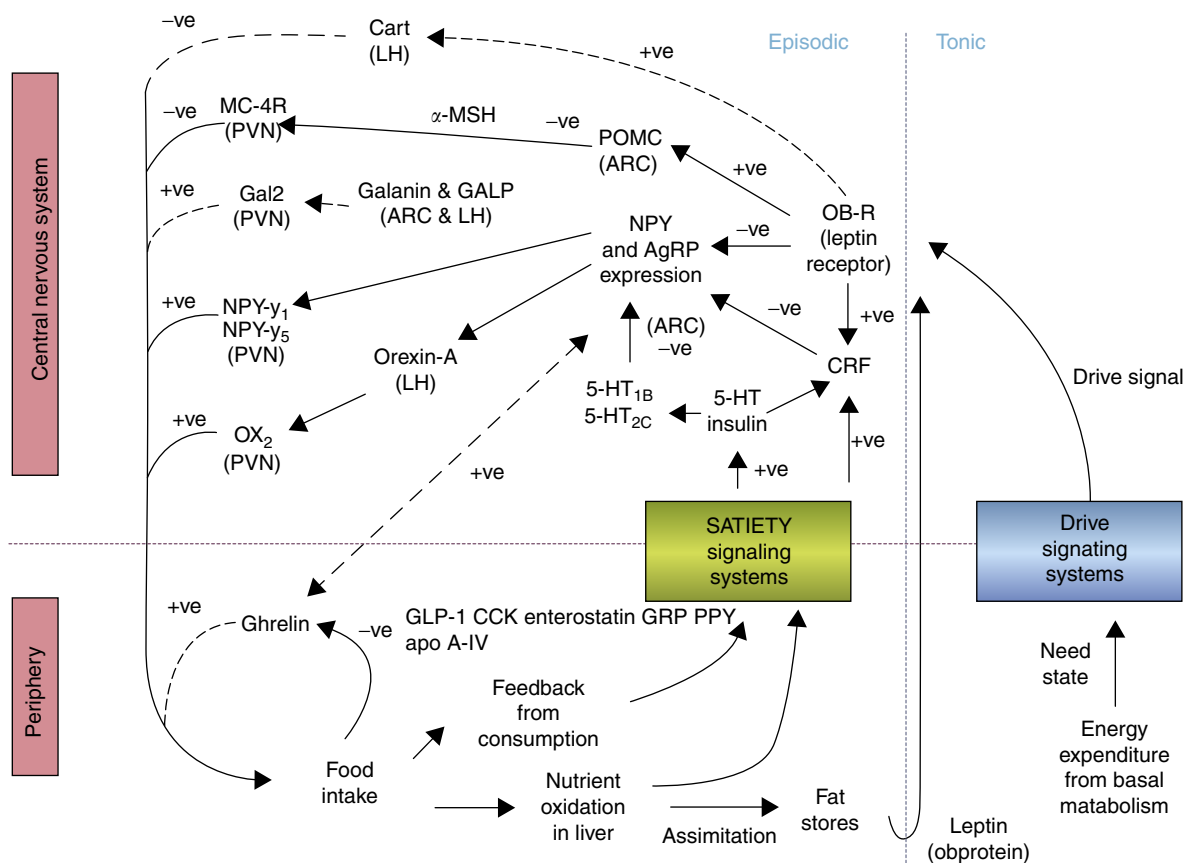


Figure 3 The integration of peripherally generated episodic and tonic signals critical to the expression of appetite. Signals generated by both meal consumption and fat deposition are integrated into a complex hypothalamic system of neuropeptides, which in turn either stimulate or inhibit subsequent food intake. Abbreviations: 5-HT, serotonin; α-MSH, alpha melanocyte stimulating hormone; AgRP, agouti-related peptide; apo A-IV, apolipoprotein-IV; ARC, arcuate nucleus; CART, cocaine and amphetamine-regulated transcript; CCK, cholecystokinin; CRF, corticotrophin releasing factor; GAL, galanin; GLP-1, glucagon-like peptide-1; GRP, gastric releasing peptide; LH, lateral hypothalamus; MC, melanocortin; NPY, neuropeptide Y; OX, orexin; PVN, paraventricular hypothalamic nucleus; POMC, proopiomelanocortin; PYY, peptide YY.

melanocortin system mediates the effects of a number of tonic regulatory factors such as leptin and insulin. However, it would seem that a functioning hypothalamic melanocortin system is also required for 5-HT drugs to alter feeding behavior, and presumably for feeding induced changes in endogenous 5-HT to influence appetite.

The melanocortin system offers one example by which tonic signals, although generated separately from episodic signals, feedback to alter subjective experiences of satiety and hunger. This is demonstrated more impressively through individuals with deficits in leptin signaling (either lacking leptin itself or downstream targets such as components of the melanocortin system). Such individuals experience constant hunger and without the tonic leptin signal this leads to constant and voracious food intake. Such extreme examples demonstrate the inability of short-term meal-generated episodic signals alone to block the overriding demand for energy generated by tonic basal metabolism.

Summary

Endogenous 5-HT and leptin represent two aspects of negative feedback integral to the appetite control system (see [Figure 3](#)). 5-HT represents short-term episodic feedback signals and as such mediates the effects of meal-derived satiety factors and promotes meals termination, thus prolonging the inter-meal interval. Through such mechanisms the body deals with the daily physiological fluxes that result from meal intake and ensures an approximately appropriate daily energy intake. In contrast, circulating leptin represents long-term tonic feedback signals. The levels of the hormone reflect the status of the body's energy stores and continually modify total daily intake to maintain a sufficient but not excessive

level of energy deposition. The net result of the action of both episodic and tonic signals will be an adjustment in the expression of appetite, adjusting subsequent feeding behavior to compensate for previous intake and energy stored.

See also: Appetite: Psychobiological and Behavioral Aspects. Brain and Nervous System: Biology, Metabolism, and Nutritional Requirements. Hunger

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Psychobiological and Behavioral Aspects

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Glossary

Appetite The subjective expression of willingness or motivation to eat. It is not necessarily solely related to situations of nutritional depletion and can be influenced by a number of physiological and nonphysiological factors. An increase in subjective hunger usually predicts meal initiation in *ad libitum* feeding subjects. It does not necessarily predict type or amount of food eaten.

Hunger The process that leads to the qualitative selection and quantitative ingestion of specific foods during an ingestive event. It is therefore specific to certain foods.

Appetites are often learned and frequently sensory specific.

Palatability The momentary subjective orosensory pleasantness of a food, which can indicate the sensory stimulation to eat. It may be influenced by the food, the physiological state of the organism, and the environment in which food and subject interact.

Satiation The events that terminate a meal.

Satiety The process of not eating and therefore should bear some reciprocal relationship to hunger. It is argued by some authors (Le Magnen, Blundell) that satiation and satiety are mechanistically distinct, the former determines meal size; the latter meal frequency.

The Nature of Feeding Behavior and Appetite Control

Mammalian feeding occurs regularly and intermittently and despite a general lack of conscious nutritional knowledge on the part of the animal, usually appears to match energy intake (EI) and nutrient intakes with requirements. How is this achieved? The common explanation is that appetite, EI or feeding behavior are regulated to ensure that physiological requirements are met. However, there is a lack of direct evidence for this regulation. Neither feeding behavior, nor appetite are regulated in a strictly physiological sense because (1) they are not held constant within certain narrow limits and (2) feeding responses are not an inevitable response to an altered physiological signal or need. Feeding behavior is responsive to a number of induced states such as pregnancy, cold exposure, growth and development, emotions, and weight loss. These responses have often been cited as an evidence of a system that is regulated. It is probable that some aspects of body size and composition are regulated and changes in feeding behavior are functionally coupled to those regulatory processes.

Hunger and satiety often have a large learned, anticipatory component rather than solely being the direct consequences of unconditioned physiological signals *per se*, such as reduced gastrointestinal content. Such physiological events can act as important cues for feeding but they do not necessarily directly determine that behavior.

The mechanism by which feeding behavior is coupled to physiological events (and other events) is the process of learning. To understand feeding behavior, hunger, and satiety processes, the mechanism by which learning links feeding behavior to physiological, sensory, nutritional, situational, and other learning cues, must be appreciated. This mechanism

is termed as associative conditioning of preferences, appetites, and satieties.

Learned Appetites, Satieties, and Feeding Behavior

Animals and humans learn (or become conditioned) to associate a given food with the physiological consequences of having ingested it. They associate certain proximal stimuli such as the smell, color, taste, or texture of a food (the conditioning stimulus), with a set of sensations that are directly felt (sensory afferent inputs), in relation to the external stimulus and to the endogenous changes such as physiological and neuroendocrine responses to food. The physiological changes that occur as a result of ingesting the food are termed as the unconditioned stimulus. The subject forms a learned or conditioned association between the conditioning stimulus and the unconditioned stimulus (the detectable consequence of eating), which informs him or her of the sensory and physiological consequences of ingesting that food. This process is summarized in **Figure 1**. Conditioned or learned associations are most efficiently established if the food is sensorially distinct, if there is a significant detectable post-ingestive consequence of ingesting the food, or if a training or learning schedule is encountered (e.g., by repeated exposure to the food under similar conditions). Learning is facilitated by social interaction.

As regards with the notion of appetite regulation, a problem arises when foods are constructed to look and taste like foods with a different composition. For sometime, after the initial exposure to the food, subjects will respond to it in a manner that is determined not by immediate exposure to the food but by what they have learned during the previous period

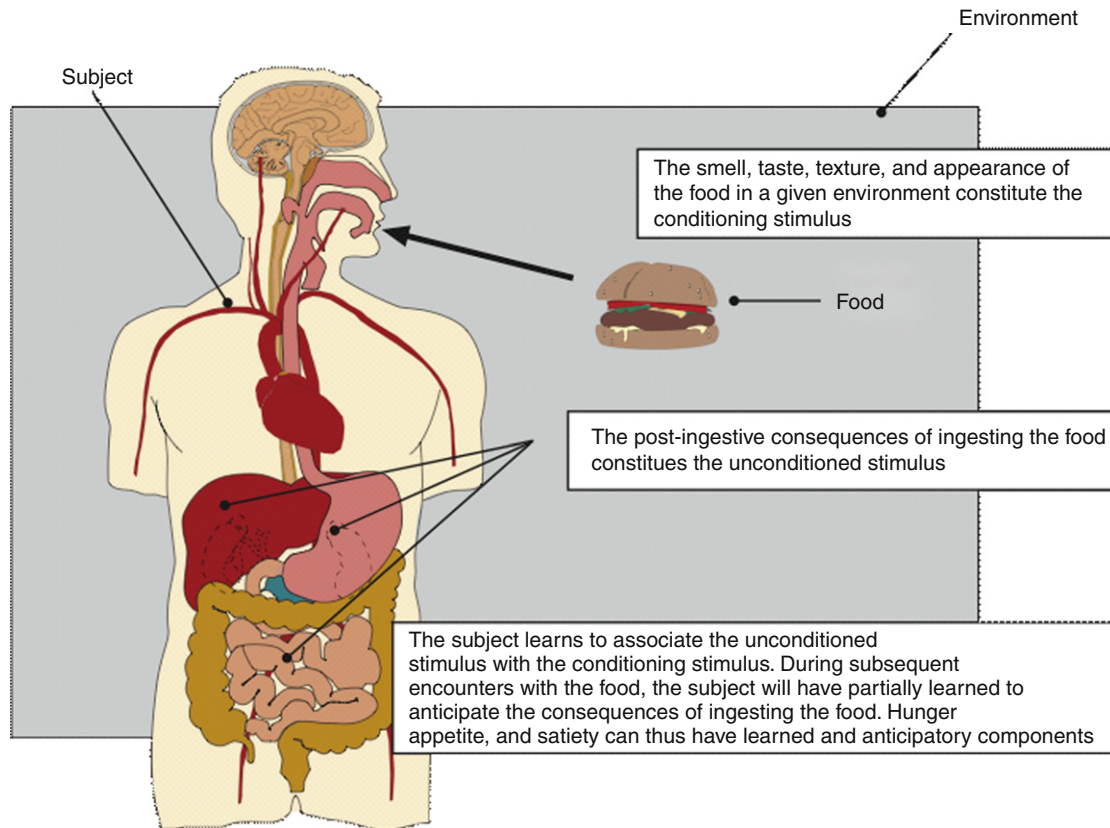


Figure 1 The process by which the subject learns to associate the postingestive consequences of eating with the food eaten and the environment in which it was eaten. Environmental influences can vary in strength from negligible effect to influences so strong that they can constitute the major factor determining a subject's subsequent response to that food.

of exposure to the similar foods upon which the learning was originally based. Only if the food produces a very large unconditioned stimulus will this previously learned response be instantly over-ridden. This raises the possibility that the use of food mimetics (e.g., artificial sweeteners) may disrupt stable patterns of learned feeding behavior in consumers at large.

The above view of the nature of feeding behavior has implications for the way the appetite system functions in lean and overweight people. Considerable time and effort has been expended by physiologists in attempting to understand how feeding behavior is geared to the regulation of a stable body weight. Obesity has therefore been seen as a consequence of defects in this regulation. The evidence from behavioral studies suggests the following: (1) feeding behavior is inherently more responsive to decreases rather than increases in body weight; (2) current secular trends in body weight suggest that, over time, it is very easy to increase body weight, which infers body weight is not tightly regulated, at least with reference to weight gain (for instance, in the National Health and Nutrition Examination Survey (NHANES) dietary surveys of American adults, average weight increases by 0.2–2.0 kg per year), and (3) the literature is remarkably short of clear lean/obese differences in feeding behavior of a type that suggest defects in a regulatory system. For example, evidence suggests that the obese tend to select a diet rich in fat, which itself facilitates over-consumption. However, the tendency to select

fat cannot be viewed as a defect in physiological regulation. There are interesting differences between some lean and obese subjects in response to food-based reward stimuli. It may be far more profitable to attempt to understand how feeding responds to environmental and endogenous stimuli and which of these responses are functional and adaptive, and which are not. In this context it is important to remember that evolution has selected us to optimize resources in uncertain environments, bank surplus energy, and compensate for energy deficits. Humans manipulate their environment to suit these design specifications. This promotes weight gain. Supermarket society is Optimal Foraging Theory taken to its logical conclusion. We do not tightly regulate energy balance. We feast in times of plenty as protection from famine. Modern industrial marketing strategies play on the constraints our species has evolved with, as an opportunistic forager. These considerations influence the methodological approaches that attempt to investigate how feeding behavior responds to endogenous and environmental influences.

Methodological Issues

Measuring Hunger

Hunger is a subjectively expressed construct that people use to express a motivation to eat. The most appropriate measure of

hunger is its subjective expression at a given time. This is achieved by asking subjects to mark a visual analog scale, which takes the form of a straight line with two extreme representations of hunger anchored at either end. It is most useful to track changes in subjective hunger over time and in relation to feeding events, diet composition, or physiological parameters. Hunger itself exhibits a large learned component (see the Section on Learned Appetites, Satiety, and Feeding Behavior, above) as reflected by the fact that most of the variation in the subjectively expressed hunger of human subjects is accounted for by time. If hunger is plotted against time in Western subjects feeding *ad libitum* then it generally exhibits 3 peaks and troughs, which broadly correspond to the 3 main meal times of a Western feeding schedule (Figure 2). Although subjective hunger is a relatively poor proxy for the amount eaten it is a reasonably good predictor of when eating will occur. It is important to recognize that hunger can be influenced by a large number of factors and so a search for a specific unitary hunger signal is likely to prove fruitless. Thus a large survey of over 600 men, women, boys, and girls could find no clear constellation of traits, sensations, or characteristics that typified hunger. A variety of hormones and drugs, the sight and smell of food, its perceived palatability, timing and social situation can all influence hunger.

A number of proxy measures of hunger, such as salivation have been made in the attempt to more objectively characterize hunger. These approaches have had relatively limited success and are difficult to compare across environmental circumstances. Satiety (or postingestive satiety (PI satiety)) is reciprocally related to hunger and can therefore be measured as such. There are now a number of hand-held electronic systems for real-time tracking of hunger, appetite, and satiety.

Measuring Appetite

Appetite is specific to foods, exhibits wide inter-subject variability, and tends to decline for a specific food as that food is eaten leading to selection of other foods. Appetite is therefore, said by Le Magnen to be sensory specific. The sensory specificity of appetite has been shown to relate *inter alia* to the

postingestive consequences (satiation and satiety) of having ingested a food. The most objective measure of appetite for a given food in a specific experimental situation is therefore the amount of that food that a subject chooses to eat. Appetite is not rigidly determined by physiological signals *per se* although they may greatly influence it. Both the palatability of a food and the appetite for it tend to co-vary and are often increased subsequent to a period of negative energy balance. Two examples are dieting and illness, both of which lead to lowered intake and a subsequent rebound in appetite. As discussed above, the appetite for a food will be learned on the basis of the consequences of having ingested that food on previous eating occasions. Because of this it is possible to use covertly manipulated foods to deceive subjects into behaving in a manner largely determined by prior learned experience. If this were not so, such deception would be impossible because the physiological signals produced by the sensorially similar yet nutritionally different food would immediately translate (through physiological signals) into behavioral compensation. This fact has implications for consumers because food technology can now dissociate the sensory and nutritional properties of foods, which may undermine the learned basis of food intake control in some people.

Measuring Feeding Behavior

There are many different techniques that can be used to measure feeding behavior in a number of different environmental situations. These techniques are used in naturalistic and/or laboratory studies of feeding. Generally in the laboratory, specific aspects of the system are manipulated at the cognitive, sensory, gastrointestinal, or even the metabolic level, for example, by deceiving subjects about the energy content of foods, altering the sensory variety of the diet, administering nasogastric infusions or parenteral infusions, respectively. A number of other manipulations can be achieved. Because the environment can have such a large influence on feeding behavior, it is important to consider a particular influence on feeding in several contexts. For instance, the effects of fat on energy intake have been considered in several

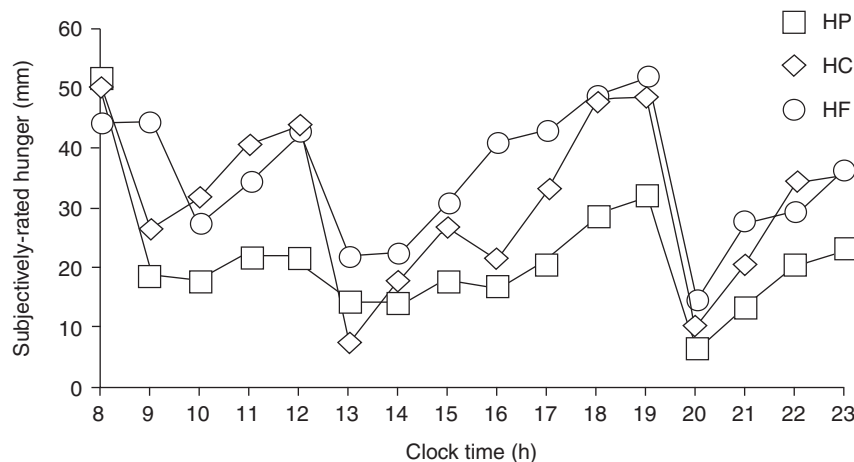


Figure 2 Subjective hunger tracked during waking hours in six subjects feeding on isoenergetically dense high-protein, high-fat, and high-carbohydrate diets. Subjects exhibit the three peaks and troughs of hunger that typify the Western feeding schedule.

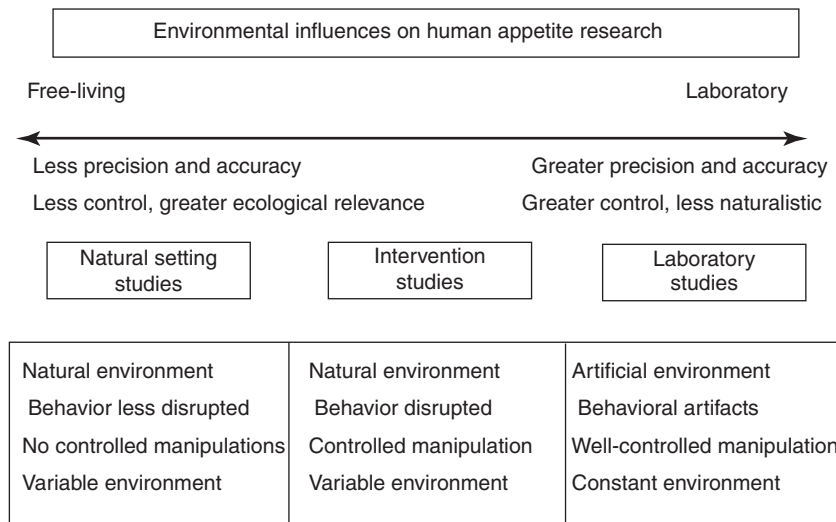


Figure 3 The constraints and limitations that the experimental environment places on studies of human feeding. In general the environment ranges from totally free living, which is realistic but very difficult to make measurements into the laboratory where measurements are easy but may be contaminated by artifacts due to the artificiality of the laboratory surroundings.

laboratory and real life contexts. Under most sets of circumstances dietary fat appears to be a risk for excess intake. The relationship between the experimental context and how it influences the investigations made is depicted in Figure 3.

Sensory Stimulation and Palatability

Palatability can be measured as the subjective preference for a food, its subjective pleasantness, or indeed the amount (in grams) of a food a subject eats. The relative palatability of a food can be determined by choice tests or taste tests relative to other standard ingestants (e.g., the 5% sugar solution). However, there has been much controversy over the actual definition of palatability. In general the palatability of a food can be thought of as (1) the momentary subjective orosensory pleasantness of a food or (2) its sensory capacity to stimulate ingestion of that food. However, the second definition should not be taken to indicate that there is a direct correlation between the perceived palatability of a food and the amount of that food, which is ingested as in case of hunger, the coupling between the expressed sensation and the amount of food or energy ingested is loose. This definition takes an account of the fact that the palatability of the food is jointly determined by the nature of the food (smell, taste, texture and state), the sensory capabilities and metabolic state of the subject, and the environment in which the food and subject interact. Palatability is therefore not stable; indeed the palatability of a food typically declines as its own ingestion proceeds. Work on military personnel suggests that the decline in preference for highly preferred foods (e.g., chocolate) is greater than that for staple foods such as bread, which exhibit more stable preference profiles. Palatability can be dissociated from sensory intensity because sensory intensity increases with the concentration of the compound or food being tasted; palatability generally shows a parabolic n-shaped curve with increasing sensory intensity of the food. Palatability can be conditioned, as can

aversions. Palatability of a food generally increases with food deprivation.

Because of the mutable nature of palatability and sensory preference the role that they play in determining EI and degree of overweight are unclear. Short-term experiments suggest that more preferred foods stimulate hunger and food intake. Work in American and French consumers suggest that on a palatability scale of 1 to 7 subjects rarely select any food below a score of 3 and that palatability does increase the size of meals. These works do not address energy balance. Indeed virtually, no published evidence supports the notion that altering dietary palatability or sensory variety *per se* will influence long-term energy balance of human subjects, despite the common perception that increasing dietary palatability will increase intake. This perception is so strong that the food manufacturing sector are unable to sacrifice the palatability of their products in developing healthier options, as the risk of decreased consumer acceptance is believed to be too high.

Certain combinations of the sensory and nutritional profiles of foods (e.g., sweet, high-fat foods) are conducive to over-consumption. This effect is often due to the combination of sensory stimuli and the postingestive effects of the food, which reinforce each other. The individual sensory and nutritional stimuli in isolation are often less effective. Thus cafeteria regimes, which can produce obesity in rats, typically alter the composition of the diet by increasing its fat content. When rats are given petroleum jelly in chow as a fat mimetic, they initially prefer this diet over normal chow. This preference soon becomes extinct, suggesting that sensory factors alone do not maintain preference.

Sensory Stimuli and Body Weight

It was originally proposed that obese subjects are more susceptible to external stimuli such as sensory stimuli than lean subjects who were more reliant upon internal physiological cues. This predicted that in a Western context the numerous

external food stimuli would promote excess EI in susceptible individuals. As can be appreciated from much of this text, the interplay between “external” and “internal” signal is much more complex and interactive than was initially supposed. Nevertheless given the multiplicity of afferent inputs that can influence feeding it is possible that some subjects have learned to base feeding predominantly on some cues rather than others. However, dividing these cues into simply internal and external sources is perhaps oversimplified. Current questionnaires which attempt to characterize subject’s responsiveness to food attempt to dissociate “externality”, “restraint”, and “emotionality”. It is becoming increasingly clear that emotional and reward-based responses to food are critically important in facilitating over-consumption.

Systematic differences have been found in sensory preference profiles, but not perceptions of intensity of various tastes between lean and obese subjects. Work has suggested that subjects with a history of weight fluctuation have an enhanced sensory preference for high-fat stimuli that are sweet. It has also recently been shown that sensory preference for fats is associated with degree of overweight in adults and children. This may be important. Although there is little evidence that sensory stimuli alone promote positive energy balances, there is evidence that people select foods they prefer. Preferential selection of energy-dense foods can lead to excess EI without any apparent change in the amount of food eaten. Thus sensory factors are likely to play a role in the selection of foods, which are conducive to weight gain.

Sensory Versus Nutritional Determinants of Intake

The major problems with the concept of sensory preference or palatability as determinants of hyperphagia and obesity in humans are that (1) there is little direct evidence for this effect *per se* (because the appropriate experiments are very difficult to do) and (2) both animals and humans appear to acquire sensory preferences for foods dense in readily available energy. Dissociation of the sensory characteristics and postingestive consequences of ingesting a food becomes difficult and perhaps artificial. It seems that at the present time the data from animal studies tends to suggest that maximal sensory preference for a food or diet is achieved when the sensory stimulus is reinforced by the metabolic consequences that form part of the satiety sequence. Indeed there is controversial evidence that ingestion of one sensory stimulus (sweetness) without the associated nutrient (carbohydrate) promotes ingestion of energy shortly afterwards. The contribution of sensory and nutritional determinants of feeding is still poorly understood in humans.

Emotional Stimuli and Over-consumption

As an intensely gregarious species we are bound together by numerous relationships ranging from mother–offspring bonds developed at birth, through kin relations to culturally defined positions in any given social structure. Positioning in social structures is also common amongst the primates and is inextricably intertwined with access to resources, safety (e.g., predatory avoidance), and food. This is critically important to

the survival of groups of animals under ecological conditions. Humans have not lost the neuroanatomical bases of our emotions and behavior with which we evolved. Hence, food is as emotionally important today as it has always been during our evolutionary heritage. However, the social and emotional meanings of food have changed. Food is still important for sharing social emotions and creating social bonds through ceremonies, rituals, and traditions. Food is a major source of reward for achievements or simply as treats. Interestingly, in children, there is evidence that more energy-dense foods are more preferred. More energy-dense foods are also generally cheaper than less energy-dense but nutritionally balanced foods, making the fast food outlet phenomenon self-perpetuating. Food is a major source of soothing for emotional distress in others, for example, when parents comfort children. In the context of this discussion it is notable that food is also a major source of reward, soothing, and comfort in binge eaters, and is a common theme that emerges, in people struggling to control their weight. Thus the emotional drives and social facilitators of excess energy intake are powerful, if not more powerful than ever in modern Western society.

Meal Patterns, Appetite, and Energy Balance

The effect of meal patterns on appetite and energy balance is also an unresolved issue. It has been noted that snacking and commercially available snack food are often believed to elevate EI. However, there is considerably less evidence that meal or snack patterns contribute to the development of obesity. It is important to note at this point that the relationship between a meal and a snack relates to timing and size of ingestive events in meal feeding animals. In nonhuman species (and indeed humans) that engage in numerous small feeding bouts throughout their diurnal cycle there is little if any distinction between a meal and a snack. Meal-feeding animals are conditioned to ingest the majority of their EI in a few large ingestive events in their diurnal cycle, at approximately the same time points. Under these conditions, a snack can be defined as an energetically small, intermeal ingestive event. To avoid confusion with a common use of the word to describe a certain type of ‘commercially available food’, we use the phrase ‘commercially available snack foods’ to describe those specific foods. Commercially available snack foods tend to differ from the rest of the diet as they are more energy dense, high in fat and carbohydrate and low in protein and usually contain a large fraction of their edible mass as dry matter. They are by no means the only food eaten as a small inter-meal ingestive event by many people at large.

The evidence in relation to meal patterns, appetite, EI, and body weight is indirect and fragmentary. On aggregate, cross-sectional studies tend to support no, or a negative, relationship between meal frequency and BMI. However, Bellisle *et al.* convincingly argue that examinations of the relationship between snacking and energy balance in free-living subjects are extensively flawed by misreporting, misclassification of meals and snacks, and potentially by reverse causality. Under these conditions it is difficult to draw clear conclusions about the effects of snacking in cross-sectional studies. It is therefore important to conduct controlled laboratory

interventions over a number of days in humans. These studies suggest that in the short-to-medium term adding mandatory snacks to the diet leads to over-consumption. This effect is most pronounced in those who do not habitually snack and least pronounced in those who do. It is also of note that rats tend to be “snackers” and Western humans tend to be meal feeders. The rat tends to adjust EI by varying meal frequency; the human by varying meal size. However, if rats are meal fed, they learn to adjust EI by varying meal size. Humans placed in time isolation begin to adjust intake by varying meal frequency. These comparisons illustrate the fact that adjustment of intake to energy or nutrient requirements occurs within a conditioned time framework, which itself is variable depending on the conditioning environment. Despite large changes in the pattern of feeding, EI can still be adjusted to satisfy requirements.

Social and Situational Influences on Feeding Behavior

There are a number of social and situational influences on food intake in humans. In general, the shorter the time period of measurement, the greater the effect of situational and social influences. Thus there are a large number of factors that can influence single meal size in humans. These factors are summarized in **Figure 4**.

Time of day appears to influence meal size in that the amount eaten and the EI increases on going from breakfast, to lunch, to the evening meal. Meal size also increases across the feeding period in rats. It has been suggested that this occurs in learned anticipation of the energy requirements in the fasting period (night for humans, day for rats). Meal size and EI tend to be greater at weekends than on weekdays, in Western adults. Meal size also varies as a power function of the number of people present at a meal. DeCastro has termed this effect

“social facilitation” of feeding. Social facilitation and daily routine account for much of this effect.

Seasonality can influence feeding. A number of studies also suggest that EI, meal size, and eating rate are all elevated in the autumn. In one particular study hunger was associated with meal size in winter and spring, but not so clearly in the autumn.

Cognitive and Social Cues

Throughout the 1960s and 1970s a large number of behavioral studies examined the effect of cognitive and social cues (perceived energy content of foods, salience of cues, eating behavior of others present) on feeding in relation to the externality hypothesis. Although a large number of studies found that so called external cues do relate to short-term feeding behavior, a large number of others did not. However, the presence of external cues alone does not reliably predict how much food people will eat. Neither does the presence of external cues always relate to lean/obese differences in feeding patterns. Some of these differences in relation to cognitive and social cues are better explained in relation to dietary restraint. Restraint is a term used to describe people who are attempting to limit or reduce their body weight by means of cognitive energy restriction (dieting). In doing so it is proposed that they are placing their motivation in relation to feeding at odds with physiological feeding stimuli. Placing cognition at odds with physiological drives can result in pathologies of eating since the normal “regulatory” processes are cognitively undermined. According to Herman and Polivy, these aberrations can extend into disturbances of emotion and cognition, which may partially underlie the increase in the prevalence of eating disorders. Furthermore it is argued that restraint will increase the probability that a person will break a diet. It has been shown that an intervention (usually a pre-load) that breaks the rules of restraint, almost paradoxically

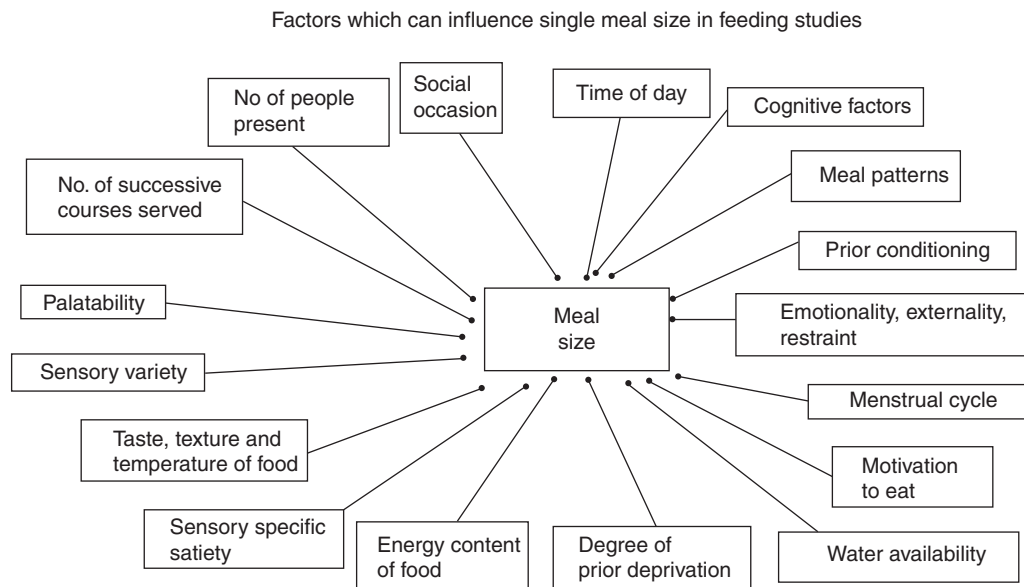


Figure 4 Major factors known to affect single meal size in humans. In general, the shorter the time interval of measurement, the greater the influence these factors have on the observed feeding behavior.

induces a greater intake. This phenomenon has been termed counter-regulation. This effect is cognitive, because it can be induced by deceiving a restrained eater into believing that a preload was high in calories. Because the concept of restraint has predictable behavioral outcomes it is a useful tool in characterizing different people with respect to their feeding behavior. However, it is now generally accepted that restraint is not a unitary construct and people who attempt to lose weight and who show flexible restraint are more successful than those who are rigid in their patterns of cognitive restraint.

Diet Composition and Appetite

Food and nutrient ingestion influence human appetite through multiple feedbacks at several levels, which can be traced through the processes of food location, ingestion, digestion, absorption, and metabolism. Satiety is therefore maintained by a functional sequence or cascade of sequential physiological events that reinforce each other. Removing parts of a food or nutrient's effects on this sequence will therefore diminish its impact on satiety.

How do Macronutrients and Energy Density Affect Satiety?

Because the control of food intake in humans is imprecise, alterations in the energy density of the diet can lead to changes in energy intake, even over quite prolonged periods. Macronutrients are not all equal when it comes to how satisfied one feels after eating them. Protein has the greatest effect on satiety, followed by carbohydrate, followed by fat. Studies using smaller preloads (typically of 250 kcal or less) do not find this effect. Although fat is the most energy-dense macronutrient it is also the least satisfying. The effect macronutrients have on satiety is illustrated in **Figure 5**. Foods that are high in protein include lean meat, fish, beans, peas, lentils, mycoprotein, tofu, and eggs. Foods rich in carbohydrates and fiber include wild rice, potatoes, cereals and oats, bread, beans, peas, lentils, and couscous. Fat-rich foods include savory snacks, pies, biscuits, cakes, cream, and most cheese.

Diet Composition and Satiety to Prevent Weight Gain

Any weight reduction strategy that focuses on one simple aspect of diet alone will have limited effects on weight control. Dietary monotherapies are very limited in what they will achieve. Several aspects of diet composition can be used simultaneously to influence satiety and help people navigate to a healthy body weight. In other words we should be taking a package definition of what constitutes a reasonable dietary approach to weight management.

- A diet should have a low fat content.
- A tolerably low energy density of $\sim 1.1\text{--}1.3 \text{ kcal g}^{-1}$.
- A high water content.
- Avoid where possible caloric beverages.

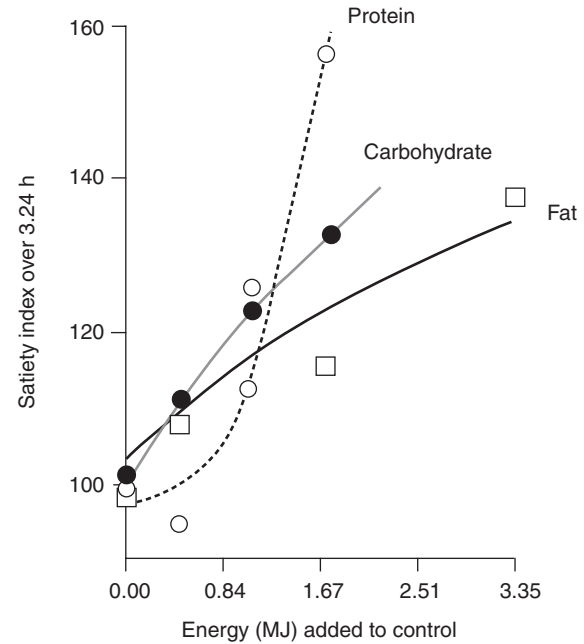


Figure 5 Effect of increasing energy content of macronutrient loads on satiety Index subjectively expressed over 3.25 h.

- Fiber content should be tolerably high (which parenthetically is not much more than 20–30 g per day).
- Fiber holds water in foods, which lowers energy density, and decreases the rates at which those foods are digested.
- To enhance satiety protein content of the diet can be relatively, tolerably, and reasonably high (probably not exceeding 25–30% of an energy-reduced diet, for example, a diet that is followed to induce a negative energy balance. This translates into less than 20–25% of energy requirements from protein). Protein intake from red and processed meats should be limited.
- The carbohydrate content depends very much on type. There is a good deal in the literature that suggests sweet, short-chain carbohydrates can elevate energy intake, especially when combined with fat. This combination is most common in commercial processed and snack foods.

The orosensory properties of foods need to be maintained to encourage people to select those foods, so that they become a practical option for the development of healthy eating habits.

See also: Dietary Intake Measurement: Methodology. Food Choice: Behavioral Aspects

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Definitions and Etiology

Over a hundred types of arthritis are currently recognized. Among the degenerative arthritides, Osteoarthritis (OA) is the most common form, and the prototype of this group. In OA there is inflammation within the joint, but there is no evidence of whole-body inflammation, a key feature in distinguishing OA from the inflammatory arthritides. In general, OA affects a few joints, usually the large weight-bearing joints of the lower extremities, such as the knees and hips. OA can also affect the hands, especially in women, but without the systemic illness that characterizes inflammatory diseases such as rheumatoid arthritis (RA). The etiology of OA is unknown, but the primary pathological problem is degradation of the cartilage leading to loss of joint space and bony overgrowth, causing pain first with weight-bearing, then with passive motion, and finally at rest.

In contrast, in an inflammatory arthritis such as RA there is a systemic illness with inflammation of many joints, usually the small joints of the hands, wrists, and feet, often spreading to include the knees and hips. There is evidence of a systemic immune response, with activation of clones of autoreactive T cells and increased production of many cytokines, including interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , IL-6, and others. There is also activation of the acute-phase response, with reduced albumin synthesis and increased production of fibrinogen, C reactive protein (CRP), and other acute-phase reactants. The systemic inflammation leads to altered energy and protein metabolisms and wasting of body cell mass and muscle mass, described as 'rheumatoid cachexia.'

Prevalence

OA is the most common joint affliction, and its prevalence increases dramatically with age. Radiographic evidence of OA is seen in 70% of people aged over 65 years, but symptoms do not necessarily correspond with X-ray changes. OA of the hip has been reported in 7–25% of adults aged 55 years and older, whereas knee OA is approximately twice as common, and OA of the hands three times as common. RA, however, affects 1–2% of the population, but generally attacks many more joints than OA and is associated with a twofold or higher increased risk of death. Other inflammatory arthritides, such as the seronegative spondyloarthropathies, are much less common.

Clinical Features

The term 'arthritis' simply means the presence of pain and inflammation (heat, swelling, redness) in a joint. Joint pain without inflammation is 'arthralgia', and may be due to disease within the joint or in the surrounding soft tissues, ligaments, and tendons. Degenerative arthritis such as OA is generally a disease of the large weight-bearing joints of the lower extremities, such as the knees and hips. In addition, OA commonly strikes the distal interphalangeal (DIP) and first carpometacarpal joints of the hands, especially in women. The affected joints have pain on motion, mild swelling, and sometimes intra-articular effusions or swelling. As the disease progresses, bony overgrowth becomes clinically apparent, coinciding with the development of osteophytes on radiographic examination. These osteophytes, together with loss of joint space, are the radiographic hallmarks of OA, and reflect new bone formation at the joint margins. Over time, the range of motion in the joint is restricted, first by pain, later by loss of joint space, and finally by the osteophytes. Treatment of OA is essentially symptomatic, using analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) to reduce pain and limit the intra-articular inflammation. However, this treatment is seldom completely satisfactory, and progression of the disease is usually seen. Joint replacement surgery has revolutionized the care of end-stage OA, allowing return of function of joints that are otherwise immobile.

In inflammatory arthritis the situation is quite different. RA is a symmetric, additive polyarthritis involving up to several dozen small joints of the hands, wrists, and feet, often with involvement of the knees, hips, and ankles, and sometimes the elbows, shoulders, and cervical spine. There is pain, swelling, and warmth in the affected joints and stiffness upon awakening or after prolonged immobility that can last for several hours. Unlike OA, in RA there is evidence of whole-body inflammation with activation of the acute-phase response. This leads to suppression of albumin gene expression and upregulation of the production of acute-phase proteins such as CRP, transferrin, and fibrinogen. In addition, there is suppression of serum iron, increased zinc, and increased whole-body protein breakdown and resting metabolic rate. Treatment begins with rest, physical therapy, and use of NSAIDs to reduce pain. Low-dose oral corticosteroids, equivalent to 5–10 mg day⁻¹ of prednisone, are often necessary to control symptoms. However, these therapies do not

alter the natural history of the disease. The best chance of doing so rests with the so-called 'slow-acting antirheumatic drugs' (SAARDs), such as methotrexate, TNF- α inhibitors, and other medications that have been shown to prevent erosions. It should be noted that some of these medications may also affect the nutritional status of individuals with RA via either altered appetite, blood sugar, plasma lipids, absorption, or protein metabolism (Table 1).

Role of Diet in the Management of Inflammatory Arthritis

Nutritional Assessment in RA

It is important to recognize that patients with RA do not have normal nutritional status. Compared with healthy persons of the same weight, age, race, gender, and height, patients with RA have lower body cell mass (especially muscle mass) and increased fat mass. This condition has been termed 'rheumatoid cachexia,' and it occurs despite adequate and even excessive dietary intake. This cachexia is generally seen in the presence of hypermetabolism (elevated resting energy expenditure) and hypercatabolism (elevated protein breakdown), along with reduced physical activity. These metabolic abnormalities are linked to increased production of the catabolic cytokines IL-1 β and TNF- α . The problems are further exacerbated by reduced physical activity, which reduces the anabolic stimulus to muscle, and disordered growth hormone kinetics. In addition, patients with RA have lower concentrations of serum albumin and other markers of visceral protein status, and often have chronic anemia disease with disordered iron metabolism.

Although many foods or food components have been considered as possible treatments for RA, most studies have focused on either supplementation (particularly the use of fish oil) or the use of an elimination diet, especially fasting or a vegetarian regimen.

Supplementation with Dietary Fatty Acids

Various dietary fatty acids have been shown to have numerous immunomodulatory effects. Arachidonic acid (AA, 20:4 n-6) is synthesized in mammalian tissues from the essential fatty acid linoleic acid (18:2 n-6), found in many plant products.

The release of AA from cell membrane phospholipids via the action of phospholipase A₂ results in the subsequent production of AA-derived eicosanoids, such as prostaglandin (PG) E₂ and leukotriene (LT) B₄, which have potent proinflammatory and chemotactic effects. Alternatively, when AA is replaced with an n-3 fatty acid in the diet, such as eicosapentaenoic acid (EPA, 20:5 n-3) or docosahexaenoic acid (DHA, 22:6 n-3), there is competitive inhibition of the use of AA as a substrate, and eicosanoids with different biological activities (PGE₃ and LTB₅) are produced through the cyclooxygenase and 5-lipoxygenase cellular metabolic pathways (Figure 1). More specifically, EPA-derived eicosanoids result in decreased platelet aggregation, reduced neutrophil chemotaxis, and anti-inflammatory effects. Omega-3 fatty acids are derived primarily from marine sources, including fish and shellfish. Because modulation of dietary fatty acids can alter cellular eicosanoid production, it has been hypothesized that increased consumption of n-3 fatty acids can affect the immunologic and inflammatory responses accompanying RA.

EPA supplementation causes modest improvement in the number of tender joints and fatigue among patients with RA although clinical benefits have generally been small, subjective, and transient. Possible mechanisms for this improvement in clinical symptoms of inflammation include decreased LTB₄ production, altered neutrophil membrane lipid composition, reduced IL-1 production, or a change in the α -tocopherol content of the diet. Overall, findings suggest that clinical benefits of dietary supplementation with n-3 polyunsaturated fatty acids (PUFAs) are more commonly observed among patients consuming higher dosages of fish oil, for longer periods than those previously studied. Indeed, beneficial clinical effects have been observed for as long as 1 year among patients with RA ingesting 2.6 g daily of n-3 PUFA supplements. In terms of optimal dosage of supplementation, however, a level of 130 mg kg⁻¹ day⁻¹ (9 g of n-3 PUFAs in a person weighing 70 kg) has been shown to result in no additional improvement compared with patients receiving doses ranging from 3 to 6 g daily. Therefore, although the optimal level of fish oil supplementation is yet to be determined, there does appear to be an upper limit beyond which no additional benefit exists for patients.

Although some studies seem to suggest modest clinical improvements as a result of dietary fish oil supplementation

Table 1 Examples of drug side effects on nutritional status

Effect	Drugs
Appetite increased	Alcohol, insulin, steroids, thyroid hormone, sulfonylureas, some psychoactive drugs, antihistamines
Appetite decreased	Bulk agents (methylcellulose, guar gum), glucagon, indometacin, morphine, cyclophosphamide, digitalis
Malabsorption	Neomycin, kanamycin, chlortetracycline, phenindione, p-aminosalicylic acid, indometacin, methotrexate
Hyperglycemia	Narcotic analgesics, phenothiazines, thiazide diuretics, probenecid, phenytoin, coumarin
Hypoglycemia	Sulfonamides, aspirin, phenacetin, β -blockers, monoamine oxidase inhibitors, phenylbutazone, barbiturates
Plasma lipids reduced	Aspirin and p-aminosalicylic acid, L-asparaginase, chlortetracycline, colchicine, dextran, fenfluramine, glucagon, phenindione, sulfapyrazone, trifluoperidol
Plasma lipids increased	Oral contraceptives (estrogen-progestogen type), adrenal corticosteroids, chlorpromazine, ethanol, thiouracil, growth hormone, vitamin D
Protein metabolism decreased	Tetracycline, chloramphenicol

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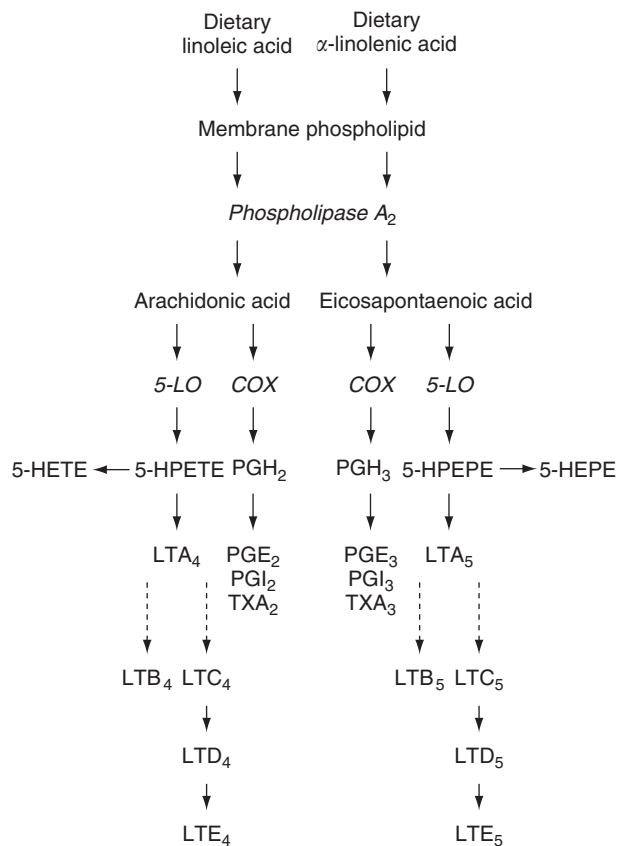


Figure 1 Simplified diagram of eicosanoid formation. Enzymes are italicized. Key intermediates are in bold. *COX*, cyclooxygenase; 5-HEPE, 5-hydroxyeicosapentaenoic acid; 5-HETE, 5-hydroxyeicosatetraenoic acid; 5-HPETE, 5-hydroperoxyeicosatetraenoic acid; 5-HPEPE, 5-hydroperoxyeicosapentaenoic acid; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

in patients with RA, the question of the effects of a patient's medical regimen on the efficacy of fish oil supplementation remains. Nonsteroidal anti-inflammatory agents are known to inhibit the cyclooxygenase enzyme system, which is the same pathway that seems to be inhibited by EPA and DHA. It is therefore possible that in studies of fish oil supplementation where patients are simultaneously maintained on NSAIDs, the effect of EPA is diminished, because the cyclooxygenase pathway is already inhibited by concurrent treatment with NSAIDs. Several studies have attempted to address this issue and have demonstrated a modest effect of n-3 fatty acid supplementation in both patients who are treated with NSAIDs and those who are not, suggesting that concurrent treatment with NSAIDs does not seem to diminish the effect of n-3 fatty acids. However, a clinically important NSAID-sparing effect of fish oil among patients who discontinue NSAIDs has not been demonstrated, suggesting that the benefits of fish oil supplementation are modest at best, relative to the effects of medication.

Although the majority of studies regarding manipulation of dietary fatty acids have focused on fish oil supplementation, other fatty acids have also been studied. The use of α -linolenic acid, the precursor of EPA and DHA, has not been shown to

be of any benefit in RA. However, γ -linolenic acid, found in blackcurrent seed, evening primrose, and borage seed oils, has resulted in clinically important reductions in the signs and symptoms of disease activity in patients with RA, perhaps via a reduction in PGE_2 , IL-1, and IL-6. Because these oils do not cause an unpleasant fishy taste and odor in recipients, they may be preferred to fish oils for chronic treatment.

In summary, most studies of dietary supplementation with n-3 fatty acids suggest a modest improvement in clinical symptoms associated with RA, which are to some extent dose- and time-dependent. The most consistent clinical benefits have been reductions in tender joint counts and morning stiffness. Studies do not suggest that benefits are great enough to warrant discontinuing patients' other medications. However, the use of fish oil supplements, or diets rich in marine fish, may further improve clinical symptoms among patients with RA. Beyond the possible benefits in terms of controlling inflammatory symptoms of RA, increases in n-3 PUFAs are also associated with reduced risk of cardiovascular disease and other health benefits. Thus, there is reason to promote fish consumption among patients with RA consistent with general healthy eating recommendations. Both the American Heart Association and World Health Organization suggest consuming a minimum of two servings of fish per week, with one or more of the servings as oily fish. Those individuals who do not consume fish regularly may consider supplementation with modest levels of fish oils; however, until more is known about optimal dosages, caution should be taken with the use of concentrated high-dose fish oil supplements. Of note, there has been concern raised over environmental contamination of some types of marine fish with methylmercury. Therefore, eating a variety of fish will help to reduce any potentially negative health effects due to environmental contaminants.

Vitamin and Mineral Supplementation

Most studies involving vitamin or mineral supplementation in RA have focused on either the antioxidant nutrients (vitamin C, vitamin E, beta carotene, selenium) or B vitamins. Various studies have examined the effects of vitamins C, E, and selenium supplementation on the management of RA. In general, results from randomized controlled trials of vitamin E supplementation have been of relatively short duration and have led to conflicting results so that there continues to be a lack of concrete evidence to support vitamin E supplementation at a particular dosage. Nonetheless, patients with RA could certainly be encouraged to increase their intake of vitamin E-rich foods, including edible vegetable oils (sunflower, safflower, canola, olive), unprocessed cereal grains, and nuts. Similarly, the effect of dietary sources of other antioxidant nutrients, such as selenium and vitamin C, on inflammatory symptoms in RA has also been ambiguous. It should be emphasized that providing individual nutrient supplements does not necessarily offer the same overall benefit as when nutrients are obtained from whole foods. It is possible that the combinations of nutrients that are present in whole foods, or even some unidentified components of a

food, are responsible for any observed beneficial effects, and that supplementing a typical diet with individual nutrients will not provide the same benefit.

We have studied vitamin B₆ levels in patients with RA and healthy controls, and found that plasma levels of pyridoxal-5-phosphate (PLP), the metabolically active form of vitamin B₆, were lower in patients with RA compared to control subjects. Furthermore, plasma levels of PLP were inversely associated with TNF- α production by peripheral blood mononuclear cells, suggesting that abnormal vitamin B₆ status may be contributing to inflammation in RA. However, there is no evidence to support the efficacy of oral vitamin B₆ supplements for treating the symptoms of RA at this time. Furthermore, large doses of vitamin B₆ can be toxic; therefore, as with the antioxidant nutrients, patients with RA would obtain the greatest benefit by increasing dietary sources of vitamin B₆, consistent with the dietary reference intake (DRI) for this nutrient. If supplementation is considered, it should not exceed twice the DRI level.

Fasting and Vegetarian Diets

An alternative approach to alleviating the symptoms associated with chronic inflammation is elimination of various foods or food components, most often by fasting or a vegetarian diet. Some studies have demonstrated a significant improvement in various objective and subjective measures of disease activity, including number of tender and swollen joints, Ritchie articular index, duration of morning stiffness, erythrocyte sedimentation rate (ESR), CRP, grip strength, and score on health assessment questionnaires, among patients with RA 6 weeks–2 years after initiating a vegetarian diet. Furthermore, these clinical improvements were accompanied by changes in biochemical and immunological parameters consistent with a substantial reduction in inflammatory activity. Other studies, however, have demonstrated no clinical improvement among patients with RA following a vegetarian diet.

Several possible mechanisms have been proposed to explain the impact of elimination diets on clinical symptoms in RA. One possibility is that RA might be the result of hypersensitivity to environmental toxins or specifically to foods or food-related products, resulting in a food allergy of sorts that exacerbates symptoms of RA. However, true food intolerance, involving a systemic humoral immune response against food items, appears to be relatively uncommon among patients with RA. Another possible mechanism that has been proposed includes an alteration in the fatty acid content of the diet. Vegetarian diets contain more linoleic acid, but less AA, EPA, and DHA than omnivorous diets. Therefore, the eicosanoid precursors (AA, EPA, and DHA) must be produced endogenously from linoleic and α -linolenic acid (see [Figure 1](#)). It has been hypothesized that if this endogenous production cannot compensate for the absence of AA in the diet, then the precursor of the proinflammatory eicosanoids would be reduced, perhaps explaining the beneficial effect of vegetarian diets in patients with RA. Furthermore, it has also been demonstrated that fasting for 7 days resulted in decreased release of LTB₄ from neutrophils, in addition to

reductions in morning stiffness, articular index, and ESR, but that this reduction in LTB₄ occurred despite an increased AA content of the serum, platelets, and neutrophils. These findings suggest that perhaps fasting may impair a metabolic step of AA conversion.

Other potential mechanisms include the possible effect of a vegetarian diet on antioxidant status, or on other dietary practices frequently associated with vegetarianism. Plant-based foods are naturally high in antioxidant nutrients (vitamin C, vitamin E, and beta-carotene) and low serum antioxidant levels have been associated with an increased risk of developing RA, although the specific mechanism involved remains unknown. Certainly, RA is associated with increased production of reactive oxygen species; these compounds seem to contribute to the inflammatory process, so a diet high in antioxidants could limit damage via their anti-inflammatory properties. Although changes in fatty acid composition or antioxidant status seem to be the most plausible explanations for the potential benefit of adhering to a vegetarian diet, there are other possible mechanisms as well. Fasting, for example, suppresses inflammation and frequently a period of fasting is recommended before initiating an elimination or vegetarian diet; it is possible that this fasting period contributes to the reduction in inflammation among patients with RA following a vegetarian diet.

In summary, the notion that food sensitivity reactions contribute significantly to clinical symptoms associated with RA remains controversial. However, it seems that at least a small subgroup of patients with RA may benefit from individualized dietary manipulation involving elimination of specific foods or food components, in combination with other medical therapies. However, fasting and other elimination diets should be used with caution in light of the prevalence of rheumatoid cachexia in this population. Such patients are prone to further loss of cell mass during restrictive diets, and the net effect may be to do more harm than good.

Conclusions

Of the two primary approaches to the dietary management of inflammatory arthritis – supplementation and elimination diets – it appears that dietary supplementation with fish oil may result in the most consistent clinical benefits, although improvements still remain modest. Elimination diets, including fasting and vegetarian regimens, may provide some benefit for a limited number of patients with RA, but consistent alleviation of disease activity by objective clinical measures has not been demonstrated.

In neither case does the use of dietary management warrant discontinuing a patient's medical regimen; rather, diet may be useful as an adjunct to other more substantiated therapies. Perhaps the most prudent approach for patients with RA interested in attempting to control their disease activity through diet is to recommend a diet consistent with current recommendations for all individuals, including an intake high in fresh fruits, vegetables, and grains, with moderate amounts of lean meats and poultry, and an emphasis on fish, particularly marine fish high in n-3 fatty acid content. More definitive research demonstrating consistent, objective clinical benefits is

needed before specific dietary manipulations for patients with RA can be recommended. In addition, there is growing evidence that exercise, both resistance and aerobic, has important beneficial effects on both inflammatory and degenerative arthritis.

Role of Diet in the Management and Prevention of Degenerative Arthritis

Much less is known about the role of diet in the treatment of OA and other degenerative arthritides. The above discussion regarding n-3 PUFAs in RA may also pertain to OA, although the strength of the effect has not been studied as thoroughly. However, the same eicosanoid metabolism occurs in OA as in RA, with the exception that the disorder is limited to the joint rather than involving the whole body. Thus, fish oils may well be of benefit in OA. Antioxidant intervention with vitamin E may also be effective in OA, with several studies showing an effect comparable with those of NSAIDs. Although not strictly nutrients, glucosamine and chondroitin sulfate, which are two of the constituents of normal cartilage that decline with arthritis, have been shown to be useful when given as an oral supplement, especially in patients with early OA.

In contrast to RA, where diet's main role is in the treatment and little is known about prevention, there is more known about dietary components that lead to OA than about nutritional management of OA. It is clear that OA of the lower extremities is largely a problem brought on by obesity, especially OA of the knee (and hip, to a much lesser extent), suggesting that obesity seems to be a mechanical rather than systemic risk factor. Thus, maintaining body weight within the recommended ranges is probably the most important nutritional intervention to prevent OA. Weight loss leads to reduction in joint stress, and often reduces symptoms. In fact, recent studies have suggested that if all overweight and obese individuals reduced their body weight by 5 kg, or until their body mass index (BMI) was within the desirable range, 24% of surgeries for knee OA could be avoided. Furthermore, studies have demonstrated that exercise can improve OA symptoms even independently of weight loss, presumably by increasing muscle strength and thus improving the shock-absorbing power of the muscles, hence sparing the cartilage and joint. However, patients with OA have a great deal of difficulty with exercise, and their sedentary life style is reinforced by their joint pain, generally leading to weight gain after the onset of OA, which in turn exacerbates the disease, creating a vicious cycle. Exercise programs that increase physical activity and strengthen the muscles surrounding afflicted joints clearly improve symptoms in OA. Thus, OA can be thought of as a disease of overnutrition, whereas RA is generally a disease of undernutrition. Interestingly, recent twin studies have examined the role of genetic versus environmental factors as mediators of the obesity-OA relationship, and have suggested that shared genetic factors are not as important as environmental factors in mediating the obesity-OA relationship. Dietary modification leading to weight loss is a critical component of the management of OA.

See also: Cytokines: Nutritional Aspects. Obesity: Complications. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Starvation and Fasting: Biochemical Aspects. Supplementation: Dietary Supplements. Vegetarian Diets

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Relevant Websites

<http://www.euro.who.int/nutrition>

World Health Organization website: CINDI (Countrywide Integrated Noncommunicable Disease Intervention) Dietary Guide. Provides a guide for healthy eating and healthy lifestyles, as suggested by the World Health Organization.

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Glossary

Cytokines These protein molecules are produced by cells of the immune system and are involved in cell–cell communication. Interleukin (IL)-4, -5, and -13 are produced by Th2 cells and are involved in development of allergic asthma.

Genetic polymorphisms Molecular biologists use this term to describe species-specific variation within the genomic DNA in the population. This includes certain point mutation of one single nucleotide, called Single Nucleotide Polymorphism (SNP). Genetic polymorphism may or may not be associated with predisposition for certain diseases.

Innate versus adaptive immunity Innate immunity provides immediate defense against pathogens, such as viruses, bacteria, and fungi. The innate response to infection includes (1) production of antibacterial and antiviral defensive molecules at the site of infection, (2) production of chemokines and other molecules at the site of infection to attract innate immune cells such as monocytes and neutrophils, (3) phagocytosis and killing of the invading pathogen, and (4) activation of the adaptive immune system by antigen presentation to lymphocytes in the draining lymph node. Adaptive immunity involves the development of pathogen-specific responses that provide

immunologic memory and pathogen-specific T and B lymphocytes that augment and supplement innate defense mechanisms.

Measurements of peak expiratory flow rates

(PEFR) PEFR occur almost immediately upon forced exhalation, and in healthy adults this number can range from 500 l min⁻¹ to 650 l min⁻¹ depending on height and sex. PEFR are recorded by patients at home with a variety of inexpensive, handheld meters. Asthma action plans that describe patient asthma management options are written based on PEFR recordings by patients.

Ovalbumin (OVA) induced mouse model of

asthma Mouse model that mimics allergic airway inflammation, typically with high eosinophil numbers found in the bronchoalveolar fluid following methacholine challenge. Firstly, the Th2 cell response is induced with intraperitoneal injections of OVA with alum (aluminum hydroxide) as vaccine adjuvant. This is followed by several exposures to nebulized OVA and intranasal or -tracheal application of OVA.

T-helper cells Subsets of T-helper cells (Th1, Th2, Th17, and Treg) develop in response to specific environmental stimuli, particularly the type of pathogenic organism. Th2 cells, which help protect against parasitic infections, are involved in the development of allergic asthma.

Epidemiology

Asthma affects an estimated 26 million people in the US, approximately 70% of them adults. For adults, asthma accounts for 13.9 million office visits, 500 000 hospitalizations and 2 million emergency room visits per year. Approximately 4000 Americans die from asthma annually and the risk of death is highest among Blacks, women, and the elderly. The national US cost for asthma is nearly \$15 billion yearly with most costs incurred from the 20% of patients with difficult-to-control asthma. Despite the reduction in asthma mortality since 1996, morbidity and healthcare resource utilization associated with asthma continue to increase. Approximately 40–60% of asthma patients do not achieve asthma control, and the fundamental asthma care is poor for large segments of the population, including African-Americans, Native Indians, Hispanics, and Asians. The majority of adult patients seen in asthma referral clinics are women, and this is in contrast to the pediatric population where boys are more prevalent. Why women make up 60–80% of adult severe asthma patients is

unclear. Genetic predisposition, hormonal effects, and patient behavior may be contributors in women. Increasingly recognized are the contributions of diet and nutrition.

Definition of Asthma

Asthma can be diagnosed in a person who presents with typical symptoms of episodic wheezing, shortness of breath, and cough and have documented reversible airflow obstruction. Lung function testing including spirometry is needed to help determine airflow abnormalities and airway hyper-reactivity. Measurements of peak expiratory flow rates (PEFR), usually with an inexpensive handheld device, can be recorded at the clinic or at home to monitor for changes in asthma control. Skin prick testing and *in vitro* IgE specific assays determine if a person has developed an antibody response to an allergen or other antigens in the environment. Surrogates of airway inflammation, like sputum eosinophilia

and exhaled breath nitric oxide, contribute to the management of patients with asthma and these markers are being evaluated.

Development and Pathophysiology of Asthma

Asthma is characterized by airway hyper-responsiveness, chronic inflammation, mucus production, and structural airway remodeling that is largely allergic in nature. It is a complex syndrome and not a singular disease. Clinical and scientific evidence indicates that multiple sub-types of asthma exist in adulthood, though this is less true in early childhood. Asthma usually develops at a young age, and it remains difficult to diagnose before age 3. Upper respiratory viral illnesses, particularly rhinovirus and respiratory syncytial virus, and environmental allergens combine to trigger an upper and lower airway inflammatory response in susceptible individuals. Several theories try to describe this phenomenon, such as the 'atopic march' that states that allergic inflammation of the airways naturally follows eczema or atopic dermatitis in young children. The 'hygiene hypothesis' suggests that allergic inflammation results from changes in the patterns of exposure – less infections, more contact with indoor allergens like house dust mite – that children experience at a very young age.

Inflammatory cell infiltration into the airways is a characteristic feature of asthma and selective recruitment by leukotrienes, cytokines, and chemokines help direct trafficking of these cells. The key effector cells in asthma include eosinophils, T-helper type 2 cells (Th2), and mast cells. The Th2 cytokines, interleukin (IL)-4, -5, and -13 have all been the focus of novel experimental therapies. Mast cells in the bronchial smooth muscle are important in the persistence of abnormal airway hyper-responsiveness and chronic airway inflammation in difficult-to-control asthma. Beyond the role of Th2 immune pathways, Th1 cells and the IL-17-producing T-cells (Th17) and regulatory T-cell (Treg) types have emerged as key players in asthma pathogenesis. It is generally accepted that Th1 cells antagonize the effects of Th2 cells in asthma, and vice versa. Similarly, Treg cells (implicated in immune tolerance and autoimmunity) also inhibit Th2 cell responses in asthma. The complex process that begins with aeroallergen-induced activation of airway dendritic cells, leads to subsequent B- and T-cell activation, which in turn leads to recruitment of mast cells, eosinophils, macrophages, and neutrophils.

Development of Asthma: The Roles of Vitamins A and D

An interesting corollary of the hygiene hypothesis is that children living in the more 'hygienic' environments in developed country settings with higher rates of asthma often have higher quality diets than do infants in developing country settings. For example, vitamin A intake is low in infants in south Asia and Africa relative to the US and Europe. Vitamin A can affect the immune system and, in animal studies, low vitamin A intake impairs Th2 immunity. Th2 cells predispose to the development of asthma and, in at least one

animal study, low vitamin A intake protected against development of asthma whereas high intake increased the development of asthma. Similar data from animal studies are available for vitamin D which, like vitamin A, can promote Th2 immunity. Directly comparing such experimental animal studies to the human experience must be tempered with recognition that such animal studies do not include the many environmental and genetic factors that may affect the development of asthma in children. For example, although vitamin D can promote adaptive Th2 immunity it can also promote antimicrobial defenses of the innate immune system, which may decrease the severity of respiratory viral infections in infants, children, and adults. Thus improving vitamin D status of infants might promote Th2 immunity and possibly increase the risk of allergic asthma but it may also enhance innate immunity and decrease severity of respiratory infections that predispose to development of asthma in infants. In adults, it is hypothesized that vitamin D may similarly decrease severity of respiratory viral infections that can cause exacerbations of asthma. The benefits of vitamin D in asthma prevention and treatment are not known with certainty but studies examining these hypotheses are in progress.

Development of Asthma: The Role of Breastfeeding

Breastfeeding is another nutritional factor that is associated with risk of asthma development and differs between developing and developed country settings. Breastfeeding provides a good source of nutrition to infants and exclusive breastfeeding (with no other liquid or solid food) is recommended through 5–6 months of age. Breast milk provides immunologic protection against infection thus completely or partially breastfed children in less hygienic environments both benefit directly from reduced rates of diarrheal and lower respiratory infections compared to children receiving less complete or no breastfeeding. Such benefits are also seen to a lesser degree (because of lower exposure to such pathogens) in more hygienic environments. For many years it has been assumed that breastfeeding in hygienic environments protects against the development of allergic asthma. The principal evidence for this conclusion came from epidemiologic studies associating breastfeeding 'level' (i.e., exclusive vs. supplemented) and duration with risk of asthma and allergic disease in infancy and later in childhood. Lower levels of breastfeeding have been associated with increased risk of asthma. However, recent studies have revealed a more mixed picture with breastfeeding not always correlating with increased protection. For example, breastfeeding is not typically associated with protection if there is a family history of asthma, thus genetic predisposition may override this protection. Evidence for an overall protective benefit is also in question. In a recent, controlled intervention study in which enhanced breastfeeding promotion was offered to new mothers in some hospitals and clinics in Belarussia and not in others. Significant differences in exclusive breastfeeding at 3 months of age were seen in the intervention sites (44% vs. 6% in the control sites) but no decrease in risk of asthma was seen at 6 years of age. Other (observational) studies point to an increased risk of asthma with breastfeeding of short duration with protection or no effect as duration of

breastfeeding increases. These and other epidemiologic studies bring into question the assumption that breastfeeding, in addition to its many known benefits, may also protect against asthma.

Development of Asthma: The Importance of Probiotics and Intestinal Flora

Breastfeeding is well known to modify the intestinal composition of bacteria that drive the development of the immune system in the infant. Breastfed infants have the most beneficial intestinal microbiota, compared to solely formula-fed infants, who possessed more colonies of *Escherichia coli*, *Clostridium difficile*, *Bacteroides*, and *Lactobacilli*. The human gastrointestinal tract is sterile at birth, followed by immediate colonization of the gut with subsequent development of the immune system. There is apparent diversity of the intestinal composition between allergic and healthy infants. In addition to environmental factors, the intestinal flora may contribute to allergic diseases due to its substantial effect on mucosal immunity. There are several studies supporting the notion that children who develop allergies harbor a different profile of microflora. Although not all reports are consistent, most data indicate that an association exists. Several reports suggest that the make-up of intestinal microflora can be different in individuals with allergic disorders and those who reside in industrialized countries where the prevalence of allergy is higher. For example, children from an industrialized country like Sweden harbor more *Staphylococcus aureus* and *Clostridia* at the expense of *Lactobacilli* and *Bifidobacteria* in their bowel compared to children who live in countries with lower asthma incidence such as Estonia, where allergies are not as common.

It is believed that in the absence of microbial exposure allergic responses still occur when the immune system is developing. Exposure to microbial gut flora early in life allows for a change in the Th1/Th2 balance promoting Th2 cytokine release, like interleukin-4 (IL-4), IL-5, IL-13, and IgE. Probiotics are considered to modulate the Toll-like receptors and the proteoglycan recognition pattern of enterocytes, leading to the activation of dendritic cells and a Th1 response, followed by Th1 cytokines release that can restrain Th2 cell responses.

A limited number of studies try to address the efficiency of probiotic supplementation in the treatment and the prevention of asthma. Most studies that have focused on the treatment rather than on the prevention of asthma produced conflicting results mainly due to different experimental designs. However, there are encouraging findings suggesting that dietary supplementation with probiotics may reduce the risk for the development of atopic dermatitis. The effect of probiotics in the prevention of asthma has not been evaluated yet and there is limited evidence available to support this idea.

Management of Chronic Asthma

The National Asthma Education and Prevention Program 3rd Expert Panel Report's guidelines from the National Institutes of Health (NAEPP-EPR3, 2007) focus on maintaining asthma control and individualizing patient treatment plans to

minimize risk of acute exacerbations. The NAEPP-EPR3 utilizes a stepwise approach to step-up or step-down asthma therapy based on control rather than disease severity. Prior guidelines had several progressive actions within different steps, but now these are separated into different steps. Furthermore, the medications have been repositioned within the six steps of care, where inhaled corticosteroids (ICS) remain the preferred long-term control therapy for all ages. The combination of long-acting β_2 -agonist (LABA) and ICS is an equally preferred option. This approach balances the established benefits of combination therapy in older children and adults with the increased risk of severe exacerbations, although uncommon, associated with daily use of LABAs. Importantly, it is emphasized that the course of asthma may change over time, and that the relevance of impairment or risk measures, and the impact of medications may all be age related.

Patient adherence with prescribed asthma therapy in the US is very poor. In one study evaluating the adherence of 48 571 adult asthma patients, <30% took inhaled ICS twice daily, <30% took inhaled LABA twice daily, and <50% took a leukotriene receptor antagonist (LTRA) once a day at 6 and 9 months. The important aspect of patient education and open communication between physician and patient must be emphasized. Greatest emphasis is placed on the two aspects of the written asthma action plan – (1) daily management and (2) how to recognize and handle worsening asthma. Discussions should also include physical, emotional, and environmental stressors that contribute to asthma symptoms, including diet and nutrition.

Management of Asthma: Nutritional Influences and Genetics

Genome-wide association studies have expanded our understanding of novel genes in asthma. There are over 100 genes associated with the clinical syndrome of asthma. Smaller subsets of these genes are found across different groups of asthmatics. Gene polymorphisms such as single nucleotide polymorphisms are key factors in the pathophysiology of asthma. Most genotype investigations in severe asthma have found an inherited predisposition to airways hyper-responsiveness and atopy, two key features of asthma. For example, one widely studied area in asthma genetics is the β -adreno-receptor, a target for inhaled bronchodilator medications. Patients who are homozygous for the glycine at position 16 (Gly-16) on the extracellular domain of this receptor have down-regulation of the β -receptor response. This genotype is more prevalent in the severe asthma population. Another example is the polymorphisms for IL-4 and its receptor, which signal Th2 lymphocyte development, and these polymorphisms correlate loosely with more severe asthma.

From a nutrition standpoint, another important set of polymorphisms that play a role in the predisposition to severe asthma are the leukotriene related genes, 5-lipoxygenase (5-LO) and the 5-LO activating protein (FLAP). Both of these are of interest because omega-3 fatty acids affect the production of proinflammatory 5-LO metabolites. 5-LO is expressed in cells of myeloid origin, including eosinophils,

monocytes, and mast cells. Increased levels of 5-LO and FLAP mRNA are found in peripheral blood leukocytes from asthmatics compared to healthy patients.

Fatty Acids in Asthma

Epidemiological studies have shown that populations that eat a large amount of fish have low incidence rates of asthma. Omega-3 polyunsaturated fatty acids (n-3 PUFA) in fish oil include both eicosapentanoic acid (EPA) and docosahexaenoic acid (DHA). EPA, in particular, appears to inhibit the allergic inflammatory pathway. EPA is a competitive inhibitor of the omega-6 PUFAs arachidonic acid (AA), and competes with the arachidonic acid as the substrate for 5-LO. 5-LO converts arachidonic acid to the inflammatory leukotrienes, enzymes that play key roles in the potentiation of airway inflammation in asthma. EPA might also inhibit IgE production through the cyclo-oxygenase (COX) pathway and EPA is capable of inhibiting both the COX and the arachidonic acid pathway. In addition to being converted to EPA, it appears that DHA also exerts beneficial effects in a manner distinct from EPA. Typically, the diet in the US contains dietary fatty acids in the range of n-6: n-3 fatty acids of approximately 10:1. The recommended ratio by the WHO is 4:1 (Figures 1 and 2).

At least 20 clinical studies that have been conducted to test the hypothesis that supplementation with n-3 PUFA, a major component of fish oil, can ameliorate the development of asthma or improve asthma outcomes. Of the 20, nine were

randomized, blinded, placebo-controlled trials that have been performed rigorously. The remainder of the studies had some methodological flaws and were not included in larger analyses. The results of these studies over the past 20 years have been conflicting, but at least one meta-analysis determined that n-3 PUFA does not affect asthma outcomes. There is a consensus that the total number of subjects in these trials is insufficient to make firm conclusions about the effects of the supplements.

Obesity and Asthma

Asthma prevalence has increased in the US at a time when the prevalence of obesity has also increased and there is ample evidence that obesity increases the risk of asthma in both children and adults and, to a lesser degree, is associated with greater asthma severity. These associations are stronger in adults than children. For example, US national survey data show that being overweight (a body mass index [BMI] of 25–29.9 kg m⁻²) as an adult is associated with approximately a 40% greater likelihood of having a diagnosis of asthma, compared to having a BMI <25 kg m⁻², whereas being obese (BMI 30–34.9 kg m⁻²) is associated with a 90% greater likelihood. Losing weight, including via surgical intervention, decreases the risk and severity of asthma thus confirming a causal association. Obesity may increase asthma risk in several ways. Mechanical differences in the chest wall and airway smooth muscle resulting from obesity can predispose to

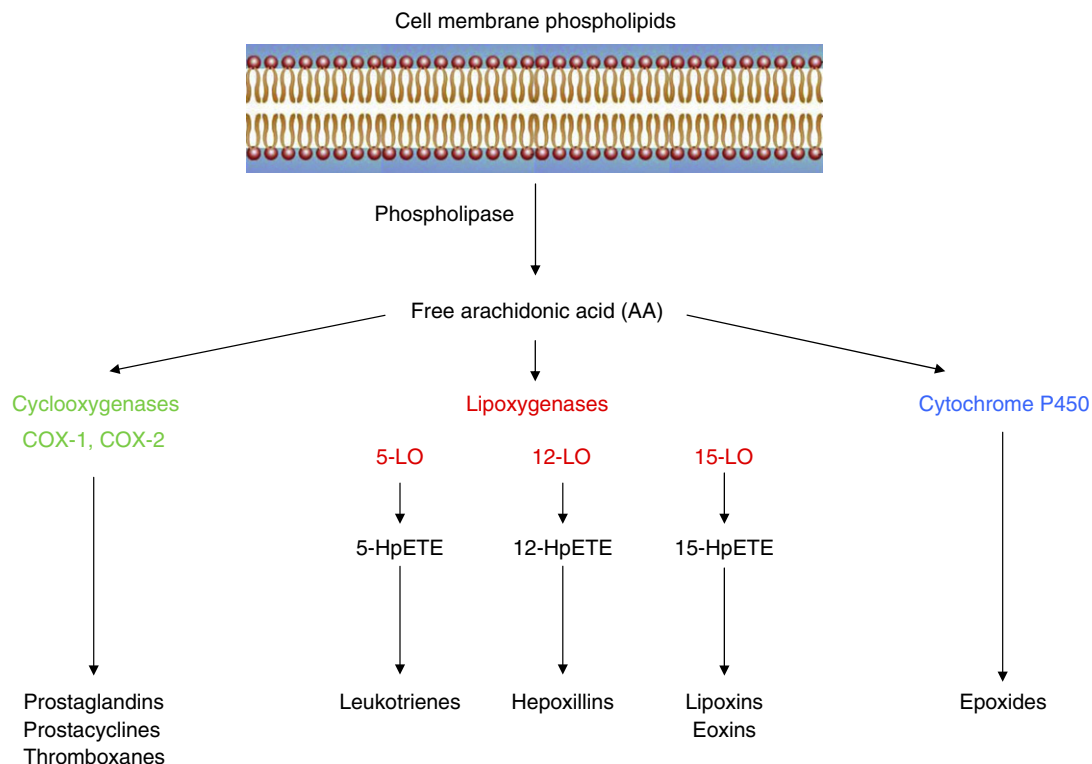


Figure 1 Bioactive products of arachidonic acid (AA) metabolism. Free AA can be converted to bioactive eicosanoids through three different enzymatic pathways: (1) cyclo-oxygenases (COX), (2) lipoxygenases (LO), or (3) P-450 cytochrome enzymes. HpETE, hydroperoxyeicosatetraenoic acid.

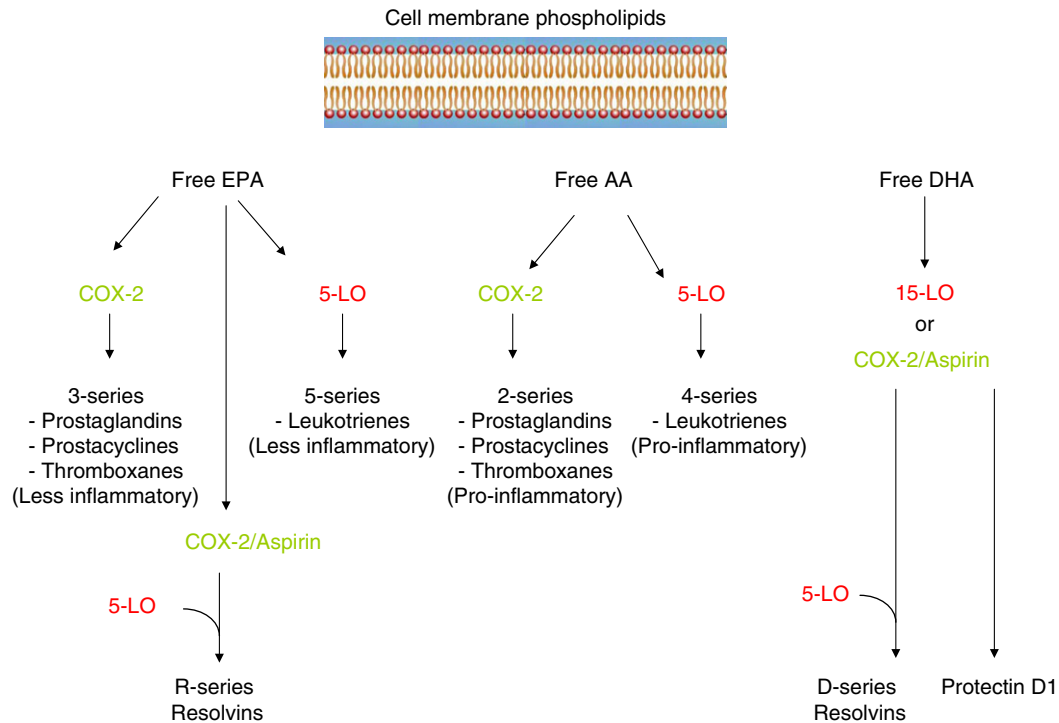


Figure 2 Cross talk between AA, EPA, and DHA. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can compete with arachidonic acid (AA) as a substrate for 5-lipoxygenase (5-LO) and cyclo-oxygenase (COX). The AA derived 4-series leukotrienes and 2-series prostaglandins can trigger airway inflammation and bronchoconstriction. Higher amounts of EPA and DHA decrease the synthesis of these potent proinflammatory lipid mediators. The EPA derived 5-series leukotrienes and 3-series prostaglandins have less proinflammatory properties. Both EPA and DHA can be converted into resolvins that have potent anti-inflammatory properties.

increased airway responsiveness and thus asthma symptoms. Systemic inflammation resulting from obesity may aggravate the airway inflammation that underlies asthma. Obesity causes an increase in the energy regulatory hormone leptin and a decrease in adiponectin. The former can have proinflammatory activity whereas the latter can be anti-inflammatory, thus directly linking body energy reserves (i.e., fat mass) to an inflammatory phenotype.

Amino Acid Supplementation and Asthma

Dietary supplementation of amino acid formulations is common practice among healthy people and those with chronic illnesses. In general, data are scant regarding the influence of supplemental amino acids on asthma. Creatine is reported to be the most popular nutritional supplement, and in an ovalbumin exposure mouse model of asthma, it was shown that creatine at a dose $0.5 \text{ mg kg}^{-1} \text{ day}^{-1}$ worsened eosinophilic lung inflammation and airway hyper-responsiveness. On the contrary, undenatured whey protein, a cysteine source, appeared to increase antioxidant capacity in the lungs and improve airway hyper-responsiveness in asthmatics with exercise-induced symptoms. Perhaps the most intriguing amino acid in asthma is L-arginine. L-arginine is a semi-essential amino acid that can be enzymatically converted into nitric oxide by the nitric oxide synthase (NOS) enzymes. Furthermore, L-arginine is metabolized by arginase enzymes in

the lung and elsewhere, and its contribution to asthma is under study. Variation in the gene that encodes arginase-1 has been associated with bronchodilator responses in children in the Childhood Asthma Management Program (CAMP) cohort. Popular as a nutritional supplement to increase muscle mass, L-arginine therapy has now been tested in numerous acute and chronic disease states, including sickle cell chest crisis, pulmonary artery hypertension, coronary heart disease, preeclampsia, and myocardial infarction, because of its bronchodilator and vasodilator actions. In these trials, L-arginine was given as an NOS substrate and as a vasodilator, either into the peripheral arterial or pulmonary arterial beds. A more recent primary therapeutic use for arginine is in sickle cell disease patients with acute chest syndrome, where a deficiency in endothelial NOS-derived NO is thought to contribute to the pulmonary vascular red cell sickling and vasoconstriction. Arginine, particularly in combination with hydroxyurea, augments NO production and improves outcomes in this disease.

Vitamins and Antioxidants in Asthma

Vitamins

Vitamins C, E, and β -carotene are broadly studied for their outcome on asthma. They are potent antioxidants and are considered to provide some anti-inflammatory properties in the pathophysiology of asthma.

Vitamin C is found in abundance in the extracellular fluid lining the lung epithelium and deficiency is associated with pulmonary dysfunction. Lower plasma levels of vitamin C and in leukocytes have been linked to asthma in children and adults, associated with increased airway hyper-responsiveness and respiratory symptoms. Several case-control and cross-sectional studies have shown that vitamin C supplementation may decrease asthma severity and frequency. It protects against bronchoconstriction induced by exposure to ozone or methacholine or by exercise. In children, most of their exacerbation of asthma has been connected with upper respiratory viral infections, especially rhinovirus. In addition, vitamin C supplementation reduces the duration and the severity of symptoms of the common cold.

The studied effects of vitamin E on asthma are less conclusive and randomized controlled clinical trials of supplemental vitamin E in asthmatics have not consistently demonstrated that higher intake of vitamin E can reduce asthma severity, though there is some evidence linking vitamin E and asthma. Higher levels of vitamin E are associated with less allergic skin sensitization, lower IgE secretion, suppressed neutrophil recruitment, and seems to be able to shift T-helper cell development towards Th1 balance. Vitamin E, like vitamin C, can lower blood histamine levels by preventing its release from mast cells and inhibit prostaglandin synthesis.

Antioxidants

Vitamin E is incorporated in the lipid membrane of lung epithelial cells and is also found in the extracellular lung fluid, where it acts as a potent antioxidant. β -Carotene, lycopene, and vitamin E are potent antioxidants that help to stabilize lung epithelia membranes and prevent oxygen-induced membrane injury by interrupting the lipid peroxidation chain reaction, though the role of β -carotene in lung protection is in question because intake of pharmacologic doses have increased the risk of lung cancer, possibly by increasing oxidative damage through an interaction with cigarette smoke. Asthma is associated with oxidative stress and especially during acute exacerbation, neutrophils may contribute to the generation of reactive oxygen species. In asthmatic patients, inflammatory cells from peripheral blood and bronchoalveolar fluid generate more superoxide than similar cells from healthy individuals. Because asthmatic patients are involved with increased levels of oxidative stress, they could benefit from higher dietary intake of food with antioxidant properties.

Apart from their antioxidant activity, flavones and flavonoids are known for their anti-inflammatory effects, which may be related to their ability to inhibit the release of histamine as well the enzymes cyclo-oxygenase and lipoxygenase, which can act on arachidonic acid in cell membranes to form potent inflammatory mediators, such as prostaglandins and leukotrienes, including LTB₄. The results from population-based studies give an inconsistent picture of potential beneficial effects to reduce asthma symptoms. Little evidence was found that dietary intake of three major subclasses of flavonoids was associated with asthma in adults, though it has been shown that their intake can improve chronic obstructive

pulmonary disorder, a disease with a number of components similar to asthma.

Dietary Minerals and Asthma

Selenium

Dietary selenium is an essential antioxidant, and because asthma is associated with impaired antioxidant capacity, it is believed that selenium may play a role in the development and outcome of this disease. Also, early case-control studies demonstrated decreased selenium intake and serum levels in patients with asthma and some data indicate selenium deficiency may increase the risk of asthma. Other studies were not in line with these findings. Selenium was even found to be positively correlated with the severity of bronchial hyper-responsiveness. Patients taking supplementary selenium, reported improved asthma control, however, there is only limited evidence of substantiated improvement in clinical assessments and measurements like testing pulmonary function and airway hyper-responsiveness.

Although well designed studies under the Global Allergy and Asthma European Network (GA²LEN) and similar projects convincingly demonstrated that the status of selenium is not significantly related to the prevalence and severity of asthma, it does not prove that asthmatic patients would not benefit from selenium intake. Selenium functions as a cofactor for glutathione peroxidase, the best known member of the selenoprotein family that reduces the synthesis and release of LTB₄. Also, selenium in combination with vitamin C can attenuate the activation of nuclear factor kappa-B, a transcription factor that mediates the proinflammatory cytokines that are linked to asthmatic immune response. Selenium may lower Th2 immune responses adding to the protective antioxidant and anti-inflammatory properties in the airways and result in an overall reduction in asthma.

Magnesium

Asthmatic patients, in general have lower circulating levels of magnesium and there is some indication that it may prevent airway constriction. Magnesium is given intravenously as an acute treatment for asthma exacerbations, because it seems to have several biological effects attenuating asthma, including relaxation of smooth muscle cells and stabilization of mast cells, and bronchodilation.

Conclusion

Optimal nutrition early in life, without over-use of supplements may be the best nutritional prescription for prevention of asthma. Breastfeeding, in addition to its other benefits, may also decrease the risk of developing asthma, though recent research questions this long-held view. Probiotic dietary supplements that optimize gut flora may also decrease asthma development and research is ongoing. For those with established asthma, intake of antioxidant nutrients, including vitamins C and E, can minimize severity. Some

studies also indicate that long-chain omega-3 fatty acid supplements may reduce asthma severity though results are not consistent. Excess body weight (in the form of adipose tissue) is associated with a greater risk and severity of asthma and weight loss can cause improvements in asthma symptoms. Current research is focusing on evaluating the possible benefits of vitamin D in decreasing the frequency and severity of asthma exacerbations but results are not yet available.

See also: Antioxidants. Breast Feeding. Cytokines: Nutritional Aspects. Fatty acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids. Lung Diseases. Nutrient–Gene Interactions: Molecular Aspects. Obesity: Childhood Obesity; Complications. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Selenium. Vitamin A: Deficiency and Interventions. Vitamin D: Physiology, Dietary Sources and Requirements

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Relevant Websites

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Weblink to Guidelines for the Diagnosis and Management of Asthma (EPR-3) from National Heart Lung and Blood Institute (NAEPP EPR3).
<http://nccam.nih.gov/>
Weblink to NIH National Center for alternative and complementary Medicine (NCCAM).

BEHAVIOR

Effects of Diet on Behavior

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Glossary

Arachidonic acid AA, is the trivial name for cis-5,8,11,14-eicosatetraenoic acid (20:4n-6). This polyunsaturated fatty acid can be biosynthesised from the omega-6 essential fatty acid, linoleic acid, and is abundant in the phospholipid membranes of several tissues, including the brain. It is a precursor of several important signaling molecules such as eicosanoids.

Attention Deficit Hyperactivity Disorder ADHD is a behavioral disorder, mainly of children, that includes a group of symptoms such as short attention span, easily distracted, restlessness, constant fidgeting, and impulsiveness. ADHD is estimated to affect 3–9% of children and about 2% of adults: it is normally diagnosed between the ages of 3 and 7 years. There is no cure, but the disorder can be managed by a combination of behavior therapy and drug therapy, in particular the use of psychostimulant drugs that enhance activity of the neurotransmitters, noradrenaline (norepinephrine) and dopamine. These may induce more focussed attention while lessening impulsivity and limiting behavioral repertoires. However, the perceived overprescription of such drugs to children remains a controversial topic.

Docosahexaenoic acid DHA, 22:6n-3, cervonic acid. This polyunsaturated fatty acid is abundant in fish oils (e.g., tuna oil) and is a significant component of the membrane phospholipids of most tissues, especially the brain, sperm, and the retina of the eye. It can be biosynthesised from the omega-3 essential fatty acid, α -linolenic acid, but the rate of conversion is very low.

Eicosanoids Metabolites of 20-carbon polyunsaturated fatty acids, such as arachidonic acid (AA), and eicosapentaenoic acid (EPA). They possess both autocrine and paracrine signaling roles, and have actions in many biological processes such as, inflammation, fever, regulation

of blood pressure, immune system modulation, and control of reproductive processes.

Eicosapentaenoic acid: EPA, 20:5n-3, timnodonic acid.

This polyunsaturated fatty acid is present in most fish oils, and is found at low levels in tissue membrane phospholipids. It is a precursor of some eicosanoids, which tend to antagonise the actions of arachidonic acid derived eicosanoids.

Essential fatty acids Polyunsaturated acids of the omega-6 and omega-3 families, which are essential for life and good health. The only true essential fatty acids are the omega-6 and omega-3 fatty acids, linoleic acid and α -linolenic acid, respectively. The other omega-6 and omega-3 fatty acids can be derived from these respective precursors, albeit at low levels.

Hypothalamic-pituitary-adrenal axis The HPA axis is a representation of a cascade of hormonal signaling known to be sensitive both to stress and nutritional state. Such events alter activity of selective nerve cells in the hypothalamus (a collection of nuclei in the base of the brain), which release corticotrophin releasing hormone (CRH). This in turn activates cells in the adjacent anterior pituitary gland to release adrenocorticotrophic hormone (ACTH) into the blood circulation. ACTH is carried to the adrenal glands, near the kidneys, where cells are stimulated to release cortisol, a glucocorticoid steroid hormone, into the circulation. Cortisol has anti-inflammatory and glucose counter-regulatory activity.

Neurotransmitters Chemical messengers that transfer neuronal activity signals across the synapse (microscopic gap) between one nerve cell and another. Electrical activity in the presynaptic nerve cell causes release of pockets (vesicles) of neurotransmitter into the synapse from where they reach receptors on the postsynaptic cell, whose activation may either excite or inhibit activity in the postsynaptic nerve cell. Neurotransmitters discussed

here include the monoamine group, serotonin (5-hydroxytryptamine, 5-HT), adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine. This group is synthesized from essential amino acids (i.e., that must be obtained from the diet): tryptophan for serotonin, and tyrosine for the others. This is of particular interest here because rate of synthesis of these neurotransmitters is sensitive to availability of their precursor amino acids, and thus to the latter's availability from the diet. Another transmitter group discussed is opioid peptides, derived from larger precursor proteins, but synthesis of these is not sensitive to diet.

Omega-3 polyunsaturated fatty acid Also called ω -3, or n-3 fatty acids. These are named after their shared structural

motif of having their terminal double bond three carbons from the methyl (or omega) end of the carbon chain. They are derived from α -linolenic acid, by carbon chain-elongation and desaturation and include eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).

Omega-6 polyunsaturated fatty acid Also called ω -6 (omega-6) acids. These are named after their shared structural motif of having their terminal double bond six carbons from the methyl (or omega) end of the carbon chain. They are derived from linoleic acid, by carbon chain-elongation and desaturation, and include arachidonic acid (AA).

Introduction

Effects of diet on behavior have long been topics of folklore superstition and popular mythology, and more recently the subject of rigorous, and not so rigorous, scientific study. Most research into dietary effects on human behavior has assessed changes in mood or mental function after eating (or drinking), or after fasting. Typical measures of mental function include tests of reaction time, attention, memory, problem solving ability and intelligence (intelligence quotient; IQ). In addition, research has addressed effects of diet on disturbed behavior, including Attention Deficit Hyperactivity Disorder (ADHD) in children, antisocial behavior and aggression, mental illness such as depression, and dementia (see Table 1).

Clearly, chronic malnutrition can seriously affect behavior by impairing brain development, and acutely by denying sufficient nutrients for optimal cognitive function. However, this article concentrates on dietary effects on behavior that are not the result of chronic malnutrition, nor of pharmacologically active ingredients of the diet such as alcohol or caffeine. Rather, they arise from more subtle effects of variation in nutrient intake within the normally nourished population.

Effects of Meals

The commonest way in which food can affect behavior is the change in mood and arousal that occurs from before to after eating a meal. This might sound trite, but it is not trivial: this general meal effect is probably the most reliable example of an effect of diet on behavior. Many animals, including human beings, tend to be aroused, alert, and even irritable when hungry. This encourages their search for food. However, their mental efforts become distracted by this task, to the detriment of other behaviors. After eating a satiating meal, we and other animals become calm, lethargic, and may even sleep.

Nevertheless, even this seemingly straightforward phenomenon can be distorted, and can vary across individuals and situations. The impact of a food or drink will depend on the person's initial state. For example, thirsty people improved

their vigilance when allowed to drink water, whereas when people were asked to drink when not thirsty, their performance deteriorated. Numerous experiments have shown that manipulation of the structure of meals results in variation in

Table 1 Examples of nutritional variables known or suspected to affect behavior, mood, and cognition

Category	Variable description
Food restriction	Early life undernutrition Chronic semistarvation Dieting to lose weight Short-term fasting (e.g., missing a meal)
Meal effects	Pre to postmeal changes Meal timing (e.g., morning, afternoon, night) Meal size Macronutrient composition (acute and chronic effects) Breast milk
Amino acids	Neurotransmitter precursors (e.g., tryptophan, tyrosine, phenylalanine) Phenylketonuria
Sugars	Sucrose (dietary intake) Glucose (supplement; tolerance)
Lipids	Essential fatty acids: Arachidonic acid (omega-6 PUFA) Eicosapentaenoic acid (omega-3 PUFA) Docosahexaenoic acid (omega-3 PUFA)
Micronutrients	Iodine Iron Selenium B-vitamins: B1, B6, B12, Folate Vitamin C Vitamin E Zinc
Diabetes	Acute effects of hypoglycemia Chronic effects
Pharmacological	Caffeine Alcohol Nutraceuticals (e.g., plant compounds)

postprandial changes in mood and mental function. One obvious facet of meals that has been investigated is what is eaten, i.e., nutrient composition; the other two main aspects of meal structure that have been studied are meal timing and meal size. Of course, the effect of a meal on appetite also represents a behavioral effect, but this aspect is covered elsewhere in this encyclopedia.

Besides any nutritional effects, two other influences on behavior are also known to interact with attempts to measure dietary effects on behavior. First, most people are very habitual in their choice of food, and size and timing of meals. As a result, they have learned a set of beliefs and expectations about the impact of their habitual dietary regime. Therefore, particularly in short-term tests, these expectations may over-ride or mitigate physiological changes. Dietary experiences that differ from a person's habitual eating could lead their behavior to change through cognitive rather than (or as well as) physiological influences.

Secondly, there are circadian rhythms and sleep-wake cycles in arousal and performance, which complicate interpretation of meal effects, as we discuss in the next section on meal timing.

Meal Timing

Does the timing of a meal in the day make a difference to any effects on behavior? In other words, do any behavioral effects differ between breakfast, midday, and evening meals, or mid-morning and afternoon snacks?

Breakfast

The potential effects of breakfast on performance and well-being continue to attract much interest, not least from industry, especially concerning performance of schoolchildren. Pollitt and colleagues have argued that children are likely to be more susceptible to the effects of fasting than adults, due to greater brain metabolic demands relative to glycogenic and gluconeogenic capacity. The numerous studies in this area have produced inconsistent results, which is partly attributable to variation in populations studied, their nutritional status, and designs used. There is a consensus that breakfast is more likely than not to benefit schoolchildren's performance, particularly if the children are already nutritionally vulnerable and have mental abilities with room for improvement. Moreover, breakfasts achieving slower release of glucose into the blood may be more effective in sustaining performance over the morning than those allowing rapidly absorbed glucose.

In all of us, there is a tendency for levels of arousal and alertness to rise during the morning, reaching a peak near midday. Some evidence suggests that breakfast may help to control this arousal, so that attention can be successfully focused on the task in hand. Conversely, omitting breakfast may increase autonomic reactivity, leading to less focused attention. This effect could explain one finding that children without breakfast showed better recall of objects to which they had not been asked to attend: such attention to irrelevant stimuli is also known to occur with increased anxiety. Furthermore, increasing hunger is likely to be distracting.

Less attention has been paid to effects of breakfast in adults. However, there are several studies of effects of giving breakfast to students that show a benefit on spatial and verbal recall tasks 1–2 h later, compared to missing breakfast. Interestingly, attention-based and reaction-time tasks were not improved by breakfast, and a logical reasoning task was even slightly impaired. Perhaps those tests benefit more from mild arousal, which could be acutely reduced by some breakfasts. These studies did not determine whether performance later in the morning would be affected by breakfast. Differential effects of breakfast content and size will be discussed below.

Midday Meal

Several studies have demonstrated a drop in performance after the midday meal, particularly for vigilance tasks requiring sustained attention. However, this 'postlunch dip' may not simply be an effect of eating, because vigilance has also been found to decline from later morning to early afternoon in subjects not eating lunch. That is, there is an underlying circadian rhythm in performance that is confounded with the effect of a midday meal. In fact, using noise stress to arouse subjects during a midday meal prevented any decline in performance due to the meal. It has also been shown that the more anxious one is feeling before lunch, the less one will experience any postlunch dip in performance. In support of this, another study found that subjects scoring highly on a personality measure of extraversion and low on neuroticism were more likely to be affected by postlunch dip. These are examples of the importance of individual differences and context on meal effects.

Evening Meal

There are few studies of effects of eating later in the day, although there has been some interest in effects of meals during nightshifts. Accuracy of performance declines with eating during a nightshift, but unlike lunch, premeal anxiety levels had no effect. One study in students of effects of eating a large freely chosen evening meal found little evidence for consistent changes in performance relative to missing the meal. Despite this, the students who omitted the meal reported feeling more feeble and incompetent and less outgoing than those who had eaten.

Snacks

One study specifically addressed whether an afternoon snack (approximately 1–1.2 MJ, 240–290 kcal, of yoghurt, or confectionery) eaten 3 h after lunch (or no lunch) would affect task performance. A beneficial effect of the snack was found on memory, arithmetic reasoning and reaction time 15–60 min later. The comparison was with performance after a 'placebo' zero-energy drink (participants were unaware of energy content). This rather different placebo does not preclude effects due to differences in sensory experience and expectations. Moreover, whether or not lunch had been eaten beforehand had little effect on the outcomes, suggesting that any nutritional effects must be due to acute impact of the snack, irrespective of prior nutritional state. It is known that snacks of this size eaten after a meal have only a small effect on blood glucose, although insulin rises sufficiently to inhibit

lipolysis and suppress the release of plasma free fatty acids later in the postprandial period.

The authors reported that these performance benefits from an afternoon snack were not found with a snack taken late morning. The most likely reason is that the beneficial effect depends on the decline in alertness normally occurring during the afternoon.

Other studies have found differential effects of macronutrient content of snacks; these are discussed below.

Meal Size

This topic has been little studied regarding behavioral effects, perhaps because there are a number of methodological difficulties and an absence of theory. For example, what counts as a large or small meal? Should the difference be in terms of absorbed energy, or weight or volume eaten, or even consumption time? If the former, then behavioral outcomes would need to be measured with sufficient delay for differences in energy absorption to be discriminable. Moreover, the influence of expectations and habit might confound experimental nutritional differences.

Two studies in adults found that large lunches (at least 4 MJ, 1000 kcal) impaired vigilance relative to eating small or medium-sized lunches. There was also evidence that this effect depended on the meal size being different from that habitually consumed. In adolescents, a larger breakfast (2.6 MJ, 634 kcal on average) resulted in poorer vigilance but better short-term memory 3 h later, compared to after a smaller breakfast (1.6 MJ, 389 kcal on average). Thus, there is some evidence that vigilance is adversely affected by a large meal.

Meal Composition

Carbohydrate Versus Protein

The effects of varying the nutrient composition of meals have been studied extensively, and rather more for mood than performance. This is largely because of evidence that plasma and brain levels of precursor amino acids for synthesis of monoamine neurotransmitters (chemicals responsible for signaling between nerve cells), strongly implicated that mood disorders, can depend on carbohydrate:protein ratios in the diet. Synthesis of the neurotransmitter serotonin (or 5-hydroxytryptamine; 5-HT) depends on dietary availability of the precursor essential amino acid, tryptophan (TRP), due to a lack of saturation of the rate-limiting enzyme, tryptophan hydroxylase, which converts TRP to 5-hydroxytryptophan (see Figure 1). An important complication is that TRP competes with several other amino acids, the large neutral, primarily branched-chain, amino acids (LNAA), for the same transport system from blood to brain. If the protein content of a meal is sufficiently low, such as 5% (or less) total energy as protein, then relatively few amino acids will be absorbed from the food in the gut. At the same time, insulin will stimulate tissue uptake of competing amino acids from the circulation, and the plasma ratio of TRP to those of amino acids (TRP/LNAA) will rise, favoring more TRP entry to the brain. Conversely, a high-protein meal, which would be less insulinogenic, results in absorption of large amounts of competing amino acids into

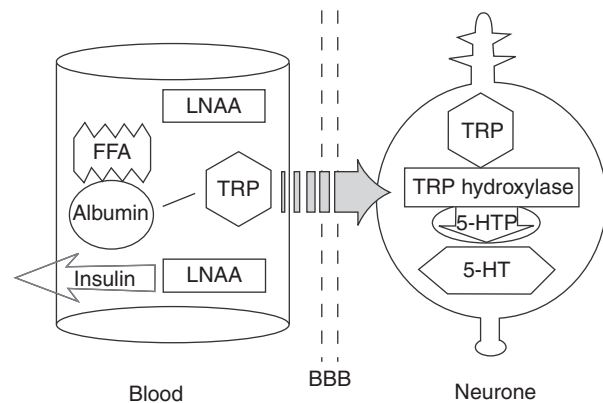


Figure 1 Diagram representing pathways to the synthesis of the neurotransmitter 5-hydroxytryptamine (5-HT, serotonin) from the precursor essential amino acid, tryptophan (TRP). TRP is taken up by neurones from blood, but its passage across the blood brain barrier (BBB) is in competition with another group of essential amino acids known as the large neutral amino acids (LNAA). Thus, the ratio of TRP to total LNAA (TRP:LNAA) determines how much TRP enters the brain. Most TRP is normally bound to albumin in plasma, so not available for uptake into the brain. However, after a carbohydrate-rich low-protein meal, increased release of insulin raises levels of free fatty acids (FFA) in plasma, and these displace TRP from albumin. In addition, insulin promotes tissue uptake of the LNAA from plasma. Hence, the TRP:LNAA ratio increases and more TRP enters the brain. Increased availability of TRP in neurones drives greater synthesis of 5-HT because the rate-limiting enzyme, TRP hydroxylase, which converts TRP to the intermediate 5-hydroxytryptophan (5-HTP), is not fully saturated.

the blood, especially the branched-chain amino acids, leucine, isoleucine, and valine. On the other hand, TRP is scarce in most protein sources, and is readily metabolized on passage through the liver: thus, the plasma ratio of TRP to competing amino acids falls after a protein-rich meal. Indeed, the protein-induced reduction in plasma TRP ratio often seems to be more marked than any carbohydrate-induced rise. Such effects also depend on the interval since, and nutrient content of the last meal.

This evidence is particularly relevant to dietary effects on mood and arousal, because 5-HT has long been implicated in sleep, as well as affective disorders such as depression and anxiety. However, cognitive performance might also be affected, given the known role of 5-HT in responsiveness to environmental stimuli and stressors, impulsivity, and information processing. Importantly, there is evidence that dietary availability of TRP can influence brain function in humans: for instance, feeding a TRP-free diet, which considerably reduced plasma TRP (and so could be expected to impair 5-HT function) induced depression in previously recovered depressives or in people with a genetic predisposition to depression. Furthermore, a TRP-free drink has been shown to impair performance on tests of visuospatial and visual discrimination learning, as well as memory. In addition, TRP depletion enhances accuracy of predicting events associated with negative consequences, and reduces accuracy of emotional face recognition, supporting the theory that 5-HT normally biases attention in favor of positive events. Typically, manipulations that should increase 5-HT synthesis and release, such as

consuming TRP-rich proteins, produce opposite behavioral effects.

There is evidence that people feel calmer and more sleepy after snacks or meals rich in carbohydrate but virtually free of protein (an unusual situation) than after protein-rich meals with little carbohydrate. This is compatible with changes in 5-HT function, but these studies did not determine whether this is due to an increase in 5-HT after the carbohydrate-rich meal, or a decrease after the protein meal, which could prevent the postprandial sleepiness. Furthermore, adding more than 5 or 6% protein (of total energy) to the carbohydrate meal has been shown to prevent the increased synthesis of central 5-HT, relative to fasted levels, in both rats and people (see Figure 2). Also, even pure carbohydrate does not appear to induce sleepiness in everyone.

Another difficulty in comparing effects of carbohydrate and protein intake is that relative changes in mood and performance might be due to protein-induced raised plasma tyrosine (TYR), the precursor amino acid for synthesis of the catecholamine neurotransmitters (adrenaline, noradrenaline, dopamine), which also competes with LNAA for entry into the brain. In catecholamine systems where the neurones are firing rapidly, acute physiological increases in brain TYR, for example, by feeding a high-protein diet, can raise TYR hydroxylation rate and catecholamine turnover. Such systems include dopaminergic neurones involved in arousal, attention, and motivation. Nevertheless, high-protein meals in human beings do not always raise the plasma TYR/LNAA ratio, depending on nutritional status or time of day.

Differential effects on performance have been seen with less extreme variations in protein and carbohydrate intake. For example, a lunch of 55% energy as protein and 15% as carbohydrate produced faster responses to peripheral stimuli,

but greater susceptibility to distraction, compared to eating the reverse proportions of protein and carbohydrate. Sleepiness was not affected by macronutrient composition in that study. However, with these protein:carbohydrate ratios, the plasma TRP/LNAA ratio could still be lowered by the protein-rich meal relative to the carbohydrate-rich one, even if TRP/LNAA does not rise from premeal levels after a carbohydrate-rich meal with much more than 5% protein (Figure 2).

A delay of at least 2 h after eating may be necessary to allow neurotransmitter precursor changes to influence behavior. Earlier effects may be related to changes in glucose availability, and levels of insulin and counter regulatory hormones such as adrenaline, glucagon, and cortisol. These changes could underlie recent results with breakfasts of 20:80, 50:50, and 80:20% protein:carbohydrate ratios (1.67 MJ, 400 kcal). A measure of central attention improved initially after the carbohydrate-rich breakfast, but then later improved after the protein-rich ones; the opposite was found for peripheral attention. This study also found that the 80% protein breakfast produced the best short-term memory performance approximately 1–2 h after eating, but not at 3.5 h.

Effects of Dietary Fat

Most studies of effects of fat have varied its level with that of carbohydrate, while keeping protein constant and so allowing equicaloric meals. Comparisons have been made for low-fat (e.g., 11–29% of energy as fat), medium-fat (e.g., 45%), and high-fat (e.g., 56–74%) breakfasts, midmorning, and midday meals, as well as intraduodenal infusions of lipid or saline. On balance, high-fat meals appear likely to increase subsequent fatigue and reduce alertness and attention, relative to high-carbohydrate/low-fat meals. However, there are inconsistencies relating to changes in specific moods and effects of

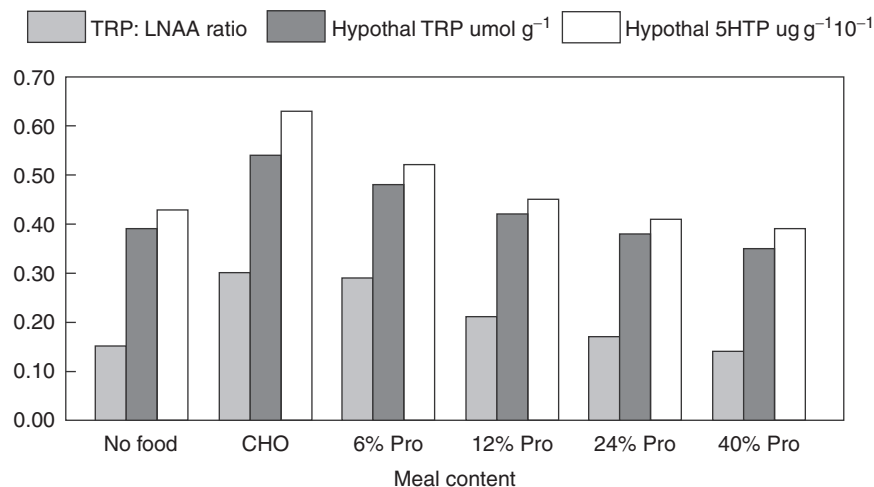


Figure 2 The data show the effect in rats of no meal, a carbohydrate meal with no protein, and one with increasing amounts of protein, on (a) the plasma ratio of tryptophan to the large neutral amino acids (TRP:LNAA) with which TRP competes for entry across the blood brain barrier (crosshatched bars) (b) levels of TRP ($\mu\text{mol g}^{-1}$) measured in the hypothalamus of rat brain (hatched bars) (c) the levels of 5-hydroxytryptophan ($\mu\text{g g}^{-1} 10^{-1}$), an intermediate precursor of serotonin synthesis, in the hypothalamus. The rise in TRP entering the brain after a carbohydrate meal drives increased serotonin synthesis, but this effect is progressively inhibited by increasing protein content. The figure is based on data obtained from Fernstrom MH and Fernstrom JD (1995) Brain tryptophan concentrations and serotonin synthesis remain responsive to food consumption after the ingestion of sequential meals. *American Journal of Clinical Nutrition* 61: 312–319, with permission from American Society for Nutrition.

meal timing: for instance, feelings of drowsiness, confusion, and uncertainty were found to increase after both low- and high-fat lunches but not after a medium-fat lunch. One possibility is that mood may be adversely affected by meals that differ substantially in macronutrient composition from habitual ones. An alternative is that similar mood effects could be induced (albeit by different mechanisms) by high carbohydrate in one meal, and high fat in the other: for example, 1.67 MJ (400 kcal) drinks of pure fat or carbohydrate taken in the morning both increased an objective measure of fatigue relative to a mixed-macronutrient drink, although the two single-nutrient drinks had opposite effects on plasma TRP/LNAA ratios; this is of course an unusual situation and may not generalize to normal mixed-nutrient meals.

In many of these studies, the meals were designed to disguise variation in fat level from participants. It is therefore possible that effects on mood may have resulted from discrepancies between subjects' expectations of certain post-ingestive effects, and the actual effects that resulted from neurohormonal responses to detection of specific nutrients in the duodenum and liver. A case in point may be the increase in tension, 90 min postlunch, with increasing fat intake, reported by predominately female subjects: this might reflect an aversive reaction to (unexpected) fat-related postingestive sensations.

Postprandial declines in arousal can be quite noticeable 2.5–3 h after high-fat meals, but fat in midmorning meals seems more sedating than at lunchtime, which might relate to expectations. By comparison, when lipid was infused directly into the duodenum, a decline in alertness was apparent much sooner, by 30–90 min after the meal. These effects of fat may result from increased release of the gastric regulatory hormone, cholecystokinin. However, in a study comparing ingestion of pure fat, carbohydrate, and protein (1.67 MJ, 400 kcal, at breakfast), measures of memory, attention, and reaction time deteriorated more after carbohydrate and protein than after fat. This beneficial effect of fat was attributed to the demonstrated relative absence of glycemic and hormonal (insulin, glucagon, and cortisol) perturbations in the 3 h following fat ingestion.

Carbohydrates, Stress, Mood, and Mental Function

Susceptibility to Mood Enhancement by Diet

The possibility that a carbohydrate-rich low-protein meal could raise 5-HT function gave rise to the proposal that some depressed people may self-medicate by eating carbohydrate, so leading to increased 5-HT release in a manner reminiscent of effects of antidepressant drugs, which enhance aspects of 5-HT function by inhibiting removal of 5-HT from the synaptic cleft between nerve cells. For the most part, however, early behavioral and pharmacological evidence for such a phenomenon was not very convincing.

Nevertheless, recent research provides some further support for beneficial effects of carbohydrate-rich/protein-poor meals on mood and emotion in some people. When participants were divided into high or low stress-prone groups, as defined by a questionnaire, carbohydrate-rich/protein-poor

meals before a stressful task were found to block task-induced depressive feelings and release of the glucocorticoid stress hormone, cortisol, but only in the high stress-prone group. This finding was replicated using high- versus low-TRP containing proteins (alpha-lactalbumin and casein, respectively). It was argued that, because stress increases 5-HT activity, the poor stress-coping of this sensitive group might indicate a deficit in 5-HT synthesis that is improved by this dietary intervention.

There is another link between macronutrient intake, stress, and mood. Chronic dysfunction of the stress-sensitive hormone, cortisol, and its controlling hypothalamic pituitary adrenal (HPA) axis, is associated with depression and anxiety, as well as abdominal obesity. Moreover, protein-rich meals that prevent a meal-induced fall in arousal also stimulate release of cortisol in unstressed people, and the size of this effect is correlated positively with poor psychological well-being. Chronically, a carbohydrate-rich diet is associated with better overall mood state and lower average plasma cortisol than a high-protein diet. Acutely, a carbohydrate preload, but not protein or fat load, enhances cortisol release during stress. This may be related to findings from both human and animal research suggesting that eating carbohydrate-rich and perhaps high-fat foods can help restore normal HPA axis function and glucocorticoid stress responses. Raised levels of cortisol in stressed people contribute to insulin resistance, which in turn promotes abdominal obesity. However, insulin resistance may increase the likelihood that high-carbohydrate/low-protein foods would raise brain TRP and 5-HT levels, because of increased levels of plasma fatty acids, which result in more unbound TRP in plasma. Conversely, it has also been found that high baseline cortisol predicts induction of depression by dietary depletion of TRP. This might underlie recent findings that insulin resistant people are less prone to suicide and depression, both of which are believed to be increased by low 5-HT function. Similarly, patients with Seasonal Affective Disorder show increased insulin resistance in the winter, together with a greater predilection for sugar-rich foods. Unfortunately, despite this protective effect, insulin resistance is a substantial risk to health by promotion of cardiovascular disease.

Sugars and Opioids

Endogenous opioids are released during stress, and are known to be important for adaptive effects such as resistance to pain. They are also involved in motivational and reward processes in eating behavior, such as stimulation of appetite by palatable foods. Perhaps the best evidence for opioid involvement in an interaction between stress and eating is the finding that, in animals and human infants, the ingestion of sweet and fatty foods, including milk, alleviates crying and other behavioral signs of stress. Recently, this effect was shown to depend on sweet taste rather than calories, as non-nutritive sweeteners also reduce crying. This stress-reducing effect can be blocked by opioid antagonists. The conclusion that adults select sweet fatty foods for opioid-mediated relief of stress is tempting, but remains speculative. Also, such behavior would need to be explained in the context of stress itself enhancing endogenous opioid release.

Glucose, Mood, and Mental Function

The possibility that ingesting glucose could alter mood and improve mental function has generated considerable research interest. However, there is only space here to summarize and interpret the key findings and controversies. The interest in glucose arises from two observations: (1) that the primary source of energy for brain function is glucose, and (2) that mental function and mood deteriorate when blood glucose concentration falls below basal physiological levels, i.e., hypoglycemia ($<3.6 \text{ mmol l}^{-1}$).

The first observation must be qualified by a recent evidence that (1) in times of metabolic demand, the brain can also use lactate very effectively as an energy source, and (2) the brain contains significant stores of glycogen in specialized cells called astrocytes, which can be metabolized for energy by neighboring neurones. Nevertheless, in rats, extracellular glucose levels in a specific region of the brain critical for memory, the hippocampus, decline to a greater extent during more demanding memory tasks, and this decline is prevented by a systemic glucose load.

As for hypoglycemia, this is rarely induced by normal food, although large amounts of sugar-rich drinks on an empty stomach might do so in some people. Yet, many studies of effects of glucose use a method similar to the Oral Glucose Tolerance Test (OGTT), in which fasted patients drink aqueous solutions containing 50–75 g of some form of glucose. This is not meant to be a normal nutritional manipulation, but a test of glucoregulation. Associations have been reported between rapid and substantial declines in blood glucose after OGTTs and aggressive thoughts and behavior: however, this might be mediated by greater counter-regulatory hormone release.

In studies comparing sugar-rich drinks with zero-energy sweet placebos, many find no effect on mood, but some report a rise in subjective energy within an hour, followed by increased calmness. In children, controlled studies failed to support the popular myth that sugar is excitatory: again, it had either no effect or was calming. However, it is worth noting that some adults, and probably children, are especially sensitive to rapid drops in blood glucose, showing counter-regulatory hormone release and 'hypoglycemic symptoms' even though actual hypoglycemic levels of glucose are not reached.

It may be that beneficial effects of glucose ingestion only become consistently apparent when demands are placed on mental function or when there is a compromised nutritional state such as food deprivation or a metabolic disorder. The findings on glucose and cognitive performance can be summarized as follows:

- The majority of studies that administered a glucose drink found subsequent improvements in some performance compared to placebo, particularly on tests of short-term memory or vigilance tasks that require a large component of 'working memory'.
- Improvements in performance can be associated with rising or falling blood glucose, even independently of consuming a glucose load.
- Young healthy subjects require more demanding tasks than the elderly to detect a beneficial effect of glucose load.

- Associations between performance and glucose may be mediated by individual glucoregulatory efficiency.
- Both glucoregulation and performance are influenced by hormones sensitive to stressful or arousing cognitive tasks, such as adrenaline and cortisol. Emotion-dependent learning resists improvement by glucose.
- Personality, stress-sensitivity, and task involvement can influence glucose uptake and disposal, and also the effects of glucose on cognition.

However, one important pattern does emerge that memory performance is worse in poorer than better glucoregulators (see Figure 3). This is true not just for elderly patients but among a healthy student population too, especially if the task is sufficiently demanding. Peak blood glucose predicts poor memory performance in elderly patients, whether or not a glucose load has been given before testing. This relationship between raised glucose levels and poor memory performance could underlie a recent finding that a snack with a high glycemic index (greater plasma glucose rise) resulted in poorer memory performance 2–3 h later, relative to a low glycemic index snack. Even so, it seems that a moderate glucose load can lessen the memory deficit present in young and old poor glucoregulators (with little consistency or no effect in good glucoregulators).

One reason why poor glucoregulation predicts poor memory performance may be that glucose intolerance is associated with higher basal and stress-induced cortisol secretion. Raised cortisol is known to impair memory, probably by an action on hippocampal neurons, including inhibition of glucose uptake. However, the substantial rise in insulin induced by a glucose load in poor glucoregulators may overcome the negative impact of cortisol in some cases: hyperinsulinemia induced independently of hyperglycemia has been shown to ameliorate memory deficits in patients with Alzheimer-type dementia.

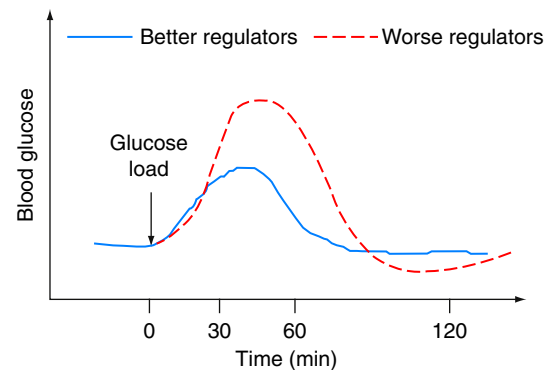


Figure 3 The graph represents a model of changes in blood glucose levels after a glucose load in people who are either good regulators (solid line) or poor regulators (broken line) of blood glucose. The increased peak and delayed recovery of blood glucose in worse regulators suggests glucose intolerance and insulin resistance. In such people, there may be a brief period of mild hypoglycemia before return to baseline levels. This type of difference in glucoregulation has been demonstrated in both young and elderly people without diabetes. Poor glucoregulation predicts poor cognitive performance in challenging tests, especially those involving memory.

Two other mechanisms might explain the ability of a glucose load to improve performance in subsequent challenging tasks. One is an increase in sympathetic activation by the glucose load: adrenaline is known to enhance memory. The other is increased synthesis and release of the neurotransmitter acetylcholine during challenging tasks: acetylcholine is also known to be critically involved in learning and memory, and is synthesized from dietary choline and acetyl CoA, which is a by-product of glucose metabolism. These complex interactions between glucose ingestion and brain function are illustrated in Figure 4.

Docosahexaenoic acid (DHA), a long-chain polyunsaturated fatty acid (PUFA) of the omega-3 series may also be an important regulator of brain energy metabolism and glucose uptake. For example, feeding rats an omega-3 PUFA deficient diet decreased brain DHA levels and reduced brain glucose uptake by 30–40% and cytochrome-c oxidase activity by 20–40% in the fronto-parietal cortex, hippocampus, and suprachiasmatic nucleus, compared to animals on the control diet. The level of expression of the GLUT-1 glucose transporters were also decreased in endothelial cells, although GLUT-3 levels were unaffected in neurons. Impairment in brain (especially hippocampal) glucose uptake is a common feature of age-related cognitive decline, and treatment with DHA may provide a potential therapeutic approach to support the maintenance of brain energy metabolism.

Hyperactivity and Antisocial Behavior

In children, there is an increasing frequency of diagnosis of ADHD, a condition characterized by inattention, impulsive,

and disruptive behavior, learning difficulties, and increased levels of gross motor activity and fidgeting. Also, prevalence of food allergies and intolerance has been increasing. Perhaps it is not surprising that dietary explanations and treatments for ADHD have been sought regularly for several decades, given theories of allergic reactions or intolerance to food additives, ingredients in chocolate, and even refined sugar (often grouped as the 'Feingold Theory', after an early instigator of unproven dietary intervention). There has also been a long-standing interest in the possibility that antisocial behavior in children and adults might in part result from poor nutrition, although early studies were poorly designed. A behavioral effect of sugar and of many additives has by and large not been supported by controlled studies: however, determining unequivocally whether behavior of young children is affected by specific dietary components is difficult to achieve. ADHD may be associated with disrupted eating behavior and poor nutrition, so that removal of a number of nutrient deficiencies might improve behavior. In addition, parents or unqualified health professionals may devise unsuitable dietary regimes that can raise the risk of undernutrition. As a result, there is little consensus as to what in the diet may or may not provoke disturbed behavior in children, other than that only a small minority of children are likely to be affected. Nevertheless, a recent British study, in which children were given a collection of food colorings and preservatives, or placebo in drinks, found deterioration in behavior reported by parents for both hyperactive and normal children given the additives, which seemed unrelated to allergic history. This effect was not detectable in a clinical setting. Clearly, a definitive answer awaits more research.

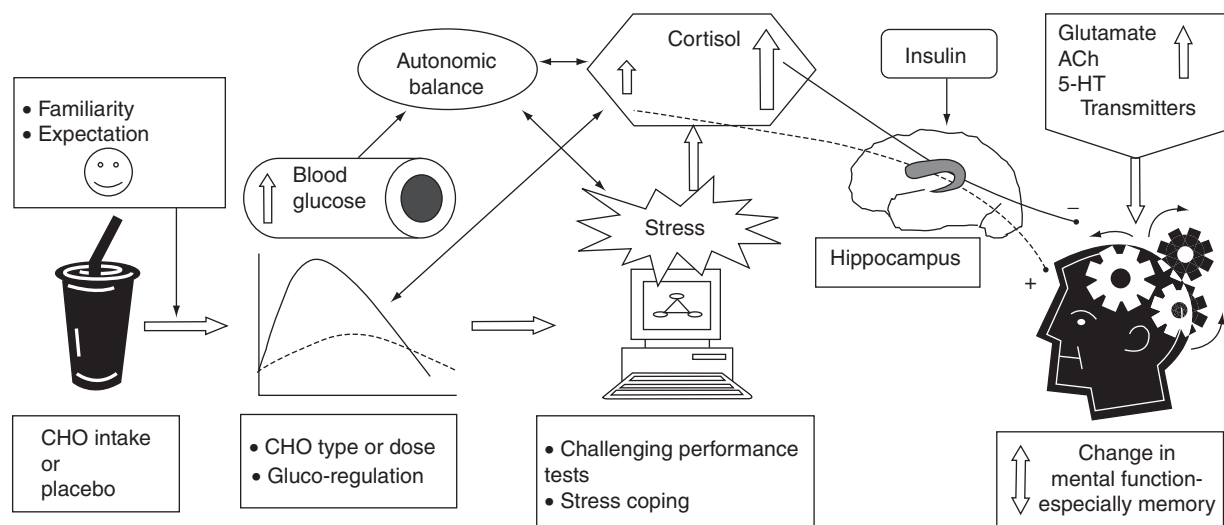


Figure 4 A summary diagram of putative pathways linking carbohydrates and mental function, with various modulatory influences. One explanation for variability in findings is that some individuals, especially if poor glucoregulators, may be much more susceptible to manipulations of mood and mental function by glucose availability, particularly when brain function is increased by a stressful challenge. However, associations between glucoregulatory ability and mental performance may be more directly mediated by differential release of cortisol than by changes in glucose availability. It is suggested that this neuroendocrine consequence of glucose absorption and stressful tasks might help explain the narrow dose-response function, and why slower glucose delivery, for example, by low-GI foods, has been reported to benefit cognition compared to faster glucose delivery. Identifying sensitive individuals, and the relevant nutritional, physiological, and psychological parameters, remains a potentially fruitful topic of research in this area. Reproduced with permission from Gibson EL (2007) Carbohydrates and mental function: Feeding or impeding the brain? *Nutrition Bulletin* (Supplement 32), 71–83.

Specific deficiencies or imbalances in blood levels of essential fatty acids, particularly omega-3 polyunsaturated fatty acids (PUFAs), have consistently been shown in developmental disorders such as ADHD, dyslexia, dyspraxia, and autistic spectrum disorders. Indeed the severity of ADHD correlates with the level of DHA deficiency in plasma, although the nature of this relationship is unclear. There is a strong rationale for investigating the effects of essential fatty acid supplementation in children with ADHD; however, so far few randomized controlled trials have been undertaken, and results have been inconclusive. For example, two double-blind trials showed no benefits following DHA treatment, whereas another, which provided a mixed omega-3 and omega-6 PUFA supplement found significant improvements in symptoms. Given the safety and tolerability of essential fatty acids, these compounds offer an intriguing prospect as a potentially new therapeutic approach and the evidence so far strongly supports the case for further research.

Other effects of essential fatty acids on cognition are discussed below.

Micronutrients and Mental Function

There has been, over the years, an increasing body of evidence suggesting that vitamin and mineral status is significantly related to both brain development in childhood and the degree of cognitive decline experienced as we age. Indeed, it is certainly the case that deficiencies of some vitamins are associated with negative neurological symptoms such as neural tube defects. The work examining vitamin and mineral supplementation comprises both cohort studies and nutritional interventions and has generated much confusing and contradictory data. To a large degree, this confusion and contradiction is dependent on a number of factors such as the methodological rigor of each study, the measures of cognitive function used and the precise nutrient being studied.

Early work in this area concentrated on the notion that supplementing the diet of schoolchildren with multi-vitamin supplements would improve both their IQ scores and academic achievement. This work was controversial and marked by a number of deficiencies, such as any clear indication as to whether participants were actually nutritionally compromised before treatment, difficulties in determining, which if any, of the vitamins in the cocktail were producing effects, and the lack of any clear hypotheses regarding mechanisms responsible. The consensus now is that supplementation will have a benefit on cognitive development and IQ (especially non-verbal) in a minority of children who are not otherwise adequately nourished. In Britain and the USA at least, there is particular concern that a significant proportion of adolescents, especially girls, are deficient in iron. There is good evidence that iron deficiency contributes to poor cognitive ability, perhaps in association with low vitamin C status, which has also been linked to reduced cognitive function. Iron is known to be essential for synthesis and function of neurotransmitters, such as dopamine, noradrenaline, and serotonin (5-HT). Selenium is another mineral, which may be important for brain function, and low levels of which have been associated with cognitive decline and depressed mood in the European population. There is also evidence that zinc deficiency is

associated with problematic cognitive development in children and that dietary supplementation with zinc leads to cognitive improvements, relative to nonsupplemented controls.

Much recent work, however, has concentrated on the use of vitamins in the treatment of age-related cognitive decline and dementia and, to varying degrees, is more scientifically rigorous than the earlier work. The overwhelming majority of the experimental work has targeted the action of two groups of micronutrients; anti-oxidant and B-complex vitamins. The work concerning the effects of antioxidant vitamins, although showing some promise with correlational studies in that levels of these vitamins (vitamin E most consistently) are associated with function in a range of cognitive domains, is more contradictory when one considers the clinical intervention trials. The work on B-complex vitamins is, however, more consistent and supported by a strong hypothetical basis. This relies on the role of vitamins B₁₂ and folate in methylation of membrane phospholipids and neurotransmitters, and in breaking down the toxic sulfur-amino acid homocysteine. High levels of homocysteine are now considered by some to be a far greater risk factor for the development of coronary and vascular problems than high levels of cholesterol. Elevated levels of homocysteine may be a cause of minor ischemic events, which cumulatively, lead to a degradation of cognitive function due either to sub-clinical deficiencies of, or problems with the absorption of, B-complex vitamins. Indeed, a large number of studies has consistently demonstrated relationships between homocysteine levels, B-complex vitamin levels and neuropsychological task performance. The number of direct intervention trials, which have supplemented the diet of the elderly with B-complex vitamins is, however, small. Although some studies have shown no net benefit of supplementation on the cognitive function of the elderly, a larger number of studies have shown a stabilization of cognitive function and reduction of homocysteine levels to result from B-complex vitamin supplementation. As with the antioxidant vitamins, however, these studies must be interpreted with a degree of caution because they use differing dosages, periods of supplementation, and measures of neuropsychological function.

Lipids

Lipids are another nutrient category, which has attracted a good deal of research interest in terms of their possible effects on psychological function. The main nutrients studied fall into three groups; these being cholesterol, omega-3 and omega-6 essential fatty acids, and phospholipids. In general, the theoretical basis underpinning the effects (or lack of) these nutrients on psychological function relates to how their relative concentrations affect cell membrane fluidity. The rigidity of lipid bilayers of cell membranes is thought to be essential for neurotransmitter function by maintaining maximum exposure of receptors at the synaptic cleft between neurons.

Cholesterol

The interest in cholesterol as a substance that is related to psychological well-being stems back to the 1980s. During

this period, a number of epidemiological studies found that individuals with low cholesterol levels were more prone to aggressive behavior, risk of suicide, and violent death. In addition, it was also found that nonhuman primates increased their incidence of aggressive behavior when kept on a low cholesterol diet. In terms of neuropsychological function, a number of studies have found associations between cholesterol levels and choice reaction time or memory function. Two of these studies to date have sought actively to reduce cholesterol levels by means of pharmacological or dietary means, both finding that lowering cholesterol produced small but statistically significant impairments in memory and attention. Conversely, however, a number of studies have also demonstrated that high cholesterol levels are also a significant risk factor for the development of Alzheimer Dementia. One mechanism for these negative effects of cholesterol lowering may be loss of rigidity in neural cell walls, thereby decreasing the relative exposure of serotonin (5-HT) membrane receptors at the synaptic cleft and impeding 5-HT signal transmission. Interestingly, omega-3 PUFA have been shown to reduce the cholesterol content of neuronal membranes.

Essential Fatty Acids

The central nervous system is highly enriched in long-chain polyunsaturated fatty acid (PUFA) of the omega-6 and omega-3 series, specifically arachidonic acid and docosahexaenoic acid (DHA), respectively. The presence of these fatty acids as

structural components of neuronal membranes influences cellular function both directly, through effects on membrane properties, and also by acting as a precursor pool for lipid-derived messengers. For example, DHA alters membrane properties and thereby influences membrane proteins, such as receptors, ion channels, and enzymes. It alters dopaminergic, serotonergic, and cholinergic neurotransmission, regulates signal transduction pathways, production of eicosanoids and other lipid-derived messengers, regulates transcription factors and gene expression, and stimulates hippocampal neurogenesis and neurite outgrowth. A summary of some of the potential mechanisms of action of omega-3 PUFA is shown in Figure 5.

An adequate intake of omega-3 PUFA is essential for optimal visual function and neural development. Fetal development has a high requirement for essential fatty acids, especially DHA. Maternal DHA levels influence infant levels, such that higher maternal levels produce higher levels in the neonate. Higher level of maternal plasma DHA during pregnancy correlates with more mature neonatal sleep-state patterns, which is a measure of maturity in the central nervous system. Such effects probably underlie evidence that higher intake of seafood during pregnancy is associated with improved social and cognitive function in the offspring, and may contribute to the advantage to cognitive development seen for breast versus formula-fed infants.

There is increasing evidence that an increased intake of the long-chain omega-3 PUFA, eicosapentaenoic acid (EPA), and

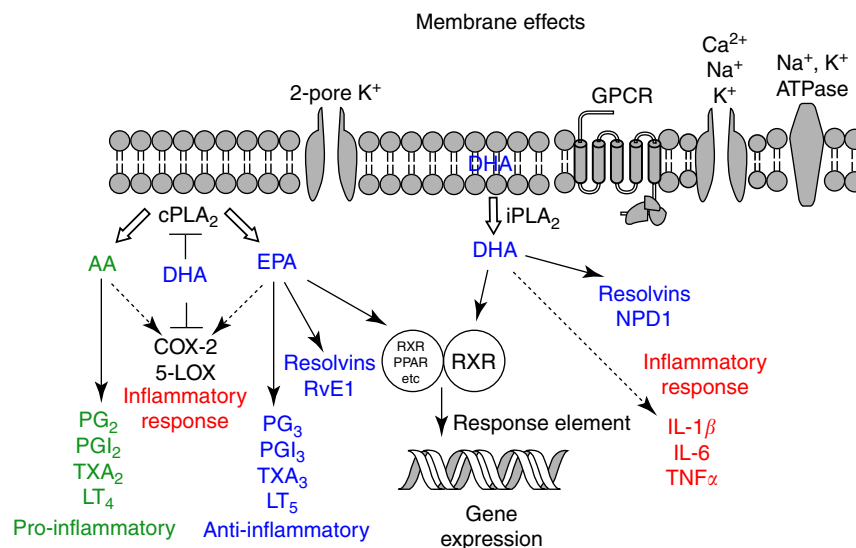


Figure 5 EPA and DHA potentially operate through a variety of overlapping mechanisms of action. These are related to direct actions on plasma membranes, altered inflammatory response and control of gene expression. The effects on membrane bound proteins such as ion channels, G-protein coupled receptors (GPCR), and the Na⁺, K⁺ ATPase appear to relate to alterations to the biophysical properties of the cell membrane. Alterations in inflammatory response are mediated through competition between AA and EPA for eicosanoid biosynthetic enzymes, with a high EPA content favouring the production of EPA derived anti-inflammatory mediators, such as series 3 prostaglandins, prostacyclins and thromboxanes, and series 5 leukotrienes. Nonesterified EPA and DHA are also the precursors of anti-inflammatory resolvins, such as RvE1 and NPD1, respectively. Nonesterified EPA and DHA may also regulate gene expression via transcription factors, such as retinoid and peroxisomal proliferator signaling pathways. Solid arrows indicate positive effects, flat arrow-heads inhibition, dotted arrows competition, and open arrows phospholipase A₂-induced release from the cell membrane. Abbreviations: 2-pore K⁺, 2-pore potassium channel; Ca²⁺, L-type calcium channel; cPLA₂, cytosolic PLA₂; iPLA₂, Ca²⁺-independent PLA₂; GPCR, G-coupled protein receptor; K⁺, K_v, and K_{ir} channels; LT, leukotriene; Na⁺, voltage-gated sodium channel; PG, prostaglandin; PGI, prostacyclin; PPAR, peroxisomal proliferator-activated receptor; RXR, retinoid X receptor and TXA, thromboxane.

DHA, may confer benefits in a variety of psychiatric and neurological disorders, and in particular neurodegenerative conditions. For example, patients suffering from depression typically exhibit reduced DHA levels in plasma, and several cross-sectional studies show associations between seafood consumption and rates of both depression and bipolar disorder, suggesting that low omega-3 PUFA levels may be a factor in the etiology of these disorders. Furthermore, a recent meta-analysis of trials involving patients with major depressive disorder and bipolar disorder has provided evidence that omega-3 PUFA supplementation reduces symptoms of depression.

It is beyond the scope of this article to discuss all of the evidence however, a number of lines of research suggest that an elevated intake of EPA and DHA may confer benefits in depression, schizophrenia, Alzheimer's disease, and Parkinson's disease. However, the mechanisms underlying these beneficial effects are still poorly understood. It is very likely that EPA and DHA operate through a number of different, potentially overlapping mechanisms, involving various cellular targets. These PUFA can act as endogenous agonists for transcription factors such as the retinoid receptors, and the peroxisome-proliferator activated receptors. Neuronal excitability can be modulated by PUFA through their effects on sodium and calcium channels or the activation of the TREK potassium channels, which are two-pore potassium channels abundantly expressed in the brain. Furthermore, it cannot be ruled out that their effects may also involve specific fatty acid receptors, such as the recently identified GPR40 receptor, which has a widespread expression in the central nervous system. Because many neurological conditions share common features, such as excitotoxicity, oxidative stress, and inflammation, which appear to be modified by omega-3 PUFA, this may explain the therapeutic potential of EPA and DHA across disease boundaries. However, the relative importance of certain mechanisms and targets may vary depending on the condition considered.

Membrane-bound DHA is also a positive modulator of biosynthesis of the membrane phospholipid phosphatidylserine in neuronal tissues. It has been suggested that the observed anti-apoptotic (i.e., cell protective) effect of DHA is due at least in part to the DHA-induced phosphatidylserine accumulation. A further potential benefit of DHA-induced phosphatidylserine accumulation may be to prevent the age-related decline in cognitive ability, because phosphatidylserine supplementation has been shown to improve age-related decline in cognitive ability and memory. Certainly, epidemiological evidence links diets higher in PUFA to saturated fat ratio to improved cognitive function in the elderly.

Food Deprivation

There is evidence to suggest psychological effects of undernutrition. In severe cases such as anorexia nervosa, neuropsychological function is impaired primarily as a result of structural changes in brain anatomy resulting from starvation. Evidence that undernutrition is associated with psychological problems in those not suffering eating disorders was first hinted in the Minnesota Study of Semi-Starvation in the

1950s. Volunteers who were kept on a half-calorie intake diet for a period of months reported mood swings, increased irritability, poorer memory, and an inability to concentrate. Although these self-reported effects were not supported by objective testing, the lack of a nondeprived control group means that this lack of effect could have been masked by a practice effect.

Dieting to lose weight is one of the most common food choice related behaviors in the Developed World and it has been consistently associated with negative psychological consequences such as preoccupation with body shape and depression. In addition, a number of investigators have also found that dieting to lose weight is associated with impairments in cognitive function, with dieters performing more poorly than nondieters on measures of reaction time, immediate memory, and the ability to sustain attention. This is unlikely to be due to pre-existing differences between individuals who happen to be dieting or not dieting at the time of testing because, within the same individuals, performance is poorer when dieting than when not dieting. It is unlikely that these effects are due to the gross physical effects of food deprivation because experimentally induced food deprivation of varying lengths fails to produce a comparable impairment in task performance, in addition to the poorer task performance being found amongst dieters who claim not to have actually lost any weight over the course of the diet.

Rather than being a function of food deprivation per se, the poorer task performance amongst current dieters appears to be a function of the preoccupying concerns with hunger and body shape, which are characteristic of dieters. Indeed, the impairments in task performance amongst dieters appear to be comparable in both structure and magnitude found to result from the preoccupying concerns of the characteristic of clinical depression and anxiety disorders. Specifically, the primary deficit appears to be a reduction in the amount of available working memory capacity, working memory being the primary cognitive system which serves to allocate processing capacity to ongoing cognitive operations. A threshold hypothesis has been formulated to account for this phenomenon. Nondietering, highly restrained eaters are characterized by an enduring, trait concern with body shape, which consumes a certain amount of working memory capacity (explaining why nondieting restrained eaters perform at a level intermediate to that of current dieters and unrestrained eaters). When they decide to diet, they then experience preoccupations with food and an increased desire to eat, this extra drain on working memory capacity reaches a point where sufficient capacity is unavailable to maintain task performance. Support for this hypothesis can be seen in a study in which highly restrained nondieters were instructed to imagine eating their favorite food or their favorite holiday while performing a reaction time task. When imagining their favorite food, but not their favorite holiday, restrained nondieters performed as poorly as current dieters on the reaction time task.

Although evidence seems to be mounting that the poor cognitive function of current dieters is due to psychological and not biological factors, work still continues to examine some of the more subtle possible biological mechanisms which, may underlie the effects. One possible mechanism is that a low dietary intake of the amino acid TRP (the precursor

for 5-HT) leads dieters to have impaired serotonergic function. However, analysis of the urine of dieters for the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA), found no evidence for this. Another possibility (not yet investigated) is that, by avoiding eating red meat, dieters experience mild iron deficiency, with deleterious consequences for hemoglobin status, brain oxygen supply, and neurotransmitter function.

The types of dieter studied so far are those who attempt to lose weight in an unsupported, unsupervised manner. Comparisons between this type of dieter and those who attempt to lose weight in the context of an organized weight loss group reveal dramatic differences. Those who diet as part of a group do not show the impairments in task performance typical of unsupported dieters. In addition, unsupervised dieters display an elevated stress response after one week of attempted weight loss (as measured by salivary cortisol levels) whereas supported dieters do not. It would appear, therefore, that the poor performance characteristic of unsupervised dieting is a result of the stress associated with this type of weight loss attempt and that the psychological manifestation of this stress is the preoccupying thoughts outlined above. The motivational processes underlying weight loss also appear to be mediating factors in any cognitive processing deficits observed, because it has been found that individuals dieting for esthetic reasons display impaired cognitive function, whereas those dieting for other, health related, reasons do not.

Functional and Pharmacological Components of Foods and Drinks

There is growing interest, particularly in the food and beverage industry, in developing foods and drinks with functional properties (nutraceuticals) attractive to the consumer. These include effects on behavior, such as improvements in cognitive function, mood, and physical performance. Components of interest include caffeine, herbal extracts such as ginkgo biloba and panax ginseng, micronutrients, essential fatty acids, amino acids, and carbohydrates. In the context of caffeine and glucose, however, there is some evidence to support the view that any beneficial effects of these substances on psychological function is, at least in part, due to an expectancy regarding their potential effects. There is some support for beneficial effects of these components, but they are not reviewed further here.

Conclusion

The scientific understanding of dietary effects on behavior has begun to move in from the fringes of respectability, indeed sufficiently to attract substantial commercial interest. Advances in nutritional and neuropsychological knowledge, experimental design, and sensitivity of measures of behavior and brain function have produced replicable findings in some areas to mollify earlier skepticism. New understanding of the impact of nutrition on brain function, and predictors of individual susceptibility, has also allowed reinterpretation of old data. Promising areas with encouraging developments in understanding include the

interactions between macronutrients, stress, and mood disorders, and the effects of vitamins, minerals, and lipids on cognition, dementia, and psychiatric disorders. Some findings, including recent awareness of poorly nourished sectors of the population, suggest useful interventions. Nevertheless, research in this field is at an early stage, and the coming years should bring further revelations on the link between diet and behavior. With industrial backing, few may escape the consequences.

See also: Appetite: Psychobiological and Behavioral Aspects. Beverages and Health. Brain and Nervous System: Biology, Metabolism, and Nutritional Requirements. Breast Feeding. Caffeine. Carbohydrates: Requirements and Dietary Importance. Children: Nutritional Requirements. Diabetes Mellitus: Etiology and Epidemiology. Eating Disorders: Anorexia Nervosa. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids. Fish and Seafood: Nutritional Value. Folic Acid. Food Choice: Behavioral Aspects. Food Culture. Food Intolerance. Functional Foods: Health Effects and Clinical Applications. Glucose: Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level. Glycemic Index. Homocysteine. Hunger. Hyperactivity: Nutritional Aspects. Hypoglycemia. Iodine: Deficiency Disorders and Prevention Programs. Iron: Physiology, Dietary Sources, and Requirements. Meal Size and Frequency: Effect on Absorption and Metabolism. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Pregnancy: Nutrient Requirements. Selenium. Sport and Exercise Nutrition. Starvation and Fasting: Biochemical Aspects. Sucrose: Dietary Sucrose and Disease. Supplementation: Dietary Supplements. Vitamin B₆: Physiology. Vitamin E: Physiology and Health Effects. Zinc: Deficiency Disorders and Prevention Programs

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Glossary

Oxygen Radical Absorbance Capacity (ORAC) A method of measuring antioxidant capacities in biological samples *in vitro*.

Introduction

Beverages, usually excluding drinking water, are referred to as fluids which are prepared for human consumption. In this article, nonalcoholic beverages have been focused on. Water and alcohol are reviewed in other articles. For clarification, definitions for different beverage categories and other terminologies that are discussed in this article are provided in Table 1.

In history, water and breast milk were the only beverages that were consumed by humans until 11 000–12 000 years ago. It is believed that other beverages appeared in the human diet no more than 11 000 years ago. Figure 1 provides a timeline for when major beverages entered the human food chain. With industrialization, more and more beverages have been introduced into the human diet. Today, beverages play a very important role in human diet and health.

Currently, a large proportion of the world’s population is consuming a significant amount of beverages on a daily basis. Globally, the availability of beverages has grown more than 20% in the past 40 years, excluding soft drinks and juices. The Coca-Cola products alone are consumed at more than 1 billion drinks per day. In the US, per capita consumption of soft drinks was 46 gallons in 2003, followed by 25 gallons of

coffee, 22 gallons of milk, 8.1 gallons of fruit juices, and 8 gallons of tea. Beverages share the same feature as fluid but vary tremendously in their energy content and nutrient composition. The nutrient composition of commonly consumed beverages is provided in Table 2. Given current consumption levels, these beverages could contribute significant amounts of calories as well as sugars, caffeine, and several other vitamins and minerals to our diet. Based on the US data in 1999, nonalcoholic beverages provided 196 mg of calcium (88% from milk), 88 mg caffeine (73% from coffee & tea and 27% from soft drinks), and 41 mg of vitamin C (60% from 100% juice) per day for the general American population.

Beverages are important sources of fluids in our diet. There is no doubt that we need fluid to provide adequate water for maintaining normal physiological function and metabolic homeostasis of the human body. It has been estimated that approximately 20% of the water we need is derived from food, and the other 80% comes from drinking water and beverages. One fundamental question we should ask is: In addition to fluids, do adults need beverages to provide energy and other nutrients? The answer is no. Historically, adults consumed predominantly water, which provided no calories. Scientifically, no evidence suggests that a healthy diet for adults relies on beverages to provide energy or other nutrients. Drinking

Table 1 Definitions of beverages

Liquid calories	All calories derived from beverages.
Fruit drinks	Sugar-sweetened drink with a small percentage of fruit juice or juice flavoring
100% juices	Beverages composed exclusively of liquids extracted from fruits and vegetables with no added calorie sweeteners
Milk	A translucent white liquid produced by the mammary glands of mammals
100% juices	Beverages composed exclusively of liquids extracted from fruits and vegetables with no added caloric sweeteners or flavorings
Soft drinks	Nonalcoholic carbonated beverages containing caloric and artificial sweeteners, including regular soft drinks and diet soft drinks
Diet beverages (diet soft drinks)	Carbonated or noncarbonated beverages sweetened with artificial sweeteners
Sugar-sweetened beverages (regular soft drinks)	Carbonated or noncarbonated beverages containing added caloric sweeteners
Added calories sweeteners	All composite sugars added to foods and beverages during production and processing, including sucrose, high-fructose corn sirup, honey, molasses, and other sirups
Artificial sweeteners	Synthetic food additives that have sweet taste but no or less energy content

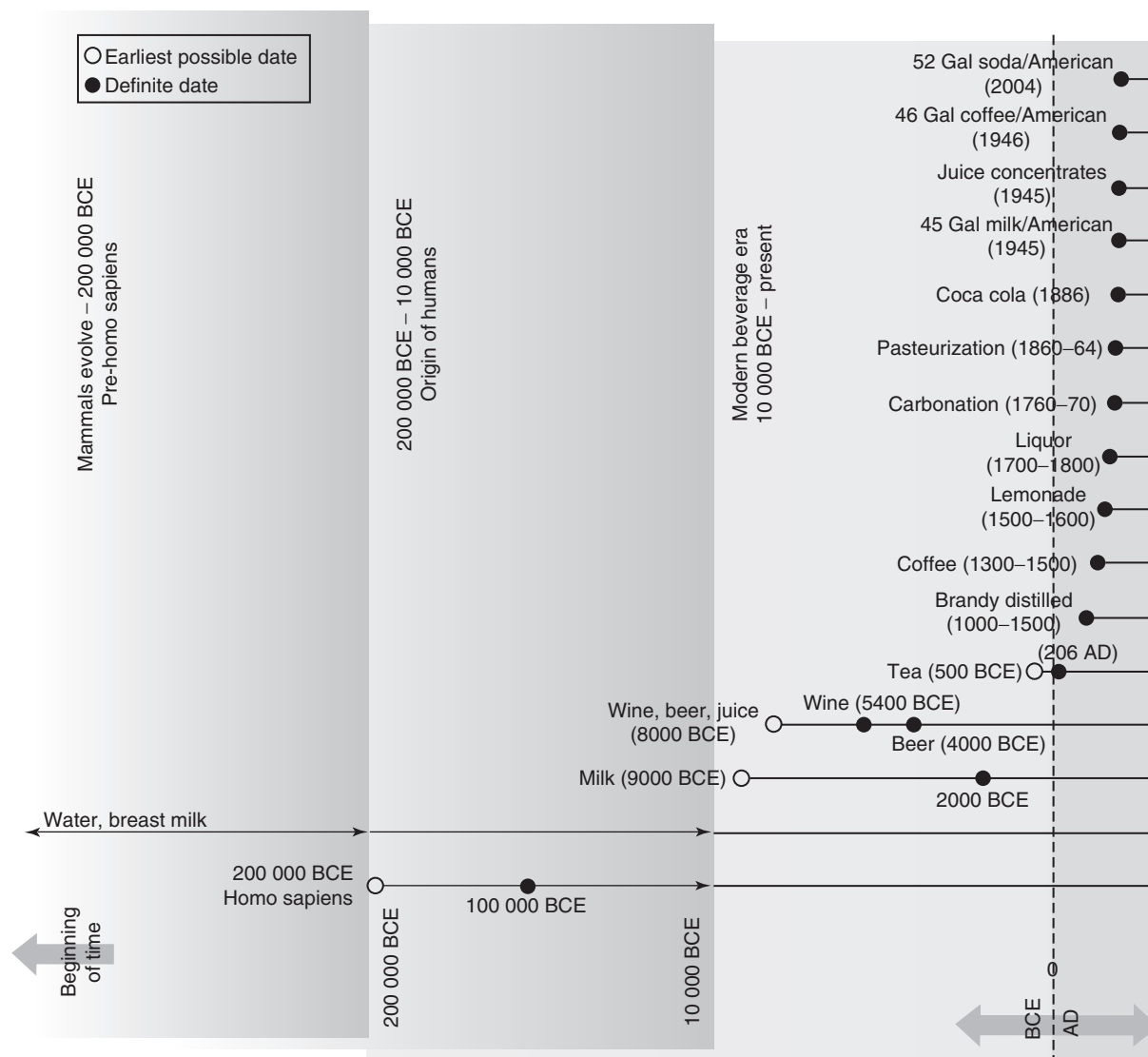


Figure 1 Beverage history timeline.

water can provide the fluids our body need as long as we maintain a healthy and balanced diet.

In the US, the average American drinks 1.5 l of beverages per day, in addition to water. The majority of these beverages contain considerable amounts of calories from sugars, high-fructose corn sirup (HFCS), or alcohol. Given such a consumption level, beverages now provide approximately 21% of total calories for the general American population. Sugar-sweetened beverages, the most popular beverages consumed in the US, are the leading source of added sugars in the Americans' diet, responsible for one-third of the total added sugar consumed by Americans. The UK and many other developed counties are heading in the same direction.

The major concern regarding such a large amount of calories from beverages is that beverages are less satiating than solid foods. Evidence suggests that compensation of energy consumed in a liquid form (liquid calories) may be less complete than energy consumed in a solid form in humans. In other

words, consumption of calorie-rich beverages does not reduce the intake of solid foods proportionally to maintain the energy balance. Therefore, when a large amount of calorie-rich beverages are consumed, the total calorie intake will increase, which may result in weight gain and other health issues over time.

In the following sections, a review of relative nutritional and health benefits and risks of various beverage categories will be provided. The beverage categories reviewed in this article are ordered according to their health benefits (from the relatively healthy to unhealthy) based on current scientific evidence.

Coffee and Tea

Tea

Tea has been widely consumed in many countries and regions for a long period of time. The origin of tea drinking can be

Table 2 Beverages nutrient composition

Beverages ^a	Black tea ^b	Coffee, brewed ^b	Whole milk (3.25% fat) ^b	1% fat milk, vitamin A & D-fortified ^b	Skim milk, vitamin A & D-fortified ^b	Soy milk, plain (Silk) ^c	Orange juice, original (Minute Maid) ^d	Apple juice ^e	Tomato juice (V8) ^e	Diet Coke ^f	Diet Pepsi ^g	Coca-Cola Classic ^f	Pepsi Cola ^g	Arizona Green Tea ^h
Calories, kcal	2	2	149	102	83	100	110	112	50	0	0	100	105	70
Protein, g	0	0.3	7.7	8.2	8.3	7	0	0.3	2	0	0	0	0	0
Total fat, g	0	0	7.9	2.4	0.2	4	0	0.2	0	0	0	0	0	0
Saturated fat, g	0	0	4.6	1.5	0.1	0.5	0	0	0	0	0	0	0	0
Carbohydrate, g	0.7	0	11.7	12.2	12.2	8	27	28	10	0	0	26	27	17
Sugars, g	0	0	12.3	12.7	12.5	6	24	26	8	0	0	26	27	17
Total dietary fiber, g	0	0	0	0	0	1	0.2	0.2	2	0	0	0	0	0
Vitamin A, IU	0	0	395	478	500	500	0	0	2000	0	0	0	0	0
Vitamin C, mg	0	0	0	0	0	0	0	1.4	60	0	0	0	0	0
Vitamin D, IU	0	0	5	117	115	120	0	0	0	0	0	0	0	0
Folate, mcg	12	5	12	12	12	24	60	0	0	0	0	0	0	0
Calcium, mg	0	5	276	305	299	300	20	14	20	0	0	0	0	0
Potassium, mg	88	116	322	366	382	300	450	301	470	0	20	0	10	0
Sodium, mg	7	0	105	107	103	85	15	17	420	70	25	33	25	20
Magnesium, mg	7	7	24	27	27	40	24	12	NA	0	0	0	0	0
Caffeine, mg	47	95	0	0	0	0	0	0	0	31	24	23	25	10

^aAmounts are per 8 fl oz (237 ml).^bData are compiled from US Department of Agriculture, Nutritional Data Laboratory: <http://www.nal.usda.gov/fnic/foodcomp/search/>^cData are compiled from <http://www.silksoy.com>^dData are compiled from <http://www.minutemaids.com>^eData are compiled from <http://www.v8juice.com>^fData are compiled from <http://www.coca-cola.com>^gData are compiled from <http://www.pepsi.com>^hData are compiled from <http://www.arizonabev.com>

traced back as early as around 500 BC in China. Tea remained primarily a Chinese beverage until the seventeenth century when Europeans started trading for teas and introducing them to a broader market. In 1908, teabags were invented in the US, which largely increased the convenience of tea consumption.

Tea is traditionally prepared by adding tea leaves to boiling water. The tea leaves are leaves, leaf buds, or internodes of the *Camellia sinensis* plant. According to how the tea leaves are produced and processed, tea can be classified into six major varieties: green tea (unwilted and unoxidized), yellow tea (unwilted and unoxidized, but allow to yellow), white tea (wilted and unoxidized), Oolong tea (wilted, bruised, and partially oxidized), black tea (wilted, crushed, and fully oxidized), and Pu-erh tea (fully fermented and composted). Tea leaves contain more than 700 bioactive compounds, including flavonoids (primarily catechins), amino acids, vitamins (C, E, and K), caffeine, and polysaccharides. Many of these compounds are antioxidants. For instance, the oxygen radical absorbance capacity (ORAC) is 1253 $\mu\text{mol TE}/100\text{ g}$ for green tea and 1128 $\mu\text{mol TE}/100\text{ g}$ for black tea.

Although beneficial effects of tea consumption in prevention of cancer and cardiovascular diseases (CVD) have been shown in animal models, it remains inconclusive whether tea consumption lowers the cancer and CVD risks in humans. Recent clinical studies found that daily consumption of 4–5 cups of black tea for 4 weeks significantly improved the coronary vessel function, which may help to explain its beneficial effects on CVD. A new large observational study also found that tea drinkers had significantly less cognitive decline than nontea drinkers during 14 years of follow-up. Tea consumption may also enhance the innate immunity, increase bone density, promote weight loss, and reduce kidney stones. However, these health benefits of tea consumption are inclusive and need to be clarified in further investigations.

Coffee

Coffee is one of the most popular beverages consumed worldwide. Coffee consumption originated in Africa around the ninth century. Early coffee was probably made using green (unroasted) coffee beans. The modern form of coffee (roasted, ground, and brewed) likely appeared in Yemen in the late fourteenth century. By the sixteenth century, coffee had reached the rest of the Middle East, and from there, spread to Italy and to the rest of Europe. Europeans began adding honey and milk to their coffee and vastly expanded the techniques for coffee brewing. In 1907, instant coffee was invented by preparing (freeze-drying or spray-drying) the soluble powder from brewed coffee beans. It rapidly gained popularity because of its convenience.

The stimulant effect of coffee is due to its caffeine content. A cup of coffee (7–8 oz), depending on the variety of coffee beans and brewed methods, may contain 80–175 mg of caffeine. The caffeine content in decaffeinated coffee is largely reduced to approximately 2–4 mg per cup. Coffee is also a good resource of antioxidants. Both caffeinated and decaf versions appear to provide similar antioxidant levels. Vitamins and minerals that can be found in coffee include folate, vitamin K, pantothenic acid, riboflavin, calcium, magnesium, and

manganese. Plain coffee contains only trace amounts of proteins and carbohydrate. The calories in coffee without any additives are only 2–3 kcal per cup. However, adding milk, cream, or sugar to coffee significantly increases the levels of calories as well as protein, fat, and carbohydrate.

Studies have consistently shown that coffee consumption was associated with lower risk of type 2 diabetes. It has been suggested that compounds other than caffeine may contribute to such benefits. High coffee and caffeine intake were also associated with a reduced risk of Parkinson disease in men, but not in women, which may be due to the modifying effect of estrogen. There is no strong evidence to suggest that high intake of coffee or caffeine is associated with increased risk of CVD. However, coffee consumption has been related to several CVD risk factors. Coffee intake was positively associated with plasma total and low density lipoprotein (LDL) cholesterol. Such cholesterol raising effects may be related to the diterpenes cafestol and kahweol in coffee. Because diterpenes cafestol and kahweol can be trapped by paper filters, their concentrations are higher in boiled and espresso than in filtered coffee. Caffeine also has a well-known acute pressor effect. However, tolerance to the caffeine-induced pressor effect develops in habitual coffee drinkers. In addition, ingredients other than caffeine may also have blood pressure control effects. Therefore, the long-term effect of habitual coffee consumption on blood pressure is still unclear. High intake of coffee has also been related to a lower risk of colorectal cancer and suicide, but the results are still inconsistent. Most beneficial effects of coffee consumption were observed from moderate drinking (2–3 cups).

Milk

Animal Milk

Animal milk may have been consumed by humans around 2000 BC or even earlier. Later, the development of pasteurization largely enhanced the safety of milk consumption and expanded the market of milk trading. By the 1930s, most milk consumed in the UK and US was pasteurized. The milk consumption in the US peaked in the 1940s and has fallen steadily since then, probably due to the competition from other beverages, such as soft drinks.

Presently, cow's milk is produced on an industrial scale and is the most commonly consumed form of milk. Milk and milk products are rich in many vitamins and minerals, including calcium, selenium, phosphorus, vitamin A, D (due to fortification), B₂ (riboflavin), B₅ (pantothenic acid), and B₁₂ (cobalamin). Lactose, a disaccharide, is the dominant carbohydrate contained in plain milk (12–13 g per cup) and gives milk its slightly sweet taste. Interestingly, only certain human populations maintain the ability to digest lactose during adulthood. Some individuals cannot produce sufficient lactase, an enzyme that aids lactose absorption, and, therefore, may suffer intestinal gas, cramps, bloating and diarrhea when drinking milks. This is usually called 'lactose intolerance' and it is more common among Asians, Africans, and Native Americans but less common among Caucasians. Processed cow's milk contains approximately 8 g proteins per cup. The

fat content of milk varies by the type of milk. A cup of whole milk (3.3% fat) contains approximately 8 g of fat, whereas reduced-fat (2% fat), low fat (1% fat), and skim milk contains 4.8, 2.4, and 0.2 g of fat, respectively. The majority of milk fats (57% for whole milk) are saturated fat acids. For this reason, whole milk contributes significantly to the saturated fat intake for Americans and many other populations. Therefore, low-fat or skim milks are recommended instead of whole milk.

For infants and children, milk is an excellent source for calcium, vitamin D, and high-quality proteins. For adults, low-fat and skim milks can contribute to a healthy diet, but are not essential. Both beneficial and detrimental health effects have been observed from milk consumption. Because milk is an important source for calcium and vitamin D, high milk consumption can be beneficial for bone health, particularly among children and adolescents. A positive association between milk consumption and bone mineral density has been consistently reported by both observational studies and randomized controlled trials. Some studies have shown that high intake of milk was associated with a reduced risk of metabolic syndrome, hypertension, and CVD, but evidence is still inconclusive. The role of milk on weight control has been suggested by some studies but not others. It has been hypothesized that milk's effect on weight regulation is attributed to its high calcium content. High calcium intake may regulate body adiposity through plasma parathyroid hormone (PTH) and $25(\text{OH})_2 \text{D}_3$ by repartitioning of dietary energy from adipose tissues to lean body mass and increasing thermogenesis. However, the 2005 American Dietary Guidelines Committee has concluded that the evidence to support that milk consumption can reduce weight gain is insufficient. The most important concern of the adverse effects of milk consumption among adults comes from cancer. Several studies have reported that high intake of milk was associated with an elevated risk of prostate cancer in men, and breast and ovarian cancers in women. It is speculated that such detrimental effects may related to the high level of insulin like growth factor I (IGF-1) in cow milk. IGF-1 has been linked with increased cell proliferation and inhibition of apoptosis, thus has been associated with cancer risk. Dairy producers have been injecting growth hormones into cows to increase the milk production, which results in an increased concentration of IGF-1 in the milk.

Soy Milk

Soy milk is an aqueous extraction of soy beans and has a milk-like appearance. Soy milk is traditionally produced by soaking the soybeans and grinding them with water. The resulting slurry is heated to boiling point for 15–20 min, followed by filtration to remove the insoluble residues (soy pulp). Soy milk was originally consumed in China and the oldest evidence can be traced to around 25–220 AD. Soy milk is widely consumed in China and other Asian countries such as Japan, Malaysia, and Singapore. The drink is slowly getting popular in Western countries. Soy milk has comparable amount of protein as cow's milk, but very little saturated fat and no cholesterol. Unlike cow's milk, soy milk does not contain lactose. Therefore, soy milk is safe for people with lactose

intolerance. For the above reasons, soy milk is considered a healthy alternative to cow's milk. However, soy milk only provides approximately 75% of calcium bioavailability from cow's milk and cannot be legally fortified with vitamin D.

100% Fruit or Vegetable Juice

Fruit Juice

Sometimes, the labels of commercial fruit juices may be misleading. In the US and several other countries, fruit juice can only be legally used to describe a product that is 100% pure fruit juice. According to US Food and Drug Administration (FDA), juices reconstituted from concentrate must be labeled as such. Beverages that contain less than 100% and more than 0% fruit juice should be called fruit beverages, fruit cocktails, fruit drinks, or juice drinks.

Fruit juice consumption has increased in recent years, probably due to the public perception of juices as a natural source of many nutrients. Fruit juices provide most of the nutrients of their natural source, including vitamins (A, C, folate, etc.), minerals (calcium, potassium, and magnesium), and other phytochemicals. However, they have relatively higher energy content and lower fiber levels compared to the whole fruits (many commercial juices are filtered to remove the fibers or pulps). Carbohydrates, including sucrose, fructose, glucose, and sorbitol, are the most prevalent nutrient in juice. The carbohydrate concentration varies from 26 g/cup (8 oz) to more than 38 g/cup, depending on the type of juices. Juices fortified with calcium have approximately the same calcium content as milk but lack other nutrients present in milk. Some juices have high contents of vitamin A, C, and flavonoids, and certain types of juices are fortified with vitamin D, which may provide beneficial health effects. However, the high energy and sugar contents in fruit juices may contribute to excessive energy intake. Studies have linked excessive intake of fruit juice to the risk of obesity and type 2 diabetes, but results are still mixed. Nevertheless, fruit juice offers no nutritional advantage over whole fruit, particularly for healthy adults. Consumption of whole fruits should be encouraged to meet an individual's nutrient needs and energy balance.

Vegetable Juice

Vegetable juices, such as tomato and multivegetable juices, are getting more and more popular in many countries. In general, vegetable juice has fewer calories and sugars than fruit juice and can be considered as a healthy alternative to fruit juice. However, commercial vegetable juices usually contain a considerable amount of sodium. As with fruit juices, whole vegetables, rather than vegetable juices, should be encouraged.

Soft Drinks

The term 'soft drinks' usually refers to nonalcoholic beverages typically containing water, sweeteners, and some flavorings. Sometime, they are also called pops, soda, or fizzy drinks. They are called 'soft' in contrast to 'hard' drinks, which are

generally referred to drinks with a significant alcohol content. Many of these beverages are carbonated and sweetened with either sugar or HFCS. Some of them may contain a small proportion of additional ingredients such as fruit juice or alcohol. If alcohol is added to a beverage, the content must be less than 0.5% of the total volume if the beverage is to be considered as nonalcoholic beverage. Widely, soft drinks include cola, lemonade, iced tea (usually sweet), sport drinks, fruit drinks, fruit punch, and root beer (nonalcoholic form). Carbonated water (also called sparkling water, seltzer, or fizzy water), which contains carbon dioxide gas and flavorings, as well as smart water (distilled water with added electrolytes), fruit water (distilled water with zero-calorie fruit flavorings), and vitamin water (distilled water with vitamins and flavorings) are usually classified between the soft drinks and plain drinking water.

The history of soft drinks can be traced back to 1760s when carbonation techniques were developed. The original purpose was to produce beverages similar to naturally occurring mineral water. Back then, no sugars or artificial flavorings were added to these beverages. It is unclear when and by whom sweeteners and/or flavorings were added to the carbonated waters. In 1886, J.S. Pemberton, an Atlanta pharmacist, combined kola (a caffeinated nut from Africa) with coca (a parent plant of cocaine from South America) to create Coca-Cola, which is considered as the landmark event in the history of soft drinks. In the following section, we divided soft drinks into two major categories based on whether they are sweetened with added caloric sweeteners (sugars or HFCSs) or noncaloric sweeteners (artificial sweeteners).

Diet Beverages

Diet beverages (diet soft drinks) are carbonated or noncarbonated beverages sweetened with artificial sweeteners that provide less than 1 kcal in one serving. Currently, five artificial sweeteners (saccharin, acesulfame, aspartame, neotame, and sucralose) have got US FDA approval. Beverages sweetened with these artificial sweeteners are considered to be safe. In the past several decades, there is an increasing trend of consumption of diet beverages, likely due to the increased concern regarding the adverse effects of added sugars. In the US, the average intake of diet beverages had increased from less than 1 oz day⁻¹ in the 1960s to 4 oz day⁻¹ in the 2000s.

Diet beverages are preferable to sugar-sweetened beverages because they provide water and sweetness but no calories. A few randomized interventions have showed that drinking diet beverages was more favorable on weight management compared to drinking sugar-sweetened beverages. Recent literature, however, is emerging to suggest that long-term consumption of these sweet beverages may increase people's desire for sweetness, resulting in increased intake of other sweet foods and beverages. Artificial sweeteners are hundreds to thousands times sweeter than sugars. Individuals who habitually consume artificial sweeteners may find less intensely sweet or unsweet foods unpalatable. In addition, diet beverages have a high degree of sweetness but no calories, which might produce a dissociation between sweet taste and calorie intake. One concern is that the dissociation of these physiological events

might disrupt the hormonal and neurobehavioral pathways regulating hunger and satiety.

Sugar-Sweetened Beverages

Sugar-sweetened beverages (regular soft drinks) include carbonated and noncarbonated beverages, which are usually sweetened with HFCS or sucrose. These beverages have relatively high calorie and sugar contents, but no or a very small amount of other nutrients. High intake of sugar-sweetened beverages has been consistently associated with higher energy intake and increased risk of dental caries, obesity, type 2 diabetes, metabolic syndrome, hypertension, and CVDs. Suggestive data have indicated that reducing sugar-sweetened beverage consumption might result in significant weight loss and blood pressure reduction. It has been proposed that sugar-sweetened beverages contribute to weight gain because of their high sugar content and incomplete energy compensation. Fructose from sucrose or HFCS may also increase blood pressure and promote the accumulation of visceral adiposity through the increase of hepatic *de novo* lipogenesis. In addition, consuming a large amount of sugar-sweetened beverages can contribute to a high glycemic load (GL) diet by providing a large amount of rapidly absorbable sugars, leading to insulin resistance, chronic inflammation, and possibly impaired β -cell function. Therefore, high sugar-sweetened beverage consumption may increase type 2 diabetes and cardiovascular risk independently of obesity. Thus, as few sugar-sweetened beverages should be consumed as possible.

Summary

In summary, the primary purpose for drinking beverages is to provide fluids rather than calories or other nutrients. Beverages with more nutritional values but few calories and sugars are encouraged to meet our body's requirement for fluids. In general, beverages should provide less than 10% of total energy for healthy adults. By considering the energy density, nutrient density, and health benefits/risks linked with each beverage category, unsweetened coffee and tea are mostly recommended, followed by low-fat and skim milk, 100% fruit and vegetable juices, and soft drinks. Among soft drinks, diet beverages are preferable to sugar-sweetened beverages.

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BIOAVAILABILITY

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Glossary

Bioavailability The fraction of absorbed and utilized micronutrient.

Bioconversion The rate at which absorbed provitamin A is cleaved to vitamin A in the intestine.

Bioefficacy Combines absorption and bioconversion and is the efficiency with which ingested dietary

provitamin A carotenoids are absorbed and converted to vitamin A.

Biofortification Agronomically improving stable crops by enhancing micronutrient levels through traditional breeding or genetic modification.

Stable isotope Nonradioactive isotope.

Introduction

Bioavailability refers to the fraction of absorbed and utilized micronutrient. This concept is particularly important for some micronutrients, e.g., nonheme iron, zinc, provitamin A carotenoids, folate, and vitamin B₁₂ as bioavailability varies widely depending on a number of factors. These factors include nutritional status, physiological factors such as gastric acid secretion, food matrix, and interactions between nutrients in addition to the presence of enhancers and inhibitors in the diet (**Figure 1**). Stable (nonradioactive) isotope techniques to assess micronutrient bioavailability have been developed over the last 20 years or so and the application of these techniques has contributed significantly to our understanding of the importance of bioavailability in micronutrient nutrition. As stable isotope techniques do not expose the study population, or the investigators, to any potential health hazard related to

ionizing radiation, studies in vulnerable population groups at high risk of developing micronutrient deficiencies are feasible. Consequently, over the last 10–15 years, crucial new information, for example, about dietary enhancers and inhibitors of nonheme iron absorption in infants and children has been made available, and important data have been generated on nonheme iron bioavailability from iron compounds used in food fortification programs. In vitamin A nutrition, the development of stable isotope techniques to estimate body pool sizes of vitamin A has provided new important information on the bioefficacy of provitamin A carotenoids and the influence of dietary composition, as well as the nutritional status of the consumer, on provitamin A bioavailability.

Nonheme Iron Bioavailability

Fractional absorption of nonheme iron varies widely in individuals, from less than 1% to 100%, depending on the nutritional status and physiological factors of the individual as well as the composition of the test meal (see **Figure 1**). From a methodological point of view, the rapid incorporation of newly absorbed iron into a target tissue, which can be sampled relatively easily, i.e., erythrocytes, is a major advantage, and a stable isotope technique to evaluate iron bioavailability has been developed based on the incorporation of stable iron isotopes into red blood cells 14 days after administration of labeled test meals. This methodology was originally developed by investigators using radioactive isotopes of iron, and the development of stable isotope technique has benefited greatly from this original work related to the development of the study protocol (defining the appropriate times for blood sampling, collecting data on incorporation rates etc.). Usually, the incorporation rate of newly absorbed iron into erythrocytes is assumed to be relatively constant, approximately

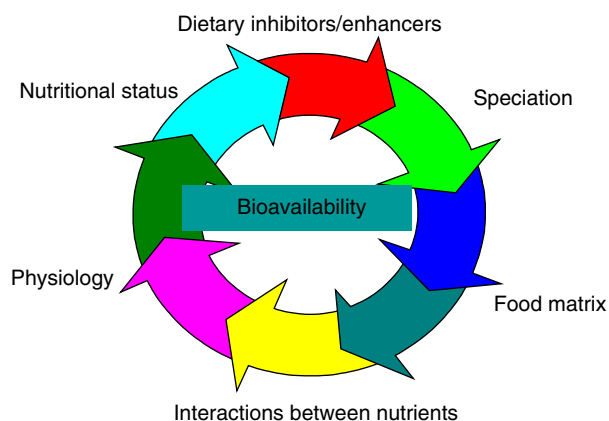


Figure 1 Factors influencing bioavailability of nonheme iron and provitamin A carotenoids.

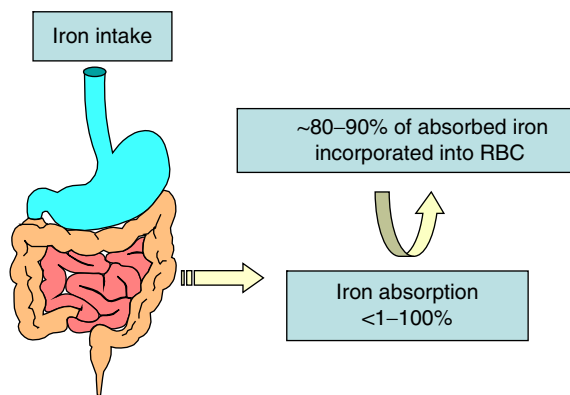


Figure 2 Newly absorbed iron is rapidly incorporated into red blood cells (RBC) at a relatively constant, high rate.

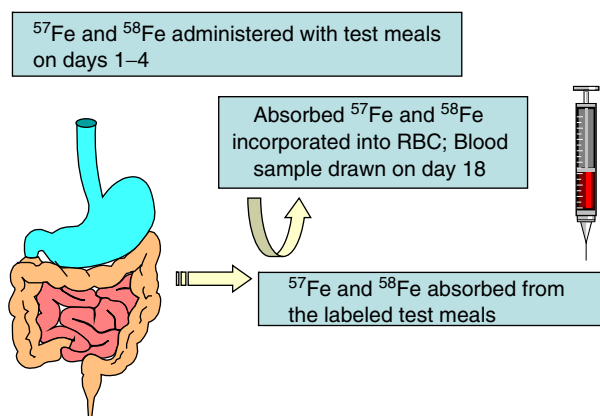


Figure 3 Basic principles of the double stable isotope technique to evaluate iron bioavailability based on incorporation into red blood cells (RBC).

80–90% (see **Figure 2**). However, when the incorporation rate cannot be assumed to remain stable, for example, during pregnancy or in individuals infected with malaria, incorporation of a stable isotope administered intravenously can be used to correct for changes in incorporation rate.

Considerable interindividual variation in iron bioavailability has been demonstrated, largely due to differences in iron status among individuals, and paired comparisons are therefore essential when evaluating iron bioavailability from different foods or food fortificants. Using a double isotope technique, i.e., administration of two stable isotopes of iron (usually ⁵⁷Fe and ⁵⁸Fe) – on consecutive days – information about iron bioavailability from two different test meals can be obtained simultaneously (**Figures 3** and **4**). When evaluating bioavailability from iron compounds used for food fortification, ferrous sulfate is typically used as the reference compound and relative bioavailability is reported, i.e., bioavailability from ferrous sulfate is set at 100% and bioavailability of other compounds are compared to this value (**Table 1**). Blood samples drawn at baseline and 14 days after administration are analyzed for ⁵⁷Fe- and ⁵⁸Fe-enrichment by Thermal Ionization Mass Spectrometry (TIMS) or High

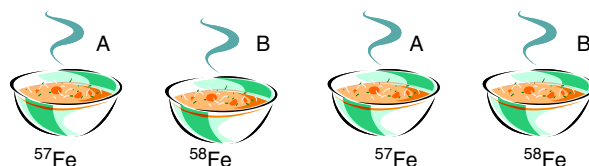
Table 1 Examples of data on relative iron bioavailability in adult humans

Compound	Relative iron bioavailability (%)
Ferrous sulfate	100
Sodium iron EDTA	> 100
Ferrous fumarate	100
Ferric pyrophosphate	21–74
Elemental iron; electrolytic	75

Source: Adapted from World Health Organization (WHO), Food and Agriculture Organization of the United Nations (FAO) (2006) In: Allen L, De Benoist B, Dary O, and Hurrell RF (eds.) *Guidelines on Food Fortification with Micronutrients*. http://www.who.int/nutrition/publications/guide_food_fortification_micronutrients.pdf

Day 1: Baseline blood sample (unenriched, i.e., normal stable isotope ratios)

Days 1–4: Intake of labeled test meals A and B



Day 18: Blood sample (enriched stable isotope ratios)

Figure 4 Study design to evaluate iron bioavailability from test meals A and B.

Resolution Inductively Coupled Plasma Mass Spectrometry. Although the number of suitable mass spectrometers dedicated to nutrition remains limited worldwide, the application of this technique has been used in a wide range of settings, based on close North–South collaboration. The recently installed TIMS in Bangalore, India, will hopefully increase the application of this technique in Asia. Successful implementation of stable isotope studies depend on joint efforts made by a group of people, including analytical chemists, nutritionists, and relevant health professionals such as pediatricians, nurses, and health workers. As many different steps are crucial – both during the preparation for the study, i.e., development of study protocols, preparation of test meals, preparation of stable isotope doses, administration of labeled test meals – as well as in the final analysis of enriched blood samples – a multidisciplinary team is clearly needed to plan and implement this kind of study.

Over the last 10–15 years, stable isotope technique has been used to generate new data on iron bioavailability from iron compounds used in food fortification programs and information about dietary enhancers and inhibitors of iron absorption in vulnerable population groups. More information about the importance of bioavailability of nonheme iron in the development of food-based strategies to combat iron deficiency, i.e., food fortification and dietary diversification, is provided below.

Bioavailability as an Important Component in the Development of Food-Based Strategies to Combat Iron Deficiency: Food Diversification

A number of dietary factors influencing nonheme iron bioavailability were identified during the earlier studies using radioactive isotopes of iron, as well as more recent data based on stable isotope technique. Inhibitors include phytic acid, polyphenols, and calcium, as well as some proteins, whereas ascorbic acid and muscle tissue (the ‘meat factor’) enhance nonheme iron absorption. More recently, the importance of host factors such as nutritional deficiencies, infection/inflammation, and genetic disorders has received more attention, and there is clearly a need to further investigate the importance of these factors in different population groups. The identification of hepcidin as a key regulator of iron homeostasis highlights the need for future studies to investigate the role of this peptide and its increased expression during chronic inflammation and obesity on iron absorption. In addition, in spite of numerous studies, including studies to evaluate the potential influence of vitamin A on nonheme iron bioavailability in healthy Western adults and African children with subclinical vitamin A deficiency, the influence of vitamin A and provitamin A carotenoids on nonheme iron absorption still remains largely unknown.

The importance of optimizing nonheme iron bioavailability from the diet can be assumed to be crucial for individuals with high requirements and consuming inhibitory diets, in particular, infants and young children in developing countries. The positive effect of adding meat to a vegetable-based complementary food on nonheme iron bioavailability has been demonstrated in European infants and the inclusion

of meat into the diet of young children would thus be beneficial by providing highly bioavailable heme iron as well as by enhancing the bioavailability of nonheme iron. However, meat is often not available or affordable and, thus, infants and young children living in resource poor communities where monotonous, cereal-based diets are consumed are of special concern.

Stable isotope technique has been used to demonstrate the potent inhibitory effect of phytic acid as well as the strong enhancing effect of ascorbic acid on nonheme iron bioavailability in infants. Dephytinization of cereals and legumes can be made by the addition of exogenous phytase or by the use of whole-grain cereals as a source of phytase, and ascorbic acid can be included into the diet by consumption of fruits, in particular citrus fruits rich in vitamin C. However, inclusion of fruits into the young child’s diet can be limited by availability, affordability, and tradition. An alternative source of ascorbic acid in the diet of African infants and young children was identified – human milk – and subsequently tested for its effect on nonheme iron bioavailability from a traditional complementary food in Bangladesh, using stable isotope technique. Although human milk contributed considerable amounts of ascorbic acid, no significant effect on iron bioavailability was observed, indicating that components in human milk modify the influence of ascorbic acid. A more recent study confirmed the potent enhancing effect of ascorbic acid on nonheme iron bioavailability in Pakistani infants and clearly demonstrated that breastfeeding immediately after intake of a complementary food with added ascorbic acid does not blunt the enhancing effect of the vitamin when evaluated by stable isotope technique.

Bioavailability as an Important Component in the Development of Food-Based Strategies to Combat Iron Deficiency: Food Fortification

Food fortification is currently implemented as a public health strategy to combat iron deficiency in many countries, either by fortification of staple foods such as milled cereal flours to reach a large proportion of the population or by targeted approaches based on fortification of products consumed by vulnerable population groups, e.g., fortified commercial infant foods. More recently, other approaches have been developed to reach individuals, in particular, infants and young children, who do not have access to centrally produced foods by providing micronutrients in sachets, crushable tablets, and spreads for ‘in-home fortification’.

The concept of bioavailability is central to the development of food fortification strategies as the overall impact of a food fortification program on the consumers’ iron status will be influenced by the bioavailability of the iron compound used as well as the presence of inhibitors and enhancers of iron absorption in the diet. Information about bioavailability of iron compounds represents an integral part of the development of food fortification strategies, together with careful selection of the food vehicle and testing of changes to the organoleptic properties of the fortified food (Figure 5). These findings include important information on significant differences in iron bioavailability between iron compounds, with different

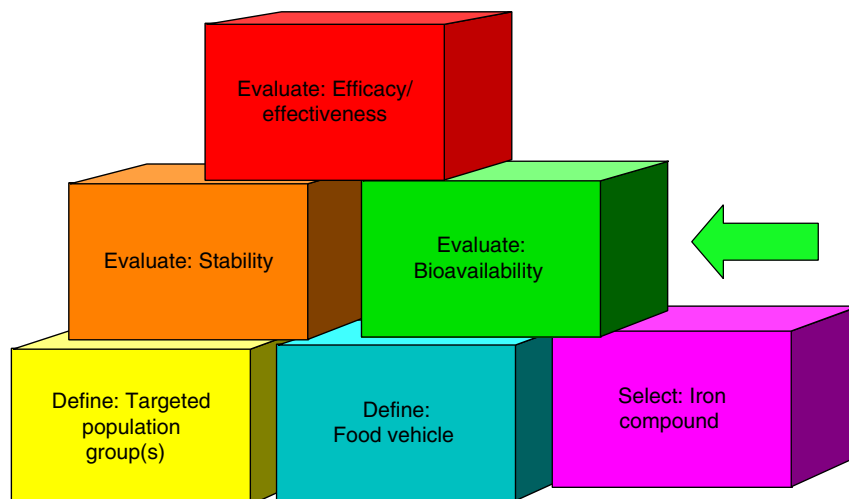


Figure 5 Components of an iron fortification program.

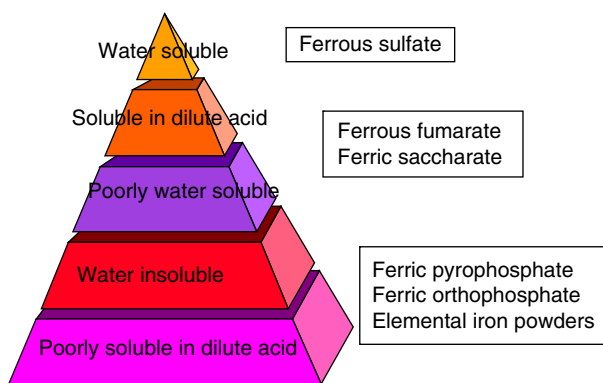


Figure 6 Examples of iron compounds with different solubility, ranging from freely soluble in water to poorly soluble in dilute acid.

physicochemical properties such as solubility in water and dilute acid (Figure 6). For example, stable isotope technique has been used to demonstrate a three-fold difference in iron bioavailability from iron compounds with different solubility properties used to fortify infant cereals in different parts of the world, i.e., ferrous fumarate and ferric pyrophosphate, in infants. In addition, substantially lower relative bioavailability of ferrous fumarate versus ferrous sulfate has been noted in pre-school children in Bangladesh (approximately 30%) as compared to that in Western adult women (100%). These observations clearly highlight the importance of studies in appropriate population groups as opposed to extrapolating data from other groups, e.g., healthy adults in the North to infants and young children in the South, who are less well-nourished and have lower gastric acid output. Furthermore, stable isotope technique has been applied to demonstrate the usefulness of NaFeEDTA for fortification of high extraction rate cereal flours and condiments such as fish sauce and soy sauce. This technique has also been used to evaluate different strategies to optimize iron bioavailability from fortified foods consumed by children, for example, by the addition of enhancers such as ascorbic acid or NaEDTA.

The World Health Organization (WHO) and the Food and Agriculture Organization of the United Nations (FAO) have developed guidelines to assist in the development and evaluation of food fortification programs. The importance of bioavailability data to optimize the impact of food fortification by selecting iron compounds with high relative bioavailability as well as by modifying the composition of the diet to increase iron absorption, is highlighted in these guidelines. In particular, the potent enhancing effect of ascorbic acid and the strong inhibitory effect of phytic acid are emphasized. Briefly, these recommendations provide a list of iron fortificants (based on relative bioavailability data) in the following order of preference: (1) ferrous sulfate, (2) ferrous fumarate, (3) encapsulated ferrous sulfate or fumarate, (4) electrolytic iron, and (5) ferric pyrophosphate. To compensate for low relative bioavailability from electrolytic iron and ferric pyrophosphate, it is recommended to add twice the amount. In addition, it is recommended to add ascorbic acid at a 2:1 molar ratio; for foods high in phytic acid, the molar ratio should be increased to 4:1 to counteract the inhibitory effect of phytic acid. NaFeEDTA is recommended for fortification of cereal flours high in phytic acid and for certain condiments such as fish sauce and soy sauce.

Bioavailability of Provitamin A Carotenoids

Carotenoids

Carotenoids are responsible for the colors of many fruits and vegetables. Bioavailability of carotenoids from food sources is an active area of research because of purported health benefits. For example, a diet high in fruit and vegetables, which are high in carotenoids, is related to a reduced risk of various types of cancer. Many factors are known to impact the bioavailability of these fat-soluble compounds from food. Evidence exists that the structure of the carotenoid impacts its ability to become part of the lipid complex (i.e., micelle) that makes it easily absorbed by the human body. The hydroxyl-group

containing carotenoids tend to be more bioavailable than the hydrocarbon carotenoids. Cooking enhances carotenoid bioavailability by disrupting the vegetable matrix, but prolonged

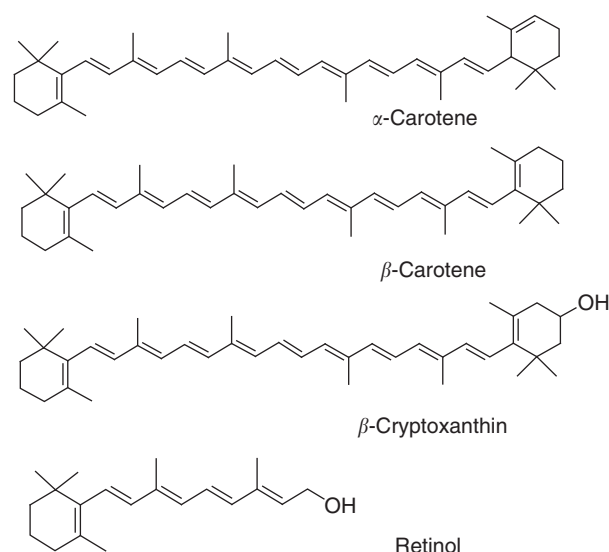
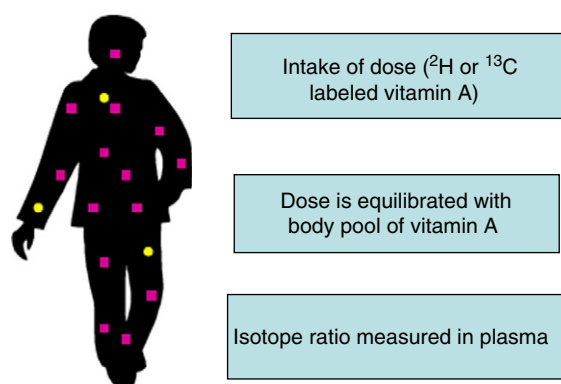


Figure 7 Chemical structures of provitamin A carotenoids in comparison to retinol. β -Carotene is able to supply two molecules of retinol, whereas α -carotene and β -cryptoxanthin supply one.



Illustration; Kerstin Gross-Helmert

Figure 8 Stable isotope dilution technique to assess vitamin A body pools.

heat destroys them or changes the structural configuration. The isomeric form of β -carotene impacts its bioavailability and the forms that are 'bent' or in the *cis* configuration are less bioavailable than the 'straight' or all-*trans* forms.

Provitamin A carotenoids number approximately 50 in nature, but only three of these are common in the human diet, namely, α -carotene, β -carotene, and β -cryptoxanthin (**Figure 7**). α -Carotene is found predominantly in carrots and some squashes, β -carotene in orange vegetables and green leafy vegetables, and β -cryptoxanthin in citrus fruits and yellow maize.

Bioconversion of Carotenoids to Vitamin A

The process of producing vitamin A from provitamin A carotenoids is termed bioconversion. The nutritional status of the person impacts whether or not the provitamin A carotenoids are converted to vitamin A. For example, if a person is vitamin A deficient they will convert more of the absorbed provitamin A carotenoids to vitamin A. However, if the person is zinc deficient, less provitamin A will be converted to vitamin A because the enzyme that cleaves the provitamin A carotenoids to vitamin A is zinc dependent.

Bioavailability of Carotenoids

Bioavailability is often measured by looking at differences in serum carotenoid concentrations. However, this only determines relative differences between two comparisons. Stable isotope techniques to evaluate vitamin A status were developed to use in vulnerable populations. The method involves administering a dose of isotopically labeled tracer and waiting for the dose to equilibrate with body pools of vitamin A, usually two to three weeks (**Figure 8**). Basically, the method is an indirect measure of liver reserves of vitamin A, and the test does require blood samples. Typically a blood sample is taken at baseline and then after equilibration to measure the change in enrichment caused by the administered isotope. The application of stable isotope techniques to evaluate changes in vitamin A body pools has contributed significantly to the evaluation of interventions based on provitamin A carotenoids. The principles of the study design used for these applications are described in **Figure 9**. In practice, two stable isotopes have been used including the heavy isotope of hydrogen, deuterium, and the heavy isotope of carbon, carbon 13. Usually, deuterated retinol esterified to acetate with four or

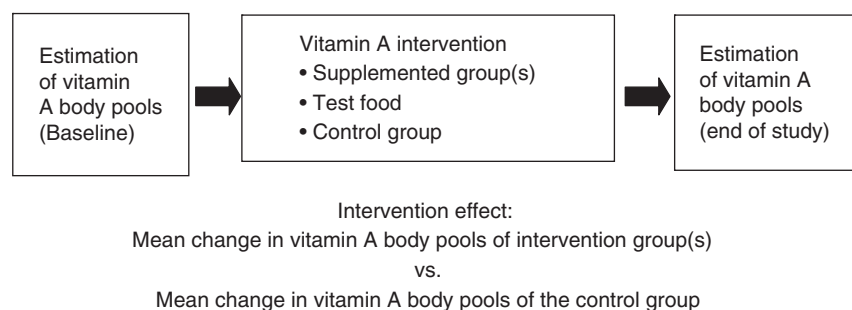


Figure 9 Principle of study design based on vitamin A body pool size.

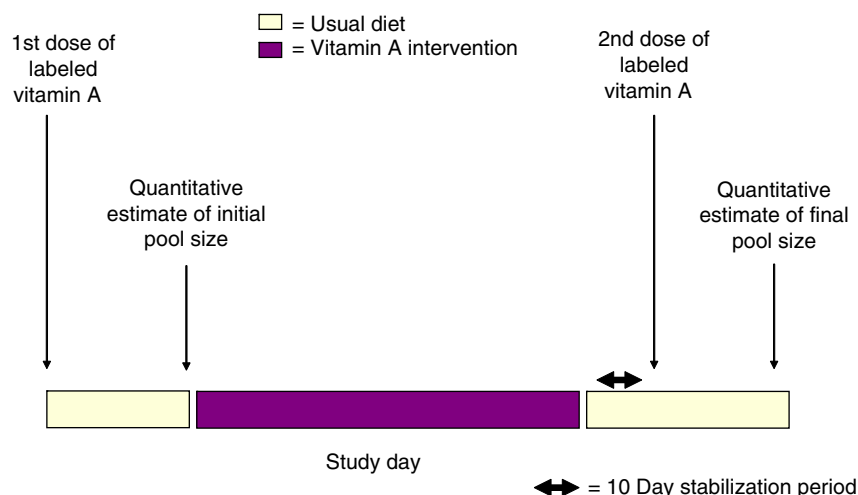


Figure 10 Design for studies based on vitamin A body pool assessment using the paired-isotope dilution technique.

eight deuterium atoms is administered, followed by analysis of deuterium enrichment in serum or plasma samples by gas chromatography-mass spectrometry (GCMS), the so-called 'paired-stable isotope dilution technique'. The study design used in 'paired-stable isotope dilution technique' studies is presented in **Figure 10**. In particular, the paired-stable isotope dilution technique has been used to generate new data on provitamin A conversion factors to retinol.

Conversion Factors

Conversion factors are a way to define how much of an ingested provitamin A carotenoid is converted to 1 μg retinol. This is also known as bioefficacy. The Institute of Medicine uses the factors of 12 μg β -carotene:1 μg retinol and 24 μg α -carotene or β -cryptoxanthin:1 μg retinol (**Table 2**). This equivalency is referred to as retinol activity equivalents (RAE) to distinguish it from the conversion factors still used by the Food and Agriculture Organization, which is 6 μg β -carotene:1 μg retinol and referred to as retinol equivalents or RE. This estimate of vitamin A formation from provitamin A assumes that the person is healthy and eating a mixed diet. In comparison to these generalized conversion factors, a mean conversion factor of 26.7 μg β -carotene:1 μg retinol was reported for green and yellow vegetables consumed by Chinese school-aged children and 13.4 μg β -carotene:1 μg retinol for sweet potato, 9.5:1 for Indian spinach (*Basella alba*), and 6.3:1 for pure β -carotene in oil were obtained in Bangladeshi men. These studies were able to determine conversion factors by applying the paired-stable isotope dilution technique to evaluate the changes in total body vitamin A pools in response to the intervention with the test food.

Other isotopic methods have also been developed to evaluate bioconversion factors. Using a different approach other than the paired-stable isotope dilution technique, intrinsically deuterium-labeled vegetables were prepared by growing carrots and spinach hydroponically in deuterated water. Humans were fed the labeled vegetables and a reference dose of $^{13}\text{C}_8$ -retinyl acetate was administered for comparison.

Table 2 The commonly used bioconversion factors of provitamin A carotenoids to yield 1 μg retinol include those published by the Institute of Medicine and the Food and Agriculture Organization of the United Nations (FAO)

<i>Institute of medicine</i>	<i>FAO</i>
2 μg β -Carotene in oil supplement	2 μg β -Carotene in oil supplement
12 μg β -Carotene	6 μg β -Carotene
24 μg β -Cryptoxanthin	12 μg β -Cryptoxanthin
24 μg α -Carotene	12 μg α -Carotene

Intake of vegetables resulted in conversion of 20.9 μg β -carotene:1 μg retinol and 14.8:1 for spinach and carrots, respectively. More recently, the intrinsic labeling technique was used to evaluate bioconversion of provitamin A carotenoids in rice that was genetically modified to contain β -carotene. The process of enhancing micronutrients in staple crops, such as rice, is called biofortification. This rice is called 'Golden Rice' due to its deep yellow hue exclusively due to β -carotene. In comparison to a reference dose, the β -carotene from Golden Rice resulted in a mean conversion factor of 3.8 μg β -carotene to 1 μg of retinol for five adults. These data highlight the potential usefulness of biofortification to impact vitamin A status. In general, animal and human studies that have tested staple crops biofortified with provitamin A have shown very good conversion rates of provitamin A to retinol demonstrating the feasibility of this agronomic technique. At this time, plant sources of provitamin A are often overlooked in infant feeding strategies in developing countries. However, a simulation based on intake of 100 g orange-fleshed sweet potato each day using the Institute of Medicine's 12:1 conversion factor resulted in a significant increase of vitamin A liver stores in infants. Furthermore, feeding sweet potato to children in South Africa and Mozambique improved vitamin A status demonstrating sweet potato β -carotene is bioavailable and converted to vitamin A.

Finally, extrinsic reference methods using stable isotopically labeled β -carotene and retinyl ester have been used to

determine bioconversion factors from supplements and foods. These methods have been compared to traditional oral–fecal balance studies and seem to be better suited to supplement studies than mixed diets.

Effectors of Bioavailability

Dietary fat is needed for absorption of carotenoids in order to facilitate the formation of micelles in the small intestine. However, fat does not seem to be a strong limiting factor in human diets when vegetables are consumed as part of a composite meal. For example, studies in Filipino school-children, based on the paired-isotope dilution test, found no difference between three levels of fat (7, 15, or 29 g fat day⁻¹) when fed with 4.2 mg provitamin A carotenoids in the form of carotenoid-rich vegetables. Studies in animals, however, did see enhancement of bioefficacy when high levels of fat were fed with orange-fleshed sweet potato. Some forms of fiber also interfere with the bioavailability of carotenoids, although the addition of dry matter in the form of white sweet potato to orange-fleshed sweet potato did not seem to affect the bioefficacy.

Influence of Vitamin A Status

From a methodological point of view, it is important to recognize that the retinol response to provitamin A carotenoids in humans and animals varies inversely with vitamin A status. Clearly, there is an urgent need for additional, well-designed studies to evaluate the bioavailability of provitamin A carotenoids consumed in settings where vitamin A deficiency remains a public health problem among infants, children, and women of child-bearing age. The WHO continues to advocate high-dose vitamin A supplements, which are oil-based, to preschool children in part due to the lack of consistent high provitamin A sources in the diets of the poor and the questionable bioavailability of the provitamin A carotenoids.

Conclusion

Stable isotope techniques provide excellent tools to assess bioavailability of nonheme iron and provitamin A carotenoids and thus contribute important information to be used in the development of nutrition interventions.

See also: Carotenoids: Chemistry, Sources and Physiology. Food Fortification: Programs. Iron: Physiology, Dietary Sources, and

Requirements. Vitamin A: Physiology, Dietary Sources, and Requirements

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BIOCHEMICAL INDICES

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Glossary

Acute phase response Various physiological processes occurring soon after the onset of infection, trauma, inflammatory processes, and some malignant conditions; an increase or decrease in acute phase proteins in serum, fever, increased vascular permeability, and metabolic and pathologic changes are part of an acute phase response.

Biochemical test Laboratory test that measures the level of an analyte or metabolite in, for e.g., serum, blood or urine.

Chromatography assay Laboratory technique that separates a compound of interest from other compounds by either passing a liquid or a gas in which the compound of interest is dissolved (mobile phase) through a stationary phase; this process separates the compound of interest from

other compounds in the mixture based on differential partitioning between the mobile and stationary phases.

Cutoff point A concentration or quantity that is selected in clinical practice for quantitative diagnostic tests and maximizes the specificity and sensitivity of the diagnostic test.

Immunoassay Laboratory assay that typically measures analytes in biological liquids such as serum and urine; the technique is based on the unique ability of an antibody to bind with high specificity to one or a very limited group of molecules, representing the antigen.

Nutritional deficiency Progression throughout various stages from adequate to inadequate nutritional body stores, leading to disturbances in metabolism and ultimately to clinical signs and symptoms.

Introduction

Biochemical methods are considered to be the most objective measures for the assessment of nutritional status of individuals or communities. Ideally, the method employed should cover various cutoff points specific and sensitive to depletion of the nutrient body pool or tissue store.

The pathophysiology of deficiency for most nutrients, particularly vitamins, progresses in successive stages. The first stage of deficiency is when nutrient body stores begin to be depleted; in this stage, urinary excretion of the nutrient decreases, whereas homeostatic regulation ensures that the level of nutrient in the blood or tissues does not change. In the next stage, depletion is more marked; nutrient excretion via urine continues to decrease and blood and other tissue concentrations are reduced. Evidence of an increase in compensatory or dependent metabolites or enzymes tends to characterize the third stage. In the last stage, morphological or functional disturbances are present, first reversible, then irreversible, which can manifest as clinical signs and symptoms when these biochemical effects accrue or worsen.

The static and functional tests most commonly used in nutritional status assessment in humans are discussed here. Static tests measure the content of nutrients, their active or inactive metabolites, or other related components in tissues or fluids. Functional tests measure the behavioral, physiological, or biochemical functions of the organism that are dependent on the adequate availability of a nutrient, or the organism's responses to the processes to maintain body stores or

homeostasis. The availability of specific and reliable chromatography-based methods in recent times has allowed for more frequent use of static compared to functional tests. The choice of tissue or fluid depends on the information required (long-term status or short-term, body pool or tissue store), operational characteristics of the biochemical test, and on the condition of the subject.

Various factors influence biochemical test results. Some are related to the individual, such as age, sex, genetics, physiological, and hormonal status; others are environmental or situational, such as seasonality, elevation, and latitude and thus cannot be eliminated; still others are behavioral and more easily modified (e.g., alcohol intake, smoking habits, and use of medicines). Major factors influencing biochemical indicators are presented in [Table 1](#). In addition, infection and inflammation are known to have confounding effects on several laboratory tests and this is discussed as part of each nutrient status section. Different approaches have been investigated to account for infection and inflammation in nutrition surveys (e.g., exclusion of participants with infection, adjustment of biomarker levels in those with infection). Although no consensus exists on the best approach, the concurrent serum determination of acute phase proteins such as C-reactive protein (CRP) and α 1-acid glycoprotein (AGP) is highly recommended. Influential factors of a technical nature can usually be reduced or eliminated by adherence to standardized sample handling protocols. [Table 2](#) provides information on preanalytical factors (e.g., fasting, storage stability,

Table 1 Factors known to influence biochemical indicators

<i>Nutritional status</i>	<i>Biochemical indicator</i>	<i>Influencing factors</i>
Protein	Serum albumin	<ul style="list-style-type: none"> ↑ ↓ Age, sex ↑ Dehydration ↓ Acute phase infection and chronic inflammation, catabolic states, protein-losing diseases, hemodilution, zinc depletion
	Serum transport proteins: TTR, TF and RBP	<ul style="list-style-type: none"> ↑ Chronic renal failure (for TTR and RBP), when estrogen is increased (for TF) ↓ Infection, protein-losing diseases, hemodilution, zinc depletion, vitamin A deficiency (for RBP), iron deficiency (for TF)
	Urinary creatinine	<ul style="list-style-type: none"> ↑ ↓ Age, sex, diet, diurnal and day-to-day variations ↑ Intensive exercise, pregnancy, catabolic states, hypothyroidism ↓ Chronic renal failure, hyperthyroidism, diseases with decreased muscle mass
	Urinary 3-methylhistidine	<ul style="list-style-type: none"> ↑ ↓ Age, sex, diet ↑ Intensive exercise, catabolic states ↓ Chronic renal failure
	Serum IGF-I	<ul style="list-style-type: none"> ↓ Stress, hormonal diseases, hepatocellular diseases
Fatty acids	Plasma fatty acids	<ul style="list-style-type: none"> ↑ ↓ Smoking, exercise, stress, pregnancy, estrogens, obesity, alcohol, diabetes, renal disease ↑ Age ↓ Fat malabsorption, catabolic states, depression
Vitamin A	Serum retinol, RBP, RDR and MRDR	<ul style="list-style-type: none"> ↑ ↓ Sex, race ↑ Age, chronic renal disease, estrogens ↓ Protein–energy malnutrition, fat malabsorption, catabolic states, zinc deficiency, liver disorders
Carotenoids	Serum carotenes, cryptoxanthin, lutein, zeaxanthin, lycopene	<ul style="list-style-type: none"> ↑ ↓ Age, sex, race, season, smoking, alcohol, BMI ↓ Fat malabsorption Diet and season, sex and age, infection, smoking, and drinking habits and circulating lipids are determinants of carotenoid concentrations.
Vitamin D	Serum 25OHD	<ul style="list-style-type: none"> ↑ ↓ Season/latitude, age, sex, race ↑ Sun exposure, dairy consumption, estrogens ↓ Skin pigmentation, liver or renal diseases, smoking, drugs, fat malabsorption, obesity
Vitamin E	Serum α -tocopherol	<ul style="list-style-type: none"> ↑ ↓ Race ↑ Age, hyperlipidemia, pregnancy ↓ Fat malabsorption, abetalipoproteinemia, premature infants
Vitamin K	Serum phyloquinone (K ₁), menaquinones (K ₂)	<ul style="list-style-type: none"> ↑ ↓ Age, sex, season ↑ Plasma triglyceride levels ↓ Fat malabsorption, osteoporosis, liver disease, hemorrhagic disease of newborn, antibiotics and other drugs
Thiamin	Erythrocyte TDP Serum thiamin Urinary thiamin Erythrocyte EKT-AC	<ul style="list-style-type: none"> ↓ Hemodialysis, storage of specimen ↓ Alcoholism ↓ Starvation ↑ Alcoholism, pernicious anemia ↓ Diabetes mellitus, polyneuritis, storage of specimen
Riboflavin	Erythrocyte or serum FAD, FMN Urinary riboflavin Erythrocyte EGR-AC	<ul style="list-style-type: none"> ↓ Alcoholism, hypothyroidism, anorexia ↑ Negative nitrogen balance, infection, drugs ↓ Starvation ↑ Alcoholism, heterozygous beta thalassemia, iron-deficiency anemia, severe uremia, liver cirrhosis ↓ Glucose-6-phosphate dehydrogenase deficiency, pyridoxine deficiency, storage of specimen
Niacin	Urinary methylated niacin metabolites	<ul style="list-style-type: none"> ↓ Drugs, alcoholism

(Continued)

Table 1 Continued

<i>Nutritional status</i>	<i>Biochemical indicator</i>	<i>Influencing factors</i>
Vitamin B ₆	Serum PLP	↑↓ Age, sex ↑ Prolonged fasting ↓ Drugs, alcohol intake, smoking, malnutrition, pregnancy, various disease states
	Urinary 4-PA	↑↓ Drugs
	Erythrocyte EAST-AC	↑↓ Drugs and diseases that affect the liver and heart ↓ Alcohol intake, pregnancy
Folate	Serum folate	↑↓ Age, sex, race ↑ Hemolysis ↓ Pregnancy, smoking, alcoholism, antifolate drugs, malnutrition, malabsorption, storage of specimen
	RBC folate	↑↓ Age, sex, race ↑ Iron deficiency ↓ Pregnancy, smoking, malnutrition, malabsorption, vitamin B ₁₂ deficiency
	Plasma tHcy	↑↓ Age, sex, race, diet ↑ Deficiencies in B ₂ , B ₆ , and B ₁₂ , impaired renal function, smoking, alcohol intake, lack of exercise, various disease states, drugs, inborn errors affecting enzymes involved in lowering tHcy level, delayed processing of blood specimen ↓ Pregnancy
Vitamin B ₁₂	Serum B ₁₂	↑↓ Age ↑ Chronic renal failure, severe congestive heart failure, diabetes, organic diseases ↓ Drugs, alcoholism, pregnancy, smoking, vegetarianism, folate deficiency, bacterial overgrowth, disease states associated with alterations in the levels of vitamin B ₁₂ -binding proteins, malabsorption, worm infestation
	Serum MMA	↑ Impaired renal function, bacterial overgrowth, cobalamin genetic defects, classical methylmalonic acidemia
Vitamin C	Serum total ascorbic acid	↑↓ Age, sex, race ↓ Smoking, low socioeconomic status, catabolic states, obesity, improper specimen handling
Sodium and potassium	Urinary sodium, potassium	↑↓ Age, sex ↑ Renal disease, conditions in which urine is alkaline ↓ Renal diseases with decreased urine flow, diarrhea, or excessive sweating
Calcium	Serum ionized calcium	↑↓ Age ↑ Hyperparathyroidism, functional hypercalcemia, hemodialysis ↓ Hypoparathyroidism, vitamin D-deficient rickets
Magnesium	Serum magnesium	↑↓ Age, sex, race, diurnal variation, hypo-/hyperalbuminemia ↑ Renal failure, hemolysis, drugs (antacids, cathartics) ↓ Pregnancy, strenuous exercise, osteoporosis, drugs (diuretics, antibiotics), GI and renal disease
Iron	Serum ferritin	↑↓ Age, sex, race ↑ Acute phase infection and chronic inflammation, liver disorders, malignant diseases, acute leukemia, Hodgkin's disease, rheumatoid arthritis, thalassemia major, alcohol intake
	Serum sTfR	↑↓ Age, sex, race ↑ Autoimmune hemolytic anemia, sickle cell anemia, folate, or vitamin B ₁₂ deficiency ↓ Chronic renal failure
	Serum iron, TIBC, TS	↑↓ Age (mainly for iron), biological variation (mainly for iron) Chronic disease states with infection, inflammation (decrease in iron and TIBC, TS low) Decreased erythropoiesis due to folate or vitamin B ₁₂ deficiency (increase in iron, decrease in TIBC, TS high)

(Continued)

Table 1 Continued

Nutritional status	Biochemical indicator	Influencing factors
	Erythrocyte zinc protoporphyrin	Increased erythropoiesis in response to vitamin B ₁₂ and folate therapy, in hemolysis, in polycythemia (decrease in iron, increase in TIBC, TS low)
	Hemoglobin	↑ ↓ Age, sex ↑ Chronic disease states with infection, inflammation and some neoplastic diseases, lead poisoning, porphyrin disorders ↑ ↓ Age, sex, race, biological variation ↑ Polycythemia, dehydration ↓ Pregnancy, iron-deficiency anemia, deficiencies of vitamin A, B ₂ , B ₆ , B ₁₂ , folate and copper, parasitic infections, chronic infection and inflammation, chronic diseases that cause overhydration or plasma volume expansion, smoking
Zinc	Serum zinc	↑ ↓ Age, sex, diurnal variation, fasting ↑ Hemolysis, delayed separation of serum from red cells ↓ Pregnancy, acute infection and inflammation, estrogen-containing preparations, malabsorption syndromes, chronic disease states resulting from hypoalbuminemia
Copper	Serum copper and ceruloplasmin	↑ ↓ Age, sex, diurnal variation, certain disease states ↑ Pregnancy, estrogen-containing preparations, smoking, acute phase infection, chronic inflammation, stress, delayed separation of serum from red cells ↓ Malabsorption syndromes
Selenium	Serum selenium	↑ ↓ Age, diet ↓ Prematurity, pregnancy and lactation, smoking, genetic defects (maple sirup urine disease, PKU), certain disease states (disorders of digestive tract, muscle disorders, neurological diseases, inflammatory diseases, chronic renal failure, cancer, CVD)
Iodine	Urinary iodine	↑ ↓ Diurnal variation ↓ Pregnancy
	Serum or whole blood TSH	↑ Congenital hypothyroidism, exposure to iodine-containing antiseptics

↑ ↓, biomarker response increased or decreased; ↑, biomarker response increased; ↓, biomarker response decreased; 25OHD, 25-hydroxyvitamin D; 4-PA, 4-pyridoxic acid; BMI, body mass index; CRP, C-reactive protein; EAST-AC, erythrocyte aspartate aminotransferase activation coefficient; EGR-AC, erythrocyte glutathione reductase activation coefficient; ETK-AC, erythrocyte transketolase activation coefficient; FAD, flavinadeninucleotide; FMN, flavinmononucleotide; IGF-1, insulin-like growth factor; MMA, methylmalonic acid; MRDR, modified relative dose response test; PLP, pyridoxal-5'-phosphate; RBC, red blood cells; RBP, retinol-binding protein; RDR, relative dose response test; sTfR, soluble transferrin receptor; TDP, thiamin diphosphate; TF, transferrin; tHcy, total homocysteine; TIBC, total iron binding capacity; TS, transferrin saturation; TSH, thyroid stimulating hormone; TTR, transthyretin; UIBC, unbound iron binding capacity.

freeze/thaw stability) that influence biochemical indicators used to assess nutritional status.

Protein Nutritional Status

Several serum or plasma proteins can be used to roughly measure the adequacy of protein intake and metabolism, including albumin and several transport proteins (transthyretin (TTR) involved in thyroid hormone transport and formerly called prealbumin, transferrin (TF), and retinol binding protein (RBP)). These proteins will decrease during acute phase response. Reduction of albumin levels may indicate a catabolic state, but is more common in chronic liver disorders. Serum albumin, easily measured by automated clinical analyzers, has a large body pool and a long half-life; it is therefore a less sensitive index of immediate nutritional status. TTR, complexed with RBP in the carriage of vitamin A, TF and RBP have smaller pool sizes and shorter half-life than serum albumin,

however, their specificity as an index of protein status is low and they are confounded by some nutritional deficiencies (vitamin A deficiency for RBP, iron deficiency for TF) and disease states. Serum transport proteins are measured by radial immunodiffusion or immunoassay including nephelometry and turbidimetry. Serum insulin-like growth factor I, or somatomedin C, is a regulator of anabolic properties. It has been proposed as a more sensitive indicator to changes in protein status than other serum proteins. It can be measured by immunoassay techniques.

Urinary creatinine, derived from the catabolism of creatine phosphate which is present mainly in muscle, can be used as a biochemical marker of muscle mass after adjustment for body mass, race, and gender. It is measured by direct colorimetric and enzymatic methods and available on automated clinical analyzers. A major difficulty in estimating urine creatinine excretion is ensuring that 24-h urine collections are complete. Various assumptions are required when estimating the muscle mass from urinary creatinine and influencing factors have to be taken into

Table 2 Preatalytical factors influencing biochemical indicators for nutritional status assessment

Nutritional status	Biochemical indicator	Specimen collection requirements	Storage stability	Freeze/thaw stability
Protein	Serum albumin	Fasting not required	Stable for a few days refrigerated; stable for several months frozen	Minimize freeze/thawing
	Urinary creatinine	Fasting not required; 24-h collection recommended (ideally for 3 consecutive days)	Stable for a few days at RT; stable for weeks refrigerated; stable for years frozen	Stable for at least 5 cycles
	Urinary 3-methylhistidine	Fasting not required; 24-h collection essential (ideally for 3 consecutive days)	Stable for a few days refrigerated; stable for several months frozen	No information
Fatty acids	Plasma fatty acids	Fasting essential	Stable for several years at -70°C	Stable for at least 4 cycles
	RBC fatty acids	Fasting not required	Stable for 7 days at RT for an omega-3 index assessment; stable for at least 4 years at -80°C	No information
Vitamin A	Serum retinol	Fasting not required	Stable for years at -70°C ; stable for weeks refrigerated	Little deterioration for at least 3 cycles
Carotenoids	Serum RBP	Fasting not required	Reported to be as or more stable than retinol	Little deterioration for at least 3 cycles
	Serum carotenes, cryptoxanthin, lutein, zeaxanthin, lycopene	Fasting recommended	Stable for years at -70°C ; stable for weeks refrigerated	Little deterioration for at least 3 cycles
Vitamin D	Serum 25OHD	Fasting not required	Stable for years at -70°C ; stable for weeks at 37°C	Little deterioration for at least 5 cycles
Vitamin E	Serum α - and γ -tocopherol	Fasting recommended; alternatively, normalization to cholesterol or lipids content	Stable for years at -70°C ; stable for weeks refrigerated	Little deterioration for at least 3 cycles
Vitamin K	Serum phyloquinone (K_1), menaquinones (K_2)	Fasting recommended; abstain from alcohol for 1 day before blood draw	Protect from light; stable for 6 months frozen	No information
Thiamin	Erythrocyte or whole blood TDP	Fasting not required; measure Hb when whole blood is used to correct for cell volume variability	Protect from light; transport frozen to avoid loss of TDP; whole blood stable at RT up to 2 days; hemolyzed erythrocytes and whole blood stable for at least 6 months at -70°C	Stable for 3 cycles (whole blood hemolysates)
	Serum thiamin	Fasting not required	Protect from light; stable for 1 year frozen	No information
	Urinary thiamin	Fasting not required; 24-h collection recommended	No information	No information
Riboflavin	Erythrocyte ETK-AC	Fasting not required; packed erythrocytes must be washed and buffy coat removed	Rapid enzyme inactivation within 2 weeks stored at -20°C ; hemolysate is stable for 1 year at -70°C	Avoid freeze/thawing
	Erythrocyte, whole blood or serum FAD, FMN	Fasting not required	Protect from light; stable for at least 5 h at RT in whole blood; endogenous vitamers stable for 14 days at RT in EDTA plasma (supplemented FAD is less stable); serum vitamers stable for 1 year frozen	No information

	Urinary riboflavin	Fasting not required; 24-h collection recommended	Protect from light; little deterioration when specimen in amber glass vial is exposed to fluorescent light at RT for up to 3 days; stable for up to 6 months refrigerated or frozen at -20°C	No information
	Erythrocyte EGR-AC	Fasting not required; packed erythrocytes must be washed	If assay is not performed immediately, hemolysate has to be frozen and is stable for 1 year at -70°C	No information
Niacin	Urinary methylated metabolites	Fasting not required; 24-h collection recommended; acidify with HCl	No information	No information
Vitamin B6	Serum PLP	Fasting not required	Protect from light; stable for several hours at RT in the dark; stable for 1 month at -20°C ; stable for years at -70°C	Stable for at least 3 cycles
	Urinary 4-PA	Fasting not required (morning fasting urine preferred)	Protect from light; stability of 4-PA in serum exceeds that of PLP and stability of 4-PA in urine is expected to be very good	No information
	Erythrocyte AST-AC	Fasting not required; packed erythrocytes must be washed	Hemolysate is stable for a few months at -70°C	No information
Folate	Serum folate	Fasting essential for individual but probably not for population	Protect from light; stable for 1 week refrigerated; stable for a few years at -70°C ; ascorbic acid can be added (0.5% w/v) before storage to improve stability	Little deterioration for at least 3 cycles
	RBC folate	Fasting not required; measure Hct to correct for packed cells; use of serum folate level in calculation of RBC folate level preferred	Protect from light; whole blood stable for several days refrigerated; hemolysate with ascorbic acid (1% w/v) stable for several years at -70°C	Little deterioration for at least 3 cycles
	Plasma tHcy	Fasting not required; separate plasma from red cells within an hour of collection to avoid artificial increase in tHcy	Stable for days at RT; stable for weeks refrigerated; stable for years frozen	Excellent stability
Vitamin B ₁₂	Serum B ₁₂	Fasting not required; avoid ascorbic acid	Protect from light; stable for several days refrigerated; stable for several years at -70°C	Stable for at least 3 cycles
	Serum MMA	Fasting not required	Stable for days at RT; stable for weeks refrigerated; stable for years frozen	Excellent stability
Vitamin C	Serum total ascorbic acid	Fasting recommended; blood must be promptly processed and serum must be acidified (metaphosphoric acid) to stabilize ascorbic acid	Acidified serum is stable for at least 10 years at -70°C	Stable for at least 3 cycles
Calcium	Serum free calcium	Fasting recommended; avoid heparin, triethanolamine, trypsin as they bind calcium; calcium standard should contain sodium and chloride at same level as test samples	Changes in pH of blood may alter measurement; adjust to pH 7.4 with CO ₂ before measurement; perform measurement as soon as possible; serum can be stored	No information

(Continued)

Table 2 Continued

Nutritional status	Biochemical indicator	Specimen collection requirements	Storage stability	Freeze/thaw stability
Magnesium	Serum magnesium	Fasting recommended; avoid hemolysis; collect in metal-free container; separate red cells immediately	anerobically refrigerated for several days or at -20°C for 6 months	No information
Iron	Serum ferritin Serum sTfR Serum iron/TIBC Erythrocyte zinc protoporphyrin	Fasting not required Fasting not required Fasting not required Fasting not required; avoid hemolysis	Stable for years at -70°C Stable for years at -70°C Stable for at least 10 years at -70°C Stable for several days if refrigerated	Stable for at least 3 cycles Stable for at least 3 cycles Stable for at least 3 cycles Do not freeze
Zinc	Serum zinc	Fasting not required; collect in prescreened metal-free container; avoid contact with rubber stopper; avoid hemolysis; remove serum promptly from red cells	Stable for years at -70°C	Stable for multiple cycles
Copper	Serum copper	Fasting not required; collect in prescreened metal-free container; remove serum promptly from red cells	Stable for years at -70°C	Stable for multiple cycles
Selenium	Serum selenium	Fasting not required; collect in prescreened metal-free container	Stable for years at -70°C	Stable for multiple cycles
Iodine	Urinary iodine	Fasting not required; 24-h collection recommended for individual; measurement of urinary creatinine allows for adjustment in casual samples but could be problematic in malnutrition; avoid contamination	Stable for months if refrigerated; stable at least 10 years at -70°C	Stable for at least 3 cycles

25OHD, 25-hydroxyvitamin D; 4-PA, 4-pyridoxic acid; AST-AC, erythrocyte aspartate aminotransferase activation coefficient; CRP, C-reactive protein; EGR-AC, erythrocyte glutathione reductase activation coefficient; ETK-AC, erythrocyte transketolase activation coefficient; FAD, flavinadeninucleotide; FMN, flavinmononucleotide; Hct, hematocrit; MMA, methylmalonic acid; PLP, pyridoxal-5'-phosphate; RBC, red blood cells; RBP, retinol binding protein; RT, room temperature; sTfR, soluble transferrin receptor; TDP, thiamin diphosphate; tHcy, total homocysteine; TIBC, total iron binding capacity.

consideration. In the clinical setting, the creatinine/height index is used to assess the degree of depletion of muscle mass in children with the marasmic form of protein-energy malnutrition and to monitor the effects of long-term nutritional intervention on repletion of lean body mass in hospital patients. Measurement of the amino acid 3-methylhistidine in urine is used as an indicator of muscle protein turnover, however, available data on various populations is limited.

Metabolic changes that occur in protein-energy malnutrition are sometimes used as less specific indices of protein status. These relate to changes in free amino acid profiles in plasma, reduced urinary hydroxyproline excretion and increased urinary nitrogen excretion. Functional indices of protein status include muscle function, handgrip strength, and immunological testing.

Essential Fatty Acid Status

Essential fatty acids are those fatty acids that are not synthesized by the body but are vital for proper growth and development; linoleic and linolenic acids are essential and must be obtained through dietary sources. Fatty acids can be measured in plasma or serum, in the phospholipid fraction of plasma, in red blood cell membranes, in whole blood, or in tissues. *In vivo*, fatty acids are almost always combined with sterols, glycerol, or phospholipids although free fatty acids circulate bound to albumin (1 mol albumin: 20 mol fatty acids) and the flux of free fatty acids through the plasma is very large and varies with metabolic demands. Fatty acid data are expressed either as concentration or as a percentage of total fatty acids. Capillary gas chromatography (GC) is the technique most frequently used to separate fatty acids for quantitative analysis. Detection methods include flame ionization or electron capture negative chemical ionization mass spectrometry. Internal standards are used to correct for losses during sample preparation and improve the accuracy and precision of measurements. Most often, the diagnosis of essential fatty acid deficiency is made from clinical findings but deficiency may be detected weeks to months before clinical manifestations are present using fatty acid profiling. Unusual or large changes in total or individual plasma fatty acids are due to underlying disease states (liver disorders), medications, or inborn errors of metabolism. General fatty acid deficiency can be detected when values of linoleic and alpha-linolenic acids are low based on total plasma fatty acids or phospholipid extracts of plasma. In addition, the ratio of triene to tetraene fatty acids is the most common laboratory indicator of essential fatty acid deficiency (Holman index). The omega-3 ($n - 3$) index, a measure of the amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in red blood cell membranes as a proportion of total fatty acids, has been suggested to be a marker of risk for sudden cardiac death.

Vitamin Nutritional Status

Vitamin A

Vitamin A (retinol) status can be assessed by testing liver, plasma, or serum. The best way to determine inadequate status

is through hepatic biopsy but this procedure is invasive and unsuitable in population studies. Serum or plasma retinol is usually measured using high performance liquid chromatography (HPLC) with ultraviolet (UV) detection after separation from its carrier RBP. Serum retinol values do not always reflect total body status because of homeostatic control and therefore are often not useful for assessing the vitamin A status of individuals. Additional tests may be required to confirm vitamin A deficiency when $0.70 \mu\text{mol l}^{-1}$ is used as a cutoff. The distribution of serum retinol values in a population together with the prevalence of individuals with serum retinol values below a given cutoff point provide important information about the vitamin A status of a population. WHO recommends using the prevalence of serum retinol $\leq 0.70 \mu\text{mol l}^{-1}$ to define public health problems involving vitamin A deficiency as mild (2–9%), moderate (10–19%) or severe ($\geq 20\%$). In the case of infection or inflammation, the degree of depression of circulating retinol can be roughly quantified by assessing the concentration of certain acute phase proteins such as CRP and AGP. Misclassification of vitamin A status is likely to occur unless subjects undergoing an acute phase response from infection, trauma, or a chronic inflammatory condition are identified and adjustments are made to account for this phenomenon.

RBP is a well-regulated transport protein for retinol. Nearly all circulating retinol is bound to a soluble RBP-transferrin (TTR) complex in equimolar amounts. Because retinol is closely correlated with RBP, the measurement of this transport protein using enzyme-linked immunosorbent assay (ELISA) has been used to assess vitamin A status. In most populations, serum RBP has been shown to be a suitable surrogate for retinol. The molar ratio of RBP to TTR was introduced to detect vitamin A deficiency in the presence of inflammation but is rarely used nowadays because of unsatisfactory results in several population groups.

The deuterated-retinol-dilution (DRD) technique is used to indirectly assess total body vitamin A reserves. In this test, a dose of deuterium-labeled retinyl acetate is given orally. After allowing time to reach equilibration (3–21 days), deuterated and nondeuterated serum retinol levels are measured using GC with mass spectrometric detection (GC-MS) and this ratio is used to estimate total body stores of vitamin A. Because of technical requirements, this method is used mostly in research. In inflammation, the release of RBP from the liver is reduced, so the test is also unreliable during the acute phase response.

The provitamin (carotenes and cryptoxanthins) and non-provitamin (lutein, zeaxanthin, and lycopene) compounds of vitamin A, the carotenoids, need consideration due to their independent and specific role in good health by preventing oxidation. Serum levels of carotenoids are correlated with vegetable and fruit intake. Lutein is the best indicator of green leafy vegetable consumption. Lycopene is a good measure of tomato-based product consumption. β -Carotene in industrialized countries is probably a biomarker of carrot consumption and in West Africa a good marker of red palm oil consumption. Serum or plasma levels of carotenoids are measured by HPLC-UV/vis; it is possible to measure in a single assay a panel of about a dozen fat-soluble micronutrients including several forms of vitamins A and E, and individual carotenoids.

Several functional tests have been developed to assess vitamin A reserves in the liver. These tests are based on the

accumulation of unbound RBP within the liver when vitamin A intake is low and reserves of retinyl esters are depleted. Once retinol becomes available as a result of uptake from food or supplements or biosynthesis from carotenoids, it is released from the liver bound to RBP within hours of availability. Vitamin A functional tests, the relative dose-response (RDR) and the modified relative dose-response (MRDR) test, take advantage of this immediate release.

Other functional testing protocols involving dark adaptometry have been used to assess night vision as an indicator of vitamin A status. In the rapid dark adaptation test, the subject is light adapted and then while working in dim light, usually takes <10 min to correctly sort colored disks while adapting to darkness. The time required to achieve a perfect score is recorded. A simpler functional test is one in which pupillary dark adaptation is assessed; it requires minimal cooperation and is suitable for very young children (≥ 2 years) who are most likely to be vitamin A deficient. The pupillary threshold test measures the tendency of the pupil to constrict in response to illumination. An impaired response is seen when vitamin A stores are depleted even if overt clinical signs of deficiency are absent.

Vitamin D

Vitamin D status is routinely assessed by measurement of serum or plasma 25-hydroxyvitamin D (25OHD). Typical methods are either antibody-based (e.g., radioisotope-, enzyme-linked- or chemiluminescence immunoassay), or chemistry-based (e.g., HPLC separation with UV or tandem mass spectrometry [MS/MS] detection). GC-MS has also been employed. Studies have shown that the chemistry-based methods are equivalent but that antibody-based methods may show significant bias compared to chemistry-based methods. Genetic variants near genes involved in cholesterol synthesis, hydroxylation, and vitamin D transport affect vitamin D status.

Several vitamin D functional tests have been suggested such as the measure of: (1) circulating parathyroid hormone (PTH); (2) bone resorption markers such as serum alkaline phosphatase activity, serum C-terminal telopeptide of type I collagen, urinary cross-linked N-telopeptides of type I collagen, and urinary deoxypyridinoline; (3) bone mineral density (BMD); and (4) intestinal calcium absorption. PTH appears to be a useful marker of vitamin D status when vitamin D is given without calcium but not with calcium. Markers of bone resorption are not good functional markers of vitamin D status, due in part to high inter-subject variability; however, bone resorption using a more sensitive calcium radioisotope method may prove to be useful. Increased BMD of some sites appears to be useful as a functional response to vitamin D supplementation in older persons but not adolescents. Intestinal calcium absorption has been shown to be responsive to vitamin D status.

Vitamin E

Vitamin E status can be assessed in serum, plasma, erythrocytes, platelets, and adipose tissue. The most common and practical measure is α -tocopherol in serum or plasma using HPLC with UV detection. Because α -tocopherol is bound to lipoproteins, circulating α -tocopherol is often expressed

relative to serum cholesterol. The determination of α -tocopherol in adipose tissue biopsy provides information on long-term nutritional status, but this test is too invasive.

Vitamin E functional tests consist of the following assays: erythrocyte hemolysis, erythrocyte malondialdehyde (MDA) release, breath pentane or ethane, susceptibility of low density lipoprotein (LDL) to oxidation using diene conjugate second derivatives, and isoprostane formation. Susceptibility to erythrocyte hemolysis is inversely correlated with α -tocopherol concentration. *In vivo*, hemolysis (>20%) occurs when α -tocopherol concentration is <4.6–11.6 $\mu\text{mol l}^{-1}$. MDA is a breakdown product of lipid peroxidation and is measured colorimetrically. These two *in vitro* erythrocyte assays are the primary vitamin E functional tests; methodological limitations including the need for freshly prepared red blood cells make them disadvantageous.

Volatile hydrocarbons (pentane and ethane) in breath, generated from oxidized lipids, and the susceptibility of LDL to oxidation are general markers of oxidative stress and also have been investigated as functional tests of vitamin E deficiency, but technical difficulties, lack of specificity, and inconsistent findings were some of the problems. More recently, vitamin E has been shown to decrease isoprostane F2 in individuals with moderate hypercholesterolemia who exhibited oxidative stress. F2-isoprostanes are formed by free radical-mediated peroxidation of arachidonic acid, an omega-6 polyunsaturated fatty acid. Plasma concentrations of F2-isoprostanes were suppressed in these patients by 35–50% in a dose-dependent manner with maximum suppression at vitamin E intake of 1600–3200 IU d⁻¹.

Vitamin K

Vitamin K status assessment is usually performed in response to abnormal bleeding. Static tests are used to measure circulating concentrations of vitamins K₁ (phyloquinone) and K₂ (menaquinone). Serum or plasma vitamin K₁ is measured using HPLC with postcolumn chemical reduction followed by fluorometric detection or with electrochemical detection. Hemolysis should be avoided when the detection method is fluorescence. Measurement of K₁ has also been performed using GC-MS or HPLC-MS/MS. It has been suggested, although not yet commonly implemented, that K₁ concentration should be expressed as a ratio of the triglyceride concentration.

Determination of the serum undercarboxylated form of prothrombin (PIVKA-II) by ELISA and urinary γ -carboxyglutamic acid by HPLC with fluorometric detection have been proposed for assessment of vitamin K status. A vitamin K functional test that has been more widely used in recent years is the determination of serum undercarboxylated osteocalcin. This test is well correlated with other indicators of vitamin K status. A number of commercial ELISA kits are available.

Thiamin (vitamin B₁)

Thiamin status can be assessed through measurement of thiamin diphosphate (TDP) – also known as thiamin pyrophosphate (TPP) – in whole blood or erythrocytes by HPLC (preferably after postcolumn derivatization and fluorometric detection). TDP is the primary active form of vitamin B₁ and

approximately 90% of thiamin in whole blood is present as TDP. Thiamin concentration in serum or plasma is small compared to that in erythrocytes and reflects recent intake rather than body stores. Urinary thiamin excretion (preferably as 24-h urine samples) under basal conditions or after thiamin loading also reflects recent dietary intake, but the within-subject variation is high. Owing to limited body stores of thiamin, deficiencies can develop within a few weeks if intake is restricted. The response to thiamin therapy is usually rapid and a reliable test for thiamin deficiency. Infections that prevent normal absorption (diarrhea and dysentery) or increase the requirement (fever) can confound tests for thiamin status.

The erythrocyte transketolase activation coefficient (ETK-AC) test is a functional test that reflects the adequacy of body stores and is sensitive to marginal thiamin deficiency. It is measured by spectrophotometry. Because transketolase is a thiamin-dependent enzyme with a specific role in the glucose oxidative pathway, decreased enzyme activity is presumed to be due to the decrease of thiamin. However, the test is somewhat nonspecific, as factors other than thiamin status, such as genetic defects, may influence the enzyme activity and thus the test results. The ETK-AC test also suffers from imprecision problems and rapid loss of enzyme activity during frozen storage and repeated freezing and thawing. There is some debate whether the ETK-AC test – due to its slower response compared to the increase of TDP in whole blood – is a better biomarker to follow thiamin supplementation.

Riboflavin (vitamin B₂)

Riboflavin status can be assessed by urinary excretion of the vitamin in fasting, random, 24-h specimens (preferable), or by loading test, and by whole blood, erythrocyte, plasma, or serum flavin concentration. Riboflavin urinary excretion is indicative of recent dietary intake and is measured by HPLC using fluorometric detection, taking advantage of the inherent fluorescent properties of flavins. Erythrocytes are considered to be a more useful sample than plasma or serum because the riboflavin cofactors flavinadeninedinucleotide (FAD) and flavinmononucleotide (FMN) are concentrated in erythrocytes. They can be measured by HPLC with fluorometric detection. Whole blood FAD is considered a reliable indicator of long-term nutritional status, whereas FMN responds more quickly to changes in riboflavin intake. The light-sensitivity of riboflavin in particular and most B vitamins in general requires careful sample handling.

A riboflavin functional test that is commonly used is the erythrocyte glutathione reductase activation coefficient (EGR-AC) test measured by spectrophotometry. Because glutathione reductase is a flavoenzyme with FAD as a prosthetic group, the EGR-AC test is an indirect measure of FAD concentration in the erythrocytes and is considered a sensitive and robust index of riboflavin deficiency, but is less suitable for the assessment of riboflavin status at high riboflavin intake.

Niacin (vitamin B₃)

Niacin intake status can be assessed by measuring the excretion of methylated metabolites in urine by HPLC. Such

metabolites are N'-methylnicotinamide (N'MN) and N'-methyl-2-pyridone-5-carboxamine (2-Py). Other biochemical markers include erythrocyte pyridine nucleotides, oral dose uptake tests, and plasma 2-pyridone derivative after an oral niacin load. Plasma concentrations of other niacin metabolites and of niacin are not useful markers of niacin status. The most reliable test for niacin deficiency is the patient's response to niacin therapy.

Niacin used as a drug has seen a sudden surge in popularity for treatment of lipid disorders and other associated clinical conditions for the prevention of cardiovascular risk. To clarify the role of metabolic pathways and evaluate pharmacokinetic studies, HPLC-MS/MS assays have recently been developed to measure the levels of niacin and its metabolites in various biological matrices.

Vitamin B₆

Vitamin B₆ status is typically assessed by measuring the level of one or more of the B₆ vitamers in serum or plasma. Serum pyridoxal-5'-phosphate (PLP) is generally viewed as the best single indicator of status. PLP is the active coenzyme form of vitamin B₆, reflects both dietary intake and tissue stores, and changes slowly in response to changes in dietary intake. 4-Pyridoxic acid (4 PA) is the end product of vitamin B₆ catabolism. 4 PA can be measured in serum, plasma, or urine and reflects recent intake. PLP and the B₆ vitamers are most commonly measured by HPLC using fluorescence detection. Chemical derivatization (sample, online, or postcolumn) is almost always used to enhance PLP fluorescence. HPLC-MS/MS methods for measuring B₆ vitamers are emerging. Plasma PLP can also be measured enzymatically, either by radioactive or nonradioactive assays.

Vitamin B₆ functional tests, such as the erythrocyte aspartate aminotransferase activation coefficient (AST-AC) test and the tryptophan load test, have been used more frequently in the past. Plasma total homocysteine (tHcy) in the absence of folate and vitamin B₁₂ deficiencies can be considered indicative of vitamin B₆ status. For its determination, see the following discussion of folate functional tests.

Folate

Folate status can be assessed by serum or plasma folate, which provides information on recent intake, and erythrocyte folate, indicative of body folate stores and long-term nutritional status. Traditionally, folate has been measured by microbiologic assay, however, in clinical settings where high throughput is needed, commercial protein-binding assays on automated clinical analyzers are used. If folate vitamers are of interest, for e.g., the measurement of free folic acid in serum or the measurement of various methyl- and nonmethyl-folate forms in erythrocytes depending on MTHFR C677T genotype, chromatography-based separation techniques need to be employed. Nowadays, they are often coupled to mass spectrometry (HPLC-MS/MS), because this detection method offers superior sensitivity, specificity, and selectivity compared to other detection methods such as fluorometric or electrochemical detection. Although the comparability of serum

folate methods has been somewhat improved recently because serum-based standard reference materials have become available, diagnostic kits are not yet sufficiently standardized and no progress has yet been made in improving the comparability of assays for erythrocyte folate. Folate is the least stable of the B vitamins; careful sample handling and use of antioxidants are required to maintain sample integrity. Dried blood spots can also be used to measure folate by microbiologic assay. This presents a field-friendly alternative when prompt specimen processing cannot be performed or blood collection is limited to a finger stick.

In the absence of vitamin B₁₂ and B₆ deficiencies, measurement of plasma tHcy is a sensitive functional test for folate status. Because an elevated plasma tHcy concentration is associated with an increased risk of cardiovascular diseases, the determination of this amino acid in plasma has become very common. Various methods are available for tHcy determination, but the most commonly used research methods are HPLC with fluorescence detection or coupled to mass spectrometry. They allow simultaneous measurement of other thiols in the same sample. Many fully-automated commercial kits are available on the basis of immunoassay and enzymatic methods. Prompt separation of the plasma from the red cells needs to be ensured to avoid artificial elevation of tHcy. Urinary formiminoglutamic acid (FIGLU) and lymphocyte deoxyuridine (dU) suppression assays are older functional tests for folate status that are no longer used routinely. Hypersegmentation of neutrophilic granulocytes are sometimes seen as a functional indicator during the examination of blood smears following routine cellular blood counts.

Vitamin B₁₂

Vitamin B₁₂ status can be assessed by measuring serum or plasma total cobalamins and serum holo-transcobalamin II. The level of plasma vitamin B₁₂ falls relatively late in depletion, limiting the utility of an isolated vitamin B₁₂ measurement. Serum or plasma total cobalamins are commonly determined by competitive protein-binding assay, but microbiologic assays have also been used earlier. Holo-transcobalamin II (holo TC) is the transport protein of absorbed cobalamin and has been considered as an early indicator of vitamin B₁₂ deficiency and possibly a marker of cobalamin malabsorption. The availability of the holo TC assay is currently limited and only recently have reliable and sensitive methods for estimating holo TC become available. A new microparticle enzyme immunoassay is available on an automated immunoassay analyzer and can measure holoTC directly without sample pretreatment.

Vitamin B₁₂ functional tests are the urinary or serum methylmalonic acid (MMA) and the plasma tHcy. MMA increases in vitamin B₁₂ deficiency; the loading with valine or isoleucine produces a marked increase in both urine and serum. MMA is measured by GC-MS or HPLC-MS/MS. In the absence of folate and vitamin B₆ deficiencies, tHcy in plasma increases in vitamin B₁₂ deficiency and decreases with B₁₂ administration. For tHcy determination, see the discussion of folate functional tests.

Biotin

Biotin status has been assessed traditionally with bioassays and microbiologic assays. Modern methods rely upon the binding of biotin by either the protein avidin or streptavidin as part of competitive binding assays. To minimize the influence of interfering substances, prior separation and purification of biotin and its metabolites can be performed by HPLC. A low plasma biotin concentration is not a sensitive indicator of inadequate biotin intake. Urinary biotin excretion, particularly when extended to the excretion of its metabolites (that is, 3-hydroxyisovaleric acid [3-HIA] and 3-methylcrotonylglycine) is a more sensitive indicator of biotin status. Biotin is abnormally decreased, whereas 3-HIA is abnormally increased in urine in deficiency. The resolution of the signs and symptoms of deficiency in response to biotin supplementation is also important in the diagnosis of biotin deficiency.

Pantothenic Acid

Pantothenic acid intake status can be assessed by measuring whole blood concentrations or urinary excretion. The widespread occurrence of releasable pantothenic acid in food however makes a dietary deficiency unlikely.

Vitamin C

Vitamin C status can be assessed by measuring total ascorbic acid (oxidized and reduced) in serum or plasma, buffy-coat, or leukocytes. Ascorbic acid in plasma is considered as an index of the circulating vitamin available to tissues, and in leukocytes (particularly polymorphonuclear) it is believed to be a good indicator of tissue stores. Concentrations in leukocytes are much higher than in serum or plasma (14-fold). Isolation of specific cells is technically challenging limiting its usefulness. The urinary excretion of ascorbic acid is an index of recent intake; but because of instability of the collected sample, the determination is limited to special cases. Serum or plasma vitamin C is the most practical indicator of vitamin C status, however preanalytical requirements must be followed to promptly generate an acidified serum sample to stabilize ascorbic acid. Ascorbic acid is measured using HPLC coupled with electrochemical detectors. Newer methods have incorporated internal standards to improve accuracy and precision. Acute and chronic infections can depress markedly the serum ascorbic acid level due to a decrease in vitamin C reserves.

There are no reliable functional tests for vitamin C status.

Essential Mineral and Trace Element Nutritional Status

Sodium and Potassium

Sodium, potassium, and chloride in serum have little meaning in nutritional terms because they are tightly regulated. However, the excretion of sodium, potassium, and chloride in urine are a good indicator of intake. A 24-h specimen is

needed to interpret concentrations in a person. Equations are available to calculate estimated electrolyte excretion from measured spot urine concentrations of electrolytes and creatinine. Electrolyte concentrations in serum and urine are typically measured by ion-selective electrodes (ISE), often available as add-ons to chemistry analyzers.

Calcium

Calcium intake cannot be assessed satisfactorily on a routine basis. Serum calcium concentrations (free plus bound calcium) are strongly homeostatically controlled and remain constant under most conditions. Serum or plasma free calcium (a.k.a., ionized calcium), the physiologically active form, is increasingly used to assess disturbances in calcium metabolism and is the most promising index of calcium status. It is measured by a calcium-selective electrode. Because one of the major roles of calcium is to be a structural component in bone and soft tissues, measurement of biochemical markers of bone remodeling (serum bone-specific alkaline phosphatase and osteocalcin; urine pyridinoline and deoxypyridinoline) or measurement of bone mass and bone density are indirect ways to assess calcium status.

Magnesium

Magnesium status can be assessed by measuring magnesium in serum, erythrocytes, leukocytes, and urine. Serum is the matrix most commonly used, mainly because of the ease of measurement by colorimetric methods or atomic absorption spectroscopy (AAS). Serum magnesium concentrations decrease rapidly in developing deficiency, followed by a slower decline of magnesium concentrations in erythrocytes. The validity of leukocyte magnesium concentrations as a biomarker of total body magnesium status is still under investigation. Urinary magnesium has been used as an indicator of magnesium status, primarily in association with a magnesium load test. However, this test is invasive and cumbersome and the protocol requires standardization. Measurement of serum ionized magnesium concentrations using ISE is promising, but its use in clinical disease states requires more investigation.

Iron

Iron status is assessed in relation to three stages of development of iron-deficiency anemia. In the first stage, to evaluate the size of body iron stores, serum ferritin can be measured using immunological methods (immunoturbidity, immunonephelometry, chemiluminescence, or ELISA). Commercial kits are available for most clinical analyzers. In the second stage, to determine the adequacy of iron supply to the erythroid marrow, the following biochemical indicators can be measured: serum iron (colorimetric methods, available as commercial kits), erythrocyte protoporphyrin (specific hematofluorometer), and serum soluble transferrin receptor (sTfR) (immunological methods, available as commercial kits). The transferrin saturation (TS) is calculated as the ratio of serum iron/TIBC (expressed as a percentage). In the third stage, iron-deficiency anemia develops, for which hemoglobin

(Hb, spectrophotometry or automated with an electronic counter) is the most common indicator. Infection is an important confounder for iron status markers. Serum ferritin and erythrocyte protoporphyrin levels increase, whereas serum iron, serum iron binding capacity and Hb decrease.

Multiple indicators should be used to assess iron deficiency. The ferritin model has been used extensively in the past. It defines iron deficiency as an abnormal value for at least two of three indicators (serum ferritin, erythrocyte protoporphyrin, and TS). An approach for estimating body iron was developed more recently. It uses two indicators, serum ferritin and sTfR, and allows the full range of the iron status to be evaluated. Furthermore, sTfR is generally not influenced by infection, inflammation, and chronic diseases. This body iron model is currently applied to the National Health and Nutrition Examination Survey (NHANES) to assess the iron status of the US population.

Zinc

In healthy individuals, plasma or serum zinc are reliable markers of zinc status, mainly reflecting zinc intake. Because the effective regulation of zinc homeostasis buffers the functional response to dietary deficiency and excess, plasma zinc levels are generally considered a poor measure of marginal zinc deficiency. Urinary zinc excretion (24-h) and hair zinc can provide useful information on zinc status in zinc-supplemented persons, but whether these reflect zinc status in depleted persons is not clear. Zinc levels are typically measured by inductively coupled plasma mass spectrometry (ICP-MS), however, they can be assessed using AAS as well. Many precautions are required during sample collection, preparation, and storage to avoid contamination of the specimen (environmental exposure and hemolysis). Newer evidence suggests that platelet, mononuclear, and polymorphonuclear cell, and erythrocyte zinc levels are ineffective as biomarkers of zinc status.

Zinc functional tests are serum or plasma alkaline phosphatase, erythrocyte metallothionein (MT), monocyte metallothionein mRNA (MTmRNA), and serum thymulin assays. Alkaline phosphatase is a zinc metalloenzyme; rather than being indicative of zinc deficiency, it is considered to be of value after zinc supplementation but with contrasting results. A commercial kit is available for plasma alkaline phosphatase determination. Alkaline phosphatase activity has low specificity and is subject to many pathophysiological conditions. Erythrocyte MT decreases in moderate and severe zinc depletion and changes in response to elevated dietary zinc intake. Erythrocyte MT is measured by sandwich ELISA assay. MTmRNA is a new approach to zinc status assessment. It responds more rapidly to zinc supplements than erythrocyte MT. MTmRNA is measured in monocytes by competitive reverse transcriptase-polymerase chain reaction. An improvement of this method is the determination of MTmRNA on blood samples spotted onto filter paper. Confounding effects are limited to infection. MT and MTmRNA assays are very promising; further studies are needed because of the difficulty in their determination. Serum thymulin activity is decreased in zinc deficiency because it requires zinc to maintain its structure. There are some indications that erythrocytes, PMNCs,

mononuclear cells, platelet zinc, and plasma alkaline phosphatase are not useful biomarkers of zinc status.

Copper

Serum copper is the most useful marker of copper status, effective in both replete and depleted persons. The tight homeostatic regulation of copper levels in circulation generally restricts major perturbations in levels to the extremes of dietary intake. Serum copper is frequently measured by ICP-MS, although AAS is a common alternative. Levels of copper in other tissues or fluids are difficult to assess or are not considered valid indices of copper status.

Different copper functional tests are available: serum ceruloplasmin, erythrocyte superoxide dismutase (SOD), and leukocyte/platelet cytochrome c oxidase assays. Serum ceruloplasmin can be measured for its oxidase activity on various substrates or by radial immunodiffusion. The majority of serum copper is bound to ceruloplasmin, resulting in a similar response for these two markers. However, ceruloplasmin is not a useful marker in copper-replete adults and levels can be affected by a range of nondietary factors, mainly by infection because this is an acute phase reactant protein. Cu/Zn SOD is a cytosolic metalloprotein that catalyzes the reduction of superoxide to hydrogen peroxide and oxygen. Newer evidence suggests that it is not a useful marker of copper status. Cytochrome c oxidase activity in platelets or leukocytes is another marker under investigation, but the paucity of data does not allow any firm conclusions about its usefulness yet.

Selenium

Short-term selenium status is usually assessed by measuring plasma or serum selenium by ICP-MS or by AAS, a more commonly-available measurement procedure. Long-term selenium status is assessed by measuring selenium in whole blood or erythrocytes. Urinary selenium has been shown to be a reliable marker for recent selenium intake rather than a robust marker for selenium status. Insufficient data currently exist that would allow for the prediction of a health effect from the concentration of selenium in hair or nails. Also the presence of selenium in hair or nails may indicate both external and internal exposures and there is a lack of reference or background ranges to help frame the interpretation of the results.

Selenium functional tests are plasma, whole blood, and platelet glutathione peroxidase activity (GSH-px) assays. The plasma GSH-px is a useful marker of selenium status in populations with low selenium intake; it responds rapidly to supplementation. Erythrocyte GSH-px has a plateau above which it is independent of selenium status. In addition, erythrocyte GSH-px responds slowly to depletion and supplementation. Platelet GSH-px responds rapidly to selenium dietary changes, accordingly, it is considered to be a sensitive indicator of changing selenium status. GSH-px can be measured with an enzyme assay or ELISA; commercial kits are available. The determination of selenoprotein P, which accounts for 50% of selenium in blood, is also a useful and sensitive test for selenium status, at least in populations with relatively low-to-moderate selenium intakes. Selenoprotein P

can be detected by isolating the protein with chromatography followed by detection of selenium with ICP-MS.

Iodine

Iodine status is most commonly assessed by measuring urinary iodine using highly specific ICP-MS methods or technically simpler spectrophotometric methods. Urinary iodine reflects iodine intake within the past few days, but the marker is generally not useful to classify intake sufficiency or deficiency in a person, but rather to define the risk of a population. If 24-h urine cannot be collected, iodine excretion can be expressed per gram of creatinine but only in areas with very low inter and intraindividual variation in urinary creatinine. In clinical settings, the measurement of uptake of radioactive iodine is used.

Iodine functional tests relate to its impact on thyroid function. The measurement of thyroid hormones and of thyroglobulin – a key precursor in the production of thyroid hormones – is performed by specific competitive radioimmunoassay or immunofluorimetric methods. When thyroid disease is not present, serum thyroxine (T4) can be a useful marker of iodine status in most age groups, whereas serum pituitary thyroid-stimulating hormone (TSH) is a good marker in pregnant and lactating women, but not useful in children and adolescents. Serum 3,5,3'-triiodothyroxine (T3) is not a useful biomarker for iodine status. Serum thyroglobulin is a useful marker in children and adolescents, but not useful during pregnancy and lactation. Infection has a confounding effect on iodine status because the synthesis of TTR – a thyroid hormone transporter – is markedly suppressed.

Choice of Laboratory Tests

The choice of laboratory tests depends on the type of study to be carried out. In field nutritional epidemiology studies, particularly in developing countries, the number and type of tests will be mainly limited by the specimen volume, the local laboratory infrastructure, and the availability of skilled personnel and financial resources. The lowest complexity profile of testing achievable with a spot urine sample and an EDTA whole blood sample from a finger stick covers representatives of iodine, iron, vitamin A, and folate status, including parameters of infection and inflammation: urinary iodine (spectrophotometric method), Hb (portable point-of-care instrument), serum ferritin/sTfR/RBP/CRP/AGP (ELISA), and RBC folate (microbiologic assay). The measurement of additional vitamins and biochemical indicators generally requires a larger volume of blood that can only be obtained through venipuncture and requires the availability of more complex laboratory tests.

In population studies carried out in developed countries with high-level laboratory facilities, the selection of laboratory tests depends on the purpose of the study, specimen volume, and financial resources. Because certain plasma proteins and urine creatinine are part of standard biochemical profiles on fully-automated clinical analyzers, they can be easily determined. Fatty acid distribution profiles are rarely assessed in population studies. However, serum cholesterol, triacylglycerols

and lipoprotein fractions are typically measured. These tests are available on fully automated clinical analyzers, have been largely standardized and are sometimes required for normalization of lipophilic compounds. The selection of micronutrient tests can be determined by the suspected deficiencies from previous dietary surveys and by the need to assess the impact of nutritional interventions such as fortification. A representation of micronutrients covered by the recent NHANES can be found in Table 3. Several analytical methods evolved in the last few years from less specific immunoassays to highly specific mass spectrometry-based techniques.

In a hospital setting, the selection of laboratory tests depends on the clinical condition of the patient at admission and during the subsequent course of injury or illness. Among hospital and institutionalized patients, deficiencies in proteins, vitamins, and trace elements are common due to underlying medical disease; suspicion of deficiency is based on history and physical examination.

Regardless of the setting, two other considerations deserve mention. First, the precision of the laboratory test largely influences the minimum detectable difference on repeat measurements or the ability to distinguish between healthy and diseased individuals or populations. It is therefore desirable to select laboratory tests with the highest achievable precision. Second, most biochemical indices do not have the required sensitivity and specificity to be used solely in the diagnosis of an abnormality. It is therefore recommended to combine findings from dietary intake assessment with static biochemical indices and functional tests whenever possible.

Most methods used to assess nutritional status have not been standardized yet which can lead to considerable differences among laboratories and methods. Where available, the selection of high-order reference methods should be favored or, in their absence, carefully validated methods with regards to sample collection, processing, and analysis should be used.

Table 3 Biochemical indicators and analytical methods used in NHANES to assess the nutritional status of the US population during some or all years of 1999–2010

<i>Nutritional status</i>	<i>Biochemical indicator</i>	<i>Analytical method</i>
Protein	Serum albumin	Colorimetric on analyzer
	Urinary creatinine	Colorimetric Jaffé reaction on analyzer
Infection/inflammation	Serum CRP	Immunonephelometry on analyzer
Lipids	Serum total cholesterol, triglycerides, HDL and LDL cholesterol (calculated)	Enzymatic and colorimetric on analyzer
Fatty acid	Plasma or serum fatty acids	GC-MS
Vitamin A	Serum retinol and retinyl esters	HPLC-UV/VIS
Carotenoids	Serum carotenes, cryptoxanthin, lutein, zeaxanthin, lycopene	HPLC-UV/VIS
Vitamin D	Serum 25OHD	Radioimmunoassay (2000–2006)
	Serum 25OHD ₂ , 25OHD ₃ , epi-25OHD ₃	LC-MS/MS (2007–2010)
Vitamin E	Serum α - and γ -tocopherol	HPLC-UV/VIS
Vitamin B6	Serum PLP	Enzymatic (2003–2004)
	Serum PLP and 4-PA	HPLC-FD (2005–2010)
Folate	Serum folate (total)	Radio protein binding assay (1999–2006)
	Serum folate (total)	Microbiologic assay (2007–2010)
	Serum folate (species)	LC-MS/MS (2007–2008)
	RBC folate (total)	Radio protein binding assay (1999–2006)
	RBC folate (total)	Microbiologic assay (2007–2010)
	Plasma tHcy	FPIA on analyzer
Vitamin B ₁₂	Serum B12	Radio protein binding assay
	Serum MMA	GC-MS
Vitamin C	Serum total ascorbic acid	HPLC-ED
Sodium, potassium	Serum sodium and potassium	ISE on analyzer
Calcium	Serum total calcium	ISE on analyzer
Iron	Serum ferritin	Radioimmunoassay (1999–2002)
		Immunoturbidity on analyzer (2003–2010)
	Serum iron, TIBC, TS (calculated)	Colorimetric manual (1999–2002)
	Serum iron, UIBC, TS (calculated)	Colorimetric on analyzer (2003–2010); iron only (2007–2010)
	Serum sTfR	Immunoturbidity on analyzer
	Erythrocyte protoporphyrin	Fluorometric manual
Selenium	Serum selenium	ICP-MS
Iodine	Urinary iodine	ICP-MS

25OHD, 25-hydroxyvitamin D; 25OHD₂, 25-hydroxyvitamin D₂; 25OHD₃, 25-hydroxyvitamin D₃; 4-PA, 4-pyridoxal-5'-phosphate; CRP, C-reactive protein; epi-25OHD₃, 3-epimer-25-hydroxyvitamin D₃; FPIA, fluorescence polarization immunoassay; GC-MS, gas chromatography with mass spectrometry detection; HDL, high-density lipoprotein; HPLC-ED, high performance liquid chromatography with electrochemical detection; HPLC-FD, HPLC with fluorometric detection; HPLC-UV/VIS, HPLC with UV and visible detection; ICP-MS, inductively coupled plasma with mass spectrometry detection; ISE, ion selective electrode; LDL, low-density lipoprotein; LC-MS/MS, liquid chromatography with tandem mass spectrometry detection; MMA, methylmalonic acid; NHANES, National Health and Nutrition Examination Survey; PLP, pyridoxal-5'-phosphate; RBC, red blood cells; sTfR, soluble transferrin receptor; tHcy, total homocysteine; TIBC, total iron binding capacity; TS, transferrin saturation; UIBC, unbound iron binding capacity.

Table 4 Available reference materials and external quality assessment programs for biochemical indicators of nutritional status

<i>Nutritional status</i>	<i>Biochemical indicator</i>	<i>Reference materials</i>	<i>Selected list of external quality assessment programs</i>
Protein	Serum albumin and transport proteins (TTR, TS and RBP)	ERM-DA470 (human serum, freeze-dried; one level; consensus value)	CAP General Chemistry Survey and Cal V/L Survey
Infection/ inflammation	Serum CRP	ERM-DA472 (human serum, frozen; one level; consensus value)	CAP CRP-Immunology Survey and Cal V/L Survey
	Serum AGP	ERM-DA470 (human serum, freeze-dried; one level; consensus value)	Not available at this time
Fatty acids	Fatty acids	NIST SRM 1950 (human plasma, frozen; one level; certified values for selected fatty acids)	Not available at this time
Vitamin A	Serum retinol	NIST SRM 968e (human serum, frozen; three levels; certified values)	NIST MMQAP; UK NEQAS
Carotenoids	Serum carotenes, cryptoxanthin, lutein, zeaxanthin, lycopene	NIST SRM 968e (human serum, frozen; three levels; certified values)	NIST MMQAP; UK NEQAS
Vitamin D	Serum 25OHD ₂ , 25OHD ₃ and epi-25OHD ₃	NIST SRM 972 (human serum, frozen; four levels; certified values for 25OHD ₂ , 25OHD ₃ , and epi-25OHD ₃); NIST SRM 2972 (solvent-based; certified values for 25OHD ₂ and 25OHD ₃)	UK DEQAS; CAP Bone and Growth Survey and ABVD Survey; and NIST/NIH VitDQAP
Vitamin E	Serum α - and γ -tocopherol	NIST SRM 968e (human serum, frozen; three levels; certified values)	NIST MMQAP; UK NEQAS
Vitamin K	Serum phyloquinone (K ₁)	UK KEQAS SRM-001 (human plasma; one level; consensus value)	UK KEQAS; NIST MMQAP
Vitamin B ₆	Serum PLP	NIST SRM 3950 (human serum, frozen; two levels; certified values)	Not available at this time
Folate	Serum folate species	NIST SRM 1955 (human plasma, frozen; three levels; certified values for 5MTHF, reference values for FA, information values for TFOL and 5FTHF); NIBSC RM 03/178 (human serum, freeze-dried; one level; certified values for 5MTHF, FA, 5FTHF and TFOL)	CAP Ligand Assay General Survey and Cal V/L Survey for TFOL; UK NEQAS for TFOL
	Whole blood folate	NIBSC RM 95/528 (human whole blood hemolysate, freeze-dried; one level; consensus value)	CAP Ligand Assay General Survey and Cal V/L Survey for TFOL; UK NEQAS for TFOL
	Plasma tHcy	NIST SRM 1955 (plasma, frozen; three levels; certified values)	CAP Homocysteine Survey and Cal V/L Survey; DEKS
Vitamin B ₁₂	Serum B12	NIBSC RM 03/178 (human serum, freeze-dried; one level; consensus values); NIBSC RM 81/563 (serum, freeze-dried; one level; consensus values)	CAP Ligand Assay General Survey and Cal V/L Survey; UK NEQAS
	Serum MMA	NIST SRM 1950 (human plasma, frozen; one level; certified value)	DEKS
Vitamin C	Serum total ascorbic acid	NIST SRM 970 (human serum, frozen; four levels; certified values)	NIST MMQAP
Sodium and potassium	Serum and urine sodium and potassium	NIST SRM 2201 (NaCl standard) and 2202 (KCl standard)	CAP General Chemistry Survey and Cal V/L Survey; CAP Urine Chemistry Survey and Cal V/L Survey
		NIST SRM 956c (human serum, frozen; three levels; certified value)	
Calcium	Serum total calcium	NIST SRM 956c (human serum, frozen; three levels; certified value)	CAP General Chemistry Survey and Cal V/L Survey

(Continued)

Table 4 Continued

<i>Nutritional status</i>	<i>Biochemical indicator</i>	<i>Reference materials</i>	<i>Selected list of external quality assessment programs</i>
Magnesium	Serum magnesium	NIST SRM 956c (human serum, frozen; three levels; certified value)	Center de Toxicology Quebec
Iron	Serum ferritin	NIBSC RM 94/572 (human plasma, freeze-dried; one level; consensus value)	CAP Chemistry Survey and Cal V/L Survey; UK NEQAS
	Serum sTfR	NIBSC RR 07/202 (human serum, freeze-dried; one level; gravimetric/spectrophotometric value assignment)	Not available at this time
	Serum iron	NIST SRM 937 (iron metal); NIST SRM 3126a (iron standard solution)	CAP Chemistry Survey and Cal V/L Survey; UK NEQAS
	Erythrocyte protoporphyrin	None available	State of New York Department of Health, Wadsworth Center
Zinc	Serum zinc	NIST SRM 1598a (bovine serum, frozen; one level; certified value)	CAP Chemistry Survey and Cal V/L Survey; Center de Toxicology Quebec
Copper	Serum copper	NIST SRM 1598a (bovine serum, frozen; one level; certified value)	CAP Chemistry Survey and Cal V/L Survey; Center de Toxicology Quebec
Selenium	Serum selenium	NIST SRM 1598a (bovine serum, frozen; one level; certified value)	CAP Chemistry Survey and Cal V/L Survey; Center de Toxicology Quebec
Iodine	Urine iodine	NIST SRM 3668 (urine-based; two levels) and NIST SRM 2668 (urine-based; two levels)	CDC EQUIP (Ensuring the quality of Urine Iodine Procedures)

25OHD, 25-hydroxyvitamin D; 25OHD₂, 25-hydroxyvitamin D₂; 25OHD₃, 25-hydroxyvitamin D₃; 5FTHF, 5-formyltetrahydrofolic acid; 5MTHF, 5-methyltetrahydrofolic acid; ABVD, Accuracy-based vitamin D; AGP, α 1-acid glycoprotein; Cal V/L, Calibration Verification and Linearity Survey; CAP, College of American Pathologists; CDC, Centers for Disease Control and Prevention; CRP, C-reactive protein; DEKS, Danish External Quality Assessment Program; DEQAS, Vitamin D External Quality Assessment Scheme; epi-25OHD₃, 3-epimer-25-hydroxyvitamin D₃; EQUIP, Ensuring the quality of Urine Iodine Procedures; FA, folic acid; KEQAS, Vitamin K External Quality Assurance Scheme; MMA, methylmalonic acid; MMQAP, Micronutrients Measurement Quality Assurance Program; NEQAS, National External Quality Assessment; NIBSC, National Institute for Biological Standards and Control; NIH, National Institutes of Health; NIST, National Institute of Standards and Technology; PLP, pyridoxal-5'-phosphate; RBP, retinol-binding protein; RM, reference material; RR, reference reagent; SRM, standard reference material; sTfR, soluble transferrin receptor; TF, transferrin; TFOL, total folate; tHcy, total homocysteine; TTR, transthyretin; UK, United Kingdom; ViTDQAP, Vitamin D Metabolites Quality Assurance Program.

An appropriate quality control (QC) system with internal and external verifications should be in place throughout the entire study. Particularly for longitudinal studies, large batches of in-house prepared QC pools are preferred to commercial QC samples where frequent lot changes can be expected. It is suggested to prepare two or three levels of QC pools and to analyze them in every assay together with the patient samples. Participation in external quality assessment programs or interlaboratory cross-comparisons as well as the regular use of reference materials for calibration verification is highly recommended. **Table 4** provides information on currently available international reference materials and gives a selected list of external quality assessment programs. The Centers for Disease Control and Prevention (CDC) also maintains an inventory of external quality assessment programs by country, some of which pertain to nutritional status indicators.

Evaluation of Laboratory Indices

In general, reference values are population specific; accordingly, each major laboratory in homogenous areas has to derive them from a clinically healthy reference population selected with very specific criteria. These values should preferably be given in percentiles. The National Report on Biochemical Indicators of Diet and Nutrition in the US Population 1999–2002 is a comprehensive CDC publication

that offers nationally representative reference information for 27 nutritional indicators derived from NHANES. A second edition of the report, providing reference information for 58 nutritional indicators from NHANES 2003–2006, as well as information on prevalence of nutritional deficiencies for selected indicators, is released in 2012.

Ideally, cutoff points are derived by determining the biochemical values that correspond to the earliest determinable physiological, metabolic, functional, and morphological alterations. Because such an approach has been followed only in very few cases, most cutoff points have been derived statistically from reference values and should therefore be considered as tentative. **Table 5** presents a list of tentative cutoff points for interpretation of nutritional status tests. In some cases, different cutoff points are used for children, pregnant and lactating women, and the elderly. These values can be found in reference texts. It is important to remember that cutoff points as well as reference intervals can vary with the method used to measure the biochemical indices. Continued efforts to standardize methods are therefore needed.

Conclusions and Future Directions

Over the last few years, scientific and public health agencies initiated renewed efforts to better define biomarkers of nutritional status, to focus on analytical method standardization

Table 5 Tentative cutoff points for interpretation of biochemical laboratory indices

Nutritional status	Biochemical indicator	Deficiency	Excess
Protein	Serum albumin ^a	<30 g l ⁻¹	
	Serum TTR ^a	<0.11 g l ⁻¹ (severe) 0.11–0.16 g l ⁻¹ (moderate)	
	Serum TF ^a	<1.0 g l ⁻¹ (severe) 1.5–2.0 g l ⁻¹ (mild)	
	Serum RBP ^a	<25 mg l ⁻¹	
Fatty acids	Plasma essential fatty acids Holman Index ^b	>0.2 (ratio of triene [C20:3n – 9 mead acid] to tetraene [C20:4n – 6 arachidonic acid] fatty acids)	
	RBC membrane fatty acids Omega-3 Index ^c (marker of risk for sudden cardiac death)	Amount of EPA and DHA as a proportion of total fatty acids: <4% (high risk) 4–8% (intermediate risk) >8% (low risk)	
Vitamin A	Serum retinol ^a	<0.35 µmol l ⁻¹ (severe) <0.70 µmol l ⁻¹ (moderate) <1.05 µmol l ⁻¹ (suboptimal)	
	Serum retinyl esters ^a		>10% of total vitamin A (fasting) (potential hypervitaminosis A)
	Serum RBP ^a	<0.70 µmol l ⁻¹ ; validation in different populations needed	
	Serum RDR ^a	>20% (marginal status)	
	Serum MRDR ^a	>0.060 (marginal status)	
Vitamin D	Serum 25OHD ^d	<30 nmol l ⁻¹ (deficient) 30–50 nmol l ⁻¹ (inadequate)	>125 nmol l ⁻¹ (reason for concern, possibility of hypervitaminosis D)
Vitamin E	Serum α-tocopherol ^a	<11.6 µmol l ⁻¹	
Thiamin	Erythrocyte TDP ^a	<120 nmol l ⁻¹ (high risk) 120–150 nmol l ⁻¹ (marginal)	
	Urinary thiamin ^a	<27 µg g ⁻¹ creat (high risk) 27–65 µg g ⁻¹ creat (medium risk)	
	Erythrocyte EKT-AC ^a	>1.25	
Riboflavin	Erythrocyte FAD ^a	<270 nmol l ⁻¹ RBC	
	Urinary riboflavin ^a	<27 µg g ⁻¹ creat (high risk) 27–79 µg g ⁻¹ creat (medium risk)	
	Erythrocyte EGR-AC ^a	>1.40 (high risk) 1.2–1.4 (medium risk)	
Niacin	Urinary N'MN, 2-Py ^a	<0.5 mg g ⁻¹ creat	
Vitamin B ₆	Serum PLP ^a	<20 nmol l ⁻¹	
	Erythrocyte EAST-AC ^a	>1.85 (deficient) 1.70–1.85 (marginal)	
Folate	Serum folate ^a	<6.8 nmol l ⁻¹ (negative balance)	
	RBC folate ^a	<317 nmol l ⁻¹ (used frequently)	
	Plasma tHcy ^a	>12–14 µmol l ⁻¹ (used frequently)	
Vitamin B ₁₂	Serum B12 ^a	<74 pmol l ⁻¹ (deficient) 100–150 pmol l ⁻¹ (moderate) 100–300 pmol l ⁻¹ (low-to-normal)	
	Serum MMA ^f	>271 nmol l ⁻¹	
Vitamin C	Serum total ascorbic acid ^a	<11.4 µmol l ⁻¹ (deficient) 11.4–23 µmol l ⁻¹ (low levels)	
Iron	Serum ferritin ^g	<12 µg l ⁻¹ (<5 years) <12 µg l ⁻¹ (≥5 years)	>150 µg l ⁻¹ (females) >200 µg l ⁻¹ (males)
	Serum sTfR ^a	Assay specific cutoff values	
	Serum TS ^a	<16%	>70%

(Continued)

Table 5 Continued

Nutritional status	Biochemical indicator	Deficiency	Excess
	Erythrocyte protoporphyrin ^a	> 80 µmol/mol heme (severe)	
	Hemoglobin ^a	60–80 µmol/mol heme (moderate) < 110 g l ⁻¹ (6–59 months) < 115 g l ⁻¹ (5–11 years) < 120 g l ⁻¹ (12–14 years) < 120 g l ⁻¹ (nonpregnant women) < 110 g l ⁻¹ (pregnant women) < 130 g l ⁻¹ (men)	
Zinc	Serum zinc ^a	Children < 10 years: 9.9 µmol l ⁻¹ (collected AM) 8.7 µmol l ⁻¹ (collected PM) Males ≥ 10 years: 11.3 µmol l ⁻¹ (collected AM fasting) 10.7 µmol l ⁻¹ (collected AM other) 9.3 µmol l ⁻¹ (collected PM) Females ≥ 10 years: 10.7 µmol l ⁻¹ (collected AM fasting) 10.1 µmol l ⁻¹ (collected AM other) 9.0 µmol l ⁻¹ (collected PM)	
Selenium	Serum selenium	< 0.1 µmol l ⁻¹ (severely depleted)	
Iodine	Urine iodine ^a (used as population statistic only; not useful as individual measure)	< 20 µg l ⁻¹ (severe) 20–49 µg l ⁻¹ (moderate) 50–99 µg l ⁻¹ (mild) < 150 µg l ⁻¹ (pregnant women)	> 300 µg l ⁻¹ (excessive) 200–299 µg l ⁻¹ (more than adequate) > 250 µg l ⁻¹ (more than adequate for pregnant women)

^aGibson RS (2005) *Principles of Nutritional Assessment*, 2nd edn. New York: Oxford University Press.

^bInstitute of Medicine (2005) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, DC: The National Academies Press.

^cHarris WS (2008) The omega-3 index as a risk factor for coronary heart disease. *American Journal of Clinical Nutrition* 87(suppl), 1997S–2002S.

^dInstitute of Medicine (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: The National Academies Press.

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^fAllen RH, Stabler SP, Savage DG, Lindenbaum J (1990) Diagnosis of cobalamin deficiency I: Usefulness of serum methylmalonic acid and total homocysteine concentrations. *American Journal of Hematology* 34, 90–98.

^gWHO. Serum ferritin concentrations for the assessment of iron deficiency in populations. *Vitamin and Mineral Nutrition Information System*. Geneva, World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.2) [cited 2012]. Available at: http://www.who.int/vmnis/indicators/serum_ferritin.pdf

2-Py, N'-methyl-2-pyridone-5-carboxamide; 25OHD, 25-hydroxyvitamin D; creat, creatinine; DHA, docosapentaenoic acid; EAST-AC, erythrocyte aspartate aminotransferase activation coefficient; EGR-AC, erythrocyte glutathione reductase activation coefficient; EPA, eicosapentaenoic acid; ETK-AC, erythrocyte transketolase activation coefficient; FAD, flavinadenin-dinucleotide; MMA, methylmalonic acid; MRDR, modified relative dose response test; N'MN, N'-methylnicotinamide; PLP, pyridoxal-5'-phosphate; RDR, relative dose response test; RBC, red blood cells; RBP, retinol-binding protein; sTfR, soluble transferrin receptor; TDP, thiamin diphosphate; TF, transferrin; tHcy, total homocysteine; TS, transferrin saturation; TTR, transthyretin.

for accurate and precise measurement of biomarkers, and to develop systematic processes for the evaluation of biomarkers and surrogate endpoints. The Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health is leading an effort to build a consensus around biomarkers of nutrition for development (BOND). The goal of BOND is to promote the discovery, development, and use of biomarkers across a range of applications and to harmonize the global health community's decision-making about what biomarkers are best suited for a given use under specific conditions and settings. In collaboration with CDC and NIH, the National Institute of Standards and Technology (NIST) has developed over the last several

years reference methods and materials for nutritional biomarkers that are essential to enhance and promote high quality laboratory measurements. The development and validation of new dietary biomarkers are also constantly evolving. The emerging field of nutritional metabonomics – the study of metabolic responses – is receiving increasing attention as an analytical method to assess metabolic profiles as measures of dietary exposures and indicators of dietary patterns, dietary changes, or effectiveness of dietary interventions.

Yet, with all these efforts ongoing, the findings of the EURRECA project to assess potential biomarkers of micronutrient status by using a systematic review methodology were that far as fewer studies were available for biomarker

assessment than initially predicted, and the risk of bias of included studies was greater than expected. The authors concluded that further research is needed to assess the usefulness of many potential biomarkers and that we still have to overcome gaps in our understanding of how well a biomarker works in particular population groups or in people with different baseline micronutrient status.

See also: Ascorbic Acid: Deficiency States. Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Biotin: Physiology, Dietary Sources, and Requirements. Calcium. Copper. Electrolytes: Acid–Base Balance. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Metabolism. Folic Acid. Homocysteine. Iodine: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements. Magnesium. Niacin and Pellagra. Pantothenic Acid. Potassium. Salt: Epidemiology. Selenium. Sodium: Physiology. Thiamin: Beriberi; Physiology. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements. Vitamin D: Physiology, Dietary Sources, and Requirements. Vitamin E: Metabolism and Requirements; Physiology and Health Effects. Vitamin K. Zinc: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements

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Relevant Websites

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CDC Inventory of external quality assessment programs by country.
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CDC National Report on Biochemical Indicators of Diet and Nutrition in the US Population.
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BIOFORTIFICATION

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Glossary

Biofortification Biofortification is the process of increasing the nutrient content of the edible portion of plant foods. Also refers to the food-based strategy to reduce micronutrient malnutrition in low-income populations by means of the large-scale introduction of biofortified staple food crops.

Conventional plant breeding Conventional plant breeding is the process by which two plants, that are genetically, closely related, are cross-pollinated to induce sexual recombination with the aim of producing new plants with favorable, selected traits. The selected traits are usually those that will result in increased production, or improved nutritional content, of economically useful plants.

Genetically engineered plants Genetically engineered plants are plants produced following direct human manipulation of the plant's genetic material that does not involve its normal sexual reproduction system. This usually involves insertion of selected genetic material, derived from a different species or organism, into the genetic material of the plant with the aim of producing a novel, desired trait in the plant. This procedure can be followed by conventional plant breeding.

Micronutrient bioavailability Micronutrient bioavailability refers to the quantity or fraction of

micronutrient ingested that is absorbed and available for physiological function in the body.

Micronutrients Micronutrients are nutrients required by humans in small quantities to support physiological functions. They include vitamins and trace elements.

Mutational breeding Mutational breeding refers to a two-step process by which induced mutagenesis is followed by conventional plant breeding to produce plants with desired traits such as increased productivity or improved nutrient content. Mutagenesis refers to the process by which mutations in the genetic material of plants are induced using chemicals or radiation to result in new, desired traits in the plant.

Provitamin A Provitamin A carotenoids are specific carotenoids synthesized by some plant tissues that are precursors to the active form of vitamin A. Pro-vitamin A carotenoids are cleaved to produce vitamin A following intestinal absorption in humans. Plant carotenoids with provitamin A activity are β -carotene, α -carotene, β -cryptoxanthin, and their isomers.

Staple foods Staple foods are foods that are regularly consumed, usually in large quantities such that they contribute importantly to the energy needs of a population. They commonly include cereals, starchy roots, tubers or fruits, and legumes, and often extend to include vegetable oils and sugar.

Biofortification as a Public Health Nutrition Intervention

Definition and Research Focus

Biofortification is a public health nutrition intervention under development that aims to improve the nutritional quality of plant foods through agricultural means. Although food fortification refers to the addition of exogenous sources of nutrients to processed food products, biofortification indicates that the additional nutrient content is endogenous, resulting from biological processes of the plant.

Research to develop biofortified foods is ongoing and has largely focused on increasing the micronutrient content of the world's most important staple food crops including maize, rice, wheat, cassava, potato, sweet potato, common beans, pearl millet, sorghum, and banana/plantain. Research has also been extended to common vegetables such as lettuce,

tomatoes, and carrots, and oilseed crops. The most concerted research efforts have focused on increasing the content of provitamin A carotenoids, iron, and zinc due to the high prevalence of deficiencies associated with these micronutrients in the developing world. A wider range of nutritional components are also being addressed, including other minerals (i.e., calcium, iodine, magnesium, selenium), vitamins (i.e., vitamins C, E, folate), nonprovitamin A carotenoids (i.e., lutein, lycopene), protein and protein quality, essential amino acids, fatty acids, other secondary nutritional components such as flavonoids and probiotic compounds (i.e., inulin and other fructans). Cereal grains tend to have a high content of phytate, a phosphorus storage compound that inhibits the intestinal absorption of minerals such as iron and zinc. As a secondary strategy, biofortification research has also sought to improve mineral bioavailability from cereals by reducing the inhibitory effect of phytate, primarily through reduction in phytate content or increase in phytase activity in the grain.

Biofortification as a Strategy to Address Global Micronutrient Deficiencies

Biofortification was principally developed as a food-based strategy to address widespread deficiencies of vitamin A, iron, and zinc that remain prevalent to the greatest extent in low-income countries. Strategies most widely implemented for the prevention of micronutrient deficiencies are the distribution of micronutrient supplements in pharmacological preparations, and food fortification, with far less investment thus far in dietary diversification strategies. In this context, there are several potential advantages and limitations to biofortification as a micronutrient intervention. Biofortification is targeted primarily to the rural poor who rely heavily on locally produced staple foods as their primary source of nutrition, and who often have restricted financial or market access to commercially processed fortified foods. The biofortification strategy has potential for sustainability as, once planting material is obtained, it can often be saved, recycled, and further disseminated to other farmers. Once initial development and dissemination are completed, recurring costs of maintaining production of biofortified crops are estimated to be low. However, the additional amount of micronutrient achievable through biofortification will be modest compared to amounts supplied in supplements, and in some cases fortified food. Hence, the potential magnitude of impact of biofortification on population nutrient status may be commensurately modest. The time required to develop a viable crop with a stable, minimum level of additional nutrient content can extend up to 6–8 years or more. As such, there is less flexibility to adjust the content and combination of nutrients according to specific needs. Given the widespread prevalence of micronutrient deficiencies, and a variety of scenarios in which any one strategy will be effective, biofortification is considered as complementary to other micronutrient deficiency prevention strategies.

The largest concerted effort to develop the biofortification strategy is led by HarvestPlus, an international consortium of researchers and implementers coordinated through the Consultative Group on International Agricultural Research (CGIAR). This consortium is addressing all aspects of the strategy development, from agricultural and biotechnology research, to food science and nutritional research, economic and policy impact research, and dissemination strategies. AgroSalud is a similar research consortium coordinating biofortification research and interventions in Latin America. National biofortification research programs have also been established in Brazil, China, and India. Biofortification has been included within the national nutrition strategies of some countries (i.e., Nicaragua, Panama, Zambia).

Biofortification as a Strategy to Prevent Other Nutrition-Related Diseases

Beyond these most common micronutrient deficiencies, biofortification may find other applications in public health nutrition. For example, oil seeds with improved fatty acid profiles, starchy staples with increased content of probiotic fructans, or vegetables with increased calcium content, may be marketed in the future as functional foods directed toward the

prevention of chronic diseases such as cancer, cardiovascular disease, and osteoporosis, in more affluent populations.

Processes Used to Achieve Biofortification

The content of nutrients in the edible portions of plant foods is determined by multiple genes and physiological processes. Organic nutrients must be synthesized directly in the target tissue requiring multiple, active, tissue-specific genes to control their metabolism and catabolism. Minerals require mechanisms to facilitate uptake by roots, translocation and loading into target tissues, and the availability of mineral storage compounds in the target tissue. Important advances have been made in identifying the genes controlling these processes using applied molecular genetics. Biofortification can be achieved through various processes, including conventional breeding, genetic engineering, mutational breeding, and agronomic approaches. The goal for all of these methods is to produce a biofortified staple food crop meeting or exceeding some predefined minimal increase in nutrient content, combined with high agronomic performance and consumer-preferred traits, suited to a particular target geographic location.

Conventional Breeding

The major focus for development of biofortified food crops has been on the use of conventional breeding, applying the same basic techniques as used to breed plants with improved agronomic traits, such as increased yield or pest resistance. The feasibility of this approach is dependent on the presence of sufficient natural variation in the nutrient content of a particular staple food crop and the heritability of traits controlling it. There are several examples of biofortified staple food crops under production using conventional breeding (Table 1).

The natural range of nutrient content is determined through screening of germplasm representing the available genetic diversity for a particular species. Selected germplasm with high nutrient content will enter what is typically a multistage breeding process. In addition to natural genetic variation, nutrient content of staple food crops is affected by environmental conditions during development. Quantification of the relative effects on nutrient content of genetics, the environment, and gene–environment interactions, is an important component of the breeding process. When

Table 1 Examples of biofortified staple food crops being developed by conventional breeding

<i>Staple food crop</i>	<i>Nutrient</i>
Beans (common)	Iron, zinc
Cassava	β -carotene
Maize	β -carotene, β -cryptoxanthin, lysine, tryptophan
Millet	Iron, zinc
Rice	Iron, zinc
Sweet potato	β -carotene
Wheat	Iron, zinc

Table 2 Examples of biofortified food crops being developed using genetic engineering

Nutrient	Food crop
Calcium	Carrot, lettuce
Iron	Maize, rice
Folate	Rice, tomato
Provitamin A (β -carotene)	Cassava, maize, rice, potato, tomato
Vitamin E	Maize, oil seed crops
Amino acids	Cassava, maize, rice, sorghum, wheat
Fatty acids	Oil seed crops

increased nutrient content is found to be genetically stable in breeding products, these genetic lines are ultimately backcrossed with new or existing varieties having the desired agronomic and consumer-preferred traits. A dominant effect of environmental conditions, or a low natural genetic variation of nutrient content, could render breeding unfeasible, but other methods of achieving biofortification may be applied.

Some examples of biofortified staple food crops derived through conventional breeding and achieving varietal release are Quality Protein Maize (QPM) and orange sweet potato. The protein quality of maize is limited by a low content of the essential amino acids, lysine and tryptophan. Following the 1960s discovery of a natural mutation of the *opaque-2* gene that was associated with a doubling of the lysine and tryptophan content in maize grain, several maize varieties were developed through conventional breeding and are collectively known as QPM. At least at the time, inadequate dietary protein and protein quality were thought to be the major causes of malnutrition in the developing world. Initially fraught with poor agronomic properties, improvement of QPM varieties has continued and multiple varieties have been formally released in more than 20 countries, mostly in Central America and Sub-Saharan Africa.

The flesh color of cultivated sweet potatoes has a wide natural range, including white, cream, pale to deep yellow, and pale to deep orange. The yellow and orange flesh color is attributed to the content of β -carotene, a provitamin A carotenoid that is converted to retinol following intestinal absorption. Increasing the intensity of the yellow or orange color is associated with increased β -carotene content ranging from 0 to $>400 \mu\text{g g}^{-1}$ fresh weight. In developing country regions, the flesh color of more commonly cultivated sweet potato varieties is white or pale yellow containing little or no β -carotene, and higher dry matter content. Although the most common sweet potato varieties available in North America are orange, the white or pale colored ones are much more common in Asia and Africa where vitamin A deficiency remains prevalent. The β -carotene trait is hence being bred into sweet potato clones suited for the latter regions. Several new orange biofortified varieties have been formally released for the purpose of vitamin A deficiency prevention.

Genetic Engineering

When sufficient natural genetic variation in nutrient content does not exist in a particular food crop, transgenic approaches

offer an alternative method in achieving biofortification. This is done by introducing new genes derived from different organisms that are novel to the target plant species, or that turn on or upregulate existing genes in the target tissue. Although much of this research is in the early stages of development, proof-of-concept has been established for biofortification with several nutrients in a variety of food crops (Table 2).

The biofortified staple food crop produced by transgenic methods that is most advanced in development is rice that contains β -carotene. Although rice plants contain the genes required to produce β -carotene, their expression is turned off in the rice grain endosperm. The insertion of two genes, a plant phytoene synthase (PSY) and bacterial carotene desaturase (CRTI) were sufficient to turn on the pathway. Referred to as Golden Rice, the initial proof-of-concept milled rice grain contained only a small amount of β -carotene ($<1.6 \mu\text{g g}^{-1}$ dry weight). However, a second generation Golden Rice using PSY derived from maize instead of daffodil has a higher β -carotene content of up to $>30 \mu\text{g g}^{-1}$ dry weight before storage, which is of greater nutritional significance.

Mutagenesis

Genetic mutations occur naturally and are a primary source of genetic variation in biology. Mutagenesis can also be induced using certain chemicals or radiation and produce desired traits in plants. As subsequent conventional breeding is still required, the process is referred to as mutational breeding. This approach has been used for decades to develop improved crop varieties and has more recently been applied to biofortification. Examples of biofortified staple food crops produced by this method are barley, maize, rice, wheat, sorghum, and soybean with a low phytic acid trait. Reductions in seed phytic acid content can reach 50–95%, but very low content is also associated with reduced agronomic performance.

Agronomic Methods

Increasing the availability of certain trace elements to plants by agronomic means can also increase their content in the edible portion of staple food plants. This can be achieved through soil application, foliar application, or by their addition to irrigation water. These approaches remain limited to a few trace elements including iodine, selenium, and, in some cases, zinc, which are deficient in the soil in some geographical regions. Human iodine and selenium deficiency is directly related to low content of these trace elements in the soil and in the local agricultural products. In Finland, selenium has been added to fertilizer since the 1990s, resulting in an increased selenium content in domestically produced plant and animal source foods. In a few remote townships in China, the short-term dosing of iodine in irrigation water also led to increased iodine in the local food supply. In Turkey, zinc-deficient soils are common and the addition of zinc to fertilizer, beginning in the 1990s, has led to important increases in wheat crop yields, and a concomitant increase in the zinc content of wheat grain. The extent to which the use of zinc-containing fertilizer or foliar applications of zinc solutions can lead to zinc biofortification appears to vary by soil type and

Table 3 Examples of estimates used to set initial minimum targets for increased content of provitamin A, iron, and zinc in selected biofortified staple food crops^a

Nutrient	Maize, whole grain	Beans, whole	Wheat, whole grain
	Provitamin A	Iron	Zinc
Average intake of staple food crop, g day ⁻¹ , raw fresh weight			
Children 4–6 years of age	200	100	200
Women, nonpregnant/nonlactating	400	200	400
Nutrient retention after processing, storage, and cooking (%)	50	85	90
Nutrient bioavailability ^b	12/1	5%	25%
Initial average nutrient content of staple food crop, µg g ⁻¹ raw, fresh weight	0	50	25
Desired minimum increment in biofortified staple food crop, µg g ⁻¹ raw, fresh weight	+ 15	+ 44	+ 8

^aEstimates apply to children 4 to 6 years of age and nonpregnant, nonlactating women. The minimum desired increases in nutrient content of biofortified staple foods was based on the provision of approximately 30–50% of Estimated Average Requirements for vitamin A Retinol Activity Equivalents, and the estimated average physiological requirement for absorbed iron and zinc. Research is required to verify or adjust these estimates for particular population groups.

^bBioavailability of provitamin A is expressed as the retinol equivalency ratio and the percent intestinal absorption of iron and zinc.

Source: Reproduced from Bouis HE, Hotz C, McClafferty B, Meenakshi JV, and Pfeiffer WH (2011) Biofortification: A new tool to reduce micronutrient malnutrition. *Food and Nutrition Bulletin* 32: S31–S40.

staple food crop. Systematic study of these methods is still required to evaluate their potential.

The Potential of Biofortification to Improve Nutritional Status

As biofortified staple food crops are in various stages of development, direct evidence for their potential to improve population nutritional status is just beginning to accumulate. As for other micronutrient interventions, there are several types of information and evidence required to fully assess the potential nutritional impact of biofortified foods. These include the prevalence or pre-existing nutrient deficiency in the target population, usual intake of the major staple food(s), nutrient retention in the biofortified food following storage, processing and cooking, nutrient bioavailability, the efficacy of the biofortified food to improve nutrient status when consumed under controlled conditions, and the effectiveness of a biofortification program to improve population nutritional status.

The nutritional impact of biofortified foods will ultimately be determined in population-based studies. However, minimum target levels for increased nutrient content in staple food crops must be estimated early on to guide the initial development process. These targets use estimates for the usual daily intake of the staple food crop to be biofortified, and nutrient retention and bioavailability. Some minimum, theoretical increases in provitamin A, iron, and zinc content for biofortified staple food crops have been suggested (Table 3). These aim to provide an additional amount of nutrient equivalent to 30–50% of dietary requirement for children and women. Empirical data derived from studies using biofortified staple food crops will allow the adequacy of the increases in nutrient content to be evaluated and adjusted as necessary for particular target populations.

Nutrient Retention in Biofortified Staple Foods

The retention of minerals in plant-based staple foods following typical household cooking methods is generally quite

high, with ≥90–95% of iron and zinc retained after cooking of cereal grains, roots, and tubers and ≥75% retained in boiled legumes. The extent to which retention of minerals with cooking will differ in mineral-biofortified staple food crops is not yet determined. Of greater concern are the lower proportions of iron and zinc retained (i.e., 20–60%) following milling of cereal grains for refinement as a large proportion of minerals occur in the outer aleurone layers. Where consumption of unrefined cereals is predominant, as for rice in most of the world, biofortification must aim to increase mineral content specifically in the starchy endosperm.

Nutrient retention is an important consideration for biofortification with vitamins, particularly provitamin A carotenoids as their stability is reduced by exposure to heat, light, and oxygen. Retention during postharvest storage of the staple food crop must also be considered, but data are lacking. In orange sweet potato, the retention of β-carotene averages 84% after boiling and 77% after steaming, which are the most common cooking methods. In biofortified orange maize, retention of β-carotene was ~75% in porridge cooked from fermented or unfermented, wet milled flour. Retention of β-carotene after drying of sweet potato or cassava to make flour or chips is moderate, but dramatic degradation occurs during storage of these dried products over weeks or months.

Bioavailability

Minerals

The bioavailability of nutrients from specific foods is modified by several factors related to the food matrix, including the chemical form or physical location of the nutrient and the presence or absence of other food factors that inhibit or promote the bioavailability of a nutrient. It is possible that the process of biofortification will result in concomitant changes in these or other properties that may alter the relative bioavailability of the additional nutrient.

There are still few studies measuring the relative bioavailability of nutrients from biofortified foods in humans. The amount of absorbed zinc was found to be significantly higher from zinc-biofortified wheat among Mexican women when

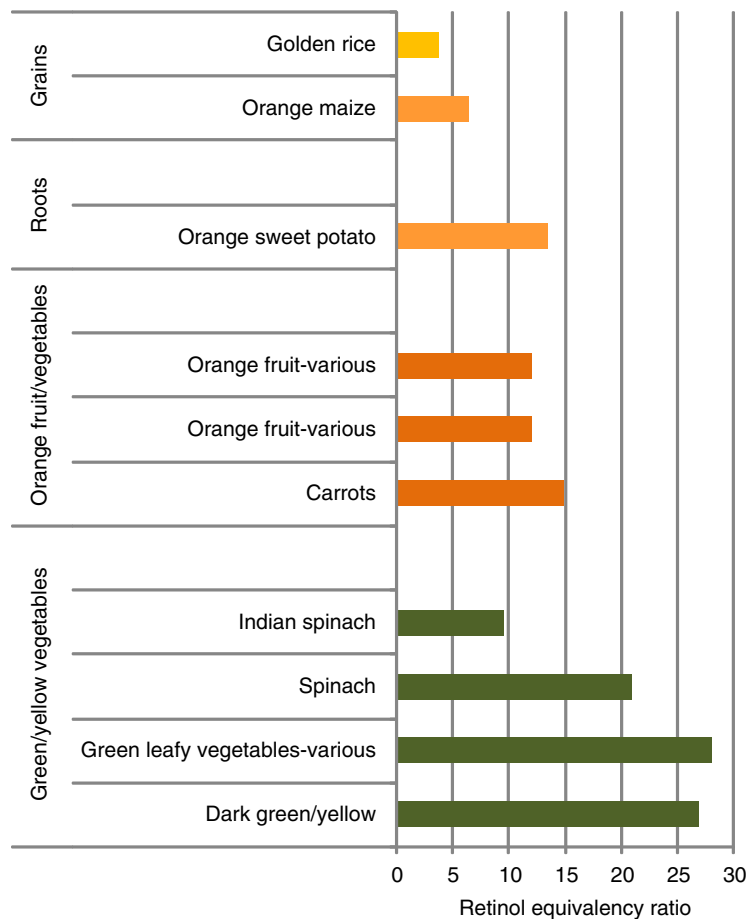


Figure 1 Retinol equivalency ratio of provitamin A from plant food sources.

provided in diets containing wheat tortillas, suggesting that the additional zinc was in an adequately bioavailable form. Although research is still limited, there is a general indication that iron biofortification of grains will not modify the relative bioavailability of additional iron from that of native iron. Ferritin is an iron storage protein in plants, and transgenic rice and wheat with increased grain ferritin have increased grain iron content. Results from animal and human studies indicate that iron from ferritin is as well absorbed as iron from exogenous ferrous sulfate.

Several studies were conducted using low-phytic acid mutant maize with 60–65% less phytic acid content than the wild type. Controlled laboratory studies measured large increases in calcium, iron, and zinc bioavailability. However, when this maize was incorporated into typical diets of Guatemalan children in a community-based study, phytic acid in the total diet was reduced by only 25–30% and no significant improvement in zinc bioavailability was observed. The difference in results highlights the importance of conducting these kinds of studies under realistic dietary conditions.

Provitamin A

Food related factors affecting the efficiency of absorption and conversion of dietary provitamin A to retinol include the

type of provitamin A carotenoid (i.e., α -carotene, β -carotene, β -cryptoxanthin), its isomeric form (i.e., *trans*-, *cis*-), and the food matrix. The amount of provitamin A ingested (μ g) required to produce 1 μ g retinol is expressed as the retinol equivalency ratio, and this varies by food type (Figure 1). The order of increasing efficiency of conversion to retinol, or decreasing ratios, is: green or yellow vegetables \ll orange fruits, vegetables, or sweet potato \ll yellow or orange biofortified cereal grains. Studies using animal and *in vitro* models also indicate that β -carotene in biofortified cassava is well absorbed and as efficiently converted to retinol as β -carotene from biofortified maize and rice. These results support the potential for provitamin A-biofortified staple foods to serve as important sources of vitamin A.

Efficacy

There is as yet very limited experience in determining the efficacy of biofortified staple food crop to improve nutritional status when consumed under controlled conditions. Randomized controlled trials in different settings have studied the efficacy of orange sweet potato consumption to improve vitamin A status. Although different methods were used to assess change in vitamin A status, consumption of orange

sweet potato resulted in increased vitamin A status by at least one indicator employed among children in Indonesia and South Africa, and men in Bangladesh. Comparing with the US Estimated Average Requirement (EAR) for vitamin A Retinol Activity Equivalents (RAE), the additional β -carotene derived from orange sweet potato in these studies represented $>100\%$ of the EAR in Indonesian children, $>300\%$ of the EAR in the South African children, and approximately 30% of the EAR in the Bangladeshi men.

A randomized controlled trial studied the efficacy of iron biofortified rice (9.8 mg iron per kg dry weight), compared to commercially available control rice (1.8 mg iron per kg dry weight), to improve iron status among Filipino women. The high-iron rice provided 1.41 mg day^{-1} of additional dietary iron and, after 9 months of consumption, resulted in an increase in estimated total body iron stores among nonanemic women, representing a 20% increase over the controls. The magnitude of increase in iron stores was greater among women with lower iron stores at baseline.

More efficacy studies are required for a greater range of biofortified crops, but these studies have provided an initial proof-of-concept for the strategy.

Effectiveness of Biofortified Crops to Improve Population Nutritional Status

For biofortification to impact population nutritional status, affected individuals must have access to and consume the available biofortified crop in adequate quantities and with adequate frequency. Beyond nutritional biology, several factors will interact to determine the consumption of the improved nutritional products by the population and their ultimate impact on population nutritional status. These include effective dissemination and marketing, consumer education, consumer acceptance of the product, and intervention implementation costs.

Dissemination

Strategies to disseminate and promote production and consumption of biofortified crops require development, and will vary depending on the crop, the target country's existing channels for introducing improved crop varieties to farmers, whether the nutrient changes the appearance of the crop (i.e., color change), and whether it was produced by transgenic means. Public seed systems tend to be more developed in Asia, and these systems could feasibly be used to widely disseminate biofortified crops at little to no additional cost. In Africa, public seed systems are presently less well developed, and effective dissemination may require agricultural extension assistance from nongovernmental organizations. The role that private seed companies will play requires exploration. Some of the major obstacles to the potential widespread use of transgenic biofortified food crops are related to regulatory issues and consumer acceptance of the technology. Many lower income countries where biofortified crops may be of greatest nutritional benefit are still lacking regulatory mechanisms for the testing and approval of genetically modified organisms.

Consumer Acceptance of Orange-Colored Staple Food Crops

The adoption of new crop varieties by farmers depends on many crop characteristics such as agronomic performance and consumer-preferred traits such as shape, size, texture, color, odor, and cooking qualities. Increased mineral content of staple food crops does not impart visual or sensory changes and it is hence anticipated that consumer acceptance will largely be driven by agronomic performance and consumer-preferred traits in the biofortified crop. However, consumer acceptance of color change of staple foods from white to yellow or orange is an important consideration.

Several research methods derived from food science (i.e., sensory panels, central location and home-use testing) and economics (i.e., willingness-to-pay and trading experiments) have been used to assess consumer acceptance of provitamin A-biofortified orange sweet potato and maize in several Sub-Saharan African countries. Collectively, results from these studies suggest that orange colored staple food crops are equally acceptable or preferred to standard white crops by a majority of individuals. The acceptance of orange-colored staple foods increases when information on its nutritional attributes is also provided. Nonetheless, these studies are short-term in nature, and the methods drawn from economics research employ some hypothetical conditions. Longer-term studies of acceptance in diverse populations are required, but experience to date suggests that these orange-colored staple foods will not be outright rejected. These findings are supported by the successful introduction of orange sweet potato for household production and consumption in rural Mozambique.

Nutritional Impact

For orange or yellow, provitamin A-biofortified crops, public education and market development campaigns will facilitate their adoption. A two-year pilot intervention combined orange sweet potato vine distribution, agricultural extension, nutrition education, social marketing, and market development, to introduce orange sweet potato in rural Mozambique farm communities. The majority of households in intervention communities (90%) produced orange sweet potato, 55% of children consumed it ≥ 3 days in the last week, children's vitamin A intakes increased more than seven-times that in the control communities, and the prevalence of vitamin A deficiency was reduced by >20 percentage points. This quasiexperimental study suggested that an orange-colored biofortified crop was acceptable and could be effective at preventing vitamin A deficiency.

Before the definition and development of the current biofortification strategy, the addition of selenium to fertilizer in Finland, and the addition of iodine to irrigation water on a small-scale in China, were implemented. In Finland, the addition of selenium to fertilizer was shown to be safe and effective in increasing the selenium content of locally produced food, and serum selenium concentrations in the population. In the remote rural townships in China with severe iodine deficiency, addition of iodine to irrigation water for 2–4 weeks led to a large, sustained increase in the content of iodine in soil, plant food crops, and animal food products, and improved the iodine status of livestock and humans. An

important decrease in infant and neonatal mortality rates as associated with the intervention. These strategies work well with selenium and iodine as they are easily mobilized from the soil to all cultivated plants, and hence become pervasive in the food chain. However, due to the pervasive nature of these interventions, the effects need to be monitored closely.

See also: Bioavailability. Vitamin A: Deficiency and Interventions

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Physiology, Dietary Sources, and Requirements

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Glossary

Avidin A glycoprotein found in egg white that binds to dietary biotin.

Biotin A water-soluble vitamin that is generally classified in the B complex group.

Biotinidase An enzyme that in humans is encoded by the *BTBD9* gene and plays a critical role in intracellular

recycling of biotin by releasing biotin from intracellular proteins.

Histones DNA binding proteins that mediate the folding of DNA into chromatin.

Sodium-dependent multivitamin transporter (SMVT) Transporter for biotin, pantothenic acid, and lipoic acid into cells. Coded for by the gene *SLC5A6*.

Biotin is a Vitamin

Biotin (also known as Vitamin B₇, Vitamin H, and Coenzyme R) is a water-soluble vitamin that is generally classified in the B complex group. Mammals (including humans) cannot synthesize biotin and depend on biotin synthesized by microbes and plants. Biotin was discovered in nutritional experiments that demonstrated a factor in many foodstuffs capable of curing the scaly dermatitis, hair loss, and neurologic signs induced in rats fed dried egg white. Avidin, a glycoprotein found in egg white, binds biotin very specifically and tightly. From an evolutionary standpoint, avidin probably serves as a bacteriostat in egg white; consistent with this hypothesis is the observation that avidin is resistant to a broad range of bacterial proteases in both the free and biotin-bound form. Because avidin is also resistant to pancreatic proteases, ingested avidin binds to dietary biotin (and probably any biotin from intestinal microbes) and prevents absorption, carrying the biotin on through the GI tract. Cooking denatures avidin, rendering avidin susceptible to digestion and unable to interfere with absorption of biotin.

Digestion of Protein-Bound Biotin

The content of free biotin and protein-bound biotin in foods is variable, but the majority of biotin in meats and cereals appears to be protein-bound via an amide bond between biotin and lysine. The determinants of biotin bioavailability have been not clearly delineated. Biotin release from covalent protein binding is likely mediated by a specific biotin-amide hydrolase, biotinidase (EC 3.5.1.12). Biotinidase mRNA is present in pancreas and intestinal mucosa. Biotinidase also similarly plays a critical role in intracellular recycling of biotin by releasing biotin from intracellular proteins during protein turnover.

Intestinal Absorption

At physiologic pH, the carboxylate group of biotin is negatively charged. Thus, biotin is at least modestly water-soluble and requires a transporter to cross cell membranes. Biotin transport must occur across two structurally and functionally different membrane domains of human intestinal epithelial cells: the brush border (apical) membrane that faces the intestinal lumen and the basolateral membrane that faces the interstitium in contact with blood that perfuses the intestine.

A biotin transporter is present in each of the membrane domains. In the brush border membrane, transport occurs via a Na⁺-dependent, electroneutral, carrier-mediated mechanism that saturates at the micromolar range, accounting for the overall limitation in nondiffusion transport. In the presence of a Na⁺ gradient, biotin transport occurs against a concentration gradient. This biotin transporter can also transport pantothenic acid and lipoic acid and hence has been named Sodium-dependent MultiVitamin Transporter (SMVT). Human SMVT is the product of the *SLC5A6* gene, which is located on chromosome 2p23 and consists of 17 exons. SMVT is exclusively targeted to the brush border membrane.

Biotin transport across the basolateral membrane is also a carrier-mediated mechanism. However, this carrier is Na⁺-independent, electrogenic, and cannot accumulate biotin against a concentration gradient.

The intestinal biotin transport upregulates in response to biotin deficiency. Upregulation likely is accomplished primarily by induction of SMVT mRNA synthesis and, hence, an increased number of SMVT transporters per cell. The increase in SMVT is likely mediated by an induction in the activity of P1, which is one of the two promoter regions upstream from the SMVT gene.

Based on a study in which biotin was administered orally in pharmacologic amounts, bioavailability of biotin is approximately 100%. Thus, both physiologic intakes and the

pharmacologic doses of biotin given to treat biotin-dependent inborn errors of metabolism are likely to be well absorbed.

The Contribution of Microbial Biotin to Absorbed Biotin

The contribution of microbial biotin to absorbed biotin, if any, remains unknown. Biotin is synthesized by many intestinal microbes. Based on rat studies, carrier-mediated transport of biotin is most active in the proximal small bowel, where microbes are the least numerous. However, biotin absorption from the proximal colon where microbes are the most numerous is still significant, supporting the potential nutritional significance of biotin synthesized and released by enteric flora.

Transport from the Intestine to Peripheral Tissues

Biotin concentrations in plasma are small relative to other water-soluble vitamins. Most biotin in plasma is free and dissolved in the aqueous phase of plasma. However, approximately 7% is reversibly bound to plasma protein, and approximately 12% is covalently bound to plasma protein. Binding to human serum albumin likely accounts for reversible binding. Biotinidase has been proposed as a biotin-binding protein or biotin-carrier protein for transport into cells. A biotin-binding plasma glycoprotein has been observed in pregnant rats. Although the importance of protein binding in the transport of biotin from the intestine to the peripheral tissues is not yet clear, the immunoneutralization of this protein led to decreased transport of biotin to a fetus and early death of the embryo.

Biotin uptake by Liver and most Peripheral Tissues

SMVT is widely expressed in human tissues. Studies by Said and coworkers provide strong evidence that biotin uptake by liver (and likely many other somatic tissues) occurs via SMVT. Metabolic trapping, (e.g., biotin bound covalently to intracellular proteins) is also important.

Studies by Zemleni and coworkers provide evidence in favor of monocarboxylate transporter 1 (**MCT1**) as the lymphocyte biotin transporter. **MCT1** may also be responsible for biotin transport in keratinocytes.

A child with biotin-responsive neurologic problems and a pattern of organic aciduria consistent with multiple carboxylase deficiency has been reported. An autosomal recessive defect in lymphocyte biotin transport was identified. SMVT gene sequence was normal. These investigators speculated that lymphocyte biotin transporter is expressed in other tissues and mediates some critical aspect of tissue biotin homeostasis.

Biotin Transport

Transport of Biotin into the Central Nervous System

Biotin is transported across the blood-brain barrier. The transporter is saturable and structurally specific for the free

carboxylate group on the valeric acid side chain. Transport into the neuron also appears to involve a specific transport system with subsequent trapping of biotin by covalent binding to brain proteins, presumably carboxylases.

Ozand and collaborators have described several patients in Saudi Arabia with biotin-responsive basal ganglia disease. Symptoms include confusion, lethargy, vomiting, seizures, dystonia, dysarthria, dysphagia, seventh nerve paralysis, quadriparesis, ataxia, hypertension, chorea, and coma. Gusella and coworkers provided evidence of a genetic defect in **SLC19A3** and speculated that this might be a biotin transporter. However, Said and coworkers conclusively proved that **SLC19A3** codes for **THTR2**, a thiamine transporter located in the apical membrane of intestinal, renal tubule, and hepatic cells. **THTR2** does not transport biotin, leaving the biotin responsiveness of these patients unexplained.

Placental Transport of Biotin

Biotin concentrations are 3–17-fold greater in plasma from human fetuses compared to their mothers in the second trimester, consistent with active placental transport. SMVT is likely responsible for placental biotin transport based on the observations that SMVT is expressed in normal human placenta and that the microvillus membrane of the placenta contains a biotin transport system that is saturable, Na^+ -dependent and actively accumulates biotin. However, in the isolated, perfused single cotyledon from placenta, transport of biotin across the placenta is relatively weak, potentially allowing greater fetal deficiency than maternal deficiency. Indeed, in mice, the degree of fetal biotin deficiency is substantially greater than maternal biotin deficiency.

Transport of Biotin into Human Milk

More than 95% of the biotin in human milk is free in the skim fraction. The concentration of biotin varies substantially in some women and exceeds the concentration in serum by 1–2 orders of magnitude, suggesting that there is a system for transport into milk. Metabolites account for more than half of the total biotin plus metabolites in early and transitional human milk. With postpartum maturation, the biotin concentration increases. No soluble biotin-binding protein has been detected in human milk.

Metabolism and Urinary Excretion of Biotin and Metabolites

Biotin is a bicyclic compound (**Figure 1**). One of the rings contains an ureido group ($-\text{N}-\text{CO}-\text{N}-$). The tetrahydrothiophene ring contains sulfur and has a valeric acid side chain. A significant proportion of biotin undergoes catabolism before excretion (**Figure 1**). Two principal pathways of biotin catabolism have been identified in mammals. In the first pathway, the valeric acid side chain of biotin is degraded by β -oxidation. β -Oxidation of biotin leads to the formation of bisnorbiotin, tetranorbiotin, and related intermediates that are known to result from β -oxidation of fatty acids. The

Table 1 Normal range of urinary excretion of biotin and major metabolites (nmol/24 h; $n = 31$ males and females)

<i>Biotin</i>	<i>Bisnorbiotin</i>	<i>Biotin sulfoxide</i>
18–77	11–39	8–19

cellular site of this β -oxidation of biotin is uncertain. Spontaneous (nonenzymatic) decarboxylation of the unstable β -ketoacids (β -ketobiotin and β -ketobisnorbiotin) leads to formation of bisnorbiotin methylketone and tetranorbiotin methylketone; these catabolites appear in urine.

In the second pathway, the sulfur in the thiophane ring of biotin is oxidized leading to the formation of biotin L-sulfoxide, biotin D-sulfoxide, and biotin sulfone. Sulfur oxidation may be catalyzed by an NADPH-dependent process in the smooth endoplasmic reticulum. Combined oxidation of the ring sulfur and β -oxidation of the side chain lead to metabolites such as bisnorbiotin sulfone. In mammals, degradation of the biotin ring to release carbon dioxide and urea is quantitatively minor.

On a molar basis, biotin accounts for approximately half of the total avidin-binding substances in human serum and urine (Table 1). Biocytin, bisnorbiotin, bisnorbiotin methylketone, biotin-D,L-sulfoxide, and biotin sulfone account for most of the balance. Biotin catabolism to these inactive forms is accelerated by anticonvulsant therapy, by cigarette smoking, and during pregnancy, thereby increasing the ratio of biotin metabolites to biotin in urine and potentially contributing to an increased requirement.

Because biotin and its metabolites are small molecules (≤ 244 Da) and mainly free in plasma, most of the biotin will pass into the glomerular filtrate. Thus, specific systems for the reabsorption of biotin from the glomerular filtrate are important to avoid substantial losses in urine. Based on work by Said and coworkers, SMVT is the principal transporter responsible for biotin reabsorption. Biotin uptake by SMVT is adaptively regulated by biotin deficiency, consistent with previous studies demonstrating reduced biotin excretion in marginal biotin deficiency induced experimentally in human subjects.

Subsequent egress of biotin from the tubular cells occurs via a basolateral membrane transport system that is not dependent on Na^+ . Studies in patients with biotinidase deficiency suggest that there may be a role for biotinidase in the renal handling of biotin.

Biliary Excretion of Biotin and Metabolites

Biliary excretion of biotin and metabolites is quantitatively negligible compared to urine based on a study in rats. Although the concentrations of biotin, bisnorbiotin, and biotin-D,L-sulfoxide were approximately 10-fold greater in bile than serum of pigs, the bile to serum ratio for biotin is still more than 10-fold less than the bile to serum ratio for bilirubin, which is actively excreted in bile.

Metabolic Functions

In mammals, biotin serves as an essential cofactor for five carboxylases, each of which catalyzes a critical step in intermediary metabolism. All five of the mammalian carboxylases catalyze the incorporation of bicarbonate as a carboxyl group into a substrate. All five employ a similar catalytic mechanism.

Biotin is attached to the apocarboxylase by a condensation reaction catalyzed by holocarboxylase synthetase (HCS) (Figure 1). An amide bond is formed between the carboxyl group of the valeric acid side chain of biotin and the ϵ -amino group of a specific lysyl residue in the apocarboxylase; these regions contain sequences of amino acids that are highly conserved for the individual carboxylases both within and between species.

In the carboxylase reaction, the carboxyl moiety is first attached to biotin at the ureido nitrogen opposite the side chain; then the carboxyl group is transferred to the substrate. Because the valeric acid side chain of biotin is coupled to the side chain of lysine in each holocarboxylase, this CO_2 is at the end of a long, flexible chain, allowing the biotinyl coenzyme to be carboxylated at one site and used as a CO_2 donor at a second site. The reaction is driven by the hydrolysis of ATP to ADP and inorganic phosphate. Subsequent reactions in the pathways of the mammalian carboxylases release carbon dioxide from the product of the carboxylase reaction. Thus, these reaction sequences rearrange the substrates into more useful intermediates but do not violate the classic observation that mammalian metabolism does not result in the net fixation of carbon dioxide.

The five biotin-dependent mammalian carboxylases (Figure 2) are acetyl-CoA carboxylase (EC 6.4.1.2) isoforms ACC-1 and ACC-2 (formerly known as α ACC and β ACC), pyruvate carboxylase (EC 6.4.1.1, PC), methylcrotonyl-CoA carboxylase (EC 6.4.1.4, MCC), and propionyl-CoA carboxylase (EC 6.4.1.3, PCC).

Both ACC-1 and ACC-2 catalyze the incorporation of bicarbonate into acetyl CoA to form malonyl CoA (Figure 2), but ACC-1 and ACC-2 are thought to have two very different roles in cellular metabolism; one controls fatty acid synthesis, and the other controls fatty acid oxidation. ACC-1 is located in the cytosol and produces malonyl CoA, because availability of malonyl CoA is rate limiting, activity of ACC-1 controls fatty acid synthesis (elongation) and is tightly regulated in a sophisticated fashion. Cytosolic ACC-1 exists as a very large polymer with a molecular mass in the millions of Daltons and is inactivated by dissociation into its protomer units. Citrate activates ACC-1 by increasing polymerization. CoA itself activates ACC-1 by lowering the K_m for acetyl CoA. ACC-1 is inhibited by the products of fatty acid synthesis, the long-chain acyl CoAs, which also act to depolymerize the enzyme. In addition, ACC-1 activity is regulated by covalent modification (phosphorylation) in response to the hormones insulin and glucagon. A high insulin-to-glucagon ratio typical of the immediate postprandial state with increased blood glucose level favors dephosphorylation of ACC-1 to an active form, whereas a low insulin-to-glucagon ratio (typical of fasting) favors phosphorylation to the inactive form. The amount of ACC-1 protein also responds to changes in dietary and hormonal conditions.

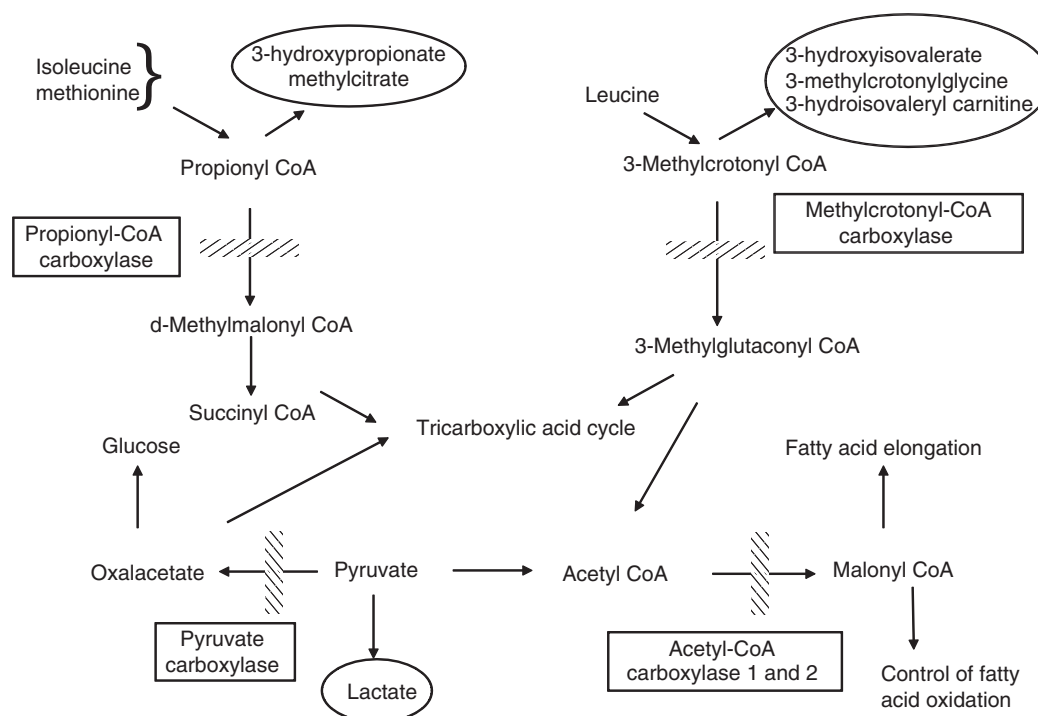


Figure 2 Interrelationship of pathways catalyzed by biotin-dependent enzymes (shown in boxes). Organic acids and odd-chain fatty acids accumulate because biotin deficiency causes reduced activity of biotin-dependent enzymes. Hatched bars denote metabolic blocks at deficient carboxylases; ovals denote accumulation of products from alternative pathways.

ACC-2 is located on the outer mitochondrial membrane and regulates the availability of fatty acids for oxidation through the inhibition of carnitine palmitoyltransferase I by malonyl CoA. Carnitine palmitoyltransferase I catalyzes the rate-limiting transfer of the fatty acid from CoA to carnitine; the fatty acyl carnitine is transported into the mitochondrial matrix converted back to the CoA derivative and oxidized.

The three remaining carboxylases are mitochondrial. PC catalyzes the incorporation of bicarbonate into pyruvate to form oxaloacetate, an intermediate in the Krebs tricarboxylic acid cycle (Figure 2). Thus, PC catalyzes an anaplerotic reaction. In gluconeogenic tissues (that is, liver and kidney), the oxaloacetate can be converted to glucose. Deficiency of PC is probably the cause of the lactic acidemia, central nervous system lactic acidosis and abnormalities in glucose regulation observed in biotin deficiency and in genetic biotinidase deficiency and HCS deficiency. PC also plays a role in the formation of the protective myelin sheath that surrounds certain nerve cells and the production of neurotransmitters, both of which may contribute to the phenotype of inherited PC deficiency.

In the 'A' form of inherited PC deficiency, affected infants present in the first few months of life with mild or moderate lactic acidemia and psychomotor retardation. Lactic acidemia can lead to vomiting, abdominal pain, fatigue, muscle weakness, and difficulty breathing. Most children die within the first few years, and survivors have severe mental retardation.

In the more severe 'B' form of PC deficiency, the initial presentation usually occurs shortly after birth with hypotonia, seizures, coma, severe lactic acidemia, and signs of liver failure

such as elevated blood ammonia concentrations. Death usually occurs before 3 months of age.

MCC catalyzes an essential step in the degradation of the branched-chain amino acid leucine (Figure 2). MCC is composed of two non identical subunits: a biotinylated α subunit that is encoded by the gene MCCC1 and a nonbiotinylated β subunit, which is encoded by the gene MCCC2. MCC is not regulated by small molecules or by dietary or hormonal factors. Deficient activity of MCC leads to metabolism of 3-methylcrotonyl CoA to 3-hydroxyisovaleric acid, 3-hydroxyisovaleryl carnitine and 3-methylcrotonylglycine by an alternate pathway. Thus, increased urinary excretion of these abnormal metabolites reflects deficient activity of MCC. The inherited deficiency of MCC characteristically presents with recurrent episodes of vomiting, diarrhea, lethargy, hypotonia, severe metabolic acidosis, hypoglycemia, and carnitine depletion. Moderate restriction of dietary protein to limit leucine intake and carnitine supplementation to correct or prevent carnitine deficiency generally result in normal development. Some cases respond to biotin supplementation. Newborn screening of acyl carnitines has identified a much higher incidence of asymptomatic MCC deficiency than expected from the number of patients ascertained by clinical symptoms; this observation suggests that many patients may have a benign clinical course.

PCC catalyzes the incorporation of bicarbonate into propionyl CoA to form methylmalonyl CoA; methylmalonyl CoA undergoes an isomerization to succinyl CoA catalyzed by the B₁₂-dependent enzyme methylmalonyl-CoA mutase and enters the tricarboxylic acid cycle (Figure 2). In a fashion analogous to MCC deficiency, deficiency of PCC leads to increased urinary excretion of 3-hydroxypropionic acid and

3-methylcitric acid. Sources of propionyl CoA include catabolism of the amino acids valine, isoleucine, threonine, and methionine; β -oxidation of odd-numbered or branched-chain fatty acids; byproducts of bile acid synthesis from cholesterol; and intestinal microflora. PCC is not rate limiting in the metabolism of propionyl CoA, and the enzyme activity is not sensitively regulated by allosteric effectors or by dietary or hormonal changes. PCC is composed of two nonidentical subunits: a biotinylated α subunit that is encoded by the gene PCCA and a nonbiotinylated β subunit, which is encoded by the gene PCCB.

Propionic acidemia is the disease caused by an inherited deficiency of PCC. Affected individuals have repeated, life-threatening episodes of severe ketosis and metabolic acidosis that often begin in infancy. Findings include vomiting, dehydration, and lethargy, which can progress to coma and death if not treated. Frequent neurological complications include developmental delay, seizures, and cerebral atrophy. The concentration of propionyl CoA increases proximal to the metabolic block caused by decreased PCC activity and results in increased urinary excretion of a constellation of propionate metabolites that are diagnostic of propionic acidemia. These include 3-hydroxypropionate, propionylglycine, propionylcarnitine, and methylcitrate.

The most important treatment for propionic acidemia is restriction of dietary protein, thereby limiting the amino acid precursors of propionate. Use of special formulas that have very low levels of isoleucine, valine, methionine, and threonine has the same goal. The minimum requirement for these essential amino acids is met by the addition of other proteins after calculation of the content of each amino acid. Loss of propionylcarnitine can lead to a secondary deficiency of carnitine. Therapy includes dietary carnitine to prevent carnitine deficiency; this promotes the conversion of propionyl CoA to propionylcarnitine, which helps restore free CoA concentrations and facilitates excretion of propionate.

Biotin Deficiency Diseases

HCS Deficiency

Genetic deficiencies of HCS and biotinidase cause the two types of multiple carboxylase deficiency that were previously designated the neonatal and juvenile forms. The inherited deficiency of HCS activity results in decreased activities of all five of the biotin-dependent carboxylases. In turn, these multiple carboxylase deficiencies result in clinical findings arising from the roles of all five carboxylases in metabolism. In patients with a severe HCS deficiency, illness often occurs in the neonatal period and includes severe ketoacidosis, seizures, and lethargy, if not recognized and treated, coma and death can ensue. In patients who have a milder form of HCS deficiency, hair loss (alopecia), and an erythematous skin rash typical of biotin deficiency can appear at several months of age. Elevated urinary excretions of the metabolites characteristic of deficiency of several of the biotin-dependent carboxylases deficiencies are seen.

Treatment with large oral doses of biotin (e.g., 10–60 mg day⁻¹), usually gives dramatic improvement of the

biochemical abnormalities, skin rash, alopecia, and neurological findings, provided irreversible neurological damage has not occurred. A variety of mutations of the HCS gene have been reported. When studied, the concentration of biotin needed to attain half of the maximal reaction rate (the K_m) generally was found to be increased far above biotin levels found in normal cells. On the contrary, the maximal enzyme activity (V_{max}) usually was importantly greater than zero and approached normal. These observations explain why treatment with very large doses of biotin that increased tissue levels of biotin far above normal can result in enough active of HCS to convert enough apocarboxylases to active holocarboxylases, correcting the multiple carboxylase deficiencies. A complete absence of HCS activity, which would mean no activity of any of the five carboxylases, would probably be fatal *in utero*.

Biotinidase Deficiency

In the normal turnover of cellular proteins, holocarboxylases are degraded to biocytin or biotin linked to an oligopeptide containing at most a few amino acid residues. (Figure 1). Biotinidase similarly plays a critical role by releasing biotin for recycling from intracellular proteins such as carboxylases and histones during protein turnover. Consistent with this global role, biotinidase is found in many tissues including heart, brain, liver, lung, skeletal muscle, kidney, plasma, and placenta in addition to pancreas and intestine. The liver is thought to be the source of serum biotinidase.

Individuals with less than 10% of normal activity in serum exhibit seizures, hypotonia, skin rash, and alopecia, usually presenting in infancy. Many children have ataxia, developmental delay, conjunctivitis, hearing loss, and visual problems, including optic atrophy as well as a characteristic organic aciduria. If untreated, some progress to coma or death. Some only manifest one or two features, or present later in life with motor limb weakness, spastic paresis, and eye problems, such as loss of visual acuity and scotomata. Once hearing loss, optic atrophy, and moderate or severe developmental delay appear, they are often irreversible despite treatment with biotin. If treatment is begun before onset of clinical findings, signs and symptoms appear to be preventable.

Thus, the clinical findings and biochemical abnormalities of biotinidase deficiency resemble those of biotin deficiency (dermatitis, alopecia, conjunctivitis, ataxia, and developmental delay) suggesting that they are caused by biotin deficiency. However, the signs and symptoms of biotin deficiency and biotinidase deficiency are not identical. Seizures, irreversible neurosensory hearing loss, and optic atrophy have been observed in biotinidase deficiency, but not in biotin deficiency. A knockout mouse model has recently been reported that recapitulates many of these findings.

Potential Role for Biotin in Gene Expression

In 1995, Hymes and Wolf discovered that biotinidase can act as a biotinyl-transferase; biocytin serves as the source of biotin, and histones are specifically biotinylated. Zemleni and coworkers demonstrated that the abundance of biotinylated

histones varies with the cell cycle and that biotinylated histones are increased approximately two-fold in activated, dividing lymphocytes compared to quiescent lymphocytes. These early observations provided the initial evidence that biotinylation of histones might play a role in regulating DNA transcription and regulation as an additional element in the histone code.

Initially, biotinylation of histones was hypothesized to be catalyzed by biotinidase. However, subsequent studies provided evidence that HCS is substantially more important than biotinidase for biotinylation of histones *in vivo*. HCS is present in both nuclear and cytoplasmic compartments; in the nucleus, HCS is associated with chromatin. Fibroblasts from patients with inherited deficiency of HCS exhibit decreased biotinylation of histones. On the contrary, biotin can be removed from histones by biotinidase, and debiotinylation of histones is decreased in samples from biotinidase-deficient patients. Current understanding is that HCS plays the predominant role in histone biotinylation and biotinidase plays the predominant role in histone debiotinylation.

Biotinylation of distinct lysine residues is now a recognized covalent modification in the histone code. Currently, about a dozen biotinylation sites have been identified in histones H2A, H3, and H4. Although the mechanisms remain to be elucidated, biotin status clearly affects gene expression. Cell culture studies suggest that cell proliferation generates an increased demand for biotin, perhaps mediated by increased synthesis of biotin-dependent carboxylases. Evidence is emerging that this demand is met by an upregulation of biotin transporter expression mediated by control of the gene by biotinylation of lysine 12 in histone H4 (H4K12bio). H4K12bio is also enriched in transcriptionally repressed genes and heterochromatin repeats such as telomeres, long-terminal repeats, and pericentromeric alpha satellite repeats; this covalent modification of H4 appears to repress expression of long-terminal repeats and thereby reduce retrotransposition. Low levels of histone biotinylation have been reported in biotin deficient cells and model organisms; reduced histone biotinylation has been linked to increased frequency of retrotransposition events, consistent with a role for histone biotinylation in chromosomal stability.

Although the mechanisms remain to be elucidated, biotin status has been shown to clearly effect gene expression. Solórzano-Vargas and coworkers have reported that biotin deficiency reduces messenger RNA levels of HCS, ACC-1, and PCC and have postulated that a cyclic GMP-dependent signaling pathway is involved in the pathogenesis. Zemleni and coworkers have demonstrated that nitric oxide in the cyclic GMP signaling pathway depends on biotin status.

Assessment of Biotin Status

Measurement of Biotin

For measuring biotin at physiological concentrations (that is, from 100 pmol l^{-1} to 100 nmol l^{-1}), most recent studies have used an avidin-binding assay to evaluate biotin status. Avidin-binding assays generally detect all avidin-binding substances after chromatographic separation of biotin analogs. This method appears to be both sensitive and chemically specific.

Table 2 Recommended intake of biotin

Age	Safe and adequate biotin intakes (μg)	Daily parenteral supplement ($\mu\text{g/kg}$)
Preterm infants	NA	8
Infants < 6 months	35	20
Infants up to 1 year	50	20
Children 1–3 years	65	20
Children 4–6 years	85	20
Older children, 7–10 years	120	20
Older children > 11 years and adults	100–200	60

Laboratory Findings of Biotin Deficiency

In humans, laboratory indicators of biotin deficiency have been validated in studies in which progressive, but asymptomatic biotin deficiency was induced experimentally by feeding diets high in egg white. The urinary excretion of biotin declines dramatically, reaching frankly abnormal values in approximately 90% of subjects after 3 weeks. Urinary excretion of 3-hydroxyisovaleric acid and plasma and urinary 3-hydroxyisovalerylcarnitine increase to greater than the normal range in approximately 90% of subjects after 2 weeks of egg-white feeding, providing evidence that biotin depletion decreases the activity of MCC. The most sensitive indicator of biotin status appears to be activity of PCC in lymphocytes isolated from venous blood samples. Unfortunately, this assay is technically demanding, and the blood samples require special handling and storage.

Serum concentrations of free biotin decrease to abnormal values in less than half of the subjects. This observation is consistent with the impression of many investigators in this field that blood biotin concentration is not an early or sensitive indicator of impaired biotin status.

Requirements and Allowances

Data providing an accurate estimate of the dietary and parenteral biotin requirements for infants, children, and adults are lacking. However, recommendations for biotin supplementation have been formulated for oral and parenteral intake for preterm infants, term infants, children, and adults (Table 2).

Dietary Sources, Deficiency, and High Intakes

Dietary Sources

There is no published evidence that biotin can be synthesized by mammals; thus, the higher animals must derive biotin from other sources. The ultimate source of biotin appears to be *de novo* synthesis by bacteria, primitive eukaryotic organisms such as yeast, molds, and algae, and some plant species.

The great majority of measurements of biotin content of foods have used bioassays. Recent publications provide evidence that the values are likely to contain substantial errors.

However, some worthwhile generalizations can be made. Biotin is widely distributed in natural foodstuffs, but the absolute content of even the richest sources is low when compared to the content of most other water-soluble vitamins. Foods relatively rich in biotin include egg yolk (4.08 µg biotin/serving), chicken liver (138 µg biotin/serving), and some vegetables (broccoli at 1.07 µg biotin/serving, canned mushrooms at 2.59 µg biotin/serving, and cooked sweet potato at 1.16 µg biotin/serving). The average daily dietary biotin intake has been estimated to be approximately 35–70 µg.

Circumstances Leading to Deficiency

That normal humans have a requirement for biotin has been clearly documented in three situations: prolonged consumption of raw egg white, parenteral nutrition without biotin supplementation in patients with short bowel syndrome, and infant feeding with an elemental formula devoid of biotin. Because biotin could not legally be added as a supplement to infant formulas in Japan until 2003, all reports related to infant formula have come from Japan. Often feeding of an elemental formula was required to treat intractable, chronic diarrhea. The infants typically developed both the classic cutaneous manifestations of biotin deficiency and the characteristic pattern of organic aciduria.

Based on lymphocyte carboxylase activities and plasma biotin levels, some children with severe protein-energy malnutrition are biotin deficient. Investigators have speculated that the effects of biotin deficiency may be responsible for part of the clinical syndrome of protein-energy malnutrition.

Long-term anticonvulsant therapy in adults can lead to biotin depletion severe enough to interfere with leucine metabolism and cause increased urinary excretion of 3-hydroxyisovaleric acid. The mechanism of biotin depletion during anticonvulsant therapy is not known, but may involve accelerated biotin catabolism, impaired biotin absorption, impaired biotin transport in plasma, impaired renal reclamation biotin, or a combination of these.

Recent studies of biotin status during pregnancy and of biotin supplementation during pregnancy provide evidence that a marginal degree of biotin deficiency develops in at least one third of women during normal pregnancy. Although the degree of biotin deficiency is not severe enough to produce overt manifestations of biotin deficiency, the deficiency is severe enough to produce metabolic derangements. A similar marginal degree of biotin deficiency causes high rates of fetal malformations in some mammals. Moreover, data from a multivitamin supplementation study provide significant, albeit indirect, evidence that the marginal degree of biotin deficiency that occurs spontaneously in normal human gestation is teratogenic.

Biotin deficiency has also been reported or inferred in several other clinical circumstances including Leiner's disease, sudden infant death syndrome, renal dialysis, gastrointestinal diseases, and alcoholism.

Clinical Findings of Frank Deficiency

The clinical findings of frank biotin deficiency in adults, older children and infants are similar. Typically, the findings appear

gradually after weeks to several years of egg-white feeding or parenteral nutrition. Thinning of hair and progression to loss of all hair including eyebrows and lashes has been reported. A scaly (seborrheic), red (eczematous) skin rash was present in the majority; in several, the rash was distributed around the eyes, nose, mouth, and perineal orifices. These cutaneous manifestations, in conjunction with an unusual distribution of facial fat, have been dubbed 'biotin deficiency facies'. Depression, lethargy, hallucinations, and paresthesia of the extremities were prominent neurologic symptoms in the majority of adults. The most striking neurologic findings in infants were hypotonia, lethargy, and developmental delay.

The clinical response to administration of biotin has been dramatic in all well-documented cases of biotin deficiency. Healing of the rash was striking within a few weeks, and growth of healthy hair was generally present by 1–2 months. Hypotonia, lethargy, and depression generally resolved within 1–2 weeks, followed by accelerated mental and motor development in infants. Pharmacological doses of biotin (e.g., 1–10 mg) have been used to treat most patients.

Pharmacology

Mounting reports of biotin deficiency in commercial animals and humans have led to several studies of plasma levels, pharmacokinetics, and bioavailability after acute or chronic oral, intramuscular, or intravenous administration of biotin in cattle, swine, fish, and humans. High doses (e.g., 1200 mg) result in high biotin concentrations in blood and the urinary excretion of a large proportion as the unchanged biotin. Increased blood concentrations of bisnorbiotin and biotin sulfoxide and increased urinary excretion of bisnorbiotin and biotin sulfoxide are also reported, providing evidence that the biotin metabolites originate from human tissues rather than enteric bacteria.

Daily doses up to 200 mg orally and up to 20 mg intravenously have been given to treat biotin-responsive inborn errors of metabolism and acquired biotin deficiency. Toxicity has not been reported.

See also: Biochemical Indices. Dietary Guidelines, International Perspectives. Eggs. Inborn Errors of Metabolism: Classification and Biochemical Aspects. Lactation: Dietary Requirements. Nutrient–Gene Interactions: Molecular Aspects. Nutritional Requirements of Infants. Pantothenic Acid. Pregnancy: Nutrient Requirements; Prevention of Neural Tube Defects

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BODY COMPOSITION

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Glossary

Anthropometry Measurement of the size and proportions of the human body.

Bioimpedance analysis A method of measuring the proportion of fat in the body by the difference in the resistance to passage of a small electric current.

Computed tomography A method of examining body organs by scanning them with X-rays and using a computer to construct a series of cross-sectional scans.

Hydrodensitometry Assessment of the composition of matter by weighing it underwater in order to determine its density.

Lipodystrophy Abnormal or degenerative conditions of the body's adipose tissue.

MRI A form of medical imaging that measures the response of the atomic nuclei of body tissues to high-frequency radio waves when placed in a strong magnetic field, and that produces images of the internal organs.

Plethysmography Measurement of the volume of a body (and hence, in conjunction with weight, its density).

Sarcopenia The degenerative loss of skeletal muscle mass and strength associated with aging.

Introduction

Historically, the measurement of the body and its components centered around cadaver analyses, where specific tissues and organs were extracted from the body for inspection. The extraction of tissue samples from the living body was a step forward in allowing for the analyses of tissue morphology in a state more closely resembling the *in vivo* state. However, both cadaver and *in vitro* tissue analyses are subject to inaccuracies when extrapolations are being made to the living body. Nevertheless, much of our understanding of human body composition in both children and adults has roots in these approaches. During the twentieth century, significant advances were made in the development of *in vivo* methods of body composition analysis thanks to the disciplines of Physics, Engineering, and Medicine. Methodologies with minimal or no risk to the participant have allowed for the assessment of body composition in growth and development, aging, and disease.

The physiological significance of knowing the composition of the body greatly depends on the question of interest. Common applications involving medical/clinical diagnoses include osteopenia/osteoporosis; muscle wasting; sarcopenia; lipodystrophy; altered states of hydration; malnutrition; obesity. There are also metabolic consequences (for example, insulin resistance) associated with high and low levels of body fat and where the fat is distributed. From a nutritional perspective, the interest in body composition has increased multifold with the global increase in the prevalence of obesity and its complications. This article will focus on our current state of body composition knowledge and how this knowledge was determined with the available most advanced methodologies.

Body Composition Determination

There is no single gold standard for body composition measurements *in vivo*. All methods incorporate assumptions that do not apply in all individuals and the more accurate models are derived using a combination of measurements, thereby reducing the importance of each assumption. The most commonly used technique today with good reproducibility in children and adults is dual-energy X-ray absorptiometry (DXA).

DXA

The DXA method evolved from earlier single and dual photon absorptiometry methods for evaluating bone mineral. DXA systems share in common an X-ray source that, after appropriate filtration, emits two photon energy peaks. The attenuation of the two energy peaks relative to each other depends on the elemental content of tissues through which the photons pass. Bone, fat, and lean soft tissues are relatively rich in calcium/phosphorus, carbon, and oxygen, respectively. DXA systems are designed to separate pixels, based on appropriate models and relative attenuation, into these three components. There are no known factors, including hydration effects, that significantly influence the validity of DXA fat and bone mineral estimates. Excessive or reduced fluid volume would be interpreted as changes in lean soft tissue. The radiation exposure is minimal and can be used in children and adults of all ages. DXA measures in persons who fit within the DXA field-of-view have good reproducibility for total body and regional components.

Hydrodensitometry/Air Plethysmography

One of the oldest methods of measuring body composition, the determination of body volume by water displacement (Archimedes principle) allows for the estimation of fat-free mass (FFM) density (where an assumption is made that densities of fat and FFM are constant) from which percent body fat is calculated using a two-compartment body composition model. Today there are a number of additional methods for measuring body volume, including air displacement plethysmography. Limitations with this approach include the assumptions of stable densities of fat and FFM across the age range where this may not be true in older individuals and across race/ethnic groups where it is now known that the density of FFM in Blacks is higher.

Dilution Techniques

Because fat is relatively anhydrous, the body's water is found primarily in the body's FFM compartment where approximately 73% of a healthy nonobese adults FFM compartment is water. The body's water pool can be measured using tracers, which after administration, dilute throughout the body. Basic assumptions involved with tracer dilution for body composition determination include equal distribution throughout the pool of interest and dilution is complete within a specific period of time without any loss. Examples of commonly used tracers include deuterium oxide for total body water and sodium bromide for extracellular water. These isotope dilution techniques allow for the evaluation of fat and FFM without making the assumption that the hydration of FFM is constant and therefore stable.

Whole-Body Counting

A small constant percentage of total body potassium (TBK) is radioactive (^{40}K) and emits a γ -ray. With appropriate shielding from background, this γ -ray can be counted using scintillation detectors. As the ratio of ^{40}K to ^{39}K is known and constant, ^{39}K or 'total body potassium' can be estimated accordingly. All of the body's potassium is within the FFM compartment and the proportion of the body FFM compartment TBK/FFM ratio is relatively stable in the same subject over time and between different subjects. However, with increasing age or when comparing young versus elderly, the TBK to FFM ratio decreases. Although the specific mechanism(s) associated with this decrease in the TBK to FFM ratio with increasing age is/are unclear, it could be explained by a small but consistent increase in extracellular fluid compared to intracellular fluid.

Magnetic Resonance Imaging (MRI)

The use of MRI has resulted in important advances in body composition phenotyping. MRI studies are safe and instruments are available in most hospital or related facilities. Expense is a limiting factor. The importance of MRI is that this method acquires cross-sectional images of the body at

predefined anatomic locations. Image analysis software then allows estimation of the adipose tissue, skeletal muscle, and organs based on pixel intensity. Acquiring images at predefined intervals and integrating the area between slices allows reconstruction of an entire organ of interest such as skeletal muscle mass. A significant advancement made possible by these imaging methods has been the characterization of a tissue distribution, such as adipose tissue where it is now possible to quantify visceral, subcutaneous, and intermuscular depots at the regional and whole-body level.

Quantitative Magnetic Resonance (QMR)

A recently available QMR system (EchoMRI-AH; Echo Medical Systems, Houston, TX) relies on proton nuclear magnetic resonance to measure human body composition. Using various pulse sequences, the QMR system provides estimates of fat mass, lean tissue mass, free water, and total body water. The QMR approach has important advantages over currently available methods as it provides body composition estimates with high precision and without the use of ionizing radiation. The system, moreover, can accommodate subjects up to 250 kg, almost double than that of the widely used DXA approach. Analyses to date from two different laboratories have revealed systematic differences in body-fat estimates between the QMR and other available research methods and further exploration is needed to identify these sources of and potential correction approaches for these differences.

Bioimpedance Analysis (BIA)

BIA is a simple, inexpensive, and noninvasive body composition measurement method. BIA is based on the electrical conductive properties of the human body. Measures of bioelectrical conductivity are proportional to total body water and the body compartments with high water concentrations such as fat free and skeletal muscle mass. BIA assumes that the body consists of two compartments, fat and FFM (Body weight = Fat + FFM). BIA is best known as a technique for the measurement of percent body fat although it has more recently been used for estimating skeletal muscle mass too. The equations developed to estimate body composition by any BIA system are population specific such that they are most valid in populations similar to the population that a specific equation was developed in. The validity of BIA in very obese persons is questioned as TBW and extracellular water relative to TBW are both greater in obese subjects compared with normal-weight individuals.

Anthropometry

For routine clinical use, anthropometric measurements (circumference measures and skinfold thickness) have been preferred due to ease of measurement and low cost. Waist circumference and the waist-hip ratio measurements are commonly used surrogates of fat distribution, especially in epidemiology studies. Waist circumference is highly correlated with visceral fat and was recently included as a clinical risk

factor in the definition of the metabolic syndrome. Specifically, waist circumferences greater than 102 cm (40 in) in men and greater than 88 cm (35 in) in women are suggestive of elevated risk.

Skinfold thicknesses which estimate the thickness of the subcutaneous fat layer are highly correlated with percent body fat. Because the subcutaneous fat layer varies in thickness throughout the body, a combination of site measures is recommended, reflecting upper and lower body distributions. Predictive percent body fat equations based on skinfold measures are age and sex specific in adults and children.

Body Mass Index (BMI)

The body mass index ($BMI = \text{weight (kg)} / \text{height (m)}^2$) continues to be the most commonly used index of weight status, where normal weight is a $BMI\ 18.5 - 25.9\ \text{kg m}^{-2}$; overweight is a $BMI\ 25.0 - 29.9\ \text{kg m}^{-2}$; obese a $BMI > 30.0\ \text{kg m}^{-2}$. BMI is a commonly used index of fatness due to the high correlation between BMI and percent body fat in children and adults. The prediction of percent body fat is dependent on age (higher in older persons), sex (higher in males), and race (higher in Asian compared to African American and Caucasian).

In Vivo Neutron Activation

Nitrogen, carbon, hydrogen, phosphorus, sodium, chlorine, calcium, and oxygen are all measurable *in vivo* by a method known as neutron activation analysis. A source emits a neutron stream that interacts with body tissues. The resulting decay products of activated elements can be counted by detectors and elemental mass established. Carbon, nitrogen, and calcium can be used to estimate total body fat, protein, and bone mineral mass using established equations. Neutron activation analysis is uniquely valuable in body composition research as there are no known age or sex effects of currently applied equations. However, facilities that provide these techniques are limited.

Models in Body Composition

The use of models in the assessment of body composition allows for the indirect assessment of compartments in the body. Typically, a compartment is homogenous in composition (for example, fat), however, the simpler the model the greater the assumptions made and the greater the likelihood of error. The sum of components in each model is equivalent to body weight (Figure 1). These models make assessments at the whole-body level and do not provide for regional or specific organ/tissue assessments.

The basic two-compartment (2C) model (Table 1) is derived from measuring the density of FFM by hydrodensitometry and subtracting FFM from total body weight thereby deriving fat mass ($\text{body weight} - \text{FFM} = \text{fat mass}$). FFM is a heterogeneous compartment consisting of numerous tissues and organs. A 2C approach becomes inadequate when the tissue of interest is included within the FFM compartment. Nevertheless, the 2C

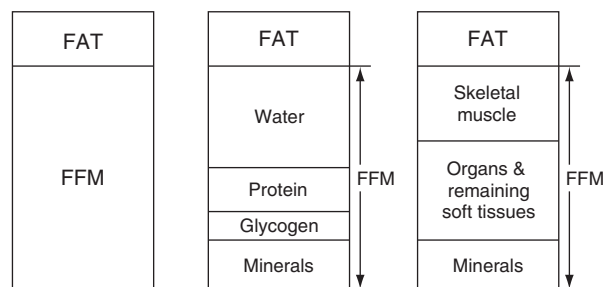


Figure 1 Three different models for characterizing body composition compartments. Components are as labeled: FFM, fat-free body mass.

model is routinely and regularly used to calculate fat mass from hydrodensitometry, total body water, and total body potassium.

A three-compartment (3C) model consists of fat, fat-free solids, and water. The water content of FFM is assumed to be between 70% and 76% for most species and results from cross-sectional studies in adult humans show no evidence of differences in the hydration of FFM with age. The fat-free solids component of FFM refers to minerals (including bone) and proteins. The 3C approach involves the measurement of body density (usually by hydrodensitometry) and total body water by an isotope dilution technique. Assumptions are made that both the hydration of FFM and the solids portion of FFM are constant. Because bone mineral content is known to decrease with age, the 3C approach is limited in its accuracy in persons or populations where these assumptions are incorrect.

A four-compartment (4C) model involves the measurement of body density (for fat), total body water, bone mineral content by DXA, and residual ($\text{residual} = \text{body weight} - (\text{fat} + \text{water} + \text{bone})$). This model allows for the assessment of several assumptions that are central to the 2C model. The 4C approach is frequently used as the criterion method against which new body composition methods are compared in both children and adults.

The more complex 4C model involves neutron activation methods for the measurement of total body nitrogen and total body calcium, where $\text{total body fat} = \text{body weight} - \text{total body protein (from total body nitrogen)} + \text{total body water (dilution volume)} + \text{total body ash (from total body calcium)}$. A six-compartment model is calculated as follows: $\text{fat mass (measured from total body carbon)} = \text{body weight} - (\text{total body protein} + \text{total body water} + \text{bone mineral} + \text{soft tissue mineral (from a combination of total body potassium, total body nitrogen, total body chloride, total body calcium)} + \text{glycogen (total body nitrogen)} + \text{unmeasured residuals})$. However, the availability of neutron activation facilities is limited and therefore the latter models are not readily obtainable by most researchers.

At the organizational level, a five-level model was developed where the body can be characterized at five levels. The following are the levels and their constituents: atomic= oxygen, carbon, hydrogen, and other (level 1); molecular= water, lipid, protein, and other (level 2); cellular= cell mass, extracellular fluid, and extracellular solids (level 3); tissue-system level= skeletal muscle, adipose tissue, bone, blood, and other (level 4); whole body (level 5).

Table 1 Multicompartment body composition models

Model	Equations for % fat	Reference
2C	$100(4.971/D_b - 4.519)$	^a
3C	$100(2.118/D_b - 0.78(TBW/W) - 1.354)$	^b
4C	$100(2.747/D_b - 0.727(TBW/W) + 1.146(BMC/W) - 2.0503)$	^c
6C	$100(2.513/D_b - 0.739(TBW/W) + 0.947(TBBM/W) - 1.79)$	^d

^aBehnke AR Jr, Feen BG, and Welham WC (1942) The specific gravity of healthy men. *Journal of the American Medical Association* 118: 495–498.

^bSiri WE (1961) Body composition from fluid spaces and density: analysis of methods. In: Brozek J and Henschel A (eds.) *Techniques for Measuring Body Composition*, pp. 223–224. Washington, DC: National Academy of Science.

^cBoileau RA, Lohman TG, and Slaughter MH (1985) Exercise and body composition of children and youth. *Scandinavian Journal of Sports Sciences* 7: 17–27.

^dHeymsfield SB, Wang ZM, and Withers RT (1996) Multicomponent molecular level models of body composition analysis. In: Roche AF, Heymsfield SB, and Lohman TG (eds.) *Human Body Composition*, pp. 129–147. Champaign: Human Kinetics.

D_b , body density; TBW, total body water; W, body weight; BMC, bone mineral content; TBBM, total body bone mineral.

Tissues and Organs

The aforementioned models do not allow for subregion or specific organ and tissue measurements. For example, skeletal muscle mass (SM) is contained within the FFM compartment. SM represents the single largest tissue in the adult body and is equivalent to ~40% of body weight in young adults, decreasing to ~30% of young values at elderly ages. SM is one of the more difficult components to quantify. Estimates of SM are commonly derived from anthropometry, total body potassium, and DXA using modeling approaches previously described. The use of MRI in body composition research has allowed for a good estimation of SM, adipose tissue (AT), and select organs *in vivo*, in all age groups with no risk to the participant (Figure 2). Moreover, AT distribution, including subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and intermuscular adipose tissue (IMAT) is also measurable using a whole-body multislice MRI protocol (Figure 3). In studies relating body composition to energy expenditure, high metabolic rate organs including liver, kidneys, heart, spleen, and brain are also measurable using MRI.

Bone mineral content and bone mineral density of specific body sites (for example, radius, hip, and lumbar spine) are most commonly measured using DXA. Bone mass and microarchitecture are important determinants of bone strength, with microarchitectural deterioration being one of the specific changes associated with osteoporosis. Using high-resolution microcomputed tomography (micro-CT) and computer software, detailed analysis of three-dimensional (3D) architecture is feasible and allows microstructural 3D bone information to be collected.

Body Composition Applications During Growth

Skeletal muscle mass has a central role in intermediary metabolism, aerobic power, and strength. Its mass increases as a portion of body weight during growth, accounting for 21% at birth and 36% at adolescence. The essential role of skeletal muscle in many physiologic processes throughout the lifespan makes understanding of factors affecting it significant. The greater incidence of type 2 diabetes mellitus in adolescents in the US (particularly in girls from minority populations) and in Japan makes evaluation of race and sex differences in pediatric

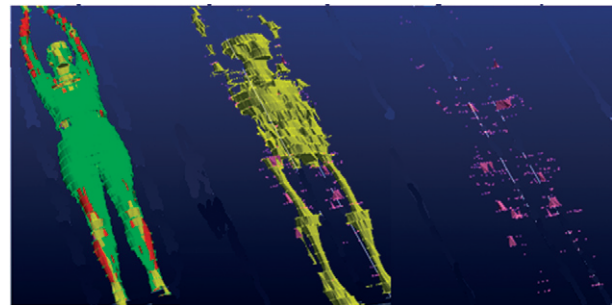


Figure 2 3D reconstructed image of whole-body scan (from MRI). Skeletal muscle (red); adipose tissue (green); bone, organs, and residual (yellow); intermuscular adipose tissue (pink).

skeletal muscle mass (and adipose tissue or fat mass) especially important. Identification and characterization of differences could form the basis for further investigation of the associated metabolic implications.

Race differences in SM are known to exist as early as pre-puberty. African-Americans have greater limb lean tissue mass compared to Asian and Caucasian children, although Caucasian children have greater amounts than Asians throughout Tanner stages 1–5. Race differences in total body bone mineral content adjusted for total body bone area, age, height, and weight have been reported in prepubertal African-American, Asian, and Caucasian females and males. African-American children had greater total body bone mineral content than Asian and Caucasian children, although differences between Asian and Caucasian children are less clear. Collectively, these findings suggest that the proportions of specific FFM sub-components may differ by race. Although mechanisms leading to bone and skeletal muscle differences between races are not well understood, endocrine factors may be involved.

Sex differences in FFM have been reported from birth throughout childhood with females having smaller amounts than males. Total body bone mineral content is less in Tanner 1 females compared to males in African-Americans, Asians, and Caucasians. The mechanism for this sex difference is unclear. Gonadal steroids are significant mediators of adult sexual dimorphism of body composition, including fat-free soft tissues. Prepubertal females have higher concentrations of circulating estradiol than prepubertal males, and gonadotropin and gonadal steroids increase gradually in both males

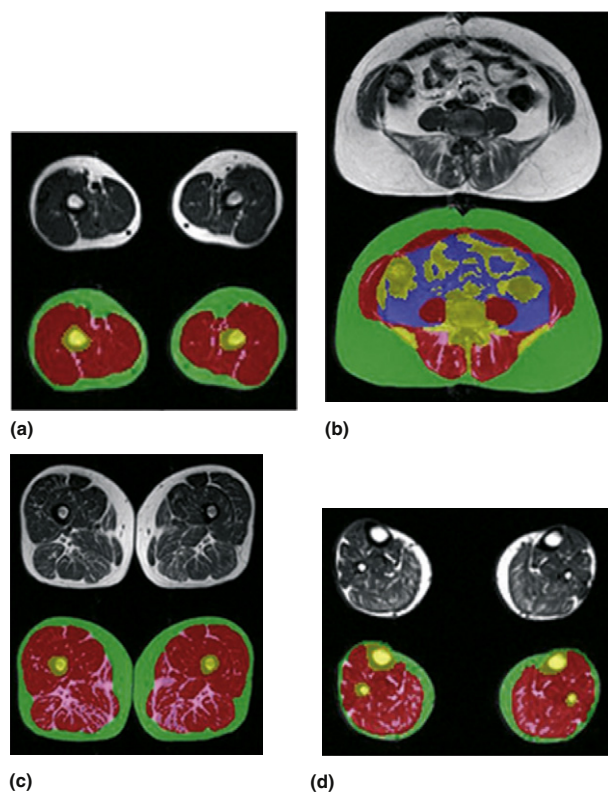


Figure 3 Cross-sectional images from (a) upper arm, (b) trunk (L4–L5 level), (c) mid-thigh, and (d) mid-calf in an elderly female volunteer. IMAT, intermuscular adipose tissue (pink); SM, skeletal muscle (red); SAT, subcutaneous adipose tissue (green); VAT, visceral adipose tissue (blue).

and females from the age of 5 years. Thus, prepuberty is a period with sex differences in circulating concentrations of sex steroids and of changes in these concentrations with advancing age. The earlier skeletal maturation of females, for example, has been attributed to the greater estradiol level in females compared to males. However, nonhormonal (possibly genetic) mechanisms may also play a role.

Fat or adipose tissue distribution is recognized as a risk factor for cardiovascular disease in both adults and children. An android or male fat pattern, with relatively greater fat in the upper body region, is associated with negative metabolic predictors whereas a gynoid or female fat pattern, with relatively greater fat in the hip and thigh areas, is associated with less metabolic risk. More and more studies are showing that the metabolic syndrome develops during childhood and is highly prevalent among overweight children and adolescents. Although the concept of the metabolic syndrome is referred initially to the presence of combined risk factors including VAT, dyslipidemia, hypertension, and insulin resistance in adults, it is now known to exist in children, especially where obesity and/or higher levels of VAT are present. Although sex-specific patterns of fat distribution had previously been thought to emerge during puberty, sex and race differences in fat distribution are now known to exist in prepubertal children. The implications are that a specific body composition pattern may differ by sex and race. An example is the

relationship of blood pressure to central fat distribution in boys compared to girls where a significant positive relationship between trunk fat and blood pressure was reported in boys but not girls, and was independent of race, height, weight, and total body fat. Understanding the predictors of blood pressure in children is important because childhood blood pressure has been shown to track into adulthood in longitudinal studies. Children whose blood pressure levels were in the highest quintile, were two times more likely to be in the highest quintile 15 years later. Identification of clinically useful body composition measures would allow for the identification of children at increased risk for hypertension, who could benefit from monitoring.

Race differences in fat distribution among prepubertal Asians, African-Americans, and Caucasians also exist. Previous reports in adolescents have suggested significantly smaller hip circumferences in Asian females at all pubertal stages compared to Caucasians and Hispanics and greater trunk subcutaneous fat in Asian females compared to Caucasians. Differences in subcutaneous fat mass and fat distribution in Asian compared to Caucasian adults have also been described. Understanding the sex- and race-specific effects of puberty on regional body composition may help delineate the developmental timing of specific health risk associations.

Body Composition Applications During Aging

During the adult life span, body weight generally increases slowly and progressively until about the seventh decade of life, and thereafter, declines into old age. An increased incidence of physical disabilities and comorbidities is likely linked to aging-associated body composition changes. Characterization of the aging processes has identified losses in muscle mass, force, and strength, which collectively are defined as 'sarcopenia.' Little is known about the overall rate at which sarcopenia develops in otherwise healthy elderly subjects, whether this rate of progression differs between women and men, and the underlying mechanisms responsible for age-related sarcopenia. Peak SM mass is attained in the young adulthood years and slowly declines thereafter. During the latter adult years, SM decreases more rapidly as body fat becomes more centralized. Anthropometric equations have been developed for predicting appendicular skeletal muscle ($ASM = SM$ of the limbs) in the elderly where sarcopenia was defined as $ASM (kg)/height^2 (m^2)$ less than two standard deviations below the mean of the young reference group. In the elderly men, the mean $ASM/height^2$ was approximately 87% of the young group. The corresponding value in women was approximately 80%. Table 2 shows the estimated prevalences of sarcopenia in the same survey sample for each ethnic group, by age and sex. The same authors have reported that obese and sarcopenic persons have worse outcomes than those who are nonobese and sarcopenic.

Even in healthy, weight-stable elderly persons, changes in body composition over a 2-year period can include decreases in SM mass and bone mineral content with corresponding increases in IMAT and VAT, after adjusting for their baseline values, despite no detectable changes in physical function or food intake.

Table 2 Prevalance (%) of sarcopenia^a in the New Mexico Elder Health Survey, by age, sex, and ethnicity, 1993–1995

Age group (years)	Men		Women	
	Hispanics (n = 221)	NonHispanic whites (n = 205)	Hispanics (n = 209)	NonHispanic whites (n = 173)
< 70	16.9	13.5	24.1	23.1
70–74	18.3	19.8	35.1	33.3
75–80	36.4	26.7	35.3	35.9
> 80	57.5	52.6	60.0	43.2

^aAppendicular skeletal muscle mass/height² (kg/m²) less than two standard deviations below the mean value for the young adults from Gallagher D, Visser M, De Meersman RE, et al. (1997) Appendicular skeletal muscle mass: effects of age, gender, and ethnicity. *Journal of Applied Physiology* 83: 229–239, with permission from APS and OUP.
Source: Adapted from Baumgartner RN, Koehler KM, Gallagher D, et al. (1998) Epidemiology of sarcopenia among the elderly in New Mexico. *American Journal of Epidemiology* 147: 755–763, with permission from APS and OUP.

In adults, excess abdominal or VAT is recognized as an important risk factor in the development of coronary heart disease and noninsulin dependent diabetes mellitus. Waist circumference and the waist : hip ratio are commonly used to predict visceral fat accumulation in epidemiological studies. However, waist circumference is unable to differentiate VAT from SAT. As a result, persons with similar waist circumferences could have markedly different quantities of VAT and abdominal SAT. Skinfold thickness has been used as a continuous variable grading adiposity or adipose tissue distribution within study populations.

The most accurate measurement of VAT requires imaging techniques (MRI and computed tomography (CT)), which are expensive and not readily available in many clinical settings. **Figure 3(b)** shows an MRI-derived cross-sectional image at the L4–L5 level with adipose tissue depots identified. The AT located between muscle bundles (IMAT; **Figure 3**) and visible by MRI and CT may be negatively associated with insulin sensitivity. In the elderly, greater IMAT (as suggested by lower skeletal muscle attenuation by CT) is associated with lower specific force production. Currently, there is no simple or clinic-based method to measure adipose tissue located between the muscle groups, defined in our laboratory as intermuscular adipose tissue (IMAT). IMAT has been reported to be significantly negatively correlated with insulin sensitivity and higher in type 2 diabetic women compared to nondiabetic women.

Sex and race differences in body composition are well established in adults. Men acquire higher peak SM mass than women and some evidence exists suggesting that men may lose SM faster than women with age. Moreover, it is well established that women have a larger amount of total body fat or total adipose tissue than men. Among races, African-American adult men and women have larger amounts of SM than Asian and Caucasians even after adjusting for differences in body weight, height, age, and skeletal limb lengths.

Efforts are ongoing to better understand variations in IMAT as a function of age, race, and level of fatness. IMAT deposits appear comparable in size in adult African-Americans, Asians, and Caucasians at low levels of adiposity but accumulate as a greater proportion of TAT in African-Americans compared to Caucasians and Asians subjects (58 g IMAT/kg TAT in African-Americans; 46 g IMAT/kg TAT in Caucasians; 44 g IMAT/kg TAT in Asians). Across race groups, VAT deposits also appear comparable in size at low levels of adiposity but with increasing adiposity VAT accumulates more in Asians and Caucasians compared to IMAT, although accumulation rates for

IMAT and VAT do not differ in African-Americans. Although the association between greater amounts of abdominal or VAT and increased insulin resistance and the metabolic syndrome is well established compared to the peripherally located SAT, the role of the IMAT compartment in the metabolic alterations leading to the development of insulin resistance warrants further investigation, especially as it may influence race/ethnicity differences in dysglycemia. Collectively, sex and race differences exist in body composition in children and adults.

Physiological Application: Two Examples

Example 1

Expressing heat production relative to body mass is required when comparing energy expenditure rates between individuals that differ in size. Age and gender-specific resting energy expenditure (REE) norms based on body weight and stature-derived were developed in the early 1900s by Kleiber and showed that adult mammals differing widely in body size had similar metabolic rates relative to body weight raised to the 0.75 power. Two components are usually considered as representative of whole-body metabolically active tissue, body cell mass (BCM), and FFM. BCM is typically estimated as the exchangeable potassium space that can be measured by total body potassium. The FFM component can be measured using two-component body composition methods.

In studies assessing REE, FFM is considered the principal contributor to energy requirements, and is commonly used as a surrogate for metabolically active tissue. However, this practice is inherently flawed as it pools together numerous organs and tissues that differ significantly in metabolic rate. The brain, liver, heart, and kidneys alone account for approximately 60% of REE in adults although their combined weight is less than 6% of total body weight or 7% of FFM. The skeletal muscle component of FFM comprises 40–50% of total body weight (or 51% of FFM) and accounts for only 18–25% of REE. REE varies in relation to body size across mammalian species. Within humans, REE per kg of body weight or FFM is highest in newborns ($\sim 56 \text{ kcal kg}^{-1} \text{ day}^{-1}$), declines sharply until 4 years, and slowly thereafter reaching adult values ($\sim 25 \text{ kcal kg}^{-1} \text{ day}^{-1}$). Among adults, REE is lower in the later adult years, to an extent beyond that explained by changes in body composition. That is, the loss of FFM cannot fully explain the decrease (5–25%) in REE in healthy elderly persons.

Table 3 Organ and tissue coefficients used in developing models

	Weight (kg) ^a	Density (kg l ⁻¹) ^a	Metabolic rate (kJ kg ⁻¹ day ⁻¹) ^b
Skeletal muscle	28.0	1.04	55
Adipose tissue	15.0	0.92	19
Liver	1.8	1.05	840
Brain	1.4	1.03	1008
Heart	0.3	1.03	1848
Kidneys	0.3	1.05	1848
Residual	23.2	*	50

^aAdapted from Snyder WS, Cook MJ, Nasset ES, *et al.* (1975) Report of the task group on reference men. *International Commission on Radiological Protection* 23. Oxford: Pergamon, with permission from OUP and LWW.

^bAdapted from Elia M (1992) Organ and tissue contribution to metabolic rate. In: Kinney JM and Tucker HN (eds.) *Energy Metabolism. Tissue Determinants and Cellular Corollaries*, pp. 61–77. New York: Raven Press, with permission from OUP and LWW.

*Residual mass was not assigned a density but was calculated as body mass minus sum of other measured mass components.

Recent attention has been given to modeling REE based on available information on organ- and tissue-specific metabolic rates combined (Table 3) with the mass of these tissues as determined by MRI. Whole-body REE can be calculated from organ-tissue mass (REE_c) and then compared to REE measured using indirect calorimetry (REE_m) for individuals or groups. REE (kJ day⁻¹) of each organ-tissue component (subscript *i*) can be calculated using the following equation:

$$REE_i = OMR_i M_i \quad [1]$$

where organ metabolic rate (OMR) (organ metabolic rate) is the metabolic rate constant (kJ per kg per day) for each organ-tissue component (Table 3) and *M* is the mass of the corresponding organ/tissue (kg). Whole-body REE (kJ per day) is calculated as the sum of the seven individual organ-tissue REE:

$$REE_c = \sum_{i=1}^7 (REE_i) \quad [2]$$

The whole-body REE equation is

$$REE_c = 1008M_{\text{brain}} + 840M_{\text{liver}} + 1848M_{\text{heart}} + 1848M_{\text{kidneys}} + 55M_{\text{SM}} + 19M_{\text{AT}} + 50M_{\text{residual}} \quad [3]$$

This approach has allowed for the hypothesis to be tested that the proportion of FFM as certain high metabolic rate organs, specifically liver and brain, is greater in children compared to young adults (Figure 4). Findings thus far have shown that after accounting for this disproportion, the specific organ/tissue metabolic constants available in the literature (Table 3) are not adequate to account for REE in children. These results therefore imply that the decline in REE per kg body weight (or per kg FFM) during the growth years is likely due to both changes in body composition and changes in the metabolic rate of individual organs/tissues. When this approach was applied to young adults (31.2 ± 7.2 years), REE_c and REE_m were highly correlated, with no significant differences between them. When this approach was applied to persons over 70 years, both older men and women had significantly lower REE_m compared to REE_c, and the magnitude of the differences were 13% and 9.5%, respectively, for men and women. These findings suggest that even after adjustment for age-related organ and tissue atrophy in the elderly, whole

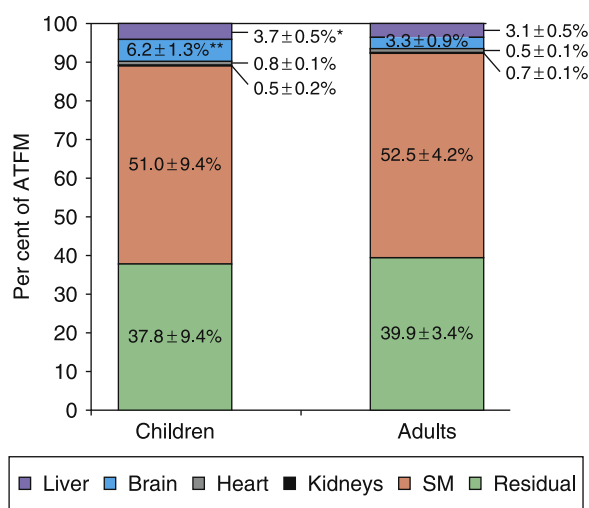


Figure 4 Proportional contribution of each organ/tissue to Adipose Tissue Free Mass (ATFM). Liver (■), brain (■), heart (■), kidneys (■), skeletal muscle mass (■), residual mass (■), * $p < .01$ and ** $p < .001$ for children vs. adults. Reproduced from Hsu A, Heshka S, Janumala I, *et al.* (2003) Larger mass of high-metabolic-rate organs does not explain higher resting energy expenditure in children. *American Journal of Clinical Nutrition* 77: 1506–1511, with permission from JCI.

body REE by indirect calorimetry continues to be lower than expected. The latter suggests that the metabolic rate constants used (Table 3) for specific organs and tissues may not be appropriate in the elderly.

At the individual or clinic level, the measurement of REE by indirect calorimetry is frequently unavailable. An alternate approach has been to estimate REE based on body weight, height, age, and sex. Many studies have examined the association between these basic and easily acquired measures and REE. A small number of studies have included FFM in their REE prediction equations. Table 4 lists published equations for the prediction of REE in healthy individuals.

Conclusion

The measurement of body composition allows for the estimation of body tissues, organs, and their distributions in living

Table 4 REE prediction equations based on anthropometrics or body composition

<i>Authors</i>	<i>Subjects/gender/ nation</i>	<i>Weight status</i>	<i>Age (years)</i>	<i>Equation</i>
Harris and Benedict (1919)	239/M–F/USA	NW	29 ± 14 (X ± SD)	F: BMR = 9.5 wt (kg) + 1.9 ht (cm) – 4.7 age (years) + 655 M: BMR = 13.8 wt (kg) + 5.0 ht (cm) – 6.8 age (years) + 66
Robertson and Reid (1952)	2310/M–F/UK	NS	Range (3–80)	RMR = BSA (m ²) × 24 × age-specific value
Altman and Dittmer (1968)	> 200/M–F/USA	NW	Range (3–16)	F: REE = 0.778 wt (kg) + 24.11 M: REE = 0.815 wt (kg) + 21.09
Dore <i>et al.</i> (1982)	140/F/UK	NW, OW, OB	Variable	REE = 8.24 wt (kg) + 0.02 FFM (kg) – 3.25 age (years) + 712
Bernstein (1983)	202/M(154)/USA	OW, OB	40 ± 12 (X ± SD)	RMR = 7.48 wt (kg) – 0.42 ht (cm) – 3.0 age (years) + 844 REE = 22 FFM (kg) + 6.4 FM (kg) – 2.1 age (years) + 251 REE = 24.2 FFM (kg) + 5.8 (% fat) + 310
Garrow and Webster (1985)	104/F/UK	NW, OW, OB	Variable	
Joint FAO/WHO/UN (1985)	11 000/M–F/Multi	NW, OW, OB	Variable	3–10 years F: REE = 22.5 wt (kg) + 499 3–10 years M: REE = 22.7 wt (kg) + 495 10–18 years F: REE = 17.5 wt (kg) + 651 10–18 years M: REE = 12.2 wt (kg) + 746 18–30 years F: BMR = 55.6 wt (kg) + 1397.4 ht (m) + 146 30–60 years F: BMR = 36.4 wt (kg) – 104.6 ht (m) + 3619 18–30 years M: BMR = 64.4 wt (kg) – 113.0 ht (m) + 3000 30–60 years M: BMR = 47.2 wt (kg) + 66.9 ht (m) + 3769
Schofield (1985)	7549/M–F/UK	NW, OW, OB	Range (<3–>60)	Under 3 years F: BMR = 0.068 wt (kg) + 4.281 ht (m) – 1.730 Under 3 years M: BMR = 0.0007 wt (kg) + 6.349 ht (m) – 2.584 3–10 years F: BMR = 0.071 wt (kg) + 0.677 ht (m) + 1.553 3–10 years M: BMR = 0.082 wt (kg) + 0.545 ht (m) + 1.736 10–18 years F: BMR = 0.035 wt (kg) + 1.948 ht (m) + 0.837 10–18 years M: BMR = 0.068 wt (kg) + 0.574 ht (m) + 2.157 18–30 years F: BMR = 0.057 wt (kg) + 1.184 ht (m) + 0.411 18–30 years M: BMR = 0.063 wt (kg) – 0.042 ht (m) + 2.953 30–60 years F: BMR = 0.034 wt (kg) + 0.006 ht (m) + 3.530 30–60 years M: BMR = 0.048 wt (kg) – 0.011 ht (m) + 3.670 Over 60 years F: BMR = 0.033 wt (kg) + 1.917 ht (m) + 0.074 Over 60 years M: BMR = 0.038 wt (kg) + 4.068 ht (m) – 3.491
Owen (1986)	44/F/USA	NW, OW, OB	29 ± 14 (X ± SD)	F: RMR = 7.18 wt (kg) + 795
Owen (1987)	60/M/USA	NW, OW, OB	29 ± 14 (X ± SD)	M: RMR = 10.2 wt (kg) + 879
Owen (1988)	104/M–F/USA	NW, OW, OB	29 ± 14 (X ± SD)	REE = 23.6 FFM (kg) + 186
Ravussin and Bogardus (1989)	249/M–F/USA	NW, OW, OB	Variable	REE = 21.8 FFM (kg) + 392
Maffei <i>et al.</i> (1990)	130/M–F/Italy	NW, OW, OB	Range (6–10)	F: REE = (35.8 wt (kg) + 15.6 ht (cm) – 36.3 age (years) + 1552)/4.18 M: REE = (28.6 wt (kg) + 23.6 ht (cm) – 69.1 age (years) + 1287)/4.18
Mifflin <i>et al.</i> (1990)	498/M–F/USA	NW, OW, OB	Range (19–78)	F: RMR = 9.99 wt (kg) + 6.25 ht (cm) – 4.92 age (years) – 161 M: RMR = 9.99 wt (kg) + 6.25 ht (cm) – 4.92 age (years) + 5 REE = 19.7 FFM (kg) + 413
Cunningham (1991)	Meta-analysis	NW, OW, OB		REE = 21.6 FFM (kg) + 370
Hayter and Henry (1994)	2999/M/UK	NW, OW, OB	Range (18–30)	M: RMR = 51.0 wt (kg) + 3500
Piers <i>et al.</i> (1997)	39/M/Australia	NW, OW	Range (18–30)	M: RMR = 51.0 wt (kg) + 3415
van der Ploeg <i>et al.</i> (2001)	38/M/Australia	NW, OW	24.3 ± 3.3 (X ± SD)	18–30 years M: RMR = 48.2 wt (kg) + 25.8 ht (cm) – 49.6 age (years) + 113 18–30 years M: RMR = 21.0 wt (kg) – 56.2 age (years) + 76.1 FFM 4C (kg) + 2202

(Continued)

Table 4 Continued

van der Ploeg <i>et al.</i> (2002)	41/M/Australia	NW, OW	44.8 ± 8.6 (X ± SD)	30–60 years M: RMR = 41.92 wt (kg) + 13.79 ht (cm) – 14.89 age (years) + 1939 30–60 years M: RMR = 91.85 FFM 4C (kg) + 1463
Siervo <i>et al.</i> (2003)	157/F/Italy	NW, OW, OB	23.8 ± 3.8 (X ± SD)	F: RMR = 11.5 wt (kg) + 542.2

M, male; F, female; NS, not specific; NW, normal weight; OW, overweight; OB, obesity; X, mean; SD, standard deviation; BSA, body surface area; wt, weight; ht, height; BMR, basal metabolic rate; RMR, resting metabolic rate; REE, resting energy expenditure; FFM, fat-free mass; FFM 4C, fat-free mass via the four-compartment body composition model; FM, fat mass.

Source: Adapted from Altman P and Dittmer D (1968) *Metabolism*. Bethesda: Federation of American Societies for Experimental Biology; Bernstein RS, Thornton JC, Yang MU, *et al.* (1983) Prediction of the resting metabolic rate in obese patients. *American Journal of Clinical Nutrition* 37: 595–602; Cunningham JJ (1991) Body composition as a determinant of energy expenditure: A synthetic review and a proposed general prediction equation. *American Journal of Clinical Nutrition* 54: 963–969; Dore C, Hesp R, Wilkins D and Garrow JS (1982) Prediction of requirements of obese patients after massive weight loss. *Human Nutrition Clinical Nutrition* 36C: 41–48; Garrow JS and Webster J (1985) Are preobese people energy thrifty? *Lancet* 1: 670–671; Harris JA and Benedict FG (1919) *A Biometric Study of Basal Metabolism in Man*, pp. 1–266. Washington DC: Carnegie Institution; Hayter JE and Henry CJK (1994)

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persons without inflicting harm. It is important to recognize that there is no single measurement method in existence that allows for the measurement of all tissues and organs and no method is error free. Furthermore, bias can be introduced if a measurement method makes assumptions related to body composition proportions and characteristics that are inaccurate across different populations. The clinical significance of the body compartment to be measured should first be determined before a measurement method is selected because the more advanced techniques are less accessible and more costly.

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See also: Obesity: Childhood Obesity. Older People: Physiological Changes

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BRAIN AND NERVOUS SYSTEM

Biology, Metabolism, and Nutritional Requirements

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Glossary

Blood–brain barrier Now not known really to be a ‘barrier’, but a set of transporter mechanisms located in the cell membranes of the endothelial cells that make up the brain capillaries, which promote or retard the exchange of certain small and large water-soluble molecules between the blood and the brain.

Central nervous system (CNS) The portion of the nervous system consisting of the brain, retina, and the spinal cord.

Glia Non-neuronal cells of the nervous system that provide physical and metabolic support for neurons, and insulate axons and nerve terminals, to ensure privacy in signaling.

Ketone bodies Small breakdown products of fatty acids, such as acetoacetate and beta-hydroxybutyrate, made in the liver when circulating fatty acids increase, such as during starvation, which are then taken up by brain (and other tissues) and used to generate ATP. Ketone bodies are a key source of energy for the brain during starvation.

Neuron The principal functional cells of the nervous system, which are organized into very complex circuits (analogous to the components of electronic circuits) that manage all of the housekeeping functions of the body and elaborate behavior and cognitive functions.

Neurotransmitter A molecule that is released by neurons that couples with specific receptors on other cells and induces a functional response, such as depolarization

(a neuron), contraction (a muscle cell), or secretion (an endocrine or exocrine cell).

Parkinson’s disease A disease of unknown etiology in which neuronal circuits in the brain that control certain features of muscle function and coordination degenerate. Patients, who are usually older, gradually develop muscle tremor, a bent posture, and difficulty in initiating voluntary movements, such as walking.

Peripheral nervous system The portion of the nervous system consisting of neurons and glial cells lying outside the CNS, which either supply sensory information to the CNS or send commands from the CNS to effector cells in the rest of the body, such as muscle and gland cells.

Rhodopsin The light-responsive pigment of the eye, which elicits phototransduction when struck by photons of light. It is synthesized in retina photoreceptor cell membranes through the covalent linkage of a protein, opsin, and a metabolite of vitamin A, 11-*cis*-retinaldehyde. Rhodopsin breaks down to opsin and a metabolite of 11-*cis*-retinaldehyde after phototransduction but is quickly regenerated.

Spina bifida A malformation of the spinal cord that occurs early during fetal development, when the spinal cord is forming, which when serious can compromise body functions controlled by nerves entering and exiting the spinal cord below the lesion. The incidence of spina bifida is markedly reduced when women take a folic acid supplement before and during pregnancy.

Design of the Nervous System

The nervous system has two principal cell types, neurons and glia. Neurons (like wires) conduct electrical signals and are organized into circuits to perform specific functions. They have a unique cellular architecture: small cellular extensions (dendrites), which receive chemical and electrical signals from other neurons; a longer extension (the axon, which can be up to a meter in length), which sends electrical signals down its length to one or more nerve terminals. Nerve terminals contain neurotransmitters, molecules released by arriving electrical signals that modify the electrical activity of the adjacent neurons. Neurons have considerable energy needs; indeed the brain, which is approximately 2% of body weight, consumes 15–20% of the body’s daily energy intake. Glial cells, which

make up approximately 60% of the brain’s cell mass, provide physical and metabolic support for neurons and insulate axons and nerve terminals to insure privacy in electrical signaling. The glial cells found in peripheral nerves serve the same functions.

The nervous system is broadly divided into two parts, the central nervous system (CNS) and peripheral nervous system. The CNS consists of the brain, retina, and the spinal cord. It also contains complex neuronal circuits that control body functions (e.g., blood pressure, breathing, hunger, and movement). The peripheral nervous system consists of groups of neurons that mostly lie outside of the CNS and either supply sensory information to the CNS or send CNS commands to effector cells, such as muscle and gland cells.

The Blood–Brain Barrier (BBB)

Each portion of the nervous system is separated from the blood (and thus the rest of the body) by a metabolic ‘barrier,’ which modulates the access of nutrients to and the removal of metabolites from the neurons and glia within it. For the brain and spinal cord, this barrier is termed the ‘blood–brain barrier’ (BBB; there is also a blood–cerebrospinal fluid (CSF) barrier; CSF is made from blood); for the retina, it is the ‘blood–retinal barrier,’ and for peripheral neurons, the ‘blood–nerve barrier.’ The functions of these barriers are very similar; the focus of the discussion will be the BBB, because it has been the most studied.

The BBB is located in the endothelial cells that make up the brain’s capillaries. Unlike the capillaries elsewhere in the body, the endothelial cells of the brain capillaries are tightly joined, such that nothing passes into (or out of) the brain without passing through these cells. The BBB thus presents a continuous lipid barrier to molecules. One implication is that the ease with which molecules in the blood gain access to brain should depend on their lipid solubility: The more lipid soluble, the greater the accessibility to brain by diffusion. However, most molecules of biologic importance to brain are not lipid soluble and thus do not easily diffuse across lipid membranes into brain. Examples include glucose, amino acids, and water-soluble vitamins. Consequently, endothelial cell membranes must be more than just lipid barriers; in fact, embedded in them are specific transport carriers that mediate the brain uptake of most nutrients.

Energy Substrates

The brain uses glucose as its primary energy substrate. Glucose is not lipid soluble and thus requires a BBB transporter. The glucose transporter has a maximal transport capacity for glucose of $1.4 \text{ mol min}^{-1} \text{ g}^{-1}$ of brain or approximately 1200 g d^{-1} for the entire brain (a human brain weighs 1400 g). The human brain consumes 15–20% of the body’s oxygen consumption; brain glucose utilization is therefore about 100 g d^{-1} . The BBB transporter thus has a maximal capacity for transporting glucose well in excess of the daily requirements of the brain.

Inside the brain, glucose is rapidly taken up into the neurons by a cellular glucose transporter. Within the neuron, glucose enters the glycolytic pathway. The initial enzyme, hexokinase, has a very high affinity for glucose and is fully saturated at normal brain glucose concentrations. Hence, overall, each step in the glucose pipeline from the blood to brain neurons is designed to maximize glucose supply for neuronal energy production. It only fails when the blood glucose supply is abruptly curtailed, such as when a diabetic patient injects too much insulin and blood glucose levels rapidly fall (the transporter cannot compensate for such abrupt drops in blood glucose). The effect is dramatic: confusion, delirium, seizures, coma, and finally death occur as blood glucose drops to very low levels. Such effects are most rapidly reversed by the infusion of glucose, suggesting that no other compound in blood

readily substitutes for glucose as the brain’s primary energy substrate.

Normally, the body carefully maintains blood glucose concentrations. During starvation, however, blood glucose falls enough to cause the brain to recruit an additional energy source, ketone bodies. Ketone bodies are liver-produced by-products of the breakdown of stored fat and provide an extended supply of energy when the input of food-derived energy is low. The brain uses ketone bodies whenever their blood levels rise; blood ketone body concentrations rise during starvation. The BBB ketone body transporter (ketone bodies are not lipid soluble) is induced during starvation, enhancing the flow of ketone bodies into the brain. During prolonged starvation, more than half of the energy used by the brain is derived from ketone bodies. However, continued use of some glucose appears obligatory and is supplied via liver gluconeogenesis.

The chronic ingestion of high-fat diets also elevates blood ketone body concentrations, promoting their use by brain for energy production. However, extremely high levels of fat must be consumed and such diets are unpalatable. Hence, diet is not thought normally to influence cerebral energy production via dietary fat manipulation of ketone body supply to brain. Very high fat diets are occasionally used clinically to treat intractable seizures. Although the beneficial effect is linked to circulating ketone bodies levels, the mechanism is presently unknown.

Amino Acids and Protein

Neurons and glial cells in brain use amino acids to produce proteins. In addition, certain amino acids are used to produce small functional molecules such as neurotransmitters. Does diet influence amino acid flow into brain and their use in generating proteins and transmitters? The path from diet to brain begins with amino acid absorption from the gastrointestinal tract, insertion into the circulation, and extraction by the brain. This extraction process involves the BBB, which contains a number of transporters of amino acids. The properties of these transporters dictate how much of each amino acid enters (and exits) the brain. Currently, six carriers have been identified. Of special interest are two carriers: (1) The large neutral amino acid (LNAA) carrier – this carrier is shared by several amino acids (some are precursors for neurotransmitters, namely phenylalanine, tyrosine, tryptophan (TRP), and histidine). This carrier is competitive, allowing changes in the plasma concentration of any one LNAA to affect not only that amino acid’s BBB transport but also that of each of its transport competitors. Glutamine, an LNAA present in the brain in high concentrations, drives the brain uptake of the other LNAA, by serving as the principal amino acid counter transported from the brain to blood each time an LNAA is taken up into brain. (2) The acidic amino acid carrier, which transports glutamic and aspartic acids. This carrier primarily transports glutamate (GLU) and aspartate from the brain to the circulation. The other transporters include one selective for basic amino acids; two selective for

subgroups of the small, neutral amino acids; and one selective for taurine.

The carriers that move amino acids into brain are those that primarily transport essential amino acids (the large, neutral and basic amino acids), whereas those that move amino acids out of brain are those transporting nonessential amino acids (the acidic and small neutral amino acids). A small, net influx of the essential amino acids into brain no doubt reflects their consumption in brain by biosynthetic and metabolic pathways. The net efflux of the nonessential amino acids, notably aspartate, GLU, glycine, and cysteine may serve to remove from brain the amino acids that act directly as excitatory transmitters or cotransmitters. The brain carefully compartmentalizes these amino acids metabolically, because they excite neurons, and a mechanism to remove them from brain may be a component of this compartmentalization design.

Changes in dietary protein intake have no effect on brain protein synthesis in adults. Indeed, the chronic ingestion of very low levels of dietary protein does not depress brain protein synthesis; brain cells may thus be efficient in retaining and reusing amino acids released during intracellular protein breakdown. In neonatal and infant animals, however, low levels of protein intake are associated with below normal rates of protein synthesis in the brain. But, the presumed mechanism of this association, reduced uptake of essential amino acids into brain, and abnormally low brain concentrations of these amino acids has not been proven. Hence, at present, there is no convincing evidence linking dietary protein intake and brain protein synthesis via a limitation of amino acid availability to brain. For neurotransmitters, the evidence of this diet-brain link is more certain and provides interesting examples of the fundamentally different manner in which the brain uses transport carriers to handle amino acids that are neurotransmitter precursors and those that are neurotransmitters themselves. Good examples are TRP (an LNAA) and GLU (an acidic amino acid), which have been most extensively studied.

TRP is the precursor for the neurotransmitter serotonin (5-HT). The TRP concentration in brain rapidly influences the rate of 5-HT synthesis: Raising brain TRP concentrations increases 5-HT synthesis, whereas lowering brain TRP decreases 5-HT synthesis. Brain TRP uptake and concentrations are directly influenced by the plasma concentrations of TRP and its BBB LNAA transport competitors. The plasma concentrations of TRP and the other LNAA are readily modified by food intake, thereby linking diet to brain 5-HT synthesis. Dietary proteins and carbohydrates are the food components that change brain TRP and 5-HT: Carbohydrate ingestion increases plasma TRP, while lowering the plasma concentrations of its LNAA competitors (an effect dependent on the release of insulin), causing BBB TRP uptake, brain TRP concentrations, and 5-HT synthesis all to increase. The effect of ingesting a meal containing protein depends on the protein: A meal containing α -lactalbumin, a milk protein rich in TRP, causes the plasma concentrations of TRP to rise much more than those of its LNAA competitors. As a consequence, TRP gains a sizeable advantage in the competition for BBB transport, and brain TRP concentrations and 5-HT production increase (considerably more than that seen after carbohydrates are

ingested). In contrast, consuming a meal containing zein, a corn protein very low in TRP, causes a marked decline in plasma TRP concentrations, while the plasma concentrations of its LNAA competitors rise. The result is considerable reduction in brain TRP uptake and 5-HT synthesis. The ingestion of meals containing proteins, such as casein, which contain moderate levels of TRP and other LNAA, modifies plasma concentrations of TRP and the other LNAA in a manner that results in no change in the competitive transport of TRP into brain. Consequently, brain TRP levels and 5-HT production are unchanged. Hence, a key feature of the LNAA transporter, its competitive nature, explains the impact of meals containing carbohydrates with or without protein on the production of a molecule important to normal brain function (5-HT).

Although there are many implications of the previously mentioned findings, one earlier hypothesis, based on the now erroneous idea that the ingestion of carbohydrate – but not protein – would raise brain TRP and stimulate 5-HT production, is no longer tenable. This hypothesis argued that a phenomenon termed ‘carbohydrate craving’ occurred in individuals who experienced no rise in brain TRP levels or 5-HT synthesis when ingesting carbohydrates (due to insulin resistance, which occurs in type-2 diabetics and in insulin-resistant obese subjects). Because increases in brain 5-HT release are known to suppress appetite, the argument was, such individuals would not experience the normal 5-HT-mediated suppression of hunger on eating carbohydrates and continue consuming them (thus ‘carbohydrate craving’). Clearly, because the ingestion of even modest amounts of common dietary proteins is now known also to raise brain TRP and 5-HT (the α -lactalbumin in milk and also egg protein), a person seeking to raise brain 5-HT by ingesting carbohydrates could just as easily accomplish this by consuming these proteins. Thus carbohydrate craving, at least as envisioned in this earlier hypothesis, should not exist.

Chronic dietary effects are also observed. For example, chronic ingestion of diets containing proteins by rats with high proportions of one or more LNAA relative to TRP cause brain TRP and 5-HT concentrations to decline. The chronic ingestion of diets low in protein also causes the plasma concentrations of all LNAA to decline (including TRP), and brain TRP and 5-HT. In this case, brain TRP falls not because of a change in BBB competition but simply because the BBB uptake of all LNAA declines with falling plasma concentrations (the transporter becomes unsaturated, eliminating competition).

Other LNAA are neurotransmitter precursors in substrate-driven pathways in brain. Phenylalanine and tyrosine are substrates for catecholamine synthesis and histidine is the precursor of histamine. Like TRP, the brain concentrations of these amino acids are influenced by their competitive BBB uptakes from the circulation, and thus the diet. However, dietary effects for these amino acids are generally less noteworthy than for TRP.

The nonessential amino acid GLU is an excitatory neurotransmitter, causing neurons that express GLU receptors to depolarize. Because GLU is excitatory, responsive neurons can become overexcited, when subjected to prolonged GLU exposure, and die. The term ‘excitotoxicity’ was coined to describe this effect and led to the concern that GLU ingested in food (as a constituent of dietary proteins or as a flavoring agent) might

cause the brain to become flooded with GLU, causing widespread neurotoxicity. The BBB acidic amino acid transporter prevents this from occurring: It primarily transports GLU out of the brain and not into it. Consequently, the BBB functions as a 'barrier' to GLU penetration from the blood.

Another mechanism also protects brain neurons from excessive exposure to GLU. Glial cells rapidly remove GLU from brain extracellular fluid and convert it to an electrically inert amino acid, glutamine. Although glial cells efficiently absorb neuronal GLU, they also readily clear any GLU that might stray into the brain from the circulation.

Fatty Acids and Choline

Fatty Acids

The brain uses fatty acids to synthesize the complex fat molecules that form neuronal and glial cell membranes. This process is more active in growing animals than in adults. The brain synthesizes some fatty acids from smaller molecules, but their uptake from the circulation is also an important source and is the only source for certain fatty acids (the essential fatty acids, which cannot be manufactured in the body). The details of the uptake process are not well understood.

From the nutritional perspective, diet influences essential fatty acid availability to the brain, with potentially important functional consequences. In almost all mammals, there are two essential fatty acids, linoleic acid and α -linolenic acid (termed polyunsaturated fatty acids (PUFAs)). In the nervous system (as elsewhere), linoleic and α -linolenic acid are incorporated into phospholipid molecules and inserted into cellular membranes, where they influence membrane fluidity and membrane-associated functions (e.g., the functionality of receptors and transporters). In addition, the linoleic acid in membrane lipids can be released and converted into arachidonic acid, a key precursor in the synthesis of prostaglandins and leukotrienes, which are families of important signaling molecules. α -Linolenic acid can be converted into docosahexanoic acid, a molecule found in very large amounts in the rods and cones of the retina and in the nerve terminal membranes of brain. Docosahexanoic acid is thought to be a key component of phototransduction and has been demonstrated to have important effects on vision. Dietary modifications in essential fatty acid intake might therefore be expected to influence membrane functions in brain, leading to alterations in brain function (as has been demonstrated for vision).

Choline

Choline occurs in the body as a constituent of lipid molecules in cell membranes, as a source of methyl groups, and as a precursor for the neurotransmitter acetylcholine (ACh). Choline is not an essential nutrient in humans, and deficiencies are rarely seen, because it is ubiquitous in the diet. However, in recent decades, dietary choline has been a focus of interest because of the possibility that changes in choline intake could influence neuronal ACh synthesis. ACh is a neurotransmitter; its synthesis and release by brain neurons is influenced by

choline availability, which in turn can be altered by dietary choline intake, either in the form of free or fat-bound choline (phosphatidylcholine). In this context, oral choline and phosphatidylcholine have found some application in human diseases thought to involve ACh. For example, they have been used successfully to treat movement disorders such as tardive dyskinesia, a drug-induced muscular disorder in schizophrenic patients linked to low ACh function. However, they proved to be of little value in controlling abnormal muscle movements associated with Huntington's disease (also linked to low ACh function). Dietary choline and phosphatidylcholine supplements have also been studied as potential memory enhancers because CNS ACh neurons play an important role in memory. Patients with Alzheimer's disease have been most studied, but in general, the disappointing outcome has been that neither choline nor phosphatidylcholine has afforded much improvement in memory.

Vitamins

Neurons and glia have the same functional demands for vitamins as do other cells in the body. Their access to brain is thus an important consideration, particularly given the existence of the BBB. Water-soluble vitamins are transported across the BBB, and in some cases, the blood-CSF barrier, most often by non-energy-requiring carriers. After they are taken up into the neurons and glial cells, most are rapidly converted into their biologically active derivatives, namely cofactors in enzyme-mediated reactions. Because cofactors are recycled, dietary deficiencies in one or another vitamin do not immediately lead to brain dysfunction, inasmuch as cofactor pools may take extended periods of time to become depleted. Although fat-soluble vitamins are lipid soluble, their passage through the BBB most likely involves more than simply diffusion.

Water-Soluble Vitamins

Folic acid is transported into brain as methylenetetrahydrofolic acid, the major form of folic acid in the circulation. It is then transported rapidly into the neurons and glia from the CSF or extracellular fluid. Once inside the cells, folates are polyglutamated. Methylenetetrahydrofolate is used by neurons and glia in reactions involving single-carbon groups, such as in the conversion of serine to glycine or homocysteine to methionine. Once methylenetetrahydrofolate is consumed in these reactions, folic acid is transported out of the brain into the circulation. Folate has become an issue of neurologic concern because of a link between folate deficiency and abnormal CNS development. The incidence of spina bifida, a serious spinal cord abnormality, rises above the population mean in the children of women who are folate deficient during pregnancy. Moreover, the incidence of spina bifida can be reduced by folic acid supplementation during pregnancy, beginning before conception. Initiating supplementation before conception is essential because the basic design of the CNS is laid down during the first trimester. At present, the mechanism(s) by which folic acid deficiency leads to the

improper formation of the spinal cord is unknown. Folate deficiency may also be linked to depression in adults, and occasional studies suggest that folate supplementation can be a mood elevator in depressed patients. The mechanism(s) by which folate modifies mood is presently unknown.

Ascorbic acid (vitamin C) is actively transported into the brain extracellular fluid through the blood–CSF barrier, from which it is actively transported into the cells. Brain ascorbate pools show minimal fluctuations over a wide range of plasma ascorbate concentrations, which presumably explains the absence of CNS signs in ascorbate deficiency. To date, the only defined biochemical function of ascorbic acid in brain is as a cofactor for the enzyme that converts dopamine to norepinephrine (although ascorbate is thought by some to function as an antioxidant).

Thiamine (vitamin B₁) is taken up into brain by a BBB transporter; small amounts also gain entry via transport from blood into CSF. It is then transported into neurons and glia; conversion to thiamine pyrophosphate effectively traps the molecule within the cell. In nervous tissue, thiamine functions as a cofactor in important enzymes of energy metabolism. Severe thiamine deficiency in animals reduces thiamine pyrophosphate levels and the activities of thiamine-dependent reactions. It causes loss of the coordinated control of muscle movement; the exact biochemical mechanism is unsettled. The functional deficits are rapidly corrected with thiamine treatment, suggesting that neurons have not been damaged or destroyed. Thiamine deficiency in humans (beri-beri and Wernicke's disease) produces similar deficits in the control of muscle movements and also mental confusion. Korsakoff's syndrome, which occurs in almost all patients with Wernicke's disease, involves short-term memory loss and mental confusion. Severe thiamine deficiency in humans appears to produce neuronal degeneration in certain brain regions. The motor abnormalities can be corrected with thiamine treatment, but the memory dysfunction is not improved.

Riboflavin enters brain via a saturable BBB transport carrier. It is then transported into neurons and glia and trapped intracellularly by phosphorylation and converted to flavin adenine dinucleotide. Flavin adenine dinucleotide functions as a cofactor in carboxylation reactions. The brain contents of riboflavin and its derivatives are not notably altered in states of dietary riboflavin deficiency or excess.

Pantothenic acid is transported into brain by a BBB transport carrier. Neurons and glial cells take up pantothenic acid slowly by a mechanism of facilitated diffusion. Inside the cell, the vitamin becomes a component of coenzyme A, the coenzyme of acyl group transfer reactions. Relative to other tissues, the brain contains a high concentration of pantothenate, mostly in the form of coenzyme A. Brain coenzyme A concentrations are not depleted in pantothenate-deficiency states.

Niacin (vitamin B₃) is transported into brain as niacinamide, primarily via the BBB. Most niacin in brain is derived from the circulation, although brain may be able to synthesize small amounts. Niacin is taken up into neurons and glia and rapidly converted to nicotinamide adenine dinucleotide. The half-life of nicotinamide adenine dinucleotide in brain is considerably longer than in the other tissues. Nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate are involved in numerous oxidation–reduction

reactions. Dietary niacin deficiency in the presence of a low intake of TRP causes pellagra in humans, a deficiency disease that includes mental depression and dementia, loss of motor coordination, and tremor. The mechanism(s) for these effects have not been identified.

Pyridoxine (vitamin B₆) is taken up into brain via a transport carrier that has not been well described. The vitamin can be transported in any of its nonphosphorylated forms (pyridoxine, pyridoxal, and pyridoxamine). Once within the brain extracellular fluid, the vitamin is readily transported into the neurons and glia and phosphorylated (primarily to pyridoxal phosphate or pyridoxine phosphate). Pyridoxal phosphate is a cofactor in a variety of neurotransmitter reactions, such as aromatic-L-amino acid decarboxylase (an enzyme of monoamine biosynthesis), glutamic acid decarboxylase (the enzyme of γ -amino butyric acid (GABA) synthesis), and GABA transaminase (the enzyme which catabolizes GABA). In humans, pyridoxine deficiency is rare because of its widespread occurrence in food. However, when identified, it has been associated with increased seizure activity, an effect disipated by pyridoxine treatment. This effect may be linked to the production of GABA, an inhibitory neurotransmitter.

Biotin is transported into brain by a BBB carrier. It is a coenzyme for a variety of key carboxylation reactions in gluconeogenesis, fatty acid synthesis, and amino acid metabolism. Normally, biotin is recycled in cells during protein (enzyme) turnover, but not in brain; brain cells are thus more immediately dependent than the other cells on circulating biotin availability. Biotin deficiency is rare; when it occurs, it can involve CNS symptoms (depression or sleepiness); the underlying basis for these effects is presently unknown.

Cobalamin (vitamin B₁₂) is thought to be transported into brain by a carrier-mediated mechanism. Little is known about this process or about the function of vitamin B₁₂ in the nervous system. Vitamin B₁₂ deficiency is associated with neurologic abnormalities, which are presumed to be derived from the demyelination of CNS axons seen in advanced deficiency cases. These effects are reversed if vitamin B₁₂ treatment is provided early enough; when it is left untreated, axonal degeneration occurs. Vitamin B₁₂ may be important in neuronal repair mechanisms, which may become compromised in deficiency states. Nervous system damage associated with vitamin B₁₂ deficiency can occur at any age.

Fat-Soluble Vitamins

Of the fat soluble vitamins, vitamin A (retinol) has been the most studied in relation to the CNS. The others have been considerably less examined, although vitamin E is currently of some interest because of its function as an antioxidant. The CNS is not thought to be a major focus of action for vitamins D and K, and thus little information is available regarding their roles in brain function.

The principal role of vitamin A in the CNS is as a component of the photoreceptive pigment of the eye, rhodopsin. In the blood, vitamin A circulates bound to the retinol-binding protein and transthyretin (prealbumin). Its transport into retinal cells occurs at the blood–retinal barrier (the retinal pigmented epithelial (RPE) cells), after the retinol–protein

complex binds to retinol-binding protein receptors. Once bound, retinol is released into the RPE cell. The retinol-binding protein and transthyretin molecules are released back into the circulation. Inside the RPE cell, retinol binds to a specific protein and ultimately is esterified to a fatty acid. This molecule serves as the substrate for the conversion of retinol into the visually active form of the molecule, 11-*cis*-retinaldehyde, which then finds its way into the photoreceptor cell to be bound to opsin to form rhodopsin, the light-responsive pigment of the eye. When light strikes rhodopsin, phototransduction occurs and 11-*cis*-retinaldehyde is isomerized to all-*trans*-retinaldehyde, hydrolyzed from opsin, and released by the photoreceptor into the extracellular space (the opsin is retained and reused). The all-*trans*-retinaldehyde is shuttled into the RPE cell, where it is reconverted into 11-*cis*-retinaldehyde, and then recycled to the photoreceptor cells again to form rhodopsin.

From the nutritional perspective, retinal cells have an efficient system for managing and maintaining vitamin A pools. Hence, depletion of retinal vitamin A pools secondary to dietary deficiency only occurs over an extended time period. Deficiency appears functionally as 'night blindness,' as rhodopsin levels decline. Extended vitamin A deficiency leads to a loss of photoreceptor elements and eventually of the photoreceptor cells themselves. The cause of this cellular degeneration is not well understood.

Vitamin E is an antioxidant and a free radical scavenger that protects fatty acids in cellular membranes. It is transported in blood associated with lipoproteins. The mechanism of its transfer into nervous tissue is unknown. Dietary vitamin E deficiency is extremely rare in humans. It occurs in association with certain abnormalities of vitamin E transport and fat absorption and sometimes in individuals with protein-calorie malnutrition. The neurological manifestations are peripheral nerve degeneration, spinocerebellar ataxia, and retinopathy. Vitamin E has been proposed to play a role in a number of CNS diseases linked to oxidative damage. One example is Parkinson's disease, a movement disorder caused by the degeneration of certain groups of brain neurons. Evidence of oxidative damage is present in the brains of Parkinsonian patients, although controlled clinical trials of vitamin E supplementation have proved to be ineffective. Such negative findings question the likelihood of a vitamin E link to the etiology of the degenerative changes. A second example is Alzheimer's dementia, which is associated with a progressive, ultimately catastrophic degeneration of the brain. Several types of oxidative damage have been found in the brains of Alzheimer's patients, although it is presently unclear if this damage is the cause or effect. Vitamin E supplementation can slow the progression of Alzheimer's disease. However, such findings do not indicate if vagaries in vitamin E intake over an extended period of time are a cause of the disease.

Minerals

All of the essential minerals are important for cellular functions in brain, as they are elsewhere in the body. These are sodium, potassium, calcium, magnesium, iron, copper, zinc, manganese, cobalt, and molybdenum. Although most

function as cofactors in enzymatic reactions, sodium and potassium are key ions in electrical conduction in neuronal membranes, calcium functions as a secondary messenger within neurons, and magnesium is an important component of certain neurotransmitter receptors. The diet normally provides more than adequate amounts of almost all minerals, except possibly for calcium, iron, magnesium, and zinc. The BBB permeability to most metals is quite low. For example, although the brain extracts 20–30% of the glucose in blood in a single capillary transit, it extracts <0.3% of any metal. The mechanisms of transport into brain for most metals are unknown. However, some details regarding the transport and/or functions of iron, calcium, and copper are available.

Iron circulates bound to a protein, transferrin. Iron uptake into brain occurs primarily at the BBB and involves a transferrin receptor-mediated endocytosis of the iron–transferrin complex by capillary endothelial cells. Iron dissociates from transferrin inside the cell and is delivered into the brain interstitial fluid; the transferrin is returned to the circulation. Brain iron associates with ferretin, a protein, and is stored intracellularly. The bulk of the iron–ferretin stored in brain resides in glial cells and is laid down early in postnatal life. Marked regional differences in iron and ferretin concentrations occur in brain; levels in some areas are as high as those in the liver. However, this distribution does not correlate with the density of transferrin receptors in brain capillaries; it is presently unknown how or why the unequal distribution of iron develops. Numerous enzymes in brain are iron-requiring, including several hydroxylases involved in neurotransmitter production, and a key metabolic enzyme, monoamine oxidase.

Iron deficiency can cause impairments in attention and cognition in children. Similar effects are seen in animals. In iron-deficient rats, brain iron concentrations decline, with newborn and infant animals showing more rapid declines than older animals. Iron repletion in brain occurs in infant and adult rats with iron supplementation but not in animals depleted at birth. While outside of the brain, the activities of many iron-dependent enzymes are depressed by iron deficiency, whereas their activities are unaffected inside the brain. However, a reduction in certain dopamine receptors occurs, along with aberrations in dopamine-dependent behaviors (dopamine is a CNS neurotransmitter). The inability of brain iron stores to recover in rats made them iron deficient as newborns coincides with a persistence of dopamine-linked behavioral deficits, despite normal repletion of iron stores elsewhere in the body. Restoration of normal behavior with iron supplementation, along with brain iron stores, is seen in animals made iron deficient at other ages.

Iron deficiency also interferes with myelination. Since marked glial proliferation and myelin formation occur early in infancy, iron deficiency during this period could prevent the optimal development of neuronal communications (glial cells provide insulation for axons and synapses). This effect could account for some of the behavioral deficits associated with neonatal iron deficiency.

Calcium is actively transported into the CNS, primarily via the blood–CSF barrier and is not sensitive to vitamin D. Because calcium concentrations in the circulation are regulated, under most circumstances, this process should also help to maintain brain calcium uptake and levels in the face of

vagaries in calcium intake. Deficiencies in brain calcium should thus be a relatively rare occurrence.

Copper functions as a cofactor for numerous enzymes, including dopamine β -hydroxylase (DBH), which converts dopamine to norepinephrine. Dietary copper deficiency in humans is fairly rare. When produced in animals, it leads to reduced DBH activity in neurons and cells anywhere in the nervous system that synthesize norepinephrine. The mechanism of copper transport into the brain is presently unknown. Copper deficiency occurs as an X-linked genetic disease of copper transport in Menkes disease, in which tissue and brain copper levels become extremely low and produce neurodegeneration. Children with Menkes disease die at a very young age.

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BREAST FEEDING

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The importance of breast feeding for infant nutrition, health, and cognitive development is well established. Although the magnitude of the effects on morbidity and mortality differ depending on the underlying risks the breastfed child faces, there is ample evidence in both developed and developing countries that breast feeding prevents mortality and morbidity and may reduce the risk of overweight and obesity. Evidence is also accumulating on the long-term benefits of breast feeding with respect to chronic disease reduction. There are also benefits for maternal health in the form of reduced risk of breast and ovarian cancer, cardiovascular disease, type-2 diabetes and postpartum weight loss. Because of these benefits, breast feeding should be promoted as a cultural and behavioral norm rather than as interchangeable with formula feeding. Policy and programs should be instituted to provide an environment that is conducive to breast feeding. In this article, the author provides a broad overview of the physiological and nutritional aspects of breast feeding and breast milk and behavioral aspects that determine whether or not a woman breastfeeds. The author also summarizes global initiatives to improve breast feeding practices and provides data on global breast feeding trends.

Breast Feeding Recommendations

The World Health Organization (WHO) recommends exclusive breast feeding for the first 6 months (180 days) of life followed by continued breast feeding for up to 2 years of age or beyond along with safe, appropriate, and hygienically prepared complementary foods. The American Academy of Pediatrics Section on Breast feeding also recommends exclusive breast feeding for 6 months. Exclusive breast feeding is defined as breast milk as the sole source of infant nutrition with no other liquids (including water) or food given, except for oral rehydration solution, drops, syrups (vitamins, minerals, or medicines). Breast feeding is defined as breast milk along with any food or liquid including nonhuman milk and formula. A comprehensive review by the WHO provided the scientific evidence for the recommended duration of exclusive breast feeding in 2001 and this recommendation has subsequently been strengthened by new publications. In 2006, the WHO released new Child Growth Standards based on a multicenter study of breastfed children as they have different growth patterns than formula-fed children. WHO also recommends that newborns initiate breast feeding within the first hour of birth and breast feed on demand day and night. Early initiation and frequent on demand breast feeding during the first several days is important in ensuring timely lactogenesis

stage II from colostrum to established breast milk production. The use of bottles and pacifiers should be avoided. WHO and United Nations Children's Fund (UNICEF) in collaboration with partners recently published updated indicators for assessing breast feeding practices, including a measurement guide and updated practices from 46 developing countries.

The public health challenge is to support women to follow global breast feeding recommendations so as to ensure the healthiest start in life for all the world's children. Adherence to the recommended practices or lack thereof results from a complex series of physiological and behavioral interactions between a mother and her infant, interactions that take place with a larger cultural context and familial, community, and global setting. Although breast feeding occurs when a mother puts her child to the breast, her decision to breast feed and to act on this decision depend on a number of determinants, not all of which favor breast feeding or are within her control. These include attitudes and norms among her family and community, the medical profession, and peers and employers as well as cultural traditions. It also depends on access to information, and if problems arise, access to skilled assistance. Lastly, it depends on her employment situation and whether or not she has access to her child during work hours or a private clean place where she can express and safely store breast milk.

Breast Milk Composition and Volume

Incomparable to any other mammalian milk, breast milk is a unique bioactive substance that changes composition, within and between feedings and over time to suit the nutritional and immunological needs of the growing infant. It contains fat, carbohydrate, proteins, vitamins, minerals, and water sufficient to satisfy infant energy and nutrient requirements for the first 6 months of life. It also contains a large number of bioactive substances that help to develop the newborn immature immune system. Knowledge about the number and function of these substances continually increases with new analytic techniques.

The 3.5 g of fat per 100 ml of breast milk provides approximately half the energy content and is the most variable constituent. The fat content increases during the feeding process and as a result, the hind milk is richer in fat than the foremilk. Fat constituents that are particularly important include long chain polyunsaturated fatty acids docosahexaenoic acid (DHA) and arachidonic acid (ARA), which are linked to enhanced retinal development, visual acuity, and neurological development in the child. Because these fatty acids are thought

to be so important to development, they are added to many infant formulas.

Lactose is the main carbohydrate in breast milk and is present at 7 g per 100 ml. It enhances calcium absorption and provides a readily available source of galactose, an essential component for the development of the central nervous system. Oligosaccharides constitute another important carbohydrate and provide important immune protection. A large number of carbohydrates are being discovered that do not have a nutritional but rather an immunological benefit.

The concentration of protein in breast milk, 0.9 g per 100 ml, is lower than in other animal milks and also differs in quality. The proteins include casein, serum albumin, alpha-lactalbumin, beta-lactoglobulins, immunoglobulins, and other glycoproteins. Not only does breast milk contain less of the protein casein, but this casein has a different molecular structure than that found in animal milks and forms more easily digested curds. Lactoferrin, an iron binding protein, inhibits the growth of certain iron-dependent bacteria in the gastrointestinal track. Although concerns have been raised about the adequacy of breast milk to satisfy protein requirements for 6 months, a number of well-controlled studies have shown that protein needs can be met through exclusive breast feeding.

The vitamins and minerals in breast milk are usually sufficient to meet requirements during exclusive breast feeding, unless the mother herself is deficient, or the newborn is preterm. The exception is vitamin D where the infant needs sunlight exposure to generate an endogenous supply or a supplement. Iron and zinc are present only in low concentrations and although their bioavailability and absorption is high they can become limiting in some exclusively breastfed infants. Adequate maternal iron stores and delayed umbilical cord clamping at birth until pulsations have stopped (approximately 2–3 min) improve total infant body iron, reducing the risk of iron deficiency before 6 months. Preterm or low birth weight infants should have supplements beginning at 2 months. Other nutrients that are affected by maternal

nutrition status are the water- and fat-soluble vitamins, including thiamin, riboflavin, vitamin B₆, vitamin B₁₂, vitamin A, iodine, and selenium. Those not affected are folate, vitamin D, calcium, iron, copper, and zinc.

The protection provided by anti-infective factors in breast milk is unique to each mother–infant dyad. They include immunoglobins, primarily secretory immunoglobulin A (IgA) that coats the intestinal mucosa and prevents bacteria from entering the cells; white blood cells that kill microorganisms; lysozyme and lactoferrin that have been shown to destroy bacteria, viruses, and fungi; and oligosaccharides that prevent bacteria from attaching to mucosal surfaces (**Box 1**). Not only do they protect without causing inflammation but they also are formed in response to bacteria or infections the mother has encountered and so also likely to be encountered by the infant. Breast milk is not sterile but rather has a unique microbial community that is specific to each woman. An emerging concept in research about the role of human milk in educating the neonatal immune system is that molecules like milk sCD14, a pattern recognition receptor, educate the neonatal immune system to respond with appropriate innate or adaptive immune responses to varying microbial and antigenic challenges.

Other bioactive factors in breast milk include bile salt stimulated lipase that helps in the digestion of fat in the small intestine and epidermal growth factor that stimulates maturation of the intestinal mucosa. This enables the infant to better digest and absorb nutrients.

Breast milk is particularly important to infants born preterm and with low birth weights. Human milk, whether from the mother or donor milk, has been shown to significantly reduce the risk of serious infections such as sepsis and necrotizing enterocolitis. A multicenter randomized prospective study of feeding of preterm infants and necrotizing enterocolitis showed that formula-fed infants were 10 times more likely to contract the disease than infants fed human milk (**Figure 1**). Human milk banks that pasteurize donor milk can play an important role in feeding at-risk newborns, particularly

Box 1 Pathogens against which colostrum and breast-milk have been shown to be effective

<i>Virus</i>	<i>Bacteria</i>	<i>Fungi/protozoa</i>	<i>Enterotoxins</i>
Coxsackie types A ₉ , B ₄ , B ₅	<i>Campylobacter flagellin</i>	<i>Candida albicans</i>	Cholera toxin
Cytomegalovirus	<i>Clostridium</i> (A and B)	<i>E. histolytica</i>	Labile toxin of <i>E. coli</i>
Echovirus types 6 and 9	Enteropathogenic <i>E. coli</i>	<i>G. lambia</i>	Shigella toxin I
Enteroviruses	Enterotoxigenic <i>E. coli</i>	<i>T. vaginalis</i>	Shiga-like toxin of <i>E. coli</i>
Enveloped viruses HIV	<i>Samonella</i>		Coxsackie types A ₉ , B ₄ , B ₅
Herpes simplex virus	<i>Shigella</i>		
<i>H. influenza</i>	<i>S. Aureus</i>		
Parainfluenzae	<i>V. cholerae</i>		
Polio virus types 1, 2, and 3			
Reovirus type 3			
Respiratory syncytial virus			
Rotavirus			
Rubella			
Simliki forest virus			
<i>S. pneumonia</i>			

Source: Reproduced from Huffman, *et al.* (2001) Can improvements in breast-feeding practices reduce neonatal mortality in developing countries? *Midwifery* 17: 80–92.

though not exclusively in developing countries, where preterm formulas are often not available putting them at increased risk of morbidity and mortality from powdered formulas.

Infant formulas are very different from breast milk quantitatively as well as qualitatively. They are made from industrially-modified cow milk or soy products and in the manufacturing process the protein, carbohydrate, and fat content adjusted to make them more comparable to breast milk. Nonetheless, the qualitative differences between the human milk fats and proteins cannot be altered nor can the anti-infective or bioactive factors added. Serious infections in newborns have been traced to contamination in powdered formulas with pathogenic bacteria, such as *Enterobacter sakazakii*. Formulas have been recalled because they lack key nutrients or have been adulterated in ways that harm infant health.

Lactation physiologically completes the reproductive cycle and the breast is prepared for full lactation without any active

intervention from the mother or health care provider. It has 3 stages: stage I begins approximately 12 weeks before birth and is characterized by the gathering of substrate including lactose, total proteins, and immunoglobins for milk production; stage II begins 2–3 days after birth and is characterized by fullness of the breast and secretion of milk in copious amounts; stage III, formerly called galactopoiesis is characterized by the maintenance of established milk secretion. Once breast feeding has been established, the volume produced depends on infant demand. Therefore, frequent nursing is critical, particularly in the early days postpartum, for stimulating optimal and continued milk production. Lactation physiology is discussed in greater detail in a separate article.

Risks of not Breast Feeding

Breast feeding contributes to short and long-term maternal and infant health and child cognitive development through a number of mechanisms (Table 1). Because of the well-established superiority of breast milk over infant formula, women cannot ethically be randomized in infant studies and as a result most data on the benefits of breast feeding and hence risks of not breast feeding are from observational studies. However, several studies in which women were randomized to breast feeding promotion versus standard of care that did not particularly promote breast feeding or involving preterm newborns that were randomized to human milk or formula show important benefits, substantiating many findings from observational studies. A number of studies, including a study where women were randomized to breast feeding promotion or not, show that not breast feeding or breast feeding for a short duration compared to a longer duration significantly

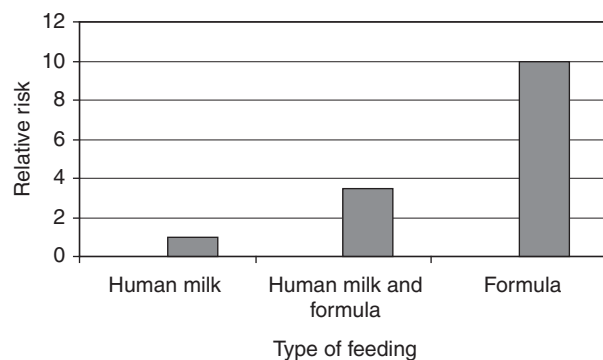


Figure 1 Feeding of preterm infants and necrotizing enterocolitis. Reproduced from PAHO/WHO.

Table 1 Summary of immediate and long-term benefits of breast feeding for the mother and infant

Immediate benefits ^a		Long-term benefits	
Infant	Mother	Infant	Mother
Prevents neonatal and infant morbidity and mortality	Stimulates oxytocin release causing uterine contractions	Decreases risk of: <ul style="list-style-type: none"> – Acute otitis media – Nonspecific gastroenteritis – Hospitalization for severe lower respiratory-tract infections – Atopic dermatitis – Obesity – Type 1 and 2 diabetes – Childhood leukemia – Sudden Infant Death Syndrome – Necrotizing enterocolitis 	Lactational amenorrhea helps to delay future pregnancies and protects maternal iron status
Early breast feeding associated with longer breast feeding duration during infancy	Possibly protective of maternal mood		Decreases risk of: <ul style="list-style-type: none"> – Type-2 diabetes – Ovarian cancer – Breast cancer – Cardiovascular disease
Early exclusive breastfeeding associated with exclusive breast feeding later in infancy		Improved motor development	More rapid weight loss

^aImmediate benefits from early initiation of exclusive breast feeding.
Source: Reproduced from PAHO/WHO.

reduced child intelligence quotient (IQ) on average approximately 7.5 points for verbal IQ and 5.9 points for full-scale IQ.

For the child, the risks of not breast feeding include increased mortality, acute illness (diarrhea, respiratory infections, ear infections, and other acute illnesses) and chronic illnesses or conditions (type-2 diabetes, blood pressure, and serum cholesterol), and reduced intelligence. Breast feeding saves lives particularly in developing countries but also in developed countries. In developing countries, infants who are not breastfed compared to those breastfed are 6, 4, and 40% more likely to die in the first 2 months, 2–3 month, and 9–11 months, respectively. These figures likely underestimate the risks of not breast feeding as exclusive breast feeding, the behavior most associated with survival, was so rare that its benefits could not be estimated. Even in the United States, breast feeding is associated with a 21% decline in infant mortality.

For the mother, the risks of not breast feeding include increased ovarian and premenopausal breast cancer, type-2 diabetes and cardiovascular disease, and reduced postpartum weight loss and birth intervals in the absence of modern contraceptives. In a prospective study of over 60 000 women who had at least one child, breast feeding women who had a close relative with breast cancer had nearly 60% less risk for premenopausal breast cancer compared with similar non breastfeeding women. Mothers who had never breastfed an infant were 90% more likely to have developed type-2 diabetes than nulliparous women and mothers who never exclusively breastfed were more 50% likely to have developed type-2 diabetes than mothers who exclusively breastfed for 1–3 months. Some though not all studies show that breast feeding, particularly exclusive breast feeding, speeds postpartum weight loss.

Promotion of Breast Feeding

Because of its many contributions to maternal and child health, breast feeding must be protected, promoted, and supported. In response to concerns about inappropriate use of infant formula in environments where lack of breast feeding resulted in large numbers of infants who became severely ill or died, a gross roots global initiative took hold in the 1970s and promoted international and national initiatives to restore breast feeding as a normative behavior. These efforts

culminated in 1981 with the nearly unanimous adoption of the World Healthy Assembly (WHA) of the International Code of Marketing of Breast-milk Substitutes. This document and subsequent relevant resolutions strengthening this original resolution are collectively known as the Code and provide guidelines for the marketing of breast milk substitutes, bottles, and teats. To ensure infant feeding decisions free from the influence of market pressures, the Code provides guidelines on a number of topics associated with increased in formula feeding, including direct promotion to the public, free supplies to mothers, and donations to health care institutions, and the use of baby images on labels that glorify bottle feeding. Its implementation is monitored by a 2-year reporting cycle by countries to the WHA. The International Baby Food Action Network (IBFAN) and Code Documentation Centre in Penang, Malaysia monitor Code implementation and compliance.

The 1990 Innocenti Declaration, signed by many governments and endorsed by the 45th WHA, set four operational targets that all governments should achieve by 1995. These included establishment of a multisectoral national breast feeding committee; appointment of a national breast feeding coordinator; ensuring that all health facilities providing maternity care fully practice the 10 steps to successful breast feeding (**Box 2**), taking action to give effect to the Code; and enacting imaginative legislation to protect the breast feeding rights of working women. This declaration provided the basis for the WHO/UNICEF Baby Friendly Hospital Initiative (BFHI), which was launched as a global initiative in 1992. BFHI promotes hospital practices consistent with early initiation of breast feeding, an environment conducive to breast feeding, appropriate clinical management of breast feeding, and compliance with certain key provisions of the Code, such as no donations of free or subsidized infant formula. Certification is awarded to hospitals that comply with a standardized set of criteria. Although over 20 000 hospitals have been certified as Baby Friendly, no process of quality control or recertification was built into the BFHI. Several evaluations show that standards lapse over time. WHO and UNICEF have recently updated the BFHI materials to include a model for hospital self-assessment. Several countries are also evaluating the extent to which hospitals that have been certified continue to meet Baby Friendly criteria and in some cases decertifying hospitals.

Concurrent with the implementation of the Code and BFHI, training materials and capacity development in

Box 2 WHO/UNICEF 10 steps to successful breast feeding

- Step 1. Have a written breast feeding policy that is routinely communicated to all health care staff.
- Step 2. Train all health care staff in skills necessary to implement this policy.
- Step 3. Inform all pregnant women about the benefits and management of breast feeding.
- Step 4. Help mothers initiate breast feeding within one hour of birth.
- Step 5. Show mothers how to breast feed and how to maintain lactation even if they should be separated from their infants.
- Step 6. Give newborn infants no food or drink other than breast milk, unless medically indicated.
- Step 7. Practice rooming-in, allow mothers and infants to remain together 24-hours a day.
- Step 8. Encourage breast feeding on demand.
- Step 9. Give no artificial teats or pacifiers (also called dummies or soothers) to breast feeding infants.
- Step 10. Foster the establishment of breast feeding support groups and refer mothers to them on discharge from the hospital or clinic.

Source: Reproduced from PAHO/WHO.

lactation management and development of national breast feeding promotion programs occurred globally. National governments also actively implemented policies, programs, and campaigns to promote breast feeding. More recently, the WHO Global Strategy for Infant and Young Child Feeding adopted by the WHA in 2003 reaffirmed the 4 targets in the Innocenti Declaration.

Breast feeding counseling has been shown to be highly effective and cost-effective (Figure 2). Numerous randomized controlled trials have shown that exclusive breast feeding can be dramatically increased through pre and postpartum counseling. WHO and UNICEF have developed a number of courses to improve health worker capacity in breast feeding. Breast feeding promotion is also a key component of both clinical and community Integrated Management of Childhood Illnesses (IMCI) programs. Because of the effectiveness of breast feeding counseling, it should be incorporated into maternity care and both well- and sick-child visits.

Human Immunodeficiency Virus (HIV) and Infant Feeding

The knowledge that the HIV virus can be transmitted through breast milk greatly complicated infant feeding recommendations, particularly in many developing countries where the risks of morbidity and mortality in the absence of breast feeding are particularly high. In developed countries, HIV-infected women are advised not to breast feed. WHO's guidance on HIV and infant feeding has been recently updated to clarify and simplify guidance on infant feeding in the context of HIV. The current recommendations are guided by a number of key principles including that recommended infant feeding practices by mothers known to be HIV-infected should support the greatest likelihood of HIV-free survival of their children and

not harm the health of mothers. Another key principle states that national or sub-national health authorities should decide whether health services will principally counsel and support mothers known to be HIV-infected and whose infants are HIV uninfected or of unknown HIV status to either breastfeed and receive antiretroviral interventions or avoid all breast feeding. This decision should be based on consideration of the socio-economic and cultural contexts of the populations served by maternal, newborn and child health services, availability and quality of health services, local epidemiology including HIV prevalence among pregnant women, main causes of maternal and child undernutrition, and main causes of infant and child mortality.

When HIV-infected mothers do breastfeed they should receive appropriate antiretroviral therapy and exclusively breastfeed their infants for the first 6 months of life, introducing appropriate complementary foods thereafter, and continue breast feeding for the first 12 months of life. Breast feeding should then only stop once a nutritionally adequate and safe diet without breast milk can be provided. Mothers of infants and young children known to be HIV-infected are strongly encouraged to exclusively breastfeed for the first 6 months of life and continue breast feeding up to two years or beyond.

Global Breast Feeding Practices

The WHO Global Data Bank on Breast feeding covers 94 countries and 65% of the world's infant population. In developing countries, breast feeding initiation in most countries is well over 90%. Based on the latest data, it is estimated that only 35% of infants are exclusively breastfed between birth and 4 months of age. The prevalence of exclusive breast feeding among infants less than 6 months of age has been increasing in many though not all regions (Figure 3).

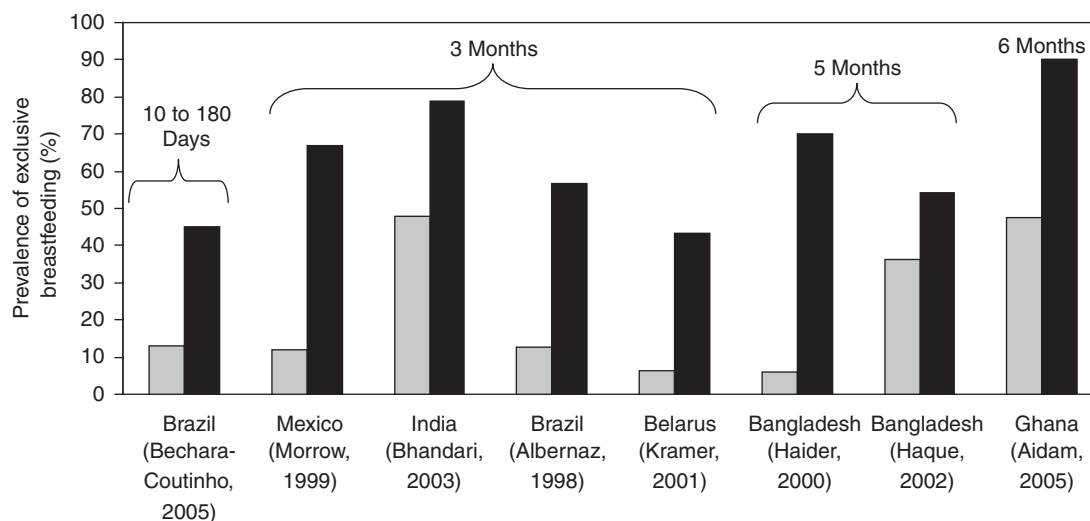


Figure 2 Effect of postpartum counseling on exclusive breast feeding. Control groups (who did not receive additional posthospital discharge breast feeding support through the study) are shown in the light bars. Intervention groups, which included some form of post-discharge peer-counseling breast feeding support (often through home visits), are shown in dark bars. The prevalence of exclusive breast feeding (EBF) at each study's endpoint is noted; for the study from Brazil, the mean aggregated prevalence over the period between 10 and 180 days is presented. Reproduced from PAHO/WHO.

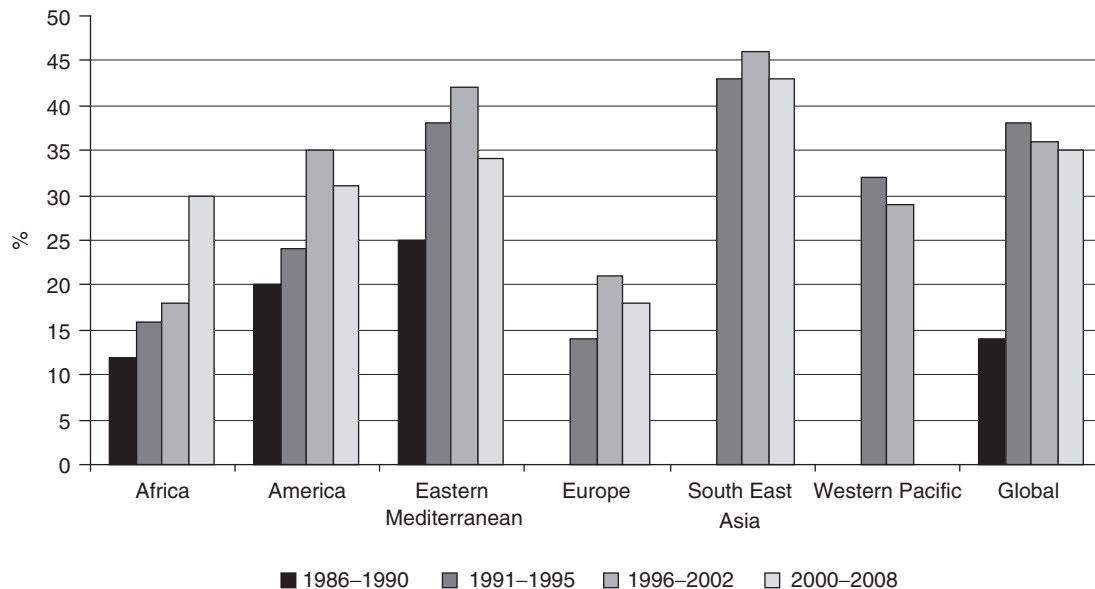


Figure 3 Prevalence of exclusive breast feeding among infants less than 6 months of age, global trends 1986–1990 to 2000–2008. WHO Global Bank on Infant and Young Child Feeding. Reproduced from PAHO/WHO.

The largest increases appear to have occurred between the years 1986–1990 and 1991–1995. Some countries, such as Ghana and Brazil experienced large increases in exclusive breast feeding through implementation of quality programs with high coverage. In Ghana, over a 20 year period, from 1988 to 2008 it increased from 4.6% to 63.4%. In Brazil, between 1986 and 2006, it increased from 2.4% to 38.6%. Many other countries have also experienced large increases. Importantly, these positive trends have occurred during a time of economic transition resulting in increased education and maternal education and employment, characteristics that have traditionally been associated with less breast feeding.

In the US, the percentage of infants who were ever breastfed increased, from 60% to 77% among infants who were born in 1993–1994 and 2006–2006, respectively. Breast feeding is lower among low-income (57%) compared to higher income (74%) women and also higher among women older than 30 years. Among non-Hispanic black women, breast feeding rose from 36% in 1993–1994 to 65% in 2005–2006. Despite these improvements in infants who were ever breastfed, there was no change in breast feeding at 6 months comparing the two time periods.

In conclusion, breast feeding promotion is a public health “best buy.” It has a large effect in reducing infant morbidity and mortality and also is highly amenable to public health intervention. Research has shown that individual maternal behaviors are amenable to change and that changes in individual behaviors collectively contribute to positive national trends in breast feeding. To ensure that virtually all newborns benefit from breast feeding and breast milk, a concerted effort by governments, health systems and employers is needed. Actions are also needed by nongovernmental organizations and communities to ensure that every mother lives and works

in an environment where the decision to breastfeed can be easily carried out.

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BURNS PATIENTS

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Glossary

Anabolism Net accumulation of protein stores in response to hormones: this may be endogenous in recovery from injury or exogenous in response to administered steroids.

Artificial skin A sheet of nonliving material that is used to cover full-thickness burns. It includes an upper protective layer and a lower collagen layer that provides a matrix into which the host cells can move to regenerate the dermal layer.

Catabolism The net breakdown of protein and fat stores, usually as a response to injury or stress.

Immunonutrition Enteral or parenteral feed containing additional amino acids that are specifically implicated in reducing infective complications such as ornithine α -ketoglutarate, arginine, or glutamine.

Indirect calorimetry A method of estimating energy expenditure by measuring oxygen uptake and carbon dioxide production.

Introduction

Of all insults to the body, burns elicit the most profound stress response. This response encompasses hormonal, metabolic, and immunologic changes, which are complicated by the loss of many protective functions of an intact skin. The initial hypermetabolic state induces intense protein catabolism, which must be checked by aggressive nutritional support in order to limit morbidity and mortality. Following this intense period of catabolism, which lasts 10–14 days, there is a gradual reduction in the metabolic rate, catabolic processes decrease, the wound heals, and the anabolic processes predominate. Nutritional support must also continue throughout these latter stages, which may take many weeks, so that anabolism can be supported and fuel reserves replenished. A diet high in carbohydrate (CHO) and protein but low in fat will

not only suppress catabolism and favor anabolism but also support an appropriate immunological response.

Hypermetabolism and Hypercatabolism

The burn wound is the focal point of all the circulatory, metabolic, and inflammatory responses associated with injury (Figure 1).

Metabolic Response

Increased glucose demand is initially met by glycogenolysis. When glycogen stores are exhausted, lipolysis and protein catabolism increase to supply gluconeogenic substrates. This hypermetabolic response is accompanied by an increase in cardiac output, oxygen consumption, and thermogenesis. The physical loss of skin cover has other major effects, including

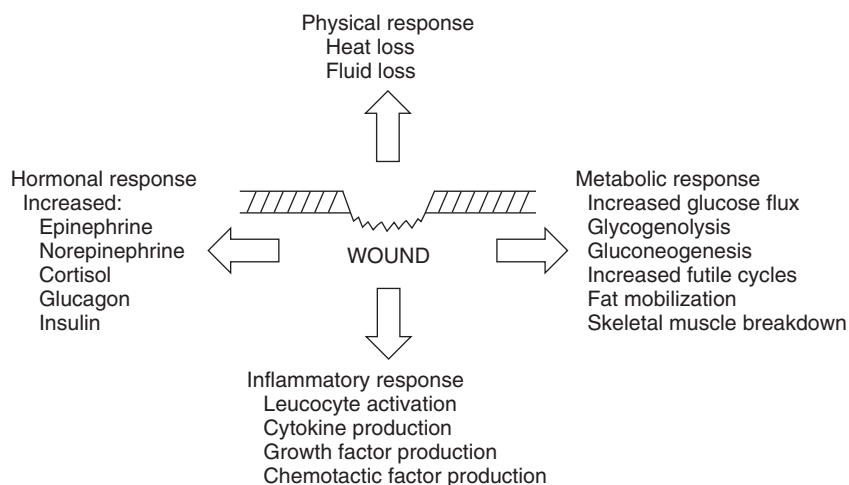


Figure 1 Physical, hormonal, metabolic, and inflammatory responses to burn injury.

fluid loss, increased heat loss by evaporation, and loss of local immune function. Skin grafting will provide some cover for burnt areas but the use of allografts increases the total area of damaged skin. Early excision and grafting is associated with increased survival in patients with more than 70% burns compared with conservative management. Donor split-skin graft sites heal within 7–14 days, unlike the burnt area, which continues to make increased metabolic demands for weeks after the initial insult. Measurements of energy requirements take into account the whole body metabolism and include any demands made by donor sites. Early coverage with artificial 'skin' can also reduce the caloric requirements of the patient, but does not entirely prevent hypercatabolism.

The sympathoadrenal axis is stimulated as a result of thermal injury, with increased plasma levels of epinephrine, norepinephrine, and cortisol. In addition, levels of both glucagon and insulin increases, although an apparent insulin resistance develops in peripheral tissues. There is an increase in core temperature, which is mediated centrally by the hypothalamus in response to the release of the cytokines interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor α (TNF- α). Plasma levels of glucose are maintained and may even increase, although glucose flux is greatly increased. Metabolic demands for glucose and amino acids increase and the body responds to meet these demands (Table 1). The degree of hypermetabolism and oxygen consumption is closely related to the extent and depth of burn injury. As a result, basal energy expenditure increases and is doubled for a 60% burn (Figure 2). Catecholamines augment cardiac and circulatory performance, which increases blood flow to the wound. Liver and kidney blood flow also increases, with increased delivery of gluconeogenic precursors, increased glucose release into the circulation, and increased nitrogen clearance. The release of fat from adipose tissue is stimulated by catecholamines. In the liver, fat metabolism to glycerol and free fatty acids produces energy as long as an adequate glucose supply replenishes oxaloacetic acid for oxidation of acetyl CoA, the product of triacylglycerol oxidation. Thus, both continued oxygen and glucose must be supplied to prevent ketoacidosis. Heat production and energy wastage occur as a result of a two- or three-fold increase in futile cycling of substrates; glucose, pyruvate, and fructose-6-phosphate are all involved in these reactions.

Gluconeogenesis can occur only in the liver and is increased by catecholamines and glucagon. The plasma levels of gluconeogenic amino acids (alanine and glutamine) initially increase during the first 2 days, when glycogen is preferentially metabolized, but subsequently decreased. Days 4–7 are associated with a maximal decrease in plasma levels of gluconeogenic amino acids, whereas muscle production and hepatic consumption are both increased.

Catabolic Response

Release of gluconeogenic amino acids, particularly glutamine, from skeletal muscle results in loss of muscle mass. Deamination of these amino acids, during the generation of carbon skeletons for glucose synthesis, increases nitrogen production with subsequent conversion to urea, which is excreted by the kidneys. Urinary nitrogen loss following thermal injury is largely from skeletal muscle breakdown, but a significant

Table 1 Metabolic and circulatory responses to burn injury

Wound	Whole body
Damage	Increase in catecholamines, cortisol, glucagon, insulin Hepatic switch to synthesis of acute phase proteins
Increased blood flow to the wound	Increased cardiac output
Increased metabolism of glucose	Increased gluconeogenesis Increased free fatty acid flux Increased oxygen consumption Futile substrate cycling of carbohydrate intermediates and fatty acids
Increased heat loss	Increased core temperature: Hypothalamic mediated
Attempted repair	Increased amino acid flux Release of arginine and glutamine from skeletal muscle Increased nitrogen loss
Inflammatory response	Inhibition of maximum inflammatory response by cortisol Cytokine and eicosanoids increase inflammation

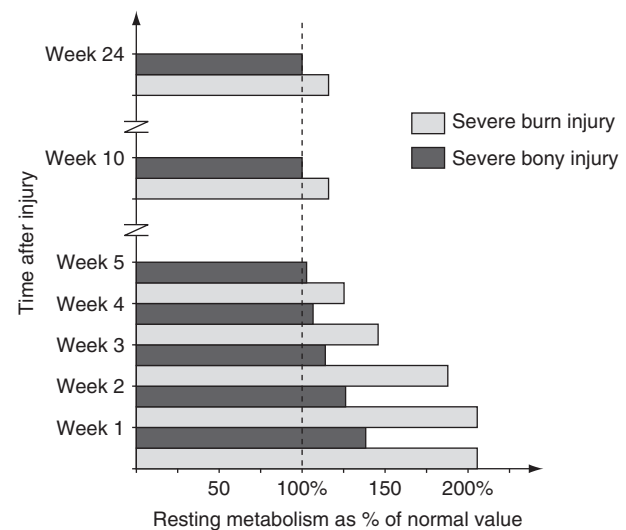


Figure 2 The time-course of metabolic changes following a burn injury compared with traumatic bony injury. Reproduced from Long CL, Schaffel N, Geiger JW, *et al.* (1979) Metabolic response to injury and illness: Estimation of energy and protein needs from indirect calorimetry and nitrogen balance. *Journal of Parenteral and Enteral Nutrition* 3(6): 452–456, with permission from Sage.

contribution of approximately 25–30% comes from the burn exudates. The rate of nitrogen loss is related to total burn area (TBA) and can be as much as 3 or 4 g kg⁻¹ day⁻¹ at its peak (Figure 3). These high rates of nitrogen loss persist for the first

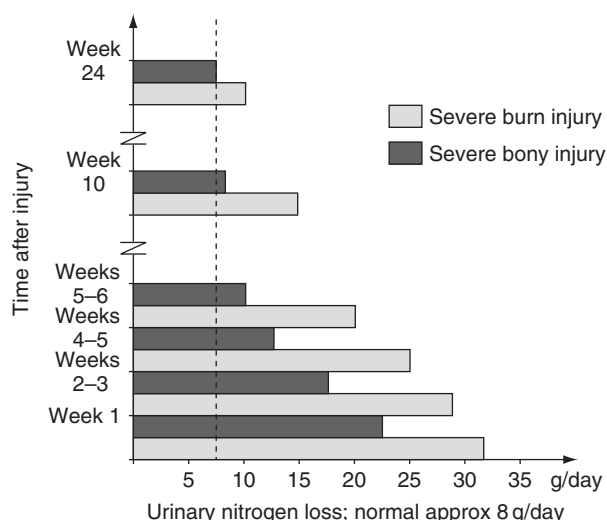


Figure 3 The time-course of urinary nitrogen loss following severe burn injury compared with traumatic bony injury. Adapted from Long CL, Schaffel N, Geiger JW, *et al.* (1979) Metabolic response to injury and illness: Estimation of energy and protein needs from indirect calorimetry and nitrogen balance. *Journal of Parenteral and Enteral Nutrition* 3(6): 452–456, with permission from Sage.

7–10 days and then gradually decline until the burnt area is healed and body stores of nitrogen are replenished; this may take many months. Nutritional support provides an exogenous source of calories and protein, which limits auto-cannibalism of skeletal muscle with a reduction in infective complications, increased survival rates, and reduced hospital stay.

Inflammatory Response

Local and systemic factors lead to an increase in inflammatory cell infiltration of the burn wound with removal of damaged tissue in preparation for epithelialization and wound healing. The cytokine cascade precipitated by stress hormones in response to injury involves TNF- α , which subsequently activates leucocyte production of IL-1, IL-6, platelet-derived growth factor, and eicosanoids (prostaglandins, thromboxanes, and leukotrienes). These in turn amplify the cellular and cytokine responses by release of chemotactic factors. TNF- α and the cytokines are also mediators of the increased metabolic and catabolic response seen in burnt patients. TNF- α will increase acute-phase protein synthesis and increase loss of amino acids from skeletal muscle. However, it is also associated with the healing process that wound healing is stimulated as a result of vascular proliferation and collagen synthesis. Excessive release of TNF- α can be harmful and is associated with muscle wasting, excessive weight loss, increased nitrogen loss, systemic inflammatory response syndrome, and debility.

These early immune reactions give way, in the second and third week postburn, to injury immunosuppression. This takes the form of reduced responsiveness of lymphocytes, impaired production of IL-2, and changes in immune cell phenotypes. Burn injury inhibits the T-helper 1 response but promotes a T-helper 2 response. As a result, IL-2 and interferon- γ (IFN- γ)

Table 2 Formulas for predicting calorie requirements in adults that compare favorably with indirect calorimetric measurements ($1.3 \times \text{REE}$)

Xie formula

$$1000 + (25 \times \text{BSAB}) \text{ kcal m}^{-2} \text{ day}^{-1} \text{ or } 4184 + (105 \times \text{BSAB}) \text{ kJ m}^{-2} \text{ day}^{-1}$$

Zawacki formula

$$1440 \text{ kcal m}^{-2} \text{ day}^{-1} \text{ or } 6025 \text{ kJ m}^{-2} \text{ day}^{-1}$$

Milner formula

$$(\text{BMR} \times 24 \times \text{BSA}) + (0.274 + (0.0079 \times \text{BSAB}) - (0.004 \times \text{DPB})) \text{ kcal m}^{-2} \text{ day}^{-1} \text{ or } (\text{BMR} \times 24 \times \text{BSA}) + (1.146 + (0.0331 \times \text{BSAB}) - (0.017 \times \text{DPB})) \text{ kJ m}^{-2} \text{ day}^{-1}$$

BMR, basal metabolic rate; BSA, body surface area; BSAB, percentage body surface area burned; DPB, day post-burn.

Source: Adapted from Xie WG, *et al.* (1993) Estimation of the calorie requirements of burned Chinese adults. *Burns* 19: 146–9; bZawacki BE, *et al.* (1970) Does increased evaporative water loss cause hypermetabolism in burned patients? *Annals of Surgery* 171: 236–40, and cMilner EA, Cioffi WG, Mason AD, McManus WF, and Pruitt BA Jr. (1994) A longitudinal study of resting energy expenditure in thermally injured patients. *Journal of Trauma-Injury Infection & Critical Care* 37(2): 167–70, with permission from LWW.

production is reduced, which increases the risk of infection. Membrane lipid composition also influences lymphocyte and macrophage functions in terms of signaling and eicosanoid production. There is a reduction in *n*-6 (mainly arachidonic) fatty acids and an increase in PGE₂, which can lead to immunosuppression. Dietary replacement of *n*-6 by *n*-3 polyunsaturated fatty acids (PUFAs) reduces immunosuppression by altering membrane composition and eicosanoid series production. Nutritional studies have focused on the influence of enteral feed composition on immune function – the so-called immune-enhancing diets. The theoretical elements of interest are *n*-3 PUFAs and the amino acids arginine and glutamine. The evidence base for a clinically measurable advantage of such immune-enhancing preparations is yet to be fully established. Small studies have shown a reduction in wound infection rate and a possible reduction in length of stay per percentage total body surface area burned.

Nutritional Requirements

In the absence of an exogenous nutrient supply, auto-cannibalism would result in major morbidity and mortality. Nutritional supply of energy and protein in excess of normal requirements prevent such complications.

Calories

Adults

Much work has focused on developing an easy-to-apply formula for predicting the number of calories required to maintain weight in the severely burnt patient. Many of these formulas are simply based on percentage burn area and body surface area, but others are complex – arrived at by regression

Table 3 Factors that may alter total energy requirements in burns patients

Number of days after burn injury
Changes in environmental temperature and humidity
Changes in core body temperature, including sepsis, infection
Inhalation injury
Activity level
Surgical interventions; grafting
Dressing changes
Pain and anxiety
Sedative drugs

analysis. Recent evaluations of these formulas, compared to indirect calorimetry, suggest that none perfectly predict a patient's true requirements. The most reliable are summarized in (Table 2). Requirements vary considerably depending on multiple factors including time after burn injury, not all of which can be taken into consideration in the formulas used (Table 3). Because of major within- as well as between-patient variation, it is agreed that indirect calorimetry should be used to predict resting energy requirements (REEs) throughout the recovery period.

By measuring oxygen uptake and carbon dioxide production, the REE of the patient can be derived. Recent work has established that although body weight can be maintained on a regimen of caloric intake of 1.3–1.5 times the REE, this reflects increased fat accumulation despite persistent catabolism of skeletal protein. The catabolism appears to persist despite nutritional manipulation. It has been suggested that lean mass can only be maintained by pharmacological means with the use of insulin, insulin-like growth factor-1 (IGF-1), or anabolic steroids such as oxandrolone.

Children

Pediatric patients, who account for at least 35% of all burn injuries, are a challenge to nutritional support teams. Compared to adults, they have lower lean body mass and fat reserves, and have a higher basal metabolic rate. Extra allowances are needed for growth and development, particularly during the infant and adolescent growth spurts. Many different pediatric formulas are used (Table 4). Indirect calorimetry indicates that those predicting lower calorie requirements may be more accurate.

Pediatric patients, for whom requirements have been particularly difficult to predict using formulas, indirect calorimetry has been of great importance in determining adequate calorie intake and measured energy expenditure should be multiplied by a factor of 1.5 to provide adequate calories for weight maintenance in children with burns.

Balance of Energy-Producing Substrates

Energy requirements may be met by glucose, fat, and protein. There has been much interest in the relative proportions of these three sources, and it now seems that 20–25% should be supplied as protein but only 15% as fat.

Table 4 Examples of formulas used to predict energy requirements in children

<i>Wolfe^a</i>			
<i>BMR × 2 kcal/24 h</i>			
<i>Males</i>	<i>BMR equation</i>	<i>Females</i>	<i>BMR equation</i>
0–3	$(60.9 \times W) - 54$	0–3	$(61 \times W) - 51$
3–10	$(22.7 \times W) + 459$	3–10	$(22.5 \times W) + 499$
10–18	$(17.5 \times W) + 651$	10–18	$(12.2 \times W) + 746$
<i>Modified Galveston formulas^b</i>			
Less than 1 year old: $2100 \text{ kcal m}^2 \text{ BSA} + 1000 \text{ kcal m}^2 \text{ BSA burned}$			
Less than 12 years old: $1800 \text{ kcal m}^2 \text{ BSA} + 1300 \text{ kcal m}^2 \text{ BSA burned}$			
12–18 years old: $1500 \text{ kcal m}^2 \text{ BSA} + 1500 \text{ kcal m}^2 \text{ BSA burned}$			
<i>Curreri junior formulas^c</i>			
<i>Daily calorie needs^d</i>			
Birth–1 year: basal RDA in kcal + (15 kcal per % burn)			
1–3 years: basal RDA in kcal + (25 kcal per % burn)			
4–15 years: basal RDA in kcal + (40 kcal per % burn)			

BMR, basal metabolic rate; BSA, body surface area; RDA, recommended daily allowance; *W*, weight in kilograms.

^aO'Neil CE, Huttsler D, and Hildreth MA (1989) Basic nutritional guidelines for pediatric burn patients. *Journal of Burn Care & Rehabilitation* 10(3): 278–284.

^bHildreth MA, Herndon DN, Desai MH, and Duke MA (1988) Reassessing caloric requirements in pediatric burn patients. *Journal of Burn Care Rehabilitation* 9(6): 616–618.

^cDay T, Dean P, Adams MC, Luteran A, Ramenofsky ML, and Curreri PW (1986) Nutritional requirements of the burned child: The Curreri junior formula. *Proceedings of the American Burn Association* 18: 86.

^dRDA (kcal) varies with age: 0–0.5 years, 320; 0.5–1 years, 500; 1–3 years, 740; 4–6 years, 950; 7–10 years, 1130; 11–14 years, 1440 (male) and 1310 (female); 15–18 years, 1760 (male) and 1370 (female).

Carbohydrate

Adults

A high carbohydrate : fat ratio is associated with better maintenance of body weight. However, this may reflect increased fat accumulation rather than an increase in protein synthesis. Hyperglycemia alone can increase alanine efflux from skeletal muscle, without stimulating protein synthesis. Euglycemia, using exogenous insulin with high glucose delivery, can inhibit amino acid oxidation and favor amino acid synthesis. This may reflect an effect of IGF-1, which is released in response to insulin. In addition, hyperglycemia stimulates hepatic lipogenesis and increased CO₂ production, which may prevent weaning from ventilatory support. Hyperglycemia must therefore be prevented.

Children

In children, carbohydrate is more effective than fat in promoting nitrogen retention by reducing the need for protein catabolism and subsequent gluconeogenesis. In infants, 5% dextrose in water parenterally can be used at $5 \text{ mg kg}^{-1} \text{ min}^{-1}$ initially and increased to a maximum of $15 \text{ mg kg}^{-1} \text{ min}^{-1}$ over the course of first few days of postinjury to provide 40–50% of calorie requirements. In older children, as in adults, glucose administration at a maximum rate of $5\text{--}7 \text{ mg kg}^{-1} \text{ min}^{-1}$ is recommended. These are parenteral recommendations; enteral feeding guidelines have not been

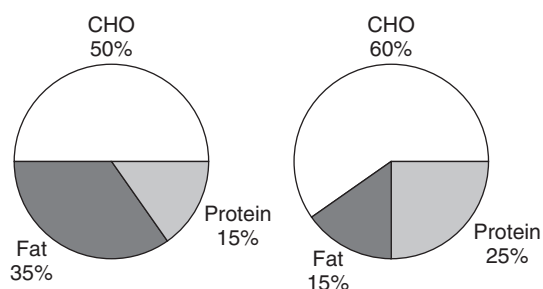


Figure 4 The pie-chart on the left shows the relative contribution of fat, carbohydrate (CHO), and protein in a normal diet. The right-hand chart shows the preferred contributions for a patient post-burn: a reduction in fat and increase in protein minimizes complications in the recovery period.

established, although in general, carbohydrate should be limited to 50% of calorie intake.

Fat

Adults

Feeding regimens that simply overfeed with a normal diet lead to problems in the recovery phase; muscle wasting persists together with central obesity. Reduction in fat administration largely prevents these problems if protein calories replace lipid calories (Figure 4). Fat cannot be excluded from the diet; a minimum fat content of 4% of total calories will ensure a supply of essential fatty acids. A diet containing 15% fat will meet such requirements as well as provide a delivery medium for fat-soluble vitamins. Dietary fat is largely composed of long-chain triacylglycerols (LCTs), and excess LCTs are associated with hepatic steatosis, reticuloendothelial system blockage, and immunosuppression. Varying the composition of fats supplied to burnt patients may alleviate some of these problems. Medium-chain fatty acids, particularly *n*-3 PUFA found in fish oil, appear beneficial in maximizing whole body protein synthesis in an animal model of burn injury. There is a decrease in plasma *n*-6 fatty acids after burn injury, so replacement with *n*-3 PUFA results in the production of prostanoid and eicosanoid series associated with less immunosuppression than those arising from *n*-6 PUFA metabolism. The use of low-fat feed, supplemented with *n*-3 PUFAs, reduces protein catabolism and increases IGF-1, particularly 2 or 3 weeks postburn. Limiting fat to 15% total calorie intake reduces wound infection rate, improves healing rate, reduces the incidence of pneumonia, improves nutritional markers, and reduces hospital stay. Such benefits have been seen both with and without the addition of *n*-3 PUFAs.

Children

A fat intake of 2% or 3% of total calories is the minimum recommended for the prevention of essential fatty acid deficiency in pediatric patients. For intravenous fat administration in infants, a maximum of 4 g kg⁻¹ lean body weight is suggested.

Protein

Adults

Protein calories comprise a significant proportion of the energy requirement of a severely burnt patient. Intact protein, rather than amino acids, is associated with better weight maintenance and improved survival. Nitrogen loss must be estimated regularly in a burn patient in order to ensure adequate nitrogen replacement. It is impossible to measure total nitrogen loss (TNL) accurately because 20–30% of nitrogen loss occurs in the exudate from wounds. There is some doubt regarding the use of urinary urea nitrogen (UUN) to estimate total urinary nitrogen (TUN), from which TNL is usually calculated. In healthy, unstressed subjects, urea comprises 80% of the TUN, but ureagenesis is inconsistent after burn injury and varies widely depending on the extent and course of illness. If measurement of TUN is available, this will reflect nitrogen loss more accurately.

$$\text{TNL} = \text{TUN} + 4 \text{ g day}^{-1} \text{ or } (\text{UUN} \times 1.25) + 4 \text{ g day}^{-1}$$

TNL must then be compared with nitrogen supply (NS) to calculate the nitrogen balance (NB).

$$\text{NB} = \text{TNL} - \text{NS}$$

The aim is to keep a positive balance, and a suitable starting point would be 2 g protein per kg lean body weight per day; 6.25 g protein is equivalent to 1 g nitrogen. Urinary excretion of 3-methyl histidine has been used as a measure of skeletal protein catabolism. Nitrogen input from blood products is appreciable, accounting for 15% of total nitrogen intake, but is often ignored when calculating NB, which therefore underestimates protein intake.

Amino acids play an important role in adaption to burn injury both as gluconeogenic substrates and as substrates for acute-phase protein synthesis and wound repair. Arginine flux appears to be increased in burnt patients, but plasma levels of arginine and glutamine appear to be greatly reduced following burn injury. These changes have prompted supplementary feeding with particular amino acids. Interest has focused on ornithine α -ketoglutarate (OAK) and its metabolites arginine and glutamine. OAK is also a precursor for proline, the incorporation of which into collagen is a rate-limiting step in collagen synthesis. Arginine also increases collagen deposition, and in animal models of burn injury, increased arginine provision has been associated with increased wound healing and improved immune function. There is evidence of a clinically important reduction in healing time and infectious episodes following OAK-, arginine-, or glutamine-supplemented feeding in human studies: this is known as immunonutrition. Glutamine is the most abundant amino acid in the body and is the major fuel source for the intestine. Its presence prevents villous atrophy and maintains mucosal integrity as well as stimulates blood flow to the gut. Glutamine supplementation of feed reduces the incidence of Gram-negative bacteremia in patients with severe burns. The proposed mechanism is the reduction in bacterial translocation across the gut wall; glutamine has been shown to reduce bacterial translocation in a rat model. The most recent guidelines for critically ill burn patients recommend enteral or parenteral supplementation with glutamine for reduced morbidity and hospital stay.

Children

Protein needs are often estimated by formulas based on lean body weight. To estimate preburn weight, the 50th centile weight for height should be used. For children younger than 1 year old, 3 or 4 g protein per kg lean body weight is suggested to provide adequate nutritional support for graft coverage and healing; this should be reduced to 2.5–3 g protein per kg lean body weight for children 1–3 years of age. In older children, protein requirements are further reduced to 1.5–2.5 g kg⁻¹ lean body weight. When NB is calculated for children, the following formulas have been suggested:

$$\text{Age 0–4 years: TNL} = \text{UUN} + 2$$

$$\text{Age 4–10 years: TNL} = \text{UUN} + 3$$

$$\text{Age >10 years: TNL} = \text{UUN} + 4$$

Protein-enriched diets, containing 25% calories as protein compared to 16% in normal diets, have been associated with improved NB, improved immune function, and fewer infective episodes in children with severe burns. Until recently, albumin was a mainstay of fluid requirements in children with severe burns, which contributes to protein provision. However, studies have shown increased morbidity and mortality in critically ill patients given albumin. It seems that the outcome for children with severe burns is no worse if they receive albumin supplementation only when albumin levels decline below 10 g l⁻¹ (or 15 g l⁻¹ in the presence of intolerance of enteral feed). Use of albumin should be reviewed regularly.

Vitamins

Specific vitamin requirements in burnt patients have not been established, but levels may decline in the hypermetabolic state. As a minimum, the recommended daily allowance should be given following injury. For minor burns of 10–20% body surface area, supplementation with a single multivitamin tablet orally should replace vitamin losses sustained during injury. For larger burns, additional supplementation is advised, especially vitamin C (ascorbic acid), which is of benefit in wound healing and has experimentally been shown to possess free radical scavenging properties that may help to limit tissue damage. A recommended dose is 1 g daily in two divided doses for all patients with major burns; children younger than age 3 years should receive half this dose daily. The essential, fat-soluble, vitamin A may also confer some advantages in wound healing and immunomodulation. A dose of 10 000 IU daily is recommended for all patients with major burns who are older than the age of 3 years; younger children should receive half this amount daily. Wherever possible, both vitamin and micronutrients should be administered by the enteral route. If such supplements are added to total parenteral nutrition (TPN), dosing schedules should take into account the increased bioavailability via this route and a dose reduction may be advisable. Monitoring of levels of micronutrients should guide replacement by the parenteral route.

Trace Elements

Trace elements are present in the body in amounts less than one part per million by weight; many are essential components of metalloenzymes. Following burn injury, significant amounts of these trace elements may be lost. The acute phase reaction is characterized by a decrease in plasma levels of copper, iron, selenium, and zinc and an increase in the plasma levels of the carrier proteins ferritin and caeruloplasmin. Although iron levels decline following burn trauma, it has been shown that excessive administration of iron is harmful and that lower plasma levels of iron appear to be of benefit in reducing microbial replication. In contrast, increased intravenous administration of copper, zinc, and selenium during the first week following burn injury resulted in fewer complications – particularly infections, improved leucocyte response, rapid return of the plasma levels of these trace minerals, as well as shorter hospital stay. Zinc, copper, and manganese are essential for wound healing; serum zinc levels decrease following stress and burn injury largely due to increased urinary loss. Zinc supplementation, 220 mg daily, should be considered for all patients with major burns. Inclusion of trace elements in both enteral and parenteral nutrition is essential.

Adjunctive Treatments

There is great interest in a pharmacological role for growth hormone (GH) and IGF-1 in reversal of the catabolic state and stimulation of anabolic processes. GH stimulates production of IGF-1, which improves amino acid transport and enhances gluconeogenesis from exogenously supplied amino acids. Blood levels of IGF-1 are markedly reduced in burn patients following injury and remain so for the first week, after which levels increase; these changes correlate with IGF binding protein-3 levels. This binding protein prevents plasma proteolysis of IGF-1. GH and IGF-1 have both been used in experimental models of burn injury, and their effectiveness at limiting catabolism and enhancing mucosal proliferation is encouraging. In children, GH treatment accelerates donor site healing and increases protein synthesis. GH has also been shown to exert immunomodulatory effects, which may contribute to a reduced incidence of infection. Other growth factors or hormones have also been used experimentally and in animal models, they improve the rate of healing and strength of burn wounds (Table 5). In clinical studies the anabolic steroid oxandrolone, 10 mg orally twice daily, improved wound healing, restored lean body mass, and

Table 5 Growth factors identified as potential adjunctive therapy in wound healing

Growth hormone
Insulin-like growth factor-1
Epidermal growth factor
Transforming growth factors (α and β)
Platelet-derived growth factor
Fibroblast growth factors (1–7)
Erythropoietin
Granulocyte macrophage colony-stimulating factor

accelerated body weight gain. However, the role for such hormonal therapies has yet to be firmly established in clinical management plans.

Nutritional Management

Nutritional management of the burn patient is an important facet of overall management. It is important to involve a multidisciplinary feeding team to manage nutrient intake and organize nutritional assessment. A warm and ambient temperature is essential for reducing fluid and heat loss and keeping the patient comfortable. Metabolic rate increases with discomfort and dressing changes can be a continual source of stress. Thus, analgesic requirements must be adequate or anesthesia administered. There has been a move toward continuing enteral feed in the immediate perioperative period. The risk of aspiration seems to be very low, particularly if jejunal feeding is used. Even when nasogastric feeding is used, starvation times can be minimized without apparent increased

risk. As a result, 60% rather than 6% of caloric requirements can be met on days of surgery/dressing changes.

Route of Feeding

Wherever possible, the enteral route should be used. The American Gastroenterological Association has strongly endorsed this view and stated that routine parenteral nutrition is contraindicated if the enteral route is available. Nasogastric, nasojejunal, and percutaneous enteral access tubes have all been used successfully when feeding is introduced as soon after burn injury as possible. Jejunal feeding is associated with a higher success rate than gastric feeding and may be continued even in the presence of gastric stasis. Increased mortality has been associated with the use of central venous catheters and TPN in patients with severe burn injury. This is related to both catheter-associated morbidity and depression of gut function. Glutamine is relatively unstable and has not been included in parenteral formulations. New preparations containing the dipeptide or acetylated form of glutamine will

Table 6 Scheme for nutritional monitoring in a patient with a burn injury

Day 0	<p>Record</p> <ul style="list-style-type: none"> a. Age b. Height c. Urine output d. Oral fluid/food intake <p>Investigate</p> <ul style="list-style-type: none"> a. Plasma prealbumin b. Electrolytes and urea c. Hemoglobin and hematocrit <p>Intervention</p> <ul style="list-style-type: none"> a. If burn area > 20%, place nasoenteral feeding tube, nasojejunal if possible, start feeding according to calculated values b. Intravenous crystalloid, blood, and colloid according to center protocol 	<p>Estimate</p> <ul style="list-style-type: none"> a. Ideal body weight b. % TBA c. Need for dressings/surgery/activity d. Fluid loss
Day 2/3	<p>Investigate</p> <ul style="list-style-type: none"> a. Indirect calorimetry, energy requirement, calorie balance b. 24 h UUN c. Urinary myoglobin d. Hematocrit <p>Intervention</p> <ul style="list-style-type: none"> a. Adjust calorie intake to match calculated need b. If intolerant of enteral feeding, reduce to 10–30 ml h 	
Day 4/5 and twice weekly	<p>Investigate</p> <ul style="list-style-type: none"> a. Plasma prealbumin b. Hematocrit c. Indirect calorimetry, as above <p>Intervention:</p> <ul style="list-style-type: none"> a. Calculate nitrogen balance; adjust nitrogen intake b. Adjust calorie intake 	
Day 6/7 and weekly	<p>Investigate</p> <ul style="list-style-type: none"> a. Weight b. Trace elements c. Weekly need for dressing changes and surgery d. Is enteral absorption improving? If not, start TPN e. Is oral intake increasing? Is enteral supplementation still needed? <p>Intervention</p> <ul style="list-style-type: none"> a. Adjust calorie intake to account for (c) above b. Add trace elements if indicated c. Review route(s) of feeding 	

be available in the future that may be of benefit to patients who are dependent on TPN.

Patients with burn injuries greater than 10% are often unable or unwilling to increase their oral intake to meet calorie needs, which are higher by a factor of 1.3 compared to normal. For patients with <20% burns, calorie intake can often be met by supplemental nocturnal feeding through a fine-bore feeding tube. For burns >20%, nasoenteral supplementation is essential. Evidence suggests that the earlier nutrition is started, the greater the attenuation of the hypermetabolic and catabolic response. Early feeding, within 6 h of injury, is optimal. Enteral delivery of glucose and glutamine maintains mucosal integrity and reduces gut ischaemia, as shown by a reduction in arterial-to-intraluminal CO₂ gap. This latter measure can identify an imbalance between calories presented and oxygen delivered to the gut and may be used to adjust enteral feeding levels to prevent excessive delivery.

Some patients are intolerant of enteral feeding, especially those needing mechanical ventilation, who require high levels of opiate analgesia and exogenous norepinephrine support. In these patients, TPN is needed. Wherever possible, a slow, continuous presentation of enteral feed should also be provided to prevent intestinal mucosal atrophy and preserve immune function. Whichever routes are required, calorie provision should be guided by nutritional monitoring of energy expenditure and NB.

Nutritional Monitoring

There are a variety of ways to monitor nutritional status in the burnt patient; body weight, nutritional intake, NB, and laboratory indices all play a role in nutritional assessment. A scheme for continuing nutritional assessment is given in **Table 6**.

Success of the chosen nutrition plan must be monitored and the plan adjusted accordingly. Energy expenditure and nitrogen loss should be measured once or twice weekly for calorie and NB calculations. The choice of additional biochemical markers to assess overall nutritional state is difficult and no one marker adequately predicts actual nutritional state at all times during the course of injury. The constitutively

produced prealbumin, with its short half-life and independence from exogenous albumin administration, is often used.

Pediatric patients often exhibit growth delays and special attention must be paid to younger children who are less than their ideal body weight at the time of injury. Nutritional support is often required for many weeks after discharge from hospital, and outpatient follow-up of growth in children is essential.

See also: Amino Acids: Metabolism. Cytokines: Nutritional Aspects. Nutritional Support: Adults, Enteral

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C

CAFFEINE

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Glossary

Catechins A group of flavonoid compounds that are found in relatively high concentrations in green tea. The most abundant and likely pharmacologically-active catechin in green tea is epigallocatechin gallate.

Dependence (i.e., 'Addiction' in common parlance) Defined by a cluster of cognitive, behavioral, and physiological features indicating that an individual continues to use a substance despite experiencing significant substance-related problems. Desire or inability to quit substance use, knowledge of harm caused by substance use, and withdrawal symptoms on quitting are common features of substance dependence.

Intoxication A reversible substance-specific syndrome consisting of clinically significant maladaptive psychological or behavioral changes that develop during or shortly after substance use. Symptoms of caffeine

intoxication include insomnia, nervousness, diuresis, gastrointestinal disturbance, muscle twitching, and tachycardia or cardiac arrhythmia.

Reinforcement The degree to which a stimulus following a response increases the frequency of subsequent responses. Drug (or caffeine) reinforcement is defined by the ability of a drug to maintain drug self-administration or choice behavior.

Tolerance An acquired decrease in responsiveness to a drug as the result of drug exposure. Tolerance can be defined as one or both of (1) the need to consume markedly increased amounts of a substance in order to achieve a desired effect or (2) a markedly diminished effect with repeated administrations of the same amount of a substance.

Withdrawal Symptoms represent the emergence of physiological, cognitive, or behavioral changes that occur following cessation or reduction in chronic (e.g., daily) substance use.

Caffeine is the most widely used psychoactive drug in the world. In the US, an estimated 87% of the population regularly consume beverages containing caffeine. Mean caffeine consumption among all users is 193 mg per day, with the highest intake among men aged 35–54 who consume an average of 336 mg of caffeine per day.

Caffeine is a natural constituent of more than 60 species of plants, including coffee, tea, cola nut, cacao, yerba maté, and guarana. Caffeine (1,3,7-trimethylxanthine) is a member of the methylxanthine class of alkaloids that include theobromine and theophylline. In its free base form, caffeine is a

bitter white powder that is moderately soluble in water (21.7 mg ml⁻¹). Worldwide, caffeine is most commonly consumed as coffee and tea. Consumption of beverages with added caffeine (i.e., soft drinks) has markedly increased over the past half century, with consumption volume of soft drinks now being approximately twice that for coffee in the US. A notable recent trend has been the increasing popularity of “energy drinks” which vary considerably in the amount of caffeine (from 50 mg to over 375 mg of caffeine per can or bottle). Hundreds of such products are now marketed in the US ([Table 1](#)).

Table 1 Caffeine content of foods and medications

Substance	Typical serving size (volume)	Typical caffeine content (mg)	Caffeine content range (mg)
<i>Coffee</i>			
Brewed/drip	12 oz ^a	200	108–420
Starbucks hot brewed coffee	16 oz	330	
Espresso	1 oz	70	60–95
Starbucks espresso (solo)	1 oz	75	
Instant	6 oz	70	20–130
Decaffeinated	12 oz	8	0–10
<i>Tea</i>			
Brewed	6 oz	40	30–90
Instant	6 oz	30	10–35
Can or bottle (typical)	12 oz	20	8–32
Arizona brand iced black tea	20 oz	37.5	
Arizona brand iced green tea	20 oz	18.75	
<i>Soft drinks (typical)</i>			
Typical caffeinated soft drink	12 oz	40	22–69
Coke Classic	12 oz	35	
Diet Coke	12 oz	47	
Pepsi-Cola	12 oz	38	
Diet Pepsi	12 oz	36	
Mountain Dew	12 oz	55	
Barq's Root Beer	12 oz	23	
<i>Energy drinks (typical)</i>			
Typical energy drink	Varies	Varies	50–375 ^b
Red Bull	8.3 oz	80	
Rockstar	16 oz	160	
Wired X344	16 oz	344	

^aHistorically, a "typical" serving size of coffee has been considered to be 6 oz. However, standard servings of coffee tend to be much larger than this. The smallest serving size offered at many fast food restaurants and coffee houses (e.g., Starbucks) is 12 oz.

^bSpecified range does not include energy shot products, which can contain as much as 500 mg caffeine per 1-oz serving.

Caffeine Pharmacokinetics

Caffeine is rapidly and completely absorbed from the gastrointestinal tract after oral administration. Caffeine is readily distributed throughout the body and is found in all body fluids. Peak plasma concentrations are typically reached 30–45 min following oral ingestion. The fraction of caffeine bound to plasma protein is 10–35%.

More than 25 caffeine metabolites have been identified in humans. The primary metabolic pathways involve the cytochrome P-450 liver enzyme system which produces three demethylated active metabolites: paraxanthine, theobromine, and theophylline, accounting for 80%, 10%, and 4% of caffeine metabolism, respectively. The average half-life of caffeine is 4–6 h, with elimination rates varying by more than 10-fold across individuals. Caffeine half-life is prolonged in individuals with liver disease and during the end of pregnancy. Caffeine metabolism is inhibited by numerous compounds including oral contraceptive steroids, cimetidine, some quinoline antibiotics, fluvoxamine, mexiletine, and high doses of caffeine itself. Neonates have a markedly increased caffeine half-life (80–100 h) due to immature liver enzyme systems which are fully developed at approximately 6 months of age. Tobacco smoking increases caffeine metabolism by stimulating the cytochrome P-450 1A2 enzyme (CYP1A2), with smokers metabolizing caffeine about twice as fast as nonsmokers.

Genetic variations in CYP1A2 activity are a significant determinant of caffeine metabolism. For more information on individual differences in genetics, see the Section on Caffeine Genetics.

Mechanisms of Action

The primary cellular site of action of caffeine is the adenosine receptor. Among the adenosine receptor subtypes that have been identified, A₁ and A_{2A} receptors are the preferential targets of caffeine. A₁ receptors are widely expressed throughout the brain, whereas A_{2A} receptors are concentrated in dopaminergic-rich areas, such as the striatum. Adenosine is an endogenous purine nucleoside that generally exerts inhibitory effects throughout the central and peripheral nervous system (e.g., excitatory neurotransmitter inhibition, suppression of motor activity, and inhibition of gastric secretion). Caffeine is a nonselective competitive A₁ and A_{2A} receptor antagonist. Thus, caffeine produces a variety of central and peripheral effects that are opposite to the effects of adenosine.

Of most relevance to caffeine's central nervous system (CNS) stimulating effects, caffeine enhances dopamine activity indirectly by competitive antagonism of adenosine receptors that are co-localized and functionally interact with dopamine. Adenosine receptors can form functional receptor

heteromers with dopamine receptors (i.e., A_1 - D_1 and A_{2A} - D_2). There is some evidence that the motor stimulant and reinforcing effects of caffeine are mediated by dopamine. Pre-clinical studies demonstrate that caffeine produces behavioral effects similar to the dopamine-mediated effects of classic stimulants, such as cocaine and amphetamine. Moreover, dopamine depletion or blockade of dopamine receptors significantly impairs the motor stimulant and discriminative stimulus effects of caffeine.

Although caffeine can inhibit phosphodiesterase and increase intracellular calcium concentration, typical dietary doses of caffeine are believed to be too low to be significantly influenced by these nonadenosine mechanisms. Thus, caffeine's effects appear to be primarily mediated by direct antagonism of adenosine and indirect enhancement of brain dopamine activity. For more information on ergogenic mechanisms of action, see the Section on Caffeine and Exercise.

Physiological and Health Effects of Caffeine

Caffeine modestly increases blood pressure but appears to have no effects on or to reduce heart rate. Hypertensive and hypertensive-prone caffeine users appear to be particularly sensitive to the pressor effects of caffeine. Caffeine produces increases in gastric acid secretion, colonic stimulation, diuresis (30% or more increased volume), respiratory stimulation, and bronchodilation. Caffeine also increases plasma epinephrine, norepinephrine, adrenocorticotrophic hormone, cortisol, renin, and free fatty acids. Acute caffeine administration produces increased cerebral blood flow velocity and electroencephalography (EEG) beta power activity. In addition, caffeine has prominent sleep-disrupting effects. For more information on the sleep disrupting effects of caffeine see the Section on Caffeine-Induced Sleep Disorder.

Although studies of the association between caffeine consumption and coronary heart disease have yielded inconsistent findings, one recent investigation demonstrated an association between slow caffeine metabolism and the incidence of coronary heart disease in moderate and heavy caffeine consumers. For more information on caffeine genetics, see the Section on Caffeine Genetics. Several studies have found that moderate coffee intake is associated with decreased risk for coronary heart disease, possibly because of protective effects of antioxidant and other protective compounds in coffee.

Over the last few decades, studies have yielded inconsistent findings regarding the effects of caffeine on reproductive and perinatal outcomes. Although some investigations have not found evidence of a significant association between caffeine exposure and adverse birth outcomes, several recent studies did show a relationship. Although equivocal findings preclude definitive conclusions regarding the effects of caffeine on pregnancy, some governmental health agencies have taken a prudent stance and issued health warnings to limit the use of caffeine during pregnancy. Health Canada recommends that women of reproductive age consume no more than 300 mg of caffeine per day. The Food Standards Agency of the UK advises that pregnant women keep their daily intake of caffeine below 200 mg.

Caffeine and Performance

Numerous investigations have examined the effects of caffeine on human performance. The most consistent finding to emerge is that caffeine restores performance that has been degraded by sleep deprivation, fatigue, or prolonged vigilance. At normal dietary doses, caffeine may improve tapping speed, reaction time, and sustained attention (vigilance), although results have been variable and sizes of the effects are often modest. A large number of experimental studies have examined the effects of caffeine on memory, but evidence is insufficient to conclude that caffeine produces acute improvements in memory.

A significant limitation of the majority of studies that have found performance-enhancing effects of caffeine is that subjects in these studies have been regular caffeine users who were required to abstain from caffeine before testing (e.g., overnight abstinence). Thus, the observed performance-enhancing effects of caffeine in these studies may reflect restoration of deficits that are produced by caffeine withdrawal. For more information on the caffeine withdrawal syndrome, see the Section on Caffeine Withdrawal. It is important to note, however, that a few studies have found performance-enhancing effects of caffeine in nondependent caffeine users and nonusers. A few studies have also demonstrated performance increases in caffeine users who were not required to abstain from usual caffeine use, suggesting that complete tolerance to the performance-enhancing effects of caffeine does not occur at usual dietary doses. However, in high-dose caffeine consumers, performance enhancement beyond withdrawal reversal is likely to be modest.

Caffeine and Exercise

A large body of research has examined the effects of caffeine on exercise performance. Numerous well-controlled studies have found that relative to placebo, caffeine enhances performance during endurance exercise. Studies have also generally found that caffeine reduces ratings of perceived exhaustion or effort during exercise. Ergogenic effects of caffeine are typically demonstrated at doses of 3–6 mg kg⁻¹; higher doses of caffeine (e.g., 9 mg kg⁻¹) appear to exert little or no additional benefit on endurance exercise. There is some evidence that caffeine produces greater endurance exercise benefits in caffeine nonusers and in athletes who abstained from caffeine for several days before dosing. Findings from studies examining the effects of caffeine on short-term, high-intensity exercise performance have generally been equivocal, however a recent review suggested that caffeine can improve performance in team-sports exercise and power-based sports, with this effect more common in elite athletes who do not regularly consume caffeine. Although not rigorously studied, findings are suggestive that tolerance occurs to the ergogenic effects of caffeine. Several nonindependent mechanisms have been proposed for caffeine's effects on exercise performance, including increased fatty acid oxidation, increased availability of muscle glycogen, mobilization of intracellular calcium, increased muscle contractile force, and direct CNS effects via adenosine antagonism.

Caffeine Genetics

Much of the variability in caffeine consumption and individual differences in response to caffeine can be accounted for by genetic factors. Findings from twin studies indicate that there may be common genetic factors underlying the use of caffeine, nicotine, and alcohol. Moreover, twins studies indicate that genetic factors may influence total caffeine consumption, heavy caffeine consumption, caffeine tolerance, caffeine withdrawal, caffeine intoxication, and caffeine-related sleep disturbances.

The CYP1A2 gene codes for the isoenzyme P-450 1A2, which is responsible for the demethylation of caffeine to paraxanthine, theobromine, and theophylline. For more information on caffeine metabolism, see the Section on Caffeine Pharmacokinetics. More than 150 CYP1A2 single-nucleotide polymorphisms have been identified. Individual variability in the pharmacokinetics of caffeine can be in large part accounted for by variations in CYP1A2 activity. Recent evidence suggests that individuals homozygous for the allele associated with slow metabolism (CYP1A2*1F) are at increased risk for nonfatal myocardial infarction associated with caffeinated coffee intake. Thus, caffeine consumption may increase risk for myocardial infarction in individuals with slow caffeine metabolism.

Genetic differences in adenosine A2A receptors have been implicated in individual differences in human caffeine responses. Variations in A2A receptor polymorphisms have been associated with caffeine sensitivity, caffeine-induced anxiety, caffeine-related sleep impairment, and caffeine consumption. One study reported evidence that a polymorphism in dopamine DRD2 receptors is associated with caffeine-induced anxiety.

Caffeine Subjective Effects

The qualitative subjective effects of caffeine depend on caffeine dose, individual differences in sensitivity, and degree of tolerance to caffeine. Low to moderate doses of caffeine typically produce positive subjective effects, including increased well-being, arousal, energy, alertness, concentration, motivation to work, and sociability, and decreased feelings of sleepiness or tiredness. Positive subjective effects are more likely to be reported in individuals who have undergone overnight caffeine abstinence.

At higher acute doses of caffeine (e.g., 400–800 mg), negative subjective effects of caffeine typically emerge. Negative subjective effects include increased anxiety, nervousness, jitteriness, tense negative mood, and upset stomach. Anxiogenic subjective effects are more likely to be reported in individuals with panic disorder or generalized anxiety disorder, and in nonclinical populations who endorse high levels of anxiety sensitivity (i.e., fear of anxiety). For more information about high dose caffeine effects, see the Section on Caffeine Intoxication.

Caffeine Reinforcement

The efficacy of a substance in establishing or maintaining self-administration behavior reflects the reinforcing effects of the

substance. The circumstantial evidence indicating that caffeine functions as a reinforcer is compelling. Caffeine is the most widely used mood-altering drug in the world. Regular daily consumption of pharmacologically active doses occurs in widely varying cultural and social contexts. Historically, efforts to restrict or eliminate consumption of caffeine-containing foods and beverages have been unsuccessful. Caffeine consumption occurs in a wide variety of vehicles (e.g., coffee, tea, maté soft drinks, energy drinks; chewing kola nuts). Finally, caffeine-containing beverages tend to be more popular than their caffeine-free counterparts. As an example, in 2009, in the US, the top six selling carbonated soft drink brands, and eight of the top 10 selling brands, contained added caffeine.

Numerous well-controlled experimental studies have demonstrated caffeine reinforcement in various subject populations. Across studies, approximately 40% of normal caffeine users demonstrate caffeine reinforcement. Higher rates of reinforcement have been observed among individuals with high levels of caffeine consumption or a history of drug or alcohol abuse. Caffeine can function as a reinforcer at very low doses (i.e., 25 mg per cup of coffee), but may produce avoidance at higher doses (e.g., 400 or 600 mg).

In habitual caffeine consumers, avoidance of caffeine withdrawal plays an important role in the reinforcing effects of caffeine. This relationship has been shown in retrospective questionnaire studies and in experimental studies that have used direct behavioral measures of reinforcement. For example, in one experimental study, moderate caffeine consumers who reported withdrawal symptoms (i.e., headaches and drowsiness) were more than twice as likely to show caffeine reinforcement. Other studies that have prospectively manipulated caffeine physical dependence have demonstrated that subjects were more than twice as likely to exhibit caffeine reinforcement when they were caffeine physically dependent (and thus prone to experiencing withdrawal symptoms when they abstain).

Studies using a conditioned flavor preference paradigm have provided indirect evidence of caffeine reinforcement. In these studies, caffeine abstinent subjects develop a liking and preference for caffeine-paired flavored beverages, relative to beverages paired with placebo. Further studies showed that, in subjects who were repeatedly exposed to a caffeine-paired flavored beverage, the development of liking and preference for the beverage was determined by alleviation of withdrawal symptoms. These studies suggest that conditioned flavor preferences (driven at least in part by alleviation of unpleasant withdrawal symptoms) likely play an important role in consumer preferences for caffeine-containing beverages.

Caffeine Withdrawal

Cessation or reduction of daily caffeine consumption results in withdrawal symptoms in many caffeine users. Caffeine withdrawal has been well characterized in numerous rigorous experimental studies and in survey studies. Caffeine withdrawal headache, which is the hallmark feature of the caffeine withdrawal syndrome, has been the most frequently assessed withdrawal symptom. Approximately half of regular caffeine users report headache when abstaining from caffeine. The

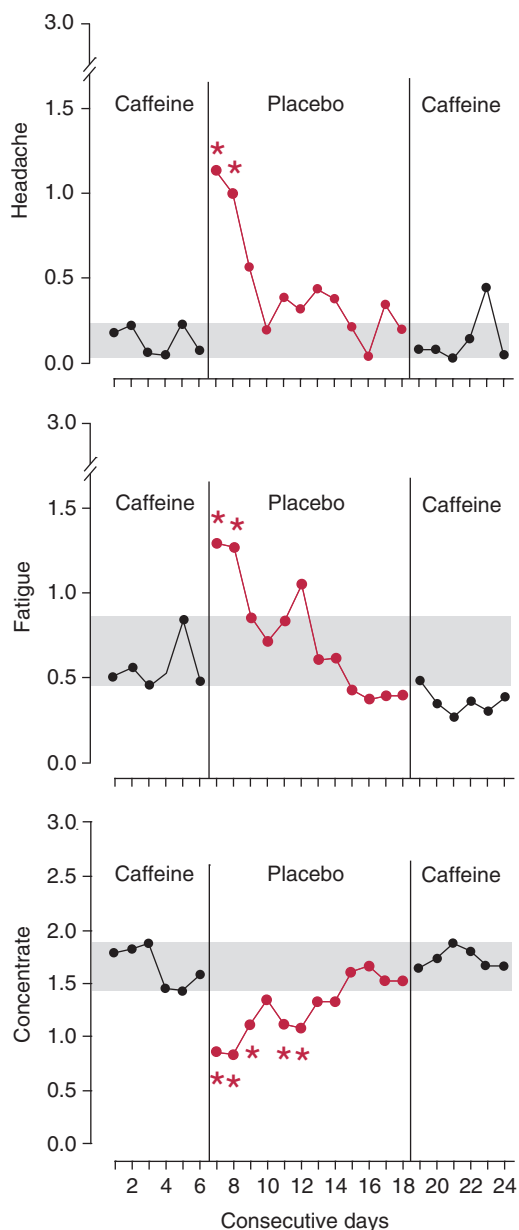


Figure 1 Time course of caffeine withdrawal symptoms in four volunteers. Under double-blind conditions volunteers received either 100 mg day^{-1} of caffeine or placebo. Assessments included subjective ratings of headache (top), feelings of lethargy/fatigue/tired/sluggish (middle), and ability to concentrate (bottom). Ratings ranged from 0 (not at all) to 3 (very much). Shaded areas indicate the range of means from the initial 6-day caffeine period. Mean ratings are presented in black for caffeine days and red for placebo days. Asterisks indicate placebo days that are significantly different from the initial caffeine period ($p \leq 0.05$). Reproduced from Griffiths RR, Evans SM, Heishman SJ, Preston KL, Sannerud CA, Wolf B, and Woodson PP (1990) Low-dose caffeine physical dependence in humans. *The Journal of Pharmacology and Experimental Therapeutics* 255: 1123–1132, with permission from ASPET.

caffeine withdrawal headache, which develops gradually, is described as diffuse, throbbing, severe, and phenomenologically distinct from migraine headache. In addition to headache, other withdrawal symptoms that have been reliably

observed across experimental and survey studies include: fatigue, decreased energy/activeness, decreased alertness, drowsiness, decreased contentedness, depressed mood, difficulty concentrating, irritability, foggy/not clearheaded, nausea/vomiting, and muscle stiffness/pain. Based on these observed symptoms, five primary clusters of withdrawal symptoms have been proposed: (1) headache, (2) fatigue and drowsiness, (3) dysphoric mood, depressed mood, or irritability, (4) difficulty concentrating, and (5) flu-like somatic symptoms, nausea, vomiting, or muscle pain/stiffness.

Onset of withdrawal typically occurs 12–24 h after abrupt caffeine cessation, although onset has been observed as early as 6 h and as late as 43 h after abstinence in some individuals. Typically, peak intensity of symptoms occurs 1 to 2 days after abstinence. The duration of caffeine withdrawal symptoms is generally 2–9 days. Re-administration of caffeine (usually within 30–60 min of onset) rapidly and often completely reverses caffeine withdrawal. Figure 1 shows the time-course of caffeine withdrawal from an illustrative double-blind experimental study.

The severity of caffeine withdrawal symptoms can range from mild to extreme and depends on several factors. Some caffeine users report clinically significant functional impairment associated with caffeine withdrawal (e.g., interference with work or child care activities). Studies show that clinically significant distress occurs in approximately 13% of caffeine users. A much higher rate (73%) of withdrawal-related clinically significant distress occurs in individuals meeting criteria for caffeine dependence. Severity of caffeine withdrawal is positively associated with increases in caffeine maintenance dose, such that greater withdrawal is experienced following cessation of higher maintenance doses. Caffeine withdrawal can occur after daily doses of caffeine as low as 100 mg day^{-1} . Caffeine withdrawal may also occur when lower doses of caffeine are substituted for the maintained caffeine dose. As the substituted dose of caffeine decreases, withdrawal severity increases. Nevertheless, even a small amount of substituted caffeine (e.g., 25 mg) can mitigate severity of caffeine withdrawal symptoms.

Caffeine Tolerance

Caffeine tolerance may occur in response to daily caffeine consumption. Tolerance can occur to the subjective, sleep disrupting, and physiological effects of caffeine. The degree of caffeine tolerance depends on several factors including the challenge and maintenance doses, frequency of administration and individual differences in elimination rate. The prevalence of self-reported tolerance among current caffeine users varies from 8% to 50%. Rates as high as 92% have been reported among caffeine-dependent individuals.

Regular caffeine users may acquire complete tolerance (i.e., no difference between placebo and caffeine after prolonged exposure to caffeine) to some, but not all, of the subjective effects of caffeine. For example, experimental studies showed that volunteers who received moderate to high doses of caffeine (400 mg to 900 mg day^{-1}) for at least two weeks developed complete tolerance to ratings of subjective stimulant effects. Other studies indicate that complete tolerance to

caffeine subjective effects does not occur at lower caffeine doses and over shorter periods of dosing.

Tolerance development may differ across different outcome measures. One study showed that tolerance or complete tolerance developed to subjective ratings but not to measures of cerebral blood flow or EEG in volunteers receiving 400 mg day⁻¹.

With regard to other physiological effects, tolerance may develop to the effects of caffeine on diuresis, parotid gland salivation, metabolic rate, plasma norepinephrine and epinephrine levels, and plasma renin activity. Findings suggest that regular caffeine users develop partial, but not complete tolerance to the effects of caffeine on cerebral blood flow and EEG measures. Two studies, which tested a small number of volunteers demonstrated the development of complete tolerance to the pressor effects of high doses of caffeine (e.g., 600 to 850 mg day⁻¹). However, several more recent studies examining a larger number of volunteers showed that tolerance to the pressor effects of caffeine is variable across individuals, with some subjects showing complete tolerance, whereas others show only incomplete tolerance.

Caffeine Intoxication

Caffeine intoxication, which is a DSM-IV-TR recognized disorder, is defined by the development of symptoms and clinical features in response to acute caffeine consumption that cause clinically significant distress or impairment. Although the DSM-IV-TR definition specifies that the diagnosis depends on recent consumption of at least 250 mg of caffeine, symptoms typically emerge at doses greater than 500 mg. Symptoms of caffeine intoxication include restlessness, nervousness, insomnia, flushed face, diuresis, gastrointestinal disturbance, muscle twitching, rambling flow of thought and speech, tachycardia or cardiac arrhythmia, periods of inexhaustibility, and psychomotor agitation. Although children and caffeine-intolerant individuals may be particularly sensitive to the acute adverse effects of caffeine, habitual caffeine users may also experience episodes of caffeine intoxication. Several case reports and experimental studies suggest that caffeine consumption may produce hallucinations in some individuals, particularly under conditions of stress.

Caffeine-Induced Sleep Disorder

Caffeine reduces total sleep time and limits latency to sleep onset, most probably by blocking the sleep promoting effects of adenosine. In addition, caffeine decreases stage 3–4 sleep and suppresses EEG slow wave activity during sleep. The sleep-disrupting effects of caffeine are well documented even at low doses (e.g., one cup of coffee). Surveys have found associations between daily dietary caffeine intake and sleep problems in both adults and adolescents.

Some caffeine users may develop caffeine-induced sleep disorder, which is a DSM-IV-TR recognized disorder typically characterized by insomnia. Some caffeine users may present with caffeine-induced hypersomnia with daytime sleepiness due to withdrawal symptoms. Sleep disturbances secondary to

caffeine may increase in severity as caffeine dose increases and proximity to caffeine administration at bedtime decreases. Individuals who are not regular caffeine users and are not tolerant, or have only partial tolerance to the sleep-disrupting effects of caffeine are more likely to experience caffeine-related sleep disruption.

Caffeine-Induced Anxiety Disorder

In addition to the symptom of anxiety that can be a component of caffeine intoxication, caffeine can also produce caffeine-induced anxiety disorder, a DSM-IV-TR disorder. Presentation of a caffeine-induced anxiety disorder may include symptoms of generalized anxiety, panic attacks, obsessive-compulsive disorder, or phobic disorder. Individuals who have an existing anxiety disorder, or who endorse symptoms of anxiety sensitivity, are at increased risk of experiencing anxiety symptoms in response to caffeine.

Caffeine Dependence

Substance dependence is characterized by a cluster of cognitive, behavioral, and physiological symptoms indicating that an individual is continuing to use a substance despite experiencing clinically significant substance-related problems. Caffeine dependence is recognized as a diagnosis in ICD-10, the official diagnostic system of the World Health Organization. In contrast, the DSM-IV-TR currently excludes caffeine from a diagnosis of substance dependence despite using very similar diagnostic criteria to ICD-10. A growing literature from experimental studies, clinical interviews, and survey studies indicates that some caffeine users manifest a pattern of symptoms consistent with a DSM-IV-TR diagnosis of substance dependence as applied to caffeine.

One population-based survey study of 162 randomly-selected caffeine users found that 9% of the sample endorsed three or more of four DSM-IV-TR substance dependence criteria that are thought to be most relevant of a meaningful diagnosis of caffeine dependence. The criteria and past-year incidence were: (1) Persistent desire or unsuccessful efforts to cut down or control use (56%); (2) Characteristic withdrawal syndrome or substance taken or relieve or avoid withdrawal (18%); (3) Use is continued despite a physical or psychological problem likely caused or exacerbated by the substance (14%); and (4) Tolerance defined by either a need for markedly increased amounts to achieve desired effect or markedly diminished effect with continued use of the same amount (8%).

Individuals meeting criteria for caffeine dependence vary considerably in the amount of caffeine consumed per day and in the types of caffeine-containing products that they regularly consume (e.g., coffee, soft drinks, tea). Importantly, the problems associated with caffeine dependence are not trivial. These include, but are not limited to anxiety, insomnia, stomach problems, and cardiovascular problems. One survey found that 13% of caffeine users had been advised by a physician or counselor to reduce or cut down caffeine in the last year. Fifteen percent of caffeine consumers were particularly resistant to modifying their use, indicating that they

would not change when or how much caffeine they used, no matter what they were doing or where they were.

Caffeine and Food

In addition to being consumed in its natural plant forms (e.g., coffee and tea), caffeine is also frequently consumed as an added ingredient in many popular sugar-sweetened soft drinks and energy drinks. The bitter taste profile of caffeine is often masked or obscured by the addition of sugar, fat, and other flavors in caffeine-containing foods and beverages. Beverage manufacturers have made the claim that caffeine is added to beverages in order to enhance flavor, but most individuals are unable to detect flavor differences between sugar-sweetened soft drinks with and without caffeine at the caffeine concentration found in most soft drink beverages. It is likely that caffeine-containing beverages are widely consumed because caffeine can function as a reinforcer, increase flavor preferences for caffeine-containing beverages, and produce physical dependence, which results in a substance dependence syndrome.

As described above, caffeine-dependent users may be unable to cut down or control caffeine use despite a persistent desire to do so, and may also continue to use caffeine despite medical problems associated with caffeine consumption. Thus, caffeine dependence may exacerbate adverse health outcomes associated with the consumption of caffeine-containing sugar-sweetened beverages. Sugary drinks have been associated with weight gain, obesity, and type-2 diabetes even after controlling for other factors. Of particular concern is that sugar-sweetened beverage consumption is associated with weight gain and obesity in children and may displace milk and other important nutrients in the diets of children and adolescents.

Caffeine is an added ingredient in many over-the-counter weight loss medications. Experimental studies have generally found that acute caffeine consumption is associated with increased energy expenditure, decreased food intake, and reduced ratings of hunger. There is also some evidence that caffeine increases fat oxidation. It is not clear whether any or all of these effects are due to acute caffeine effects *per se* versus reversal of caffeine withdrawal (e.g., the observed increased energy expenditure may be due to the reversal of suppressed energy expenditure in the caffeine-deprived comparison condition). Prospective longitudinal studies have shown that caffeine consumption is negatively associated with weight gain, but few well-controlled studies have examined the long-term efficacy of caffeine alone as an intervention for weight loss and weight loss maintenance.

Experimental studies have shown that the co-ingestion of caffeine and catechins enhances energy expenditure and fat oxidation more than an equivalent amount of caffeine

without added catechins. Numerous investigations have examined the combined effects of caffeine and catechins, most commonly co-ingested in green tea, on weight loss outcomes. A recent meta-analysis of studies comparing a caffeine/catechin condition with either a placebo condition or a low-dose caffeine/catechin condition found that the caffeine-catechin combination had small positive effects on weight loss and maintenance of weight loss after a period of negative energy balance. There is some evidence that effects are attenuated in habitual caffeine consumers with high daily caffeine intake (e.g., >300 mg day⁻¹). This effect may be mediated through caffeine tolerance. Several studies have found that caffeine in combination with ephedrine is efficacious for weight loss. However, ephedrine alkaloid supplements have been associated with adverse events and have been banned by the Food and Drug Administration (FDA) in the US.

Acknowledgments

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See also: Energy Expenditure: Indirect Calorimetry. Obesity: Childhood Obesity

Further Reading

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Glossary

Calbindin D Transcellular calcium transport protein involved in calcium absorption (9 kD form) and renal calcium reabsorption (28 kD form).

Calmodulin Calcium binding protein that undergoes conformational changes to affect cell signalling.

Osteopenia Defect in bone mass less severe but potentially leading to osteoporosis.

Osteoporosis Defect in bone mass and microarchitecture resulting in porous and brittle bones with normal mineral to collagen ratio.

Parathyroid hormone Eighty-four amino acid polypeptide hormone released from the parathyroid gland in response to low circulating calcium concentrations.

Rickets Disorder of bone characterized by poor mineralization.

TRPV5, TRPV6 Calcium channels for transcellular calcium transport, members of the Transient Receptor Protein superfamily.

Vitamin D Family of fat-soluble secosteroids, comprised of cholecalciferol (synthesized from dermal conversion of 7-dehydrocholesterol) and ergocalciferol (from irradiated plant sources); the form of vitamin D that reflects nutritional status is 25(OH) vitamin D, derived from the hydroxylation of cholecalciferol or ergocalciferol in the liver; another hydroxylation step in the kidney results in 1,25(OH)₂ vitamin D, or calcitriol.

Introduction

Calcium is a divalent cation with a molecular weight of 40 g mol⁻¹. It is the fifth-most abundant element in the human body, making up approximately 1000–1200 g of total body weight in an adult. Over 99% of calcium is present in bones and teeth of the body, whereas less than 1% is distributed among cells and extracellular fluid. Calcium circulates bound to albumin (40%), sulfates, phosphate and citrates (10%), or as ionized calcium (50%) in concentrations that are tightly maintained at ~10 mg dl⁻¹ (2.5 mmol l⁻¹) by sensitive homeostatic processes. A steep gradient between extracellular or cellular organelle and cytosolic calcium concentrations allows for a rapid flux of calcium into the cell to activate cell-signalling. Calcium is lost from the body daily in urine and through the gastrointestinal tract and skin, so calcium intakes must be sufficient to balance these obligate losses. The calcium economy is regulated through parathyroid hormone (PTH) and the conversion of 25(OH) vitamin D to 1,25(OH)₂ vitamin D that it promotes, resulting in increased renal reabsorption of calcium, resorption of calcium from bone, and more efficient absorption of dietary calcium when circulating calcium concentrations are low. Adequate calcium intake is critical for the achievement of peak bone mass in the first several decades of life, the retention of bone during middle adulthood, and the minimization of bone loss during the last several decades of life. Both skeletal and nonskeletal roles for calcium in human health continue to be explored.

Functions

Over 99% of the body's calcium occurs in bone where it is deposited on a cartilage matrix as hydroxyapatite (Ca₁₀(PO₄)₆(OH)₂); over 30% of bone mineral is calcium. Bone provides structural integrity and also serves as a reservoir of calcium. The remaining <1% of the body's calcium is present in blood, extracellular space, muscle, and other tissues. Maintenance of constant serum calcium concentrations at approximately 10 mg dl⁻¹ (2.5 mmol l) is critical for a number of cellular functions. Intracellular calcium is bound within organelles such as the nucleus, endoplasmic reticulum, and vesicles, with cytosolic calcium approximately 10⁴ times less concentrated than that of the extracellular fluid and organelles. Therefore, rapid changes in cytosolic calcium concentrations occur with the influx of extracellular or sequestered calcium. In the best known cell signalling pathway, intracellular calcium binds to calmodulin, resulting in conformational changes in the protein to trigger cellular events. Resumption of normal cell concentrations requires extrusion of calcium via pumps or sequestration via calcium binding proteins. Muscle contraction, nerve conduction, cell movement and differentiation, cell division, cell-to-cell communication, and secretion of hormones such as insulin are all dependent on intracellular calcium signalling. These functions are largely protected from dietary fluctuations in calcium intake until deficiency becomes extreme. Nonetheless, marginal levels of intake may not only affect skeletal health but predispose to subtle changes in cellular conditions that contribute to alterations in cellular metabolism.

Dietary Calcium Intake

Sources

Dietary intake of calcium in the US is typically 900–1200 mg (22.5–30 mmol) per day unless supplements are consumed. Approximately 73% of dietary calcium is consumed in dairy products, 9% in fruits and vegetables, 5% in grains, and the remaining 12% from all other sources. One serving of dairy products (i.e., 250 ml milk or yogurt or 40 g cheese) contains approximately 300 mg (7.5 mmol) calcium. Grains consumed in substantial amounts in bread or maize products can be important sources of calcium, although this is a less bio-available source than dairy products. Other foods high in calcium include tofu set with a calcium salt, kale, broccoli, and calcium-fortified juices and cereals. Supplement use is reported in over 40% of the US population overall, and in nearly 70% of older women. Calcium intakes in some regions of the world average less than 400 mg (10 mmol) per day. In countries or regions where calcium intake is low, milk-based products tend not to be a major component of the diet, and thus a higher percent of total calcium intake is supplied by plant products. To some extent calcium requirements may depend on other nutrients that enhance or inhibit calcium absorption or utilization.

The Institute of Medicine of the National Academy of Sciences recently reviewed guidelines for calcium and vitamin D intake and updated recommendations for US and Canadian populations (Table 1). These recommendations are based on the most current data available regarding the amount of dietary calcium i.e., required to optimize bone calcium deposition or maintenance, accounting for estimates of fractional absorption and usual losses. In contrast to previous

reports suggesting that a considerable number of people in the US were at risk of consuming inadequate amounts of calcium, more recent national data suggest that most population groups, other than adolescent girls, are generally consuming adequate amounts of calcium with reference to the new Estimated Average Requirements.

Metabolism

Balance

Calcium balance can be calculated as the difference between calcium entering the body through absorption of dietary calcium and obligate losses of calcium through urine, gastrointestinal tract, and skin. When uptake exceeds losses, an individual is in positive calcium balance, and net bone calcium accrual occurs, although bone continually remodels such that bone calcium turns over entirely every 5 or 6 years. Negative balance occurs when dietary intake is insufficient to cover losses, despite mechanisms to conserve body calcium. Typically, approximately 25% of dietary calcium is absorbed in adults and delivered to the exchangeable calcium pool, which turns over 20–30 times per day. A remarkably large amount of calcium is filtered through the kidneys, approximately 10 000 mg (250 mmol) per day, of which approximately 98% is reabsorbed, so that urinary excretion of the mineral is only 100–200 mg (2.5–5 mmol) per day. Calcium balance benefits from increased dietary intake up to a ‘threshold’, above which excess calcium is excreted rather than contributing to bone mass. These thresholds are highest during periods of growth. Differences in calcium economy also exist between racial/ethnic groups, such that the lower urinary calcium and better calcium conservation in African-Americans relative to Caucasians probably contributes to their higher bone mineral density. Aspects of calcium balance for a typical adult and pubertal female are shown in Figure 1.

Absorption

Mechanisms

Dietary calcium is complexed to food constituents such as proteins, phosphate, and oxalate, from which it needs to be released before absorption. Conditions that promote the solubility of calcium may enhance its absorption, and achlorhydria may therefore impair calcium absorption, as has been shown with some calcium supplements when consumed in a fasted state.

Calcium crosses the intestinal mucosa by both active and passive transport. The active process is saturable, transcellular, and occurs primarily in the duodenum. This pathway is upregulated during calcium deficiency and predominates when calcium intakes are low. Luminal calcium enters the enterocyte from the microvillus border of the apical plasma membrane via a calcium channel and is translocated to the basolateral membrane where it is released through another calcium channel. Calbindin D_{9k} , a calcium binding protein regulated by the hormonal form of vitamin D, $1,25(\text{OH})_2\text{D}$, transports calcium across the enterocyte following its uptake through an apical membrane channel, TRPV6, and calcium is

Table 1 Current intake recommendations for calcium

Group	Age range	EAR (mg)	RDA/AI (mg)	UL (mg)
Infants	0–6 months	–	200	1000
	6–12 months	–	260	1500
Children	1–3 years	500	700	2500
	4–8 years	800	1000	2500
Males	9–18 years	1100	1300	3000
	19–50 years	800	1000	2500
	51–70 years	800	1000	2000
	> 70 years	1000	1200	2000
Females	9–18 years	1100	1300	3000
	19–50 years	800	1000	2500
	51–70 years	1000	1200	2000
	> 70 years	1000	1200	2000
Pregnant Lactating	No changes in age-appropriate intakes advocated			

Abbreviations: EAR, estimated average requirement; RDA, recommended dietary allowance; AI, adequate intake recommendation; UL, tolerable upper intake level. RDAs are established for all age groups except infants.

Source: Reprinted with permission from the Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board, Institute of Medicine (Food and Nutrition Board, Institute of Medicine, 2011) Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press.

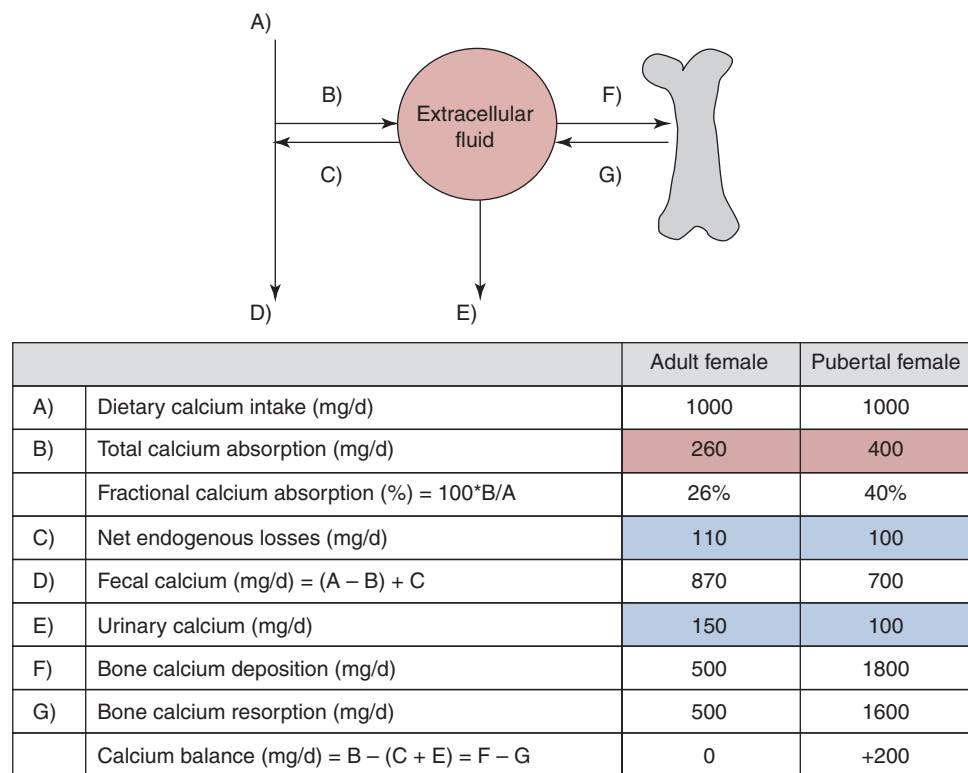


Figure 1 Hypothetical example of calcium balance on 1000 mg day^{-1} dietary calcium intakes in an adult woman and pubertal girl. Calcium uptake by the body is indicated by the red fill; losses by the blue fill. The difference between calcium intake and losses must equal the difference between bone deposition and resorption, as the extracellular fluid compartment calcium concentration remains constant, although bone turnover rates are higher during growth. Calcium absorption efficiency is higher in the girl, and endogenous losses are lower due to smaller body size. Note that the girl is not consuming the RDA for calcium, and a higher intake would favor a greater calcium balance.

extruded via an ATPase, PMCA1. Details of this process are still under review, and other transcellular processes may exist.

The passive transport pathway is nonsaturable and paracellular. It occurs throughout the small intestine and is unaffected by PTH and relatively independent of $1,25(\text{OH})_2$ vitamin D, although this metabolite has been found by some investigators to increase the permeability of the paracellular pathway. A substantial amount of calcium is absorbed by passive transport in the ileum due to the relatively slow passage of food through this section of the intestine. The amount of calcium absorbed by passive transport is proportional to the intake and the bioavailability of calcium consumed.

Although fractional calcium absorption increases in response to low calcium intake, this compensatory mechanism is incomplete, and total calcium absorption increases in relation to calcium intake. The efficiency of calcium absorption also varies throughout life. It is highest during infancy (60%) and puberty (25–35%), stable at approximately 25% in adults, and then declines with age (by approximately 2% per decade after menopause), although inter-individual variation is high. Thus, the active transport pathway is most efficient when calcium requirements are high: during infancy, adolescence, and pregnancy. Increased absorption is also observed during primary hyperparathyroidism, sarcoidosis, and estrogen and growth hormone administration. Physiological/pathological factors, which decrease intestinal calcium absorption include

low serum $1,25(\text{OH})_2$ vitamin D, chronic renal insufficiency, and hypoparathyroidism.

Bioavailability

Dietary factors that may inhibit calcium absorption include phosphate, oxalate, phytate, and fiber. Fermentation of bread during leavening reduces phytate content, making calcium more bioavailable. Fiber in fruits and vegetables can also inhibit calcium absorption; the uronic acids in hemicellulose are strong calcium binders, as is the oxalic acid present in high concentrations in foods such as spinach. Calcium bioavailability from beans is approximately half and that from spinach approximately one-tenth of the bioavailability from milk. In contrast, calcium absorption from low-oxalate vegetables, such as kale, broccoli, and collard greens, is comparable to that of milk. The difference in calcium absorption between the various forms of supplements is not large, although calcium citrate maleate may be recommended instead of the less costly calcium carbonate in individuals with achlorhydria or if supplements cannot be taken with a meal.

Dietary fat does not affect calcium absorption except in individuals with diseases that impair fat absorption (e.g., short bowel syndrome, celiac disease, and pancreatitis), where calcium may form an insoluble 'soap' with the unabsorbed fat in the alkaline lumen of the small intestine. Neither dietary

phosphorus nor a wide range of phosphorus-to-calcium ratios affect intestinal calcium absorption.

Factors that increase calcium absorption include protein (or specific amino acids, lysine, and arginine). Lactose improves calcium absorption in young infants, in whom absorption of calcium is predominantly by passive transport, but in adults lactose has little effect on the efficiency of calcium absorption. New research also suggests that certain oligosaccharides, such as inulin, may enhance absorption of calcium. It is possible that these prebiotics function through a variety of mechanisms to enhance the solubility of luminal calcium and improve the ability of the enterocyte to bind and take up calcium.

Nutrient Interactions

Calcium can inhibit iron and zinc absorption, although this interaction may not be an issue at typical levels of calcium intake. The mechanism by which this occurs remains controversial, but the inhibition probably occurs within the mucosal cells rather than in the intestinal lumen. This interaction is of concern because calcium supplements are taken by many women who may have difficulty maintaining adequate iron stores. The inhibitory effect on iron absorption is relatively unimportant when iron stores are adequate (ferritin $50\text{--}60\ \mu\text{g l}^{-1}$), but consideration should be given to monitoring the iron status of menstruating women with low iron stores who take calcium supplements. There is no inhibitory effect when calcium and iron supplements are consumed together in the absence of food, and inhibition may be less with calcium citrate.

Earlier, it was common to restrict dietary calcium in patients with a history of calcium oxalate stones. More recent studies have suggested that dietary calcium, at least below $2000\ \text{mg day}^{-1}$ ($50\ \text{mmol day}^{-1}$), is not associated with stone formation, although supplement use may contribute to the risk of calcium oxalate stones. This may be due to intraluminal effects of dietary calcium in binding and precipitating oxalates in the gastrointestinal tract.

Calcium Losses

Very large amounts of calcium are filtered by the kidney each day, but nearly all is reabsorbed throughout the nephron, such that typically, $100\text{--}240\ \text{mg}$ ($2.5\text{--}6\ \text{mmol}$) of calcium is excreted in urine daily. Most calcium is reabsorbed passively in the proximal tubule, whereas active absorption takes place in the distal convoluted tubule via a calcium channel, TRPV5, and transcellular movement via calbindin D_{28kr} , a calcium transport protein. Dietary calcium has a relatively small impact on urinary calcium in adults (e.g., only $6\text{--}8\%$ of an increased dietary calcium intake is excreted in the urine); in children, increased intake is utilized for bone accretion rather than excreted. The major food components that affect urinary calcium are protein, phosphorus, caffeine, and sodium. For each 50-g increment in dietary protein, approximately $60\ \text{mg}$ ($1.5\ \text{mmol}$) of additional calcium is lost in urine. The higher amounts of phosphorus consumed concurrently with a high-protein diet can blunt, but not eliminate, this phenomenon. Dietary phosphorus (as well as intravenously administered

phosphorus) increases PTH synthesis and subsequently stimulates renal calcium reabsorption, reducing the urinary excretion of calcium. Caffeine causes a transient loss of urinary calcium through diuresis. It has been shown repeatedly in animals and humans that dietary sodium, in the form of salt (NaCl), increases urinary calcium excretion. On average, for every $2300\ \text{mg}$ ($100\ \text{mmol}$) of sodium excreted in urine, there is an approximately $24\text{--}40\ \text{mg}$ ($0.6\text{--}1\ \text{mmol}$) loss of calcium in free-living healthy populations of various ages. Thus, when urinary calcium excretion is excessive it is often recommended to limit salt intake. Diets that produce more alkaline metabolic conditions, such as those with potassium-rich fruits and vegetables, may reduce calcium losses through the buffering effects of potassium bicarbonate on metabolic pH.

Endogenous losses of calcium occur in the gastrointestinal tract as cells are sloughed and calcium-containing pancreatic and bile secretions are released during digestion. Endogenous losses are proportional to body size and average approximately $2\ \text{mg kg}^{-1}\ \text{day}^{-1}$ in adults and $1.5\ \text{mg kg}^{-1}\ \text{day}^{-1}$ in children. Greater losses may occur under some conditions that affect gut integrity, such as protein-losing enteropathies. Because of the difficulties in measuring endogenous losses, requiring balance studies that utilize calcium stable isotope tracers, these losses are typically estimated rather than directly measured. Dermal losses of calcium occur on the order of approximately $25\ \text{mg day}^{-1}$, but may increase with sweating, and are as well typically estimated rather than measured.

Hormonal Control

The principal regulators of calcium homeostasis in humans and most terrestrial vertebrates are PTH and $1,25(\text{OH})_2$ vitamin D, the active form of the vitamin (Figure 2). PTH is a single-chain polypeptide i.e., released from the parathyroid when a decrease in the circulating calcium concentration is detected by parathyroid gland calcium-sensing receptors. It restores extracellular calcium concentrations by increasing the renal reabsorption of calcium and decreasing phosphate reabsorption, and by enhancing the renal conversion of $25(\text{OH})$ vitamin D to the active, hormonal form of the vitamin, $1,25(\text{OH})_2$ vitamin D, and by stimulating the resorption of bone to release calcium. In turn, $1,25(\text{OH})_2$ vitamin D enhances calcium absorption through the active pathway. PTH release is inhibited when serum calcium and $1,25(\text{OH})_2$ vitamin D increase or when serum phosphate is decreased. The highly regulated interactions among PTH, calcium, $1,25(\text{OH})_2$ vitamin D, and phosphate maintain blood calcium levels at remarkably constant levels despite significant changes in calcium intake or absorption, bone metabolism, or renal function. PTH regulation of circulating calcium concentration occurs on a minute-by-minute basis, and acute PTH administration leads to release of the rapidly turning over pool of calcium near the bone surface. Chronic administration of PTH increases osteoclast cell number and activity. Interestingly and paradoxically, intermittent PTH administration is anabolic, increasing formation of trabecular bone.

There are two sources of vitamin D: the diet (where it is found as the fortificant vitamin D_2 or natural D_3) or synthesis in skin during exposure to ultraviolet radiation (sunlight). The vitamin enters the circulation and is

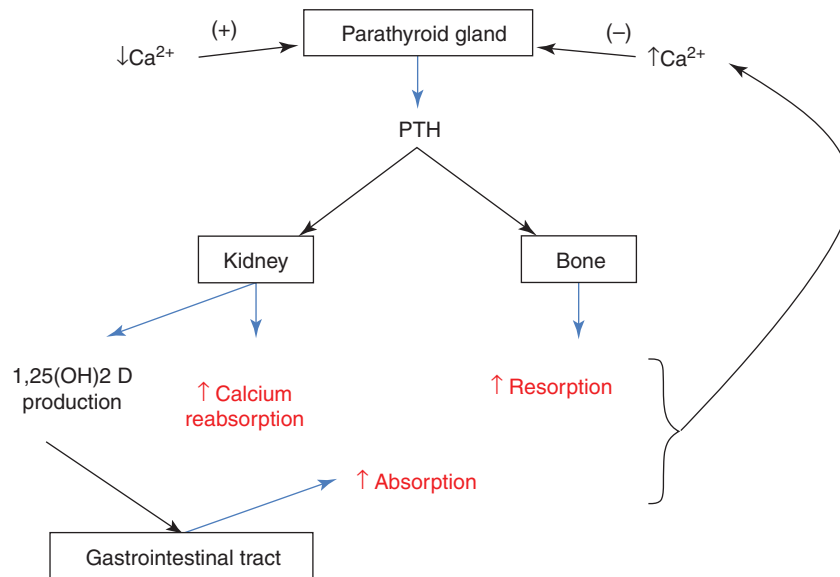


Figure 2 Regulatory system for maintaining calcium homeostasis via the PTH-vitamin D axis. Boxed items represent organs involved in calcium homeostasis; items in *italics* are consequences of action; red items are those directly resulting in increased circulating calcium. 1,25(OH)₂ vitamin D works with PTH to enhance calcium availability from bone and kidney (not depicted).

transported on a vitamin D binding protein to the liver, where it is hydroxylated to 25(OH) vitamin D – the major circulating form of the vitamin. It is hydroxylated again in the kidney to 1,25(OH)₂ vitamin D, or calcitriol, the most active metabolite of the vitamin. Vitamin D acts through nuclear receptors to enhance production of proteins involved in calcium absorption, as well as renal calcium reabsorption and bone calcium resorption in concert with PTH. Because dietary sources of vitamin D are relatively uncommon (e.g., fatty fish) and endogenous production through cutaneous exposure of 7-dehydrocholesterol to UVB radiation may be limited by latitude, season, skin tone, or availability of cutaneous precursors (i.e., in the elderly), inadequate vitamin D status has become an area of concern globally. Although debate remains regarding optimal 25(OH) vitamin D concentrations, sustained elevated PTH levels occur at low 25(OH)D concentrations, contributing to the bone loss that occurs in vitamin D deficiency. This secondary hyperparathyroidism causes increased osteoclastic activity and calcium loss from bone, and contributes to osteoporosis.

Other hormones also affect calcium metabolism, although whether they function directly or via the PTH-vitamin D pathway is not always delineated. Notably, estrogens are necessary for the maintenance of balance between bone resorption and accretion. The decrease in serum estrogen concentrations at menopause is the primary factor contributing to the elevated rate of bone resorption that occurs at this stage of life, contributing to osteoporosis. Testosterone also inhibits bone resorption, and lack of this hormone with aging can contribute to osteoporosis in men. It may work through the growth hormone/insulin-like growth factor-1 (IGF-1) axis, which stimulates cartilage formation, the formation of 1,25(OH)₂D, and intestinal calcium absorption. IGF-1 stimulates osteocalcin production, which is required for bone mineralization. Thyroid hormones stimulate bone resorption,

and calcium metabolism abnormalities occur in both hyper- and hypothyroidism. Insulin stimulates collagen production by osteoblasts and impairs the renal reabsorption of calcium. Glucocorticoids, sometimes used to treat conditions such as osteoid arthritis, inflammatory bowel disease, and asthma, inhibit both osteoclastic and osteoblastic activity, impair collagen and cartilage synthesis, and reduce calcium absorption. Excessive bone loss often occurs with glucocorticoid treatment or when excessive amounts of the hormone are secreted, such as in Cushing's disease.

Influence of Life Stages

The total body calcium content of the newborn infant is approximately 30 g (0.75 mol), with most skeletal calcium accrued during the third trimester of pregnancy. The efficiency of calcium absorption is highest during infancy (approximately 60%), and the amount absorbed from breast milk does not appear to be affected by calcium consumed in solid foods.

Childhood remains a time of bone mineral accrual, culminating in the pubertal growth spurt, when calcium absorption increases and bone calcium retention peaks at 200–300 mg (5–7.5 mmol) per day under the orchestration of growth hormone, IGF-1, and sex steroids. Rates of bone calcium deposition and net calcium accrual peak in girls just before menarche and decline thereafter; peak rates of calcium accrual occur approximately 1.5 years later in boys and bone mineralization persists later in boys as well. Forty percent of adult bone mass is acquired during pubertal growth, and while bone mass may continue to accumulate until approximately 30 years of age, relatively little is gained after 18 years of age. Thus, it is important to optimize bone mineralization during growth to ensure adequate bone mineral stores are present to defer the risk of osteoporosis later in life.

During pregnancy, a relatively small amount of calcium, approximately 625–750 mmol, is transported to the fetus. Most of this calcium is thought to be obtained through greater efficiency of maternal intestinal calcium absorption, possibly induced by increases in $1,25(\text{OH})_2\text{D}$ production. For this reason, a greater calcium intake during pregnancy is probably not required, although urinary calcium excretion also increases as plasma volume expansion increases the filtered load of the kidneys. Most studies have reported that there is no increase in intestinal calcium absorption during lactation even when dietary intake of the mineral is relatively low. Changes in biochemical markers and kinetic studies using isotopes indicate that the source of much of the calcium secreted in breast milk is the maternal skeleton, as well as more efficient renal reabsorption and subsequently lower urinary excretion of the mineral. Bone calcium is restored at the end of lactation as the infant is weaned, when ovarian function returns and menstruation resumes. At this time, intestinal calcium absorption increases, urinary calcium remains low, and bone turnover rates decline to normal levels. There is no strong evidence that lactation per se or maternal calcium intake during lactation affect later risk of osteoporosis in women. Thus, there is no strong rationale for increasing maternal calcium intake during lactation. Breast milk calcium concentration is relatively unaffected by maternal intake, and it remains stable throughout lactation.

Menopause begins a period of bone loss that extends until the end of life. It is the major contributor to higher rates of osteoporotic fractures in older women. The decrease in estrogen at menopause is associated with accelerated bone loss, particularly of the spine; in the first five postmenopausal years, approximately 15% of skeletal calcium may be lost. Calcium absorption becomes less efficient, and urinary calcium excretion increases, resulting in a declining calcium balance of approximately 30 mg day^{-1} . Hormone replacement therapy was an effective means of improving calcium balance and bone health, but is generally no longer recommended in this age group due to other potential health risks (e.g., increased risk of cardiovascular outcomes such as coronary heart disease and stroke). Calcium supplements alone have only a moderate impact in preventing postmenopausal bone loss, although calcium and vitamin D supplements are recommended to complement other therapies to treat osteoporosis.

Health Consequences of Calcium Deficiency

Skeletal

Between 60% and 80% of the variance in peak bone mass is explained by genetics, including polymorphisms in the vitamin D-receptor gene and in genes responsible for IGF-1 and collagen production. Moreover, dietary and lifestyle factors besides calcium contribute to bone health, and studies that have provided calcium supplements have often concurrently provided vitamin D, making it difficult to distinguish calcium-specific effects. Nonetheless, it is accepted that calcium intake is critical for assuring optimal bone mass and its preservation. Studies of calcium supplementation in children have shown modest improvements in skeletal mineralization, and

although these gains may be transient, with unsupplemented children acquiring similar levels of bone mineral content later, they may help prevent fractures. Studies of calcium or calcium and vitamin D supplementation during menopause have also demonstrated modest gains in bone mineral density, particularly among those whose calcium intakes are lowest.

Other skeletal effects of low calcium diets include rickets observed in equatorial regions of the world, where calcium intakes are extremely low and vitamin D status is typically adequate. Calcium deficiency occurs in children who are somewhat older than those in whom vitamin D-deficient rickets might appear (i.e., 1–3 years of age versus infancy), and is resolved with provision of calcium rather than vitamin D. It is possible that susceptibility of some children to rickets is due to efficiency of calcium utilization that might be explained by different vitamin D receptor types.

Nonskeletal

Calcium has been investigated as a protective agent in a variety of chronic diseases, including cancer, cardiovascular disease, hypertension, and metabolic syndrome. Most data come from observational studies linking dietary calcium intake to these outcomes, and associations may therefore be explained by overall healthful dietary patterns that are associated with increased calcium intake. Data from intervention trials have been less consistent in demonstrating benefits of calcium on these health outcomes, but data from high quality trials with chronic disease incidence as primary outcomes are lacking. The best evidence to date is for a protective effect of calcium on colorectal cancer. There is debate about the role of calcium on cardiovascular outcomes, with some metaanalyses showing an increased risk with increased calcium intake, whereas other data demonstrate no effect or protective effects. Calcium supplementation reduces risk of pre-eclampsia among pregnant women on low calcium intakes.

Calcium-rich diets may play a role in energy regulation and reduce the risk of obesity by maintaining low intracellular calcium concentrations in adipocytes, reducing lipogenesis. Although this metabolic pathway has been elucidated, application of calcium-rich diets has not always favored weight loss in controlled trials.

Health Consequences of Calcium Excess

Excessive calcium intakes can rarely be achieved through diet, but with increasing utilization of supplements some groups may be particularly susceptible to consequences of calcium excess. Hypercalcemia, circulating calcium above 12 mg dl^{-1} (3 mmol l^{-1}), is the acute effect of calcium excess and is associated with weight loss, fatigue, heart arrhythmias, and soft tissue calcification. In addition, it may result in hypercalcuria and affect renal function, resulting in the so-called milk-alkali syndrome. Kidney stones may result from the precipitation of calcium oxalate in renal tissue, and are more likely to be associated with supplemental rather than dietary calcium, which may be protective. An association of prostate cancer with excess calcium intake is being explored.

See also: Bioavailability. Lactation: Dietary Requirements. Pregnancy: Nutrient Requirements. **Safe Diet for Pregnancy.** Vitamin D: Physiology, Dietary Sources, and Requirements

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Contents

Carcinogenic Substances in Food

Dietary Management

Epidemiology and Associations Between Diet and Cancer

Epidemiology of Gastrointestinal Cancers Other than Colorectal Cancers

Epidemiology of Lung Cancer

Carcinogenic Substances in Food

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Glossary

Aneugen Agent that affects cell division and the mitotic spindle apparatus of a cell.

Bioassay Test of biological activity using living organisms.

Carcinogen Agent capable of initiating the development of malignant changes leading to tumors.

Epigenetic Heritable change in the activity of a gene without affecting structure.

Genome The complete set of gene(s) of an organism.

Hazard Potential of an agent to cause an adverse effect.

Mutation A detectable and heritable change in the genetic material of an organism.

Neoplasia Growth of new tissue.

Risk Probability that an (adverse) event will occur.

Introduction

Chemicals that are known or suspected to be carcinogenic to experimental animals and man are widespread throughout the environment. They occur naturally in the physical environment and are found in a very large number of higher plants, fungi, and microorganisms, many of which are part of the human diet. Some carcinogens have also been introduced into the human diet as a result of traditional cooking and preserving practices. Although carcinogens act through a wide variety of mechanisms, a substantial number have a common mechanism of action in that they react with the genetic material of the body, DNA. These so-called genotoxic carcinogens generally require metabolic activation by the 'host' animal to express their carcinogenicity. Although substantial efforts are being made to develop short-term, non-animal tests to predict the carcinogenicity of chemicals, animal bioassays remain the only reliable method for establishing the potential of a chemical to be a carcinogen, and form the basis of current approaches for the control of potentially carcinogenic chemicals in the human diet.

naturally occurring. Although only a very small proportion (perhaps less than 0.01%) of these chemicals have been tested for carcinogenic potential in laboratory studies, a high proportion (as high as 50% in some evaluations) have been found to be positive. Therefore, even allowing for the imperfect selection and testing process, it is likely that there are a very large number of naturally occurring carcinogenic chemicals in the universe of chemicals, and therefore in the food we eat.

Naturally occurring substances identified as carcinogens in animals and humans by the range of approaches available for this purpose include inorganic compounds, organometallic compounds, and both simple and complex organic chemicals (Table 1). These materials are present in the environment either as naturally occurring minerals or as a result of natural processes acting in the environment such as combustion, radioactive decay, or biodegradation of plant materials to oils. They are also widespread throughout the plant kingdom in both edible and nonedible plants and in many fungi and unicellular organisms.

Naturally Occurring Carcinogens

It has been estimated that the total number of known chemicals exceeds 7 million, and that the great majority are

Inorganic Chemical Carcinogens

Many metallic elements are present as contaminants in food, being derived from a range of sources including the water used in food processing, soil residues, packaging, and cooking

Table 1 Examples of naturally occurring carcinogens

<i>Inorganic chemicals</i>
Arsenic; beryllium; chromium; cobalt; cadmium; lead; manganese; nickel
Polonium; radium; uranium; radon (gas)
Asbestos; silica (glassfiber); talc
<i>Organic chemicals – complex mixtures</i>
Mineral oils; shale oil; soot; wood shaving/dust
<i>Organic chemicals in higher plants</i>
Cycasin (betel nuts); saffrole (sassafras); pyrrolizidine alkaloids (Boraginaceae, Compositae); ptaquilosides (bracken); nitrosoalkaloids (tobacco)
<i>Organic chemicals in lower order plants and microorganisms</i>
Agaricine (mushrooms); aflatoxin, ochratoxin, sterigmatocystin (<i>Aspergillus</i> spp. and others); mitomycin, streptozotocin, daunomycin, actinomycin (<i>Streptomyces</i> spp.)

equipment. A number of metals and some of their salts have been shown to be carcinogenic in animals and humans, particularly to the lungs. These include arsenic, beryllium, cadmium, chromium, and nickel. Little is known about the mechanism by which metals cause cancer, although evidence is emerging that some metal ions affect the fidelity of an enzyme involved in the biosynthesis of DNA resulting in abnormal DNA being produced. A number of naturally occurring radioactive elements are also carcinogenic, particularly to the lungs. These include uranium, radium, and radon gas and may act by damaging DNA directly or by increasing oxidative damage as a result of an increase in reactive radical species. In addition, some naturally occurring minerals such as asbestos, silica, and talc are known to be carcinogenic to animals and humans under some circumstances, probably acting by activating macrophages to generate damaging active oxygen species.

Organic Chemicals – Complex Natural Mixtures

The earliest association made between the development of cancer in humans and exposure to an essentially natural rather than man-made chemical was that between scrotal (skin) cancer and soot, by Percival Pott in 1775. However, the specific chemical(s) responsible (polycyclic aromatic hydrocarbons such as benzo(a)pyrene and 7,12-dimethylbenzanthracene) were not identified until more than a century later. Since then, a number of other naturally occurring materials have been shown to be carcinogenic. These have included mineral oils, shale oils, and wood dust/shavings, the oils being carcinogenic to the skin and wood dust to the nasal cavity. Inadvertent ingestion of small amounts of such materials with food may be difficult to avoid.

Organic Chemicals in Higher Plants

Although the acute toxicity of many plant species has been known since written records first appeared, only comparatively recently has the carcinogenicity of plant-derived products been recognized. The list of confirmed animal

Table 2 Some naturally occurring carcinogenic plant pesticides (a) and their sources (b)

(a) Chemical class	Examples
Aldehyde	Crotonaldehyde; benzaldehyde; hexanal
Hydrazine/hydrazone	<i>N</i> -methyl- <i>N</i> -formylhydrazine; methylhydrazine; pentanal methylformylhydrazone
Alcohol	Methylbenzyl alcohol; catechol
Ester	Ethyl acrylate; benzyl acetate
Simple heterocycles	Coumarin; hydroquinone; saffrole; sesamol; 8-methoxypsoralen
Polyphenols	Quercetin
(b) Generic source	
Fruit	Apple; apricot; cherry; grapefruit; lemon; melon; peach pear; pineapple
Root vegetables	Carrot; onion; parsnip; radish; turnip
Brassica	Broccoli; Brussel sprout; cabbage
Herbs	Coriander; dill; fennel; mint; sage; tarragon
Spices	Allspice; caraway; cardamom; nutmeg; paprika; turmeric

carcinogens present in plants is still relatively small, and few, if any, are confirmed or suspected human carcinogens. However, developments in analytical chemistry will allow an increasingly detailed inventory to be made of chemicals in plants, which will undoubtedly result in the discovery of many more carcinogens in our foodstuffs. The recent identification of over 1000 chemicals in coffee beans and the observation that whereas only 3% of the chemicals had been tested for carcinogenicity, nearly 70% of these tested positive, is a clear pointer to future directions. Although it can be argued that the majority of these compounds are present at very low levels in plants, and so the hazard to man from any individual compound may be small, reliable methods for assessing both hazard and risk of low-level exposure are not well developed. In addition, methods for assessing the hazard from complex mixtures of chemicals are also poorly developed, resulting in additional uncertainty in evaluating the hazard posed by natural materials. The identified chemical carcinogens in plants tend to be secondary metabolites, often present as part of the plant's natural defense mechanism against predation (i.e., natural pesticides), and as such are widespread in fruit, vegetables, herbs, and spices (see Table 2).

One of the first classes of toxic compounds in plants to be identified were the pyrrolizidine alkaloids from the genus *Senecio*. Subsequently, more than 200 related compounds have been isolated from numerous families and species, many of which are potent liver toxins and liver carcinogens. Other classes of alkaloids found in the plant kingdom include derivatives of the nicotine alkaloids, such as *N*-nitroso-nornicotine, which are present in tobacco leaves and are known to be carcinogenic to animals. Tobacco leaves also contain a range of compounds that have been shown to potentiate the carcinogenic effect of the alkaloids present.

Many other classes of carcinogenic plant products have been identified. These include glycosides of azoxy alcohols such as cycasin from betel nuts, a colon carcinogen; isoprene glycosides such as ptaquiloside found in bracken, a liver carcinogen; and phenolic alkylbenzenes such as saffrole present in many herbs and vegetables, which are also principally liver

carcinogens. Other phenolic compounds including flavanoids such as quercetin, rutin, and kaemferol and tannins such as trapain and brevifolin are potent mutagens but evidence for their carcinogenicity is lacking. In fact many of these compounds have been shown to exert anti-carcinogenic effects.

Organic Chemical Carcinogens in Other Edible Plants and in Microorganisms

Chemical carcinogens are also found in a wide range of lower plants, such as fungi, and in microorganisms. Simple and complex hydrazines are found in many species of mushroom and have been shown to produce tumors in many tissues of experimental animals. Mycotoxins, such as aflatoxin B₁ and the related polynuclear compound produced by *Aspergillus* species are some of the most potent carcinogens known, being active at dose levels in the nanogram per kilogram range. Human exposure to such compounds occurs when cereal crops and nuts are stored in humid conditions, as they are in many parts of equatorial Africa and China. Aflatoxin B₁ is one of the few established human carcinogens found in the plant kingdom. Other carcinogenic compounds produced as natural products include the antibiotics adriamycin and daunomycin and the antineoplastic agent streptozotocin isolated from microorganisms of the genus *Streptomyces*.

Carcinogens Produced by Food Processing

Despite the widespread occurrence of potentially carcinogenic chemicals in the plant kingdom, most foodstuffs contain only low levels of these chemicals. However, it has now been recognized that a number of processes used in food preparation/processing can introduce significant amounts of carcinogens into the food or the local environment. The most widely studied of these processes are preservation of meats and fish by salting or smoking, grilling or broiling of meats, and cooking in vegetable oils.

Traditional methods for preserving meat and fish involve either salting or smoking. Epidemiological evidence has been found for an association between an increased incidence of cancer of the mouth and pharynx and intake of salted meat and fish. It seems likely that a reaction between sodium nitrate or nitrite used for preserving the meat and alkylamides present in the meat results in the formation of *N*-nitrosamines and nitrosamides. These compounds have been shown to be potent carcinogens in animal experiments to the mouth, pharynx, and other sites. Levels of nitrosamines in cured meats and fish can be as high as 100–200 ppb (parts per billion) for the simple alkyl nitrosamines and between 10 and 100 ppb for volatile heterocyclic nitrosamines. Although dose levels required to induce tumor formation in animal studies are substantially higher than those likely to be ingested by man, there is a concern that the presence of nitrosamines in food presents a significant hazard to man.

Preservation of meats and fish by smoking has also been shown to introduce chemicals known to be carcinogenic to animals, particularly polycyclic aromatic hydrocarbons

(PAHs), although direct evidence for an association between an increased incidence of human cancers and consumption of smoked meat and fish is lacking.

The frying or grilling of meats and fish has been found to generate significant quantities of heterocyclic nitrogenous compounds derived from amino acids present in foods. These so-called cooked food mutagens include 2-amino-3-methylimidazo[4,5-f]quinoline (IQ), 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline (methyl-IQ_x), 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP), 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1), and 2-aminodipyrido[1,2-a:3',2-d]imidazole (Glu-P-2). They are some of the most potent bacterial mutagens known and have been shown to induce a wide range of tumors in animals. Levels as high as 500 ppb have been found in grilled chicken and it has been suggested that they may be implicated in the induction of colon and breast cancer in humans. PAHs can also be generated by the grilling of meat and fish and both carcinogenic and non-carcinogenic compounds have been identified. Levels of one particular PAH in foods, the carcinogen benzo(a)pyrene, have been reported to vary from < 1 ppb in grain to more than 30 ppb in singed meat.

Heating oil to cook foods has also been found to generate a range of carcinogenic chemicals, including PAHs. However, many of the compounds produced are volatile and may therefore represent more of a hazard to the cook than to the food consumer. Thus, cooking with unrefined rapeseed or soya bean oil, which contain significant levels of the polyunsaturated fatty acid linolenic acid, has been shown to result in the release of aldehydes including formaldehyde, acetaldehyde, and acrolein, hydrocarbons including 1,3-butadiene and benzene, and other chemicals. Many of these compounds are mutagenic to bacteria and carcinogenic in animals, and in areas of the world where such cooking practices are common (e.g., China), the incidence of lung cancer in the exposed population is high. The International Agency for Research in Cancer considers that emissions from high temperature frying are 'probably carcinogenic to humans'.

Mechanisms of Carcinogenicity

It is well established that cancer is a multi-step process, and that chemical carcinogens can induce neoplasia by a wide range of mechanisms involving either interaction with the hereditary material of the organism or interference with one of the many cellular control systems. The former compounds, known as genotoxic carcinogens, interact directly with DNA, resulting in a permanent heritable change to a cell following replication (i.e., an altered genotype). In contrast, nongenotoxic (so-called epigenetic) carcinogens do not interact directly with DNA but cause cancer by other mechanisms.

Chemicals that react with DNA are invariably electrophiles (i.e., they possess one or more electron-deficient centers in the molecule) that target the nucleophilic (electron-rich) sites in the DNA. The electrophilic center may be present in the molecule itself (activation independent) as in β -propiolactone, dimethyl sulfate, and α,β -unsaturated aldehydes or be generated following metabolism (activation dependent) in the target species.

Examples of classes of compounds that are converted to reactive electrophiles by oxidative metabolism include nitrosamines, chlorinated alkanes, hydrazines, and polycyclic aromatic hydrocarbons. Because of the inherent reactivity of these species, they react not only with DNA, but also with other cellular macromolecules such as RNA and proteins. These reactions protect the cell against the carcinogenicity of the chemical by reducing the amount of electrophile available to react with DNA, but may lead to other forms of damage and ultimately cell death.

The enzyme system considered to be mainly involved in the activation of chemicals to carcinogenic species is the so-called mixed function oxidase system. This enzyme complex is centered on cytochrome P-450 and is present in most, if not all, of the organs of the body. The enzyme system consists of a very large family of related isoenzymes of differing substrate specificity and has a widespread distribution in the animal kingdom. Early work with this enzyme system suggested that only certain isoenzymes were responsible for the activation of carcinogens, although it is now clear that different isoenzymes may activate the same compound in different species.

Most chemical carcinogens appear to be substrates of one particular isoenzyme called CYP1A1. Molecular modeling has shown that only relatively flat (planar) molecules are oxygenated by this cytochrome. Common carcinogens activated by this isoenzyme include PAHs, aflatoxin, and 9-hydroxyellipticine, whereas the related isoenzyme CYP1A2 activates arylamines and amides such as 2-acetylaminofluorene and the cooked food mutagens. Other subfamilies of cytochromes involved in activating carcinogens include CYP2E1, which is known to act on a wide range of small molecules, such as dialkyl nitrosamines, urethane, vinyl monomers and haloalkanes, and CYP3A, which also activates PAHs, aflatoxins, and cooked food mutagens.

The chemistry of the activation process varies with the type of carcinogen. The oxidation of aflatoxin B₁, for example, results in the formation of the 8,9-epoxide in a single step whereas the activation of PAHs, such as benzo(a)pyrene, is a multi-step process involving an epoxide that is converted to a diol by epoxide hydrolase, which is then converted to the proximate carcinogenic species, a diol-epoxide. Activation of arylamines and amides to DNA reactive species, in contrast, frequently involves an initial oxidation step to an *N*-hydroxy derivative, which is then further metabolized to a highly reactive *N*-O-ester. This latter reaction is catalyzed by a transferase enzyme, usually sulfotransferase or acetyltransferase for arylamines and glucuronotransferase for arylamides. Other oxidative reactions result in the formation of unstable compounds that decompose spontaneously to the ultimate carcinogenic species. Thus, simple nitrosamines are oxidized by CYP2E1 to an α -hydroxy intermediate, which breaks down to the electrophilic alkyl diazonium ion.

Enzyme systems other than the mixed function oxidase system may also be involved in the metabolic activation of carcinogens. Thus, for aflatoxins, there is an evidence that prostaglandin H synthetase can activate this group of compounds and for arylamines, oxidation may be carried out by prostaglandin peroxidase, myeloperoxidase, or by flavin-containing monooxygenases.

The direct metabolic activation of compounds to carcinogenic species by phase II metabolism, a process normally associated with detoxification, can also occur. Thus safrole and related compounds are converted to their sulfate esters, the ultimate carcinogenic species by the phase II enzyme, sulfotransferase.

Metabolic Activation of Epigenetic Carcinogens

Since there is no common mechanism describing the action of epigenetic carcinogens, making predictions as to the likely carcinogenic potential of these chemicals is extremely challenging and generalizations concerning the effect of metabolism on the activity of chemicals acting by a nongenotoxic mechanism are not possible. The activity of a number of epigenetic carcinogens is reduced as a result of metabolic activation, although in the case of one group of epigenetic carcinogens that produce renal tumors in the rat by binding to and preventing the degradation of a specific kidney protein, α -2-microglobulin, metabolic activation is required for carcinogenic activity. Compounds acting by this mechanism include isophorone and D-limonene, which are present naturally in many fruits. Similarly, a wide range of structurally diverse chemicals induce liver tumors in rodents due to their ability to induce the proliferation of hepatic peroxisomes. Food contaminants such as phthalate diesters, which leach out of packaging materials, fall into this category, although no naturally occurring food chemical has yet been found to be a peroxisome proliferator. Some examples of nongenotoxic mechanisms of carcinogenesis are shown in Table 3.

Carcinogenicity Tests

Animal Bioassays

As the mechanism of carcinogenesis in both humans and animals is not well understood, the only acceptable procedure for determining whether a chemical is likely to be a carcinogen is the examination of experimental animals exposed to the suspect material under carefully controlled conditions. This procedure relies on the assumption that animals will behave in essentially the same way as humans to carcinogen exposure, i.e., the mechanism of tumor induction will be similar in both

Table 3 Some examples of nongenotoxic mechanisms of carcinogenesis

<i>Mechanism</i>	<i>Examples of chemical classes</i>
Promotion	Phorbol esters; barbiturates; chlorinated hydrocarbons
Receptor-mediated (e.g., peroxisome proliferation)	Phthalate diesters; hypolipidemic drugs; chlorinated herbicides
Endocrine modulation	Androgens and estrogens; antithyroid agents (e.g., 17 β -estradiol)
Immunosuppression	E.g., Cyclosporine
Tissue specific toxicity	Metals (e.g., arsenic, beryllium)
Cytotoxicity	Metal chelators; branched chain hydrocarbons

animals and humans. Mechanistically based, short-term tests for carcinogenicity prediction not involving experimental animals are still a distant and elusive goal.

The basic approach for carcinogenicity testing involves administering the test material to two suitable animal species for a considerable proportion of their natural lifespan. Because of their small size and relatively short life expectancy, the rat and mouse are the species of choice, although the hamster is occasionally used. In the US, inbred strains of animals are widely used (the F344 rat and the B6C3F₁ hybrid mouse), although outbred strains are commonly used in Europe. To examine the carcinogenic potential of food components, the test substance is usually given in the diet, although in some circumstances administration may be in the drinking water or by gavage. The study continues until a certain proportion in one or other of the treatment groups has died or has been killed in a moribund state. As a minimum, 50 animals are allocated at random to each of the experimental groups, allowing a statistically significant carcinogenic effect to be detected if five animals in a test group develop tumors and no animals in the control group do. During the study, the animal's clinical state is regularly monitored and at the end of the study a complete necropsy is performed on all surviving animals. Any tumors found are classified as either neoplastic or non-neoplastic and some attempt is made to determine whether any tumors seen were the cause of the (early) death of the animal (fatal tumors) or were unrelated to the death (incidental tumors). The procedures of these bioassays are conducted under rigorous conditions defined by the Code of Good Laboratory Practice (GLP).

Tests are essentially of two types: The first, used widely under the National Toxicology Program (NTP) in the US, is designed to examine the ability of the test material to induce cancer in the species used; the second, is aimed at determining the cancer incidence in respect of dose – a classical dose-response study. The former requires a few treatment groups, including a relatively high-dose group to maximize the chance of detecting a carcinogenic effect, whereas the latter requires a wide range of dose groups to define accurately the dose-response relationship.

The analysis of a carcinogenicity bioassay is aimed at determining whether the administration of the test chemical has resulted in an increase in the incidence of tumors at one or more sites compared with the normal background level. In order to accomplish this analysis, two major confounding factors may have to be taken into consideration. The first is the effect of differences in mortality rates between the control and treated groups and the second is the effect of differences in food intake and its consequence on body weight. Both factors can substantially alter the tumor pattern observed in different groups. Early deaths may prevent the animals reaching tumor-bearing age, and reduced food intake and the associated reduction in body weight may result in a considerable reduction in tumor incidence.

The interpretation of the results of a bioassay are complex but most authorities work to the 'weight of evidence' principle. This evidence is taken in the light of the 'adequacy' of the bioassay, which is dependent on some of the factors previously discussed. Strong evidence for the compound being a genotoxic carcinogen would be increased malignant tumor

incidence in two species, with tumors at multiple sites showing a clear dose-response relationship. Rare or unusual tumors at a site would be given added weight. Equivocal evidence may result from a statistically marginal result or only an increase in commonly occurring benign tumors. Tumor development in only one species and in association with species-specific toxicity is a characteristic of nongenotoxic (or epigenetic) carcinogens. Sometimes, problems associated with such findings may be clarified by further mechanistic studies or by reference to historical data. When the data from bioassays are considered in human risk assessment, other factors must clearly also be taken into consideration. These may include evidence of genotoxicity in short-term tests and data on metabolism and potential human exposure. Furthermore, a measure of risk at doses substantially below the bioassay dose may be needed. This may require an extrapolation using mathematical models. As yet no general agreement has been reached as to the most appropriate method, and so the calculated risk given by different methods may vary considerably. Thus, the final assessment may be made on quite pragmatic grounds, in which the experience and expertise of a number of individuals are drawn on to reach a consensus opinion.

Short-term Predictive Tests

A large number of test systems have been developed to detect damage to the genetic material of cells in an attempt to predict carcinogenic potential and thereby reduce the reliance on animal tests. *In vitro* assays for detecting genotoxicity (i.e., damage to the cells genome, which may be directly or indirectly heritable) include tests to detect gene mutation using bacterial or mammalian cells, and so-called indicator tests that detect mechanistic changes associated with the formation of mutations, such as the binding of foreign molecules to the DNA bases. *In vitro* tests are backed up by short-term *in vivo* tests to confirm that the effects seen *in vitro* are realised in the whole animal. These tests are usually undertaken in rats, mice (ordinary and transgenic), and hamsters, but can also be in fruit flies (Table 4). Since many of the cell systems used are unable to activate metabolically the majority of test chemicals, an exogenous mammalian metabolizing system, the so-called S-9 mix, is incorporated into the assay. Chromosome damage seen in such tests includes chromosome and chromatid gaps and breaks, rings, fragments, dicentric, translocations, and inversions. A short-term *in vivo* assay measuring unscheduled DNA synthesis (UDS) in rat liver or gut is recommended by most regulatory authorities if there is a positive response in any *in vitro* assay and a negative response in an *in vivo* cytogenetics assay. Other test methods and end points are under consideration by regulatory authorities as indicators of genotoxic potential including the Comet assay for assessing DNA damage, and aneuploidy, the change in chromosome number resulting from damage to the cellular architecture (spindle) controlling chromosome replication.

The last two decades have seen extensive efforts to determine whether short-term tests are suitable for predicting carcinogenic potential. The early validation studies suggested good predictability, with correct identification of over 90% of carcinogens (high sensitivity) and over 90% of

Table 4 Short-term test systems for predicting carcinogenic potential

<i>Test system</i>	<i>Cell used</i>	<i>End point</i>
Bacterial mutation	<i>Salmonella typhimurium</i> TA strains <i>Escherichia coli</i> WP2	Reversion to histidine independence
Mammalian gene mutation	Chinese hamster lung (V79) Chinese hamster ovary (CHO) Mouse lymphoma (L5178Y) Human transformed lymphoblastoid (TK6)	Loss of HPRT, TK, or Na ⁺ /K ⁺ ATPase expression
Chromosome aberration <i>in vitro</i>	Chinese hamster fibroblast (CHL) Chinese hamster ovary (CHO) Human peripheral blood lymphocytes(PBL)	Chromosome/chromatid aberration (gaps, breaks, deletions)
Chromosome damage <i>in vivo</i>	Bone marrow erythrocytes (mouse)	Micronuclei induction
Heritable damage <i>in vivo</i>	Rodent germ cells	Dominant/lethal mutations; heritable translocations, etc.

HPRT, hypoxanthine phosphoribosyl transferase; TK, thymidine kinase.

noncarcinogens (high specificity). In later evaluations, a much lower figure (60%) was obtained. However, when carcinogens known to react by nongenotoxic mechanisms (e.g., hormones or peroxisome proliferators) were excluded, the predictability was improved suggesting that short-term tests are suitable for detecting those carcinogens that act by a genotoxic mechanism.

Although many regulatory authorities have guidelines for carcinogenicity evaluation, which include short-term tests, they all still require animal studies as the ultimate test for carcinogenicity. However, the use made of short-term tests varies. In the US, the Food and Drugs Administration (FDA) recommends a battery of short-term tests for all 'additives' for which cumulative dietary intake is expected to exceed 1.5 µg per person per day to assist in the interpretation of animal feeding studies. Some expert bodies, such as The International Agency for Research in Cancer, use short-term tests as an adjunct to animal carcinogenicity studies in their evaluation process, giving added weighting in their assessment of likely human hazard to an animal carcinogen that is also positive in short-term tests.

However, until a consensus can be reached as to what a positive or negative result in an animal feeding study means in terms of whether the compound may or may not be a human carcinogen, the further development of better (faster/cheaper) short-term tests may be a futile exercise.

Monitoring and Control of Hazards

The complex mixture of chemicals that constitute food, together with the uncertainty of the specific role of the various components in the diet, has made the control of potential carcinogens in food difficult. In particular, the realization that animal carcinogens, as identified by standard animal bioassays, are widely distributed in the general environment, including food, has made control by total elimination impossible.

Control of toxic agents in food, particularly contaminants and additives has been achieved by examining their hazard in animal studies. Thus, the establishment of a no-observable-adverse-effect-levels (NOAEL) is followed by the setting of an

allowable daily intake (ADI) through extrapolation based on the relative sensitivity of animals and humans to toxic events. This extrapolation may also take into consideration other properties of the chemical concerned, such as genotoxic potential. For genotoxic carcinogens, however, it is generally considered that there is no 'no-effect-level' and therefore acceptable intake is based on estimation of likely risk. A maximum risk of between 10⁻⁵ and 10⁻⁶ cancers in a lifetime is considered as an acceptable risk by most authorities, particularly those in the US (in California 10⁻⁵ is called the 'no-significant-risk-level'), and acceptable exposure estimates are determined by extrapolation from animal data. In California the extrapolation (scaling) factor used to estimate human potency from rat potency is 5.5. For nongenotoxic carcinogens (and for some genotoxic carcinogens, particularly those that act as aneugens), no effect levels are accepted, because the carcinogenic response is the result of a prior toxic event for which a no-effect-level can be determined. For additional safety, an arbitrary factor of 100 was applied to the NOAEL, to allow for interspecies variation (× 10) and interindividual variability (× 10). More recently, the two factors have been subdivided into variable factors (pharmacokinetic and pharmacodynamic) to reflect increased understanding of the mechanisms underlying the development of toxicity and allow for factors associated with special groups such as infants and children. It must be said that the scientific basis to support either of these approaches (acceptable risk or no effect levels) is quite limited as even for the best documented cases, the mechanism of the carcinogenic effect is poorly understood.

The unequivocal identification of human carcinogens is difficult because direct experimental approaches are precluded. Thus, epidemiological data involving both prospective and retrospective studies, and using case controls in certain investigations, has to be employed. These techniques have limited applications to diet associated carcinogenesis and have proved most useful in identifying specific carcinogens in the work place or those used as therapeutic agents. The specific problem in identifying dietary carcinogens relate to the complexity of diet, the difficulty in identifying specific components, and the sensitivity of the epidemiological methods themselves. It would seem likely that epidemiological data will only be able to link specific chemical carcinogens in food with

a carcinogenic effect in a few favorable circumstances, because such chemicals are likely to be present at low levels and induce only a small increase in tumor incidence over background levels. One such example was the identification of a carcinogenic hydrazone in the mushroom *Gyromitra esculenta*, as a result of an epidemiological study in Finland. Such methods have also indicated the relative importance of 'life style' factors in carcinogenesis: In particular, associations have been made between lack of dietary fiber and colon cancer, between a low intake of fresh fruit and vegetables and stomach cancer, and between excess dietary fat and colon and breast cancer, although the specific chemicals responsible have not been identified with any certainty.

Most of the activity aimed at controlling carcinogens in food has been directed at preventing addition of potentially carcinogenic substances to the existing background level of natural carcinogens. This has been tackled through the application of laws governing the adulteration of food, the first of which were enacted in the mid nineteenth century in the UK. The current UK legislation is the 1990 Food Safety Act, governing the nature and quality of food and its nutritive value. This Act, like its forerunner, the 1955 Food and Drug Act, requires that the constituents of food should not be injurious to health. Thus, while there is no specific requirement for carcinogenicity testing in the current act, consideration is given to all available data, including the result of mutagenicity tests and long-term tests in animals.

The position in the US up to 1958 was similar to that in the UK. Food was considered adulterated if injury could arise from its use. Legislation was based on traditional food, added substances, and unavoidable added substances (contaminants). For added substances, listed in an inventory of over 3000 chemicals and often referred to as 'Everything Added to Food in the US' (EAFUS), the food was considered adulterated if the added substance could render the food injurious to health; for unavoidably added substances, a balance was applied between the essential nature of the food material and the degree of contamination. These strictures applied to both carcinogenic and noncarcinogenic toxicants. In 1958 a change in emphasis was introduced through the Food Additives Amendment. This established a licensing scheme for substances deliberately added to foods or for substances that could migrate into food, but excluded materials that were generally, through usage, regarded as safe (GRAS). For licensing purposes, the material has to be shown to be 'safe' for its intended use, although in theory at least the GRAS substance could be a carcinogen.

In 1958 the Delaney Clause was enacted; this required that if there was evidence of carcinogenicity in any test system, the material should be prohibited from food usage. Improved analytical techniques have shown that many foods contain both unintentionally added and natural carcinogens, such as polynuclear aromatic hydrocarbons, nitrosamines, mycotoxins, and arylamines and no form of regulation could

control these materials. Furthermore, bulk components of food may themselves play an important role in the development of carcinogenesis. The recognition that the exclusion of all potentially carcinogenic additives (under the Delaney Clause) is a practical impossibility has given way to the concept of 'safe' tolerance, and that 'safe levels' may be set by appropriate, conservative risk assessment in which an 'insignificant lifetime risk' of developing tumors of, for example, 10^{-6} is considered acceptable.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Fish and Seafood: Nutritional Value. Food Safety: Mycotoxins – Occurrence and Toxic Effects. Meat, Poultry, and Meat Products: Nutritional Value. Salt: Epidemiology

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Dietary Management

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Glossary

Anorexia Loss of appetite. In cancer patients it is frequently secondary to chemotherapy, pain medication, or tumor factors.

Cachexia Severe weight loss associated with disease states (catabolic state), frequent in certain terminal cancer patients.

Dysphagia Difficulty to swallow. May be related to tumor location or as a consequence of surgery in the esophagus.

Enteral feeding Refers to specialized form of feeding in which food (usually a special formula) is delivered directly into the intestine *via* a tube.

Nutritional Support

Many cancer patients will require some form of nutritional support during the course of their illness (Table 1). When patients have an eating difficulty, the first course of action is to assess their oral intake. If patients are able to eat, then they should be given appropriate advice to maximize their oral intake. If patients are unable to swallow enough nourishment to maintain their weight, an enteral tube feed should be considered. The type of tube placed will depend on the following:

- (1) The anticipated length of time the feed will be required.
- (2) The physical state of the patient; for example, a nasogastric tube or percutaneous endoscopically placed gastrostomy tube may not be suitable for patients with complete esophageal obstruction. A jejunostomy tube may be preferred following upper gastrointestinal tract surgery.
- (3) The wishes of the patient concerning the physical appearance of different tubes and the invasiveness of the procedure required to place them.

Table 1 Methods of nutritional support

Method	Route
Oral feeding	Oral feeding can be facilitated by Altering the consistency or timing of food or drink Fortifying food and drinks with protein and energy Altering the flavoring added to food Using sip feeds and dietary supplements
Enteral tube feeding	Nasogastric or nasojejunal tube gastrostomy Percutaneous endoscopically guided Gastrostomy Radiologically inserted gastrostomy Percutaneous gastrostomy with a jejunal extension Jejunostomy
Parenteral nutrition	Central line Peripheral line

Numerous brands of enteral feeds are available. Most cancer patients will require complete, whole protein feeds providing $4\text{--}6 \text{ kJ ml}^{-1}$ ($1\text{--}1.5 \text{ kcal ml}^{-1}$). Only in cases of severe malabsorption, gastrointestinal fistula, or pancreatic insufficiency may elemental, peptide, or low-fat feeds be necessary. The choice of feeding regimen will depend on the patient's mobility and activity during the day and on the volume of feed tolerated. It may be administered in the following ways: pump feeding overnight or during the day; gravity feeding, which usually provides a faster rate of feeding that does not require the precision of a pump; and bolus feeding. Parenteral nutrition is required where the gastrointestinal tract cannot be used, such as in patients with complete bowel obstruction or severe malabsorption.

Practical Management of Eating Difficulties

Anorexia

Anorexia (loss of appetite) is often associated with other eating difficulties, such as nausea, taste changes, and constipation, and addressing these problems may improve the patient's appetite. Pain may also contribute to anorexia, and regular analgesia for pain may, in turn, help improve appetite, as may dietary alterations (Table 2). For patients who have severe anorexia, an appetite stimulant should be considered, such as dexamethasone, medroxyprogesterone acetate, or megestrol acetate.

Table 2 Dietary management of anorexia

Give small, frequent meals and snacks in preference to three meals daily.
Serve food on a small plate.
Ensure food looks appetizing.
Encourage any food the person prefers, even if it is all of one type (e.g., puddings).
Distract from eating (e.g., by conversation, watching television, or listening to music).
Give an alcoholic drink to be sipped before meals or with food.

Taste Changes

Cancer patients may suffer from lack of taste or 'taste blindness,' they may find that foods taste metallic or excessively salty or sweet, or they may find that foods taste abnormal. Depending on the taste change experienced, it is often worth excluding certain foods from the diet or using certain flavorings to try to stimulate the taste buds (Table 3).

Nausea and Vomiting

Nausea and vomiting must be controlled with antiemetic drugs. Some dietary suggestions may help patients with food choice when they are feeling nauseous (Table 4).

Dysphagia

Dysphagia (difficulty swallowing) may occur with solid food, and with semisolid foods, such as porridge, or liquids. For the person who cannot manage solid food but is able to eat semisolids, altering the consistency of the food may be the only dietary change needed, encouraging food with extra sauce, soft puddings, and nourishing drinks. For the patient who is only able to swallow fluids, close attention must be paid to their intake and dietary supplements are likely to be necessary. Some people who can only manage liquids choose to liquidize their food; this dilutes the nutrients, so meals should be fortified with butter, cream, glucose,

cheese, etc. to add protein and energy. If there is complete dysphagia to both solids and liquids, feeding by an enteral tube should be considered. In some instances, people can swallow solid food but aspirate liquids. Patients should undergo a complete assessment from a speech and language therapist to ascertain which textures are safe to swallow. It may be that thickened liquids such as milk shakes or those thickened with a commercial thickener are suitable, whereas thin liquids, such as tea and water, are aspirated. If thick fluids are also aspirated, it is usually safer to give nothing by mouth and to maintain hydration and nutrition through an enteral tube.

Mucositis and Stomatitis

If the mouth or throat is sore, eating can become very difficult. An analgesic taken before meals can help ease the pain and enable the person to eat a little more. Modifying the diet is also helpful (Table 5).

Xerostomia

Xerostomia (dry mouth) may be a long-term side effect of cancer treatment, and patients may need to use extra sauce with their foods or have soft food, and they usually need to sip a drink while eating. Chewing gum, preferably sugar-free, can stimulate saliva, although it should be avoided by those with no saliva because it will stick to their teeth. Pineapple can also stimulate saliva and eating it between meals may make the mouth more comfortable.

Good dental hygiene is particularly important because saliva protects the mouth against infection. If people with xerostomia also get mouth infections, the resulting mucositis makes it increasingly difficult for them to eat.

Trismus and Difficulty Chewing

Trismus (difficulty opening the mouth) and difficulty chewing may be overcome with soft food or, failing that, with nourishing drinks and dietary supplements. If the person loses weight and can manage very little orally, an enteral tube feed should be considered.

Gastrointestinal Fistulas

A fistula may develop anywhere in the gastrointestinal tract. The site of the fistula will determine the dietary management (Table 6).

Table 3 Suggestions for overcoming taste changes

<i>Taste change</i>	<i>Suggestions</i>
Excessively sweet	Reduce sugar content of food and drink. Add a pinch of salt to drinks and puddings.
Excessively salty	Avoid packet soups, gravy, and sauces. Avoid salted snacks (e.g., crisps and nuts) or try unsalted varieties. Avoid bacon and other cured or tinned meat. Add a pinch of sugar to sauces or soups.
Metallic taste	Soak red meat in acidic marinade (e.g., vinegar and wine). Eat white meat, fish, eggs, and cheese in preference to red meat. Avoid tea, coffee, and chocolate.
Taste blindness	Use extra flavorings: salt, pepper, pickles, mustard, herbs, and spices. Eat highly flavored food (e.g., curry).

Table 4 Suggestions for food and fluids when person has nausea

Have cold food and drink in preference to hot because these have less odor.
Sip fizzy drinks.
Drink through a straw.
Try ginger flavors (e.g., ginger ale and ginger biscuits).
Eat small, frequent snacks to avoid the stomach from becoming completely empty.

Table 5 Suggestions to relieve mucositis and stomatitis

Avoid citrus fruits and drinks.
Avoid salty, spicy food, vinegar, pickles, and other strong flavors.
Avoid carbonated drinks.
Have tepid food and drinks.
Iced drinks may be soothing (or may increase the pain).
Avoid dry foods that need extra chewing (e.g., toast).
Eat soft food and use extra sauce.

Table 6 Sites of fistulas and their management

Site	Management
Neck, salivary fistula	'Nil by mouth' and enteral tube feed until healed
Chyle leak (e.g., in neck)	Low-fat diet initially; if unsuccessful, a low-fat, medium-chain triglyceride enteral tube feed If unsuccessful, consider parenteral nutrition
Large bowel	Low-residue diet or elemental enteral tube feed
Small bowel	See Table 7

Constipation

The cause of constipation must be considered initially. If it is due to a tumor pressing on the bowel (e.g., cancer of the ovary or colon), a low-fiber diet may be helpful. Low-fiber food is less bulky and may pass through the bowel more easily, particularly if accompanied by appropriate laxatives (e.g., stool softener).

If constipation is due to lack of fiber in the diet, then an increased fiber and fluid intake may be helpful. If constipation is due to analgesia, then appropriate laxatives need to be used in conjunction with any changes in the diet. In addition to fiber, a good fluid intake must be maintained to avoid constipation; approximately 2 litres per day is recommended.

Diarrhea

Diarrhea may be due to overflow from constipation, in which case the advice for constipation should be followed. Diarrhea due to intestinal hurry caused by bowel disease or drugs may be controlled with drugs and by avoiding excessive intake of high-fiber foods, which naturally pass through the bowel quickly. When malabsorption is suspected, a low-fat, elemental enteral tube feed should be considered. When diarrhea is severe, it is important to replace the fluid lost to prevent dehydration. Oral rehydration solution is useful to replace fluid losses. Diarrhea caused by radiotherapy needs to be controlled with drugs, and a low-fiber diet is not thought to be helpful in this instance.

Intestinal Failure

A long-term side effect of pelvic radiotherapy may be enteritis resulting in intestinal failure. Extensive gastrointestinal surgery leaving less than 100 cm of small bowel, or a fistula in the small bowel causing high stoma losses, may also cause intestinal failure. Previous chemotherapy that may affect the function of the bowel can contribute to this condition. Intestinal failure is more likely to occur when the patient does not have a functioning colon (e.g., in the case of ileostomists or when the ileo-cecal valve is absent). Dietary manipulation can greatly alleviate the symptoms of intestinal failure, such as thirst, dehydration, and high stoma losses or large volumes of diarrhea (**Table 7**).

An oral rehydration solution consisting of 20 g glucose, 3.5 g sodium chloride, 2.5 g sodium bicarbonate, and 1000 ml water provides 90 mmol of sodium per liter. It may be used chilled and to dilute weak fruit squashes. If the patient remains dehydrated despite following the advice detailed in **Table 7**, intravenous fluid replacement is necessary. Drugs may be given to increase gut transit time or reduce fluid losses. If

Table 7 Dietary management to reduce gut losses in intestinal failure

Restrict fluids to 500–1000 ml daily, increasing to 1500 ml.
Avoid drinks for 30 min before and 45 min after meals.
Avoid foods that are particularly high in fiber.
Sprinkle salt liberally on food.
Consider fat restriction if patient has a colon and there is evidence of steatorrhea.
Take salt and carbohydrate foods together to help sodium absorption.
If gut losses are 1000 ml or more, part or all of fluid intake should consist of an oral rehydration solution.

medication is in the form of capsules, these should be opened and the drugs given 60 min before meals. Suitable drugs include codeine phosphate, loperamide, rantidine, and octreotide. In the longer term, the following should be monitored: Plasma electrolytes, ferritin, vitamin D, serum albumin, magnesium, zinc, calcium, phosphate, alkaline phosphate, folate and vitamin B₁₂ concentrations, prothrombin time, body weight, and urinary sodium concentration.

Bowel Obstruction

Bowel obstruction may be subacute or complete. In cases of complete bowel obstruction, the clinical condition of the patient must be considered. If it is anticipated that the obstruction will resolve, or if aggressive treatment such as surgery is planned, parenteral nutritional support should be considered. Total parenteral nutrition may be inappropriate, and is unlikely to be useful in cases in which the prognosis is poor and no treatment is possible. Depending on the degree of obstruction, in cases of subacute obstruction, the following action may be taken under medical supervision: First day: sips of clear fluid, approximately 10 ml h⁻¹; Second day: 30 ml h⁻¹ clear fluid; Third day: 60 ml h⁻¹ clear fluid; Fourth day: free clear fluids; Fifth day: free fluids, including milk, low-fiber soup, custard, and jelly; Sixth day: low-fiber diet, avoiding all fruit and vegetables, nuts, pulses, and whole grain cereals, whole meal bread, etc. A patient who starts to vomit should return to the diet prescribed for the preceding day. If symptoms of bowel obstruction, such as abdominal pain and indigestion, remain controlled, fruit and vegetables may be introduced as tolerated, starting with small amounts.

Weight Loss

Weight loss is often the consequence of the dietary problems described previously. The measures in **Table 8** should be considered to help prevent weight loss or encourage weight gain. It must be remembered that energy requirements may be elevated due to the physiological effects of malignancy. Much interest has focused on attempts to influence the metabolic alterations in cachexia *via* nutrients. Research has examined the possible role of eicosapentaenoic acid (EPA), an *n*-3 fatty acid, in reducing the inflammatory response in cachexia. A randomized trial in pancreatic cancer patients compared a high-energy drink fortified with EPA to a standard high-energy drink to examine whether this was more effective at

Table 8 Dietary advice to help prevent weight loss

Fortify food with cream, butter, cheese, oil, sugar, honey, glucose, jam, etc.
Have small, frequent snacks.
Use full-fat and full-sugar products.
Avoid large amounts of lower energy foods (e.g., fruit and vegetables).
Try dietary supplements, such as milky drinks and glucose polymer power.
Consider an overnight enteral tube feed to supplement the diet if weight loss continues despite following the previous advice.

promoting weight gain. The study failed to show any additional benefit of EPA on weight gain.

Palliative Care

In some people, cancer will not be cured. Palliative care focuses on the relief of symptoms rather than aggressive curative treatment. The majority of people receiving palliative care will suffer from at least one eating difficulty. Much of the advice detailed previously for overcoming dietary problems is relevant, but it is often upsetting for these patients to have to pay close attention to their dietary intake. If patients are unconcerned about their poor dietary intake, it may be appropriate not to offer any advice; conversely, for those who are very concerned, the problem should be addressed seriously.

Alternative and Complementary Diets

The alternative and complementary diets considered here are modifications of a normal diet that are claimed to cure or treat cancer. Such diets are often followed for their anticipated antitumor effect. Often, they have not been tested or demonstrated to be effective in scientifically acceptable clinical trials. Patients may use other complementary therapies, such as healing, relaxation, visualization, homeopathy, and herbalism, in addition to making dietary changes. Dietary regimens may share common features: Mainly vegetarian or vegan – alternatively, diets may limit red meat and allow limited free-range chicken and deep-sea fish; no manufactured or processed foods; low salt, low sugar; low fat, high fiber, including raw fruit and vegetables and whole grains (these may be organic); fruit and vegetable juices; high-dose vitamins and minerals. Nutritional inadequacies may arise in the patient who has a poor appetite. The diets may cause weight loss and are restrictive and time-consuming to prepare. Some ingredients may be difficult to obtain and are often costly. Studies appear to show no difference in survival rates between patients following complementary therapies and patients receiving conventional treatment alone. Patients who use complementary therapies, however, do report psychological benefits, such as feelings of hope and optimism. Patients should have enough information about the possible advantages and disadvantages before embarking on strict complementary or alternative diets.

The Potential Therapeutic Role of Vitamins

Much interest has been expressed in the therapeutic role of vitamins in cancer patients. This has led a number of

alternative and complementary practitioners to advocate the use of high-dose vitamins for cancer patients. It has been known for some time that some vitamin-deficiency states may predispose some individuals to develop cancer. In a study of 29 000 vegetarian Chinese with a high frequency of esophageal cancer, subjects were given supplements of β -carotene and vitamin E. Raising their daily intake above the minimum requirement reduced the incidence of deficiencies and reduced the number of esophageal cancers. This type of study on vitamins and the etiology of cancer has led many practitioners and lay people to extrapolate the role of vitamins into cancer treatment. Although vitamins in food, especially vegetables and fruits, have been shown to be beneficial in reducing the incidence of particular types of cancer when included in the diet, the beneficial effects have not always been shown with vitamin and mineral supplements. Some supplements may promote tumor growth, as was seen in a study using β -carotene supplementation in patients with lung cancer. Supplementation increased the rate of tumor recurrence in such patients. The potential therapeutic role of vitamins, such as vitamins D, K, B₆, B₁₂, and folate, has been investigated. However, additional studies are required to determine the role, if any, of such vitamins. It may be that some vitamins help protect against the side effects of tumor therapy, whereas some may modify tumor growth. Excessive dietary supplementation in cancer patients should be avoided until further evidence is available on the effects of vitamins on tumor growth.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer; Epidemiology of Gastrointestinal Cancers Other than Colorectal Cancers; Epidemiology of Lung Cancer. Cobalamins: Physiology, Dietary Sources and Requirements. Diarrheal Diseases. Eating Disorders: Anorexia Nervosa. Folic Acid. Nutritional Support: Adults, Enteral; Infants and Children, Parenteral. Parenteral Nutrition. Supplementation: Dietary Supplements. Vitamin B₆: Physiology. Vitamin D: Physiology, Dietary Sources, and Requirements. Vitamin E: Metabolism and Requirements; Physiology and Health Effects. Vitamin K

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Epidemiology and Associations Between Diet and Cancer

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Glossary¹

Case-control study A study that compares two groups of people: those with the disease or condition under study (cases) and a very similar group of people who do not have the disease or condition (controls). Researchers study the medical and lifestyle histories of the people in each group to learn what factors may be associated with the disease or condition. For example, one group may have been exposed to a particular substance that the other was not. Also called retrospective study.

Cohort study A research study that compares a particular outcome (such as lung cancer) in groups of individuals who are alike in many ways but differ by a certain characteristic (e.g., female nurses who smoke compared with those who do not smoke).

Ecologic study A study that compares large groups of people instead of individuals for differences in things such as cancer rates. The groups can differ by location (e.g., city, county, or country). They can also differ by time (a few days, years, or decades). Groups can be immigrants (compared with people who are native to the country) or people with different types of jobs.

Randomized clinical trial A study in which the participants are assigned by chance to separate groups that

compare different treatments; neither the researchers nor the participants can choose which group. Using chance to assign people to groups means that the groups will be similar and that the treatments they receive can be compared objectively. At the time of the trial, it is not known which treatment is best. It is the patient's choice to be in a randomized trial.

Relative risk A measure of the risk of a certain event happening in one group compared to the risk of the same event happening in another group. In cancer research, relative risk is used in prospective (forward looking) studies, such as cohort studies and clinical trials. A relative risk of one means there is no difference between two groups in terms of their risk of cancer, based on whether or not they were exposed to a certain substance or factor, or how they responded to two treatments being compared. A relative risk of greater than one or of less than one usually means that being exposed to a certain substance or factor either increases (relative risk greater than one) or decreases (relative risk less than one) the risk of cancer, or that the treatments being compared do not have the same effects. Also called risk ratio.

Sources of Evidence Linking Diet and Cancer

Laboratory scientists have known since the early 20th century that various nutritional manipulations can influence the occurrence of tumors in animals. Despite this discovery of the relationship between diet and cancer in animals, widespread interest in the study of diet and cancer in humans did not develop until more recently when the large international differences in cancer rates were correlated with variations in dietary factors. In fact, investigators have found strong correlations between estimated per capita fat consumption and breast cancer rates internationally, raising the possibility that dietary fat may have an important role in the etiology of breast cancer. Other observations such as those demonstrating that migrating populations adopted, sooner or later, the cancer rates of their new host population strengthened the evidence that international differences were the result not of genes, but of noninherited factors, including diet. The study designs used to investigate diet and cancer in humans are discussed below.

Descriptive Studies

Rates of cancer show large differences between countries for many malignancies. International correlations compare disease rates with lifestyle factors such as per capita consumption of specific dietary factors.

Age-adjusted rates of colon and breast cancer are up to five times higher in some countries than others. Dakar in Senegal (0.6) and Poona (3.1) and Bombay (3.5) in India, have the lowest incidence rates of colon cancer per 100 000 males; in contrast, the USA has the highest recorded rates of 32.2 in Connecticut and 31.4 in New York.

Strong nutritional correlates exist for specific cancers. These studies, also known as ecological studies, use the country or other geographic area as the unit of measure rather than the individual. For example, Armstrong and Doll in 1975 compared per capita total fat intake and national breast cancer mortality rates among women and found a correlation of 0.89: Countries with higher fat intake had higher breast cancer mortality. They also compared per capita fat intake and mortality from colon cancer and observed a correlation of 0.85 for men and 0.81 for women.

The most important of the existing strengths of correlation studies is that the contrasts in dietary intake are very large.

¹Source: NCI Cancer Glossary (www.cancer.gov/dictionary).

For example, the range of fat intake within a population tends to be small compared with the range of fat intake among different populations.

Although correlation studies have opened the door to new leads in the study of diet and cancer, certain limitations have prevented these investigations from advancing past the level of hypothesis generation. First and foremost, there are many factors other than dietary differences that distinguish countries with a high incidence from those with a low incidence. This makes it difficult to identify dietary factors as the primary explanation for the differences in the etiology of cancers. For example, besides consuming a diet with a higher proportion of energy from fat, populations of countries that are more industrialized will also have shifted from an agrarian to an urbanized, sedentary society with lower total energy expenditure. Therefore, with increasing industrialization, exposure to many aspects of life will decrease exercise and increase fat intake. Consider the example of colon cancer. The international correlation between fat and colon cancer mortality in men is 0.85, and for meat it is 0.85. There is also a correlation between gross national product and colon cancer mortality (0.77 for men); more industrialized countries have higher economic production and higher rates of cancer. Owing to the many factors that are associated with industrialization it is not possible to separate out which factor is important in the etiology of colon cancer, lack of physical activity or increased consumption of fat or meat. Studies with data on lifestyle factors at the individual level are needed to clarify which of these variables is important (see Analytical Studies below).

Special-Exposure Groups

Within populations there are groups that have atypical dietary patterns which may provide valuable information in the probe for further information on the relationship of diet and cancer. These groups are called special-exposure groups and are often defined by ethnic or religious characteristics. In addition to offering many of the advantages of correlation studies, the number of alternative explanations for any observations may be reduced if the special-exposure group lives in the same area as the comparison group.

As a largely vegetarian group, the Seventh-Day Adventists have been used in studies of meat eating and cancer. Studies of these groups, however, are limited in the same ways that other ecological studies are limited. For example, although lower rates of colon cancer have been observed among Seventh-Day Adventists – supporting the hypothesis that meat is related to colon cancer – there are other lifestyle choices that characterize the group, such as low rates of tobacco use and alcohol intake, which could also modify their rates of colon cancer.

Evidence from Descriptive Studies

In 1981, Doll and Peto made an estimate based largely on descriptive studies that 35% of cancers in the USA may be attributable to dietary factors; but reflecting uncertainty in the sources of data used for this estimate, they noted that the range of possible dietary contribution was from as low as 10% to as high as 70%. The marked variation in the rates of most

Table 1 Examples of suspected dietary factors influencing cancer risk

<i>Dietary Factor</i>	<i>Site of Cancer</i>
<i>Increased risk</i>	
Overnutrition/obesity	Endometrium, gallbladder, breast, colon
Alcohol	Liver, oesophagus, larynx, pharynx, breast, colon
Beer	Rectum
Fat (especially saturated)	Colorectum, prostate
Red meat	Colorectum
Salt	Stomach
Heterocyclic amines (from cooked meat)	Colorectum
<i>Decreased risk</i>	
Fiber	Colorectum, oesophagus
Vitamins A, C, E	Many sites
Protease inhibitors	Colorectum
Calcium, vitamin D	Colorectum
Folate	Colorectum
Lycopene	Prostate
Carotenoids	Lung
Phytoestrogens	Breast

cancers among countries is evidence that dietary factors may influence the development of cancer. The potential range of dietary factors that may influence cancer risk are presented in **Table 1**. Despite the fact that descriptive studies provide an excellent source of hypotheses, it is necessary to conduct analytical studies to collect data that will provide more definitive evidence.

Time Trends Within Countries

The analysis of cancer trends over time can lead to useful findings in the study of diet and cancer. By looking at the change in cancer rates in a specific population over time and comparing these rates with changes in specific factors over the same period (e.g., changes in dietary habits), investigators can uncover possible associations supporting the dietary factors hypotheses. For example, researchers have examined vital statistics for Japanese natives and US whites to unveil changes in cancer mortality and related antecedent patterns of lifestyle in the two populations. These investigations have uncovered that animal fat consumption in Japan steadily increased from a daily level of 6.5 g per person in 1955 to 27.6 g in 1987; at the same time the Japanese rate of colon cancer in men rose at a rapid pace; in fact, the mortality rates owing to colon cancer in men almost trebled over this time. This evidence lends more support to the hypothesis that mortality from colon cancer in men is influenced by high dietary fat consumption.

Similar data were collected in Singapore to determine trends in the incidence of breast cancer: in 1996 an average annual increase in breast cancer incidence of 3.6% over a 25-year period for all women was reported. The most convincing evidence that the observed trend was real was that it was clearly cohort-related rather than period-related. The risk was observed to increase in successive birth cohorts from the

1890s to the 1960s. Changes in dietary consumption patterns (e.g., the adoption of a more Western diet) fall among other factors cited as having a possible effect on the continuing increase in rates of breast cancer among women in Singapore. Like descriptive studies, time-trend studies are a valuable source for hypotheses generation, but more definitive evidence is required from analytical epidemiology to uncover any real associations between dietary factors and cancer rates.

Migrant Studies

Migrant studies examine the rates of specific diseases in migrating populations. These studies are important in addressing the possibility that observed correlations in ecological studies are owing to genetic factors. Generally, results from migrant studies have so far found that the migrating group takes on the rate of cancer of the new country. Hence genetic factors are excluded as the dominant cause for varying rates of cancer between countries. A good example of this is seen in the Japanese migrant population to the USA. Japan has low rates of cancers of the breast, colon, and prostate, whereas the rates of these cancers among Japanese migrants to the USA move toward the higher US rates. The increased risk of breast cancer among migrants occurs primarily in later generations, leading investigators to believe that the causal factors operate early in life. Investigators also consider major changes in the rate of disease that occur within a population over time as evidence that nongenetic factors play an integral role in the etiology of cancer. The limitations of migrant studies are similar to those of ecological studies.

Analytical Studies

Cohort Studies

Cohort studies involve the collection of information from healthy participants who are followed over time and observed for the occurrence of new cases of disease (incident cases). During or at the end of follow-up, the disease frequency within a cohort may be measured as either a cumulative incidence rate (the number of cases divided by the entire base population) or an incidence density rate (the number of cases divided by the total follow-up time accumulated by all members of the population, or 'person-time' follow-up). The relative risk is the rate of disease (cumulative incidence rate or incidence density rate) in the exposed (e.g., those with a high intake of dietary fat) divided by the rate of disease in the unexposed (e.g., those on a low-fat diet). A relative risk of 2 implies that the exposed group has twice the rate of disease compared with the unexposed group.

For illustration, in a study of 121 700 women, a group of participants who completed dietary questionnaires and had no previous diagnosis of cancer in 1980, were followed through 1988 to address the hypothesis that dietary fat increases and fiber intake decreases the risk of breast cancer. This outcome was defined by histologically confirmed cases of breast cancer. In one analysis, the primary exposure of interest was energy-adjusted intake of total dietary fiber. Among the women in the highest quintile of energy-adjusted dietary fiber intake there were 299 cases of breast cancer compared with

305 cases among the women in the lowest quintile. This gave a relative risk (with adjustment for established breast cancer risk factors as well as alcohol intake) of 1.02 for those in the highest quintile of energy-adjusted dietary fiber intake compared with those in the lowest quintile.

There is also a growing body of evidence available from cohort studies for the assessment of dietary fat intake and breast cancer in developed countries. The average relative risk was 1.05, which was not statistically significant. This observation was based on the results from seven prospective studies with at least 150 incident breast cancer cases each ($n = 4980$) and a large comparison series (i.e., noncases). At the same time, as these results suggest no overall association for total fat intake, emerging evidence suggests that monounsaturated fat may be protective against breast cancer.

The use of cohort studies can be advantageous in many ways when studying the relationship between diet and cancer. A cohort study allows the assessment of multiple effects of a given dietary exposure. Dietary data can be updated during follow-up and the temporal relation between diet and cancer can be addressed. For example, the beneficial effects of alcohol in reducing the risk of gallstone formation and coronary heart disease, and the potentially deleterious effects of alcohol on cancer and hemorrhagic stroke, can be weighed against each other in a cohort study. It is also possible to measure the absolute rates of disease according to the level of food or nutrient intake.

Among the limitations of cohort studies is the concern that current practice, usage, or exposure may change over the duration of the follow-up, limiting the ability to come to any relevant conclusions in studies of diet and cancer that have measured exposure just once at the beginning of the study. Controlling for extraneous variables such as smoking, which are related both to risk of cancer and to dietary intake, and separating the effects of specific dietary factors from those that exist together, also limit the range of knowledge that can be extracted from cohort studies.

Some investigators believe that the large number of subjects required to study rare disease and the high expense of management and maintenance also limit the usefulness of cohort studies. Others believe that the larger overall monetary investment most cohort studies require can be advantageous: more variables can be studied and in the long run further hypotheses can be generated and more conclusions produced than in a single case-control study that relies on recall of past habits.

Case-Control Studies

In case-control studies information is obtained from diseased participants and compared with information provided by disease-free controls with respect to a possible risk factor (e.g., level of a dietary factor). Data collected from these studies can be used to evaluate the hypothesis that the risk factor is a cause of the disease. The cases are selected from a defined population, such as a country population. The population represents those at risk of developing the disease under study. Each time someone in the defined population is diagnosed with the disease during the duration of the study, this individual joins the case series. As each case arises from the population, one or more controls should be sampled to estimate the prevalence of the exposures among those remaining free from disease. The controls may be chosen from

any population of individuals that provides valid information about those at risk for the disease. It is important to choose controls so that their probability of selection is unrelated to the exposure being studied.

In the study of the relationship of diet and cancer, case-control studies may be used to evaluate the hypotheses that individual or multiple dietary factors are the cause of the cancer under investigation. For example, a study in 1977 identified all cases of lung cancer diagnosed during an 18-month period from 1972 in three Singapore hospitals. Controls were chosen from other hospital patients free of any smoking-related diseases. There were a total of 233 cases and 300 controls interviewed regarding their frequency of consumption of dark-green leafy vegetables and food preparation habits. The investigation found a substantially increased risk of lung cancer among those reporting a low consumption of dark-green leafy vegetables.

Case-control studies are better suited to the study of rare diseases because in cohort studies tens of thousands of individuals must be followed in order to study the most common cancers. It is also thought that case-control studies may be quicker and less expensive to conduct because they require fewer subjects, and they are therefore often employed as an alternate mode of investigation to cohort studies.

Among the limitations of case-control studies is the comparability of information between the cases and the controls. Although in a cohort study the exposure of interest is measured before the onset of disease, in case-control studies the exposure is assessed in individuals who (in most cases) already know their own disease status. Often the person collecting the data will also know the disease status of the patient. This may influence the accuracy of the data collected, either through differential recall by cases and controls, or by an interviewer being more persistent in questioning cases than controls. In cohort studies neither the participant nor the investigator knows whether or not the subject will be a case or noncase by the end of the follow-up period.

Intervention Studies

In principle, the most powerful means of determining the effects of dietary factors on cancer risk is an intervention study (i.e., a randomized trial). In randomized trials bias is removed because of the equal distribution of risk factors in each group. For example, it has been proposed that a randomized trial of fat reduction could help uncover the mystery of the relationship between dietary fat intake and breast cancer. The Women's Health Initiative was started by the US National Institutes of Health with the goal of enrolling and randomizing several tens of thousands of women, half of whom would be trained to follow a diet deriving less than 20% of energy from fat. Unfortunately, such a trial would not be able to address the most promising modification of the dietary fat hypothesis – that dietary fat reduction at an early age may reduce breast cancer risk several decades later. Other problems with such a randomized trial include the difficulty of maintaining compliance with a diet incompatible with prevailing food consumption habits, and the gradual secular decline in total fat consumption which reduced the size of the comparison of fat intake between the intervention group and the control groups. The Women's Health Initiative Trial

counselled the women in the intervention group to adopt a diet that was high in fruits, vegetables, and grain products as well as low in total and saturated fat, therefore making it more difficult to distinguish between the effect of the fat reduction and that of increasing intake of fruits, vegetables, and grain products. The trial failed to show any significant benefit of reducing fat intake on breast cancer risk. All in all, intervention studies may in principle have a great chance of determining effects of dietary factors on cancer risk, but trials of sufficient duration and size may not be feasible because of long-term compliance and cost.

Epidemiological Issues in the Study of Diet and Cancer

Resolved and Unresolved Issues

Some of the issues that researchers have encountered in their attempt to uncover the mystery of the dietary factors linked to cancer include the difficulty of distinguishing the importance of parts of dietary factors from the overall effect of each dietary factor (e.g., total dietary fat intake compared with type of dietary fat intake). In a meta-analysis in 1990 of 12 case-control studies of dietary fat intake and cancer, four studies observed a significant positive association, six uncovered nonsignificant positive associations, and two saw inverse associations. When the data were analyzed together there was a positive association observed for both total fat intake and saturated fat intake. Investigators must ask themselves which factor has larger implications in the study of diet and cancer, as not all studies have included analyses of the individual types of fats along with their data on overall fat consumption. In the study of the influence of dietary fiber intake (which includes crude fiber and many soluble fiber fractions) on cancer rates, there is debate about the most appropriate method of biochemical analysis for determining fiber content of individual foods. This same issue arises with the study of most dietary factors and could affect any important advances in the study of diet and cancer.

Biochemical indicators of food and nutrient intake have two fundamental uses in epidemiological studies. Most often they serve as a 'surrogate' for actual dietary intake in studies of disease occurrence. For nutrients that vary widely in concentration within foods and for which food composition tables are inaccurate, biochemical indicators may be the most feasible way of measuring intake. Within-food variation may occur owing to differences in food storage, processing, or preparation, or may be owing to geographical differences in soil nutrient content. For example, it has been found that selenium content in US soil can vary by as much as 100-fold, which in turn causes the selenium content of swine muscle to vary more than 15-fold. Another example is that of fat. When the composition of fats in commercial food products is not known to study participants, it is possible to assess the fat components of the diet by subcutaneous fat aspirates which reflect long-term dietary patterns.

Like most exposures in chronic disease, nutrient exposures relevant to disease are usually long-term. As the promotion period for cancers may be years or decades, it is usually

desirable that a biomarker indicates the cumulative effect of diet over an extended period of time. There are a couple of methods to surpass the barrier of an indicator that is only sensitive to short-term intake, and to overcome the day-to-day intake fluctuations that occur with most nutrients: (1) experimental studies, in which nutrient levels are manipulated; and (2) sampling levels in individuals longitudinally. Biomarkers of nutrient levels in blood or other tissues can provide a useful assessment of intake of certain nutrients, although the above considerations must be acknowledged, and careful attention must be given to specimen collection, storage, and analysis in order to avoid misclassification or bias. With an expanding array of biochemical indicators that have been validated as measures of dietary intake, their use in nutritional epidemiology will continue to grow.

The limited range of diet within most populations adds its own set of complexities to the epidemiological study of nutrition and cancer. For example, in the majority of populations where foods high in fat are readily available, very few individuals consume less than 30% of their energy from fat. This makes it difficult to study the impact of reducing fat intake to less than 30% of total energy intake. At the same time, some individuals of a relatively homogeneous population may have very different dietary patterns: For example, a range of dietary fat intake from 25% to 40% of total energy was seen within a cohort of 52 000 male health professionals in the USA.

Given that most neoplasms have a long induction period (the time from an exposure to a carcinogen to the development of cancer), often spanning several decades, accurate measure of long-term dietary intake is of utmost importance in the study

Table 2 Levels of evidence for major forms of cancer by foods, energy-generating nutrients, dietary exposure to selected nonnutrients, and nutrition-related indicators

	<i>Suggestive Evidence</i>	<i>Strong Evidence</i>	<i>Convincing Evidence</i>
<i>Increased risk</i>			
Major food groups			
Meat	Pancreas, oesophagus, lung, endometrium, stomach, prostate		Colorectum
Sugars	Colorectum		
Macronutrients			
Saturated fat (animal)	Colorectum, lung, endometrium,		Prostate
Nonnutrients			
Alcohol		Liver	Oral cavity, pharynx, larynx, colorectum, breast, oesophagus
Salt (NaCl)		Stomach	
Nutritional covariates			
Height	Endometrium	Breast (premenopausal), ovary, pancreas, prostate	Breast (postmenopausal), colorectum
Obesity	Liver	Gallbladder	Colorectum, breast (postmenopausal), endometrium, kidney, oesophagus, pancreas
Hot drinks	Oral cavity, pharynx, larynx, oesophagus		
Calcium		Prostate	
<i>Decreased risk</i>			
Major food groups			
Vegetables	Endometrium, cervix uteri, ovary, nasopharynx	Oesophagus, stomach, pharynx, larynx, lung, pancreas, colorectum, oral cavity, prostate	
Fruits	Liver, pancreas, nasopharynx, colorectum	Oral cavity, oesophagus, stomach, larynx, pharynx, lung	
Fish	Colorectum		
Macronutrients			
Fiber	Oesophagus	Colorectum	
Monounsaturated fat	Breast		
Nutritional covariates			
Obesity			Breast (premenopausal)
Physical activity	Lung, pancreas	Endometrium	Colorectum, breast
Folate			Colorectum, breast
Calcium	Bladder		Colorectum
Vitamin D		Colorectum	

of the implications of diet on cancer. Therefore, short-term methods of dietary assessment such as 24-h recalls are usually insufficient. In the context of case-control studies these short-term methods are inappropriate because they measure current diet, and it has been found that individuals alter their diet after the diagnosis of cancer. The most feasible method of measuring long-term intakes in large numbers of individuals is the food frequency questionnaire: These questionnaires measure the usual frequency of a selected list of foods.

Food frequency questionnaires to assess dietary intake need to be carefully designed. First of all, the food items on the questionnaire must represent the major source of nutrients of interest within the study population. Depending on the consistency of the concentration of a nutrient in a given food, the precision of dietary questionnaires varies. Food frequency questionnaires may provide rankings by level of intake but they do not quantify actual intake. A dietary questionnaire may efficiently distinguish between participants with low-fiber and high-fiber intakes in a given population, but it will not necessarily provide a precise assessment of the absolute fiber intake. In the case of larger studies, it is possible for a random sample of participants to provide a more comprehensive assessment of intake by keeping several weeks of dietary records. This additional information will provide a more precise quantification of dietary intake by helping estimate true dose-response relationships between a nutrient and diet expressed in absolute intake.

Summary of Known Relations Between Diet and Cancer

A wealth of studies since the 1970s have clearly documented the relations between diet and a growing number of cancers (Table 2). Convincing evidence based on consistent findings from epidemiological studies conducted in diverse populations now shows that diet is an established cause of prostate, breast, digestive tract, airway, and urinary tract cancers. With these rich epidemiological data we can more confidently conclude that some 30% of cancer is attributable to diet. Public health officials have taken the accumulated evidence and developed strategies for minimizing cancer risk. Among these recommendations is a diet high in vegetables, fruits, and legumes and low in red meat, saturated fat, salt, and sugar. They suggest that carbohydrates be consumed as whole grains such as whole meal bread and brown rice rather than as white bread and rice. Any added fats should come from plant sources and should be unhydrogenated, an example being olive oil, which may potentially be beneficial. Given the

evergrowing knowledge of the association between diet and cancer, and the subsequent recommended prevention strategies, it is time that researchers and public health officials combined their efforts not only to uncover the mysteries of diet and cancer but also to balance the 'war on cancer' treatment with more extensive efforts in prevention.

See also: Cancer: Epidemiology of Gastrointestinal Cancers Other than Colorectal Cancers; Epidemiology of Lung Cancer. Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Dietary Fiber: Physiological Effects Leading to Health Outcomes. Vegetarian Diets

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Epidemiology of Gastrointestinal Cancers Other than Colorectal Cancers

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This article addresses the epidemiology of esophageal cancer, stomach cancer, pancreatic cancer, and small intestine cancer. People with any of these cancers are often diagnosed at 60–80 years of age. The incidences are higher among men than among women and vary widely with geographic location and population, suggesting that environmental factors are important in the development of these cancers.

Esophageal Cancer

The esophagus is a hollow tube, approximately 10 inches long in adults. It conveys food from the pharynx to the stomach. Mucous glands in the lining of the esophagus secrete mucus to aid in lubrication. Absorption in the esophagus is nil.

Epidemiology

Worldwide, esophageal cancer is the eighth most common cancer and the sixth most common cause of cancer death, accounting for approximately 481 000 new cases and 406 000 deaths in 2008. More than 70% of esophageal cancer is squamous cell carcinoma, and approximately 20% is adenocarcinoma. Squamous cell carcinoma arises from dysplasia in the middle and lower third of the esophagus epithelial lining, whereas adenocarcinoma usually develops in the glandular tissue in the distal esophagus. The incidence of esophageal cancer varies tremendously with geographic location and populations throughout the world, with a maximum ratio of 500 to 1. In central and Southeast Asia, the Far East, and the Middle East, squamous cell carcinoma is the predominant form of esophageal cancer with stable incidence rates for both squamous cell carcinoma and adenocarcinoma, whereas in the USA and Europe, adenocarcinoma of the esophagus has been rapidly increasing since 1970s, particularly in Caucasian men, to approach or surpass the rate of squamous cell carcinoma. In the USA, the incidence of adenocarcinoma of the esophagus has increased from 0.5–0.9 per 100 000 to 3.2–4.0 per 100 000 since the 1970s. African Americans have an approximately twofold increased risk for esophageal cancer compared with Caucasians, possibly because of an unhealthy lifestyle.

Disease Process

There may be no symptoms of esophagus cancer during the early stages. As the cancer develops, nonspecific symptoms occur, including dysphagia, weight loss, chronic cough, and pain in the retrosternal, back, or right upper abdomen. In more than 50% of esophagus cancer cases, the cancer is either unresectable or has metastasized at the time of diagnosis. The prognosis of esophagus cancer depends on disease stages and tumor sizes. For resectable esophagus cancer, the 5-year survival rate ranges from 15% to 24%. For metastasized esophagus cancer, the 5-year survival rate is less than 5%.

Although both squamous cell carcinoma and adenocarcinoma of the esophagus are responsive to chemotherapy, the treatment effect rarely lasts more than 1 year. Radiotherapy may reduce the chance of perioperative morbidity and mortality, but it may increase the risk for local and regional complications such as esophagotracheal fistulas. Research is under way to determine whether an improved treatment efficacy can be achieved by combined chemotherapy, radiotherapy, and surgery.

Risk Factors

Squamous Cell Carcinoma

Factors that cause chronic irritation and esophageal mucosa inflammation may increase the risk for esophageal squamous cell carcinoma. These factors include moderate-to-heavy alcohol drinking, smoking, achalasia, diverticuli, and consumption of extremely hot beverages, coarse grains or seeds, lye, and caustic spices.

The importance of alcohol consumption in the carcinogenesis of esophageal squamous cell carcinoma is well recognized. However, the mechanisms by which alcohol increases cancer risk have not been elucidated. Alcohol may cause chronic irritation to the esophagus, and it may increase cell proliferation and enhance the permeability of carcinogens to cells. An alcohol metabolite, acetaldehyde, is known to be a carcinogen. Risk for esophageal squamous cell carcinoma is higher for spirits drinkers, followed by wine and beer drinkers.

Cigarette smoke is a rich source of carcinogens, such as benzo(a)pyrene and volatile nitrosamines. It also contains free radicals, reactive oxygen species, and reactive nitrogen species that are capable of initiating and propagating oxidative damage to lipids, proteins, and DNA, leading to several degenerative diseases, including cancer. Alcohol drinking may account for approximately 80% of squamous cell esophageal cancer cases, whereas tobacco use may account for approximately 60%. Simultaneous use of alcohol and tobacco further increases esophageal cancer risk.

Achalasia is a swallowing disorder caused by degeneration of the intrinsic autonomic nerves in the esophagus wall and lower esophageal sphincter, leading to decreased or absent peristalsis in the esophageal smooth muscle or impaired relaxation of the lower esophageal sphincter. Approximately 20–29% of achalasia patients may develop esophageal cancer within 15–20 years, predominantly squamous cell carcinoma, possibly because of increased inflammation, bacterial growth, and chemical irritation caused by prolonged contact of food ingredients with esophageal mucosa. In contrast, the likelihood of malignant transformation from diverticuli is less than 1%, although the mechanisms of carcinogenesis are speculated to be the same as those for achalasia.

Low income is associated with squamous cell carcinoma of the esophagus, independent of alcohol and tobacco use, suggesting that other factors associated with poverty may play a

role. In Africa and Far East countries, incidences of esophageal cancer are high in regions where starchy food is the predominant food in the diet, and this may have been an indication of poor nutritional status. Several studies have reported that very low intake of fresh fruits and vegetables is associated with higher risk of esophagus cancer. Conversely, high intake of fruits and vegetables, particularly citrus fruits, may confer preventive benefits. Frequent consumption of highly salted meat, pickled vegetables, cured meat, and smoked meat was found to be associated with esophageal cancer risk; these foods contain carcinogenic compounds such as heterocyclic amines and *N*-nitroso compounds.

Familial aggregation of esophageal squamous cell carcinoma has been reported, but it may reflect genetic predisposition as well as common environmental exposures. Hereditary squamous cell carcinoma of the esophagus develops in approximately 95% of people with a genetic abnormality at chromosome 17q25 that causes a rare autosomal dominant disorder, nonepidermolytic palmoplantar keratoderma.

Adenocarcinoma

The risk factor profile of esophageal adenocarcinoma is quite different from that of squamous cell carcinoma. Tobacco use is associated with adenocarcinoma of the esophagus, but the association is less strong than that with squamous cell carcinoma. High intakes of fiber, vitamin C, vitamin B₆, folate, and β -carotene were found to be associated with a lower risk. However, unlike squamous cell carcinoma, esophageal adenocarcinoma does not consistently develop more often in people with low income.

Gastroesophageal reflux disease (GERD) is strongly associated with adenocarcinoma of the esophagus. In the process of gastroesophageal reflux, acid fluid regurgitates into the gastroesophageal junction and causes a sensation of heartburn. GERD can be caused by hiatal hernia, esophageal ulcer, and use of drugs that relax the lower gastroesophageal sphincter and increase reflux. Alcohol, tobacco, obesity, and pregnancy may also contribute to GERD.

Barrett's esophagus represents intestinal metaplasia of the squamous epithelium in the distal esophagus. Barrett's esophagus develops in approximately 5–10% of people with GERD and is associated with a 30- to 125-fold increased risk for esophageal adenocarcinoma.

Obesity has been hypothesized to be one of the factors responsible for this increase by augmenting abdominal pressure and gastroesophageal reflux frequency. However, evidence has not been consistent to support this hypothesis.

Prevention

For primary prevention, smoking cessation and avoidance of heavy alcohol intake may significantly reduce the risk for squamous cell carcinoma. A healthy diet with fresh fruits and vegetables but no highly salted, preserved, or smoked food should lead to a reduction in the risk for both of the major forms of esophageal cancer. For secondary prevention, routine screenings by endoscopes may confer benefits to individuals with Barrett's esophagus. Treatment with endoscopic ablation combined with proton pump inhibitors may retard Barrett's esophagus to normal squamous mucosa.

Research has been under way to determine the chemopreventive effects of 13-*cis*-retinoic acid, nonsteroidal antiinflammatory drugs (e.g., aspirin and sulindac), selenium, and ornithine decarboxylase inhibitor, α -difluoromethylornithine, in patients with Barrett's esophagus. These agents also hold promise for preventing squamous cell carcinoma. Other chemopreventive agents that may be useful for reducing both types of esophageal cancer include ascorbic acid, polyphenols (e.g., ellagic acid and epigallocatechin-3-gallate), and sulphydryl compounds. These agents have been shown in animal models to inhibit nitrosamine formation and enhance the activities of detoxifying enzymes such as glutathione-S-transferase and glutathione peroxidase, but evidence from humans is sparse.

Stomach Cancer

The stomach is located between the esophagus and the duodenum on the left side of the abdominal cavity. It serves as a short-term reservoir of foodstuff and provides digestive functions. Gastric epithelium secretes mucus, hydrochloric acid, hormones (e.g., gastrin), protease, lipase, gelatinase, and other enzymes. The movement of the stomach is controlled by the autonomic nervous system and several hormones in the digestive system.

Epidemiology

Worldwide, stomach cancer is the fourth most common cancer and the second most common cause of cancer deaths, accounting for approximately 988 000 new cases and 736 000 deaths in 2008. Stomach cancer can be classified as diffuse or intestinal. The former has an earlier onset with similar occurrences by sex and by geographic areas, whereas the latter has a later onset and develops more often in men than women. There is a wide variation (more than 10-fold) in the incidence of the intestinal type, suggesting that environmental factors are important determinants. Japan, Korea, China, Eastern Europe, Central America, and South America have higher incidences, whereas southern Asia, India, North America, and Africa have lower incidences. A wide range of incidence also occurs within countries.

The incidence and mortality rates of stomach cancer have been declining for several decades because of a reduction in childhood *Helicobacter pylori* infection, improved nutritional status, and reductions in exposures to carcinogens in preserved food. However, because of increases in life expectancy, the absolute number of stomach cancer cases has been increasing.

Disease Process

Approximately 90% of stomach cancer is adenocarcinoma. Other forms of stomach cancer include lymphomas and sarcomas. Symptoms such as excessive belching, heartburn, stomachache, and back pain may occur. Internal bleeding may appear as blood in the vomit or as black, tar-like feces, or it may be so slight that it is undetected. The prognosis of stomach cancer is poor and depends on disease stages; 5-year survival rate is approximately 20% in USA.

Risk Factors

H. pylori Infection

Helicobacter pylori infection can cause inflammatory responses that induce atrophic gastritis and intestinal metaplasia of gastric mucosa, resulting in reduced gastric acidity, which in turn facilitates *in vivo* formation of carcinogenic *N*-nitroso compounds and leads to the intestinal type of stomach cancer. In addition, *H. pylori* infection can trigger a cascade of inflammatory responses and oxidative damage to induce cell proliferation and malignant transformation, leading to the diffuse type of stomach cancer. It was estimated that *H. pylori* infection accounted for approximately 50–60% of stomach cancer cases and was associated with an approximately sixfold increased risk at least 10 years before diagnosis. These may have been underestimated because of the possibility of loss of the infection or antibody due to extensive replacement of gastric mucosa with intestinal metaplasia in people with stomach cancer.

Infection of *H. pylori* is common – 50% worldwide and 90% in developing countries. However, only a small percentage develops into stomach cancer, suggesting that factors such as diet and genetic susceptibility modify risk.

Dietary Factors

Pickled vegetables and smoked, cured, salted, or dried fish or meat contain nitrite or *N*-nitroso compounds. These preserved foods, as well as grilled or charcoal flame-broiled food that contains polycyclic aromatic hydrocarbons, have been shown to be associated with increased risk of stomach cancer in most studies. Despite the fact that vegetables are a major source of nitrate, evidence suggests an inverse association between fresh fruits and vegetables and stomach cancer risk; the associations for yellow- or green-colored vegetables and citrus fruits are particularly strong. A few studies have reported a lower risk for stomach cancer among people consuming more allium vegetables, onions, and garlic. Some, but not all, studies have found a positive association between starchy food consumption and stomach cancer risk.

Vitamin C intake is consistently found to be inversely associated with stomach cancer risk in observational studies. Vitamin C can act as a powerful water-soluble antioxidant as well as an effective scavenger of nitrite. Protective roles of α -tocopherol and β -carotene are suggestive but less strong. These micronutrients may also be surrogate markers of healthy dietary pattern or lifestyle.

Evidence is inconsistent regarding the role of alcohol, coffee, or black tea consumption in the development of stomach cancer. However, green tea consumption was associated with a lower risk in several studies, presumably because of its polyphenol content.

High intake of salt is associated with a higher risk of stomach cancer. Animal studies have demonstrated that salt *per se* can damage gastric mucosa and induce gastritis. However, in humans, high salt intake correlates positively with intake of processed meat or fish that contains nitrosamines. Hence, it is unclear whether salt evokes stomach cancer or is merely a marker of other exposures.

Cigarette Smoking

Tobacco use is associated with a 1.5- to 2.0-fold increased risk for stomach cancer, and it has been estimated to account for 10–17% of stomach cancer cases. These estimates may have been confounded by other factors such as poor diet.

Familial Factors

Familial aggregation of stomach cancer derives mostly from common environmental exposures and lifestyle factors. Hereditary stomach cancer is rare. Germline mutations in the gene coding for cell adhesion protein E-cadherin (CDH1) were found to be associated with stomach cancer of the diffuse type. Germline mutation of *p53* has also been reported. People with hereditary nonpolyposis colorectal cancer are also at higher risk for stomach cancer.

Prevention

Evidence points to the importance of improving diet and eradicating *H. pylori* infection. A diet rich in fresh fruits and vegetables without highly salted, preserved, or smoked food will theoretically offer benefits in primary prevention. In countries where the incidence of stomach cancer is high, screening for *H. pylori* may be effective for secondary prevention. To this end, programs of mass screening and eradication of *H. pylori* by antibiotics are being performed in Japan. However, because only a small proportion of individuals with *H. pylori* colonization develop stomach cancer, concerns have been raised regarding the possibility of antibiotic resistance by a mass *H. pylori* eradication program. Use of vaccines against *H. pylori* may be an alternative approach.

Pancreatic Cancer

The pancreas is an elongated organ locating in close proximity to the duodenum. It consists of three parts – head, body, and tail – and is partitioned into lobules by connective tissue. Approximately 85% of the pancreas is composed of exocrine cells called acini that secrete digestive enzymes, such as proteases, lipase and amylase, ribonuclease, gelatinase, deoxyribonuclease, and elastase. These digestive enzymes, together with bicarbonate secreted from the epithelial cells lining small pancreatic ducts, enter into pancreatic ducts and subsequently to the lumen of the duodenum. Embedded in the exocrine tissue are endocrine tissues called Islets of Langerhans that secrete endocrine enzymes, such as insulin and glucagon.

Epidemiology

Worldwide, there were approximately 278 000 pancreatic cancer cases and a similar number of deaths due to pancreatic cancer in 2008. Pancreatic cancer is the fifth and sixth most common cause of cancer death in men and women, respectively, in most Western countries. The incidence of pancreatic cancer has declined slightly, with an average annual change of –0.04%, from 1975 to 1998 in USA, presumably as a result of smoking cessation. In contrast, the incidences in Japan and European countries are increasing.

Disease Process

Adenocarcinoma of the head of the pancreas accounts for 80–90% of pancreatic cancer. Pancreatic cancer is rapidly fatal; the case fatality ratio is 0.99, median survival is 6 months or less, 1-year survival is approximately 20–30%, and 5-year survival is less than 5%. There is no effective screening modality for pancreatic cancer. The disease is difficult to diagnose and detect because the disease process is either silent or present with nonspecific symptoms, such as unexplained weight loss, back pain, nausea, jaundice, and altered intestinal habits. In approximately 80–90% of cases, the cancer is diagnosed at a nonresectable stage, when even small tumors may have already metastasized to other organs, most commonly the liver. Patients undergo cachexia, a complex metabolic syndrome clinically presenting with progressive weight loss and depletion of reserves of adipose tissue and skeletal muscle. Pancreatic cancer cells are particularly resistant to radiotherapy and chemotherapy, rendering the treatment unsuccessful. The lack of a useful screening tool and the poor prognosis of this disease highlight the importance of primary prevention.

Risk Factors

The etiology of pancreatic cancer is largely unknown. Prospective follow-up epidemiologic studies are the better study designs for determining a temporal relationship between exposures and disease outcomes. However, the rarity of pancreatic cancer makes it difficult to examine an association with sufficient statistical power. Most studies are case-control designs in which information on lifestyle and environmental exposures is collected from pancreatic cancer cases or proxies after cancer diagnoses and from selected controls with no pancreatic cancer. Such study design is prone to recall biases and information biases, and a temporal relationship cannot be determined. Once nonspecific symptoms occur, the aggressive disease processes make it difficult to complete data collection before a patient dies of the disease.

To date, the only risk factors of pancreatic cancer that have been well accepted are old age and cigarette smoking. Pancreatic cancer is more common in men than women, possibly because of differences in lifestyle factors and environmental exposures. African Americans, New Zealand Maoris, native Hawaiians, and Jews have higher incidences, whereas individuals in India or Nigeria and Seventh-Day Adventists have lower incidences. Hereditary pancreatitis and germline mutations may account for 10–15% of pancreatic cancer cases. Purported but unproven risk factors include diet, obesity, diabetes mellitus, chronic pancreatitis, *H. pylori* colonization, gastric or duodenal acidity, and occupational exposures to carcinogens. Socioeconomic status is not associated with pancreatic cancer risk.

Cigarette Smoking

Cigarette smoking has been consistently shown to be associated with a two- or threefold increased risk and accounts for 25–30% of pancreatic cancer cases. Higher risk has been associated with increased numbers of cigarettes smoked. Cigarette smoking may interact with hereditary factors to increase pancreatic cancer risk. It was estimated that smokers

who had a family history of pancreatic cancer had a sixfold increased risk compared with nonsmokers who did not have a family history, whereas a three- or fourfold increased risk was found in nonsmokers who had a family history or smokers who did not have a family history compared with nonsmokers with no family history.

Inherited Gene Mutations

Inherited mutations of genes account for approximately 10% of pancreatic cancer cases. *BRCA2* mutations are the most common, accounting for approximately 7%. Pancreatic cancer caused by these mutations often presents as apparently sporadic because of the low penetrance of *BRCA2* mutations. High-penetrance germline mutations in the *CDKN2A* (*p16*) gene that cause the familial atypical multiple mole and melanoma syndrome are also associated with higher risk for pancreatic cancer. Inherited mutations of *LKB/STK11* gene cause the Peutz-Jeghers syndrome, characterized by hamartomatous gastrointestinal polyps; mucocutaneous melanotic spots; and, in 30% of patients, pancreatic cancer. Inherited defects in a DNA mismatched repair gene causing hereditary nonpolyposis colorectal cancer and inherited mutations of the cationic trypsinogen gene causing acute pancreatitis at young age may also cause pancreatic cancer.

Dietary Factors

Higher intakes of fat, carbohydrate, animal protein, fried food, cured meat, or smoked meat have been associated with a higher risk for pancreatic cancer. In contrast, higher intakes of vitamin C; fiber; or, more generally, fresh fruits and vegetables and higher serum concentrations of folate and pyridoxine are associated with lower risk for pancreatic cancer. Alcohol, tea, or coffee consumption is not associated with pancreatic cancer.

Diabetes Mellitus

The temporal relationship between diabetes and pancreatic cancer is uncertain. A twofold increased risk of pancreatic cancer has been reported for people diagnosed with diabetes at least 5 years before pancreatic cancer diagnosis. The latency period of pancreatic cancer is unknown, but an estimate of at least 10 years has been reported. Hence, diabetes may be a consequence rather than a cause of pancreatic cancer. Interestingly, familial pancreatic cancer was not found to be associated with diabetes. In addition, approximately 50% of individuals who have noninsulin-dependent diabetes mellitus are not aware of the disease, and many pancreatic cancer patients are diagnosed with diabetes at the time of the cancer diagnosis.

Chronic Pancreatitis

Both hereditary and sporadic forms of chronic pancreatitis have been found to increase pancreatic cancer risk. In the inflammatory processes of chronic pancreatitis, cytokines, reactive oxygen species, and mediators of the inflammatory pathway (e.g., NF- κ B and COX-2) may increase cell turnover, cause loss of tumor suppressor genes, stimulate oncogene expression, and lead to pancreatic malignancy. Heavy alcohol consumption may increase the risk of chronic pancreatitis.

Prevention

Smoking cessation may be the first choice for the primary prevention of pancreatic cancer. However, evidence to support this rationale is lacking. An effective screening modality for pancreatic cancer has not been developed.

Cancer of the Small Intestine

The small intestine is approximately 20 ft long and consists of three sections: the duodenum, jejunum, and ileum. The small intestine performs extensive digestion and absorption functions. It also secretes secretin, which stimulates the pancreas to produce digestive enzymes.

Epidemiology

Cancer of the small intestine is very rare; the age-adjusted incidence is approximately 1.4 per 100 000 – less than 2% of all gastrointestinal malignancies. The incidence of small intestine cancer is higher in Maori of New Zealand and Hawaiians, and it is lower in India, Romania, and other areas of Eastern Europe. In the USA, the incidences of adenocarcinoma, lymphoma, and carcinoid have only slightly increased since 1980s; even for lymphoma, which has had the largest increase, the annual rate of increase has been no more than 1 per 1 million.

Disease Process

There are four types of small intestine cancer, each with unique characteristics: adenocarcinoma, carcinoid, lymphoma, and sarcoma. In Western developed countries, approximately 30–40% of small intestine cancer is adenocarcinoma, predominantly in the duodenum, and carcinoid and lymphoma occur more often in the jejunum or ileum, whereas sarcoma may develop anywhere in the small intestine. In developed countries, lymphoma is very rare and occurs more often in older people with relatively good survival. In contrast, in developing countries, lymphoma is the main type of small intestine cancer, and it occurs more often in younger individuals, anywhere in the small intestine, with poor survival. Hence, prognosis of small intestine cancer depends on the type, geographic location (which may be an indication of etiology and/or the advancement of treatment), and disease stages. Clinical presentation may include abdominal pain, weight loss, abdominal mass, anemia, nausea/vomiting, bleeding, obstruction, jaundice, and anorexia before diagnosis. Overall, the 5-year survival rate is approximately 80% for carcinoid, 60% for lymphoma, 45% for sarcoma, and 20% for adenocarcinoma.

Risk Factors

Owing to the rarity of small intestine cancer, etiologic investigation has relied on only a few small case-control studies. A lack of histology data has further undermined the strength of the evidence.

Tobacco use, alcohol consumption, and dietary factors such as high animal protein; high animal fat; sugar; and salted, cured, or smoked food were associated with small intestine

cancer in some but not all studies. Small intestine adenoma, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer, peptic ulcer, celiac sprue, and cholecystectomy have been found to be associated with increased risk for small intestine adenocarcinoma. In people with Crohn's disease, a 16- to more than 100-fold increased risk for small intestine adenocarcinoma has been reported, but unlike most adenocarcinomas that occur in the duodenum, these patients tend to have adenocarcinomas in the ileum. The reasons for the increased risk are uncertain, but it has been hypothesized to be due to the medication for treating Crohn's disease.

Prevention

Because very little is known about the etiology of small intestine cancer, no preventive strategy has been proposed.

Conclusion

The wide variation in the incidences of cancers of the esophagus, stomach, pancreas, and small intestine by geographic location and by population suggest that environmental factors play an important role in the etiology. Indeed, several risk factors are commonly shared by these cancer sites, including tobacco use, a diet low in fresh fruits and vegetables, and a diet high in salted, cured, or smoked food. Strategies for gastrointestinal cancer prevention should aim to counteract these risk factors. In addition, avoidance of heavy alcohol consumption and eradication of *H. pylori* may significantly reduce the incidence of esophageal cancer and stomach cancer, respectively. Studies are under way to test the efficacy of chemoprevention agents in the prevention of esophageal cancer and stomach cancer in high-risk populations. The development of noninvasive screening tests, such as molecular or imaging technology, is needed for early detection and better prognosis.

See also: Diabetes Mellitus: Etiology and Epidemiology

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Epidemiology of Lung Cancer

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Glossary

Biomarker A compound measured in human tissue as a marker of the body's exposure; for example, measurement of concentrations of beta-carotene in blood to measure dietary intake of beta-carotene.

Case-control study An observational (nonexperimental) study that starts with patients of disease (lung cancer) and controls without disease and attempts to measure exposures that occurred in the past.

Confounding When an extraneous variable (e.g., cigarette smoking) affects the association between the independent

variable (e.g., dietary factor) and dependent variable (e.g., lung cancer).

Epidemiology The study of health and disease in human populations.

Phytochemical Low molecular weight molecules produced by plants.

Prospective study An observational (nonexperimental) study that starts with measurement of exposure and follows participants up over time for occurrence of the disease (lung cancer).

Lung Cancer

Respiratory Carcinogenesis

The term 'lung cancer' refers to a histologically and clinically diverse group of malignancies arising in the respiratory tract, primarily but not exclusively from cells lining the airways of the lung. Beginning with the trachea, the airways branch dichotomously through 20 or more generations. Most cancers arise in the larger airways of the lung, typically at the fourth through the eighth generations. There, the airways are lined by a ciliated epithelium that includes secretory cells and glands, and also neuroepithelial cells. The specific cells of origin of lung cancer are still unknown; candidates include the secretory cells, pluripotential basal cells, and the neuroepithelial cells. Only a small proportion of lung cancers in smokers have been considered as originating in the lung's periphery, but with the increase in adenocarcinoma that has occurred this proportion may have increased.

Lung cancer is thought to arise from a sequence of genetic changes that move a cell from a normal to a malignant state. Diverse genetic alterations in oncogenes and tumor suppressor genes have been found in lung cancers, but the specific longitudinal sequence of these changes is unknown. The evolving understanding of respiratory carcinogenesis as a sequential progression from normal cells to clinical cancer implies there may be multiple points for interrupting the sequence and thereby preventing cancer.

Risk Factors for Lung Cancer

Cigarette smoking is the major cause of lung cancer, making it the primary culprit for the worldwide lung cancer epidemic. In smokers, the risk of lung cancer increases with both the duration of smoking and the number of cigarettes smoked. There is no known safe level of smoking, as even the secondhand tobacco smoke involuntarily inhaled by nonsmokers is

causally associated with lung cancer. Lung cancer risk decreases in those who quit smoking compared to persistent smokers, but not to the level of those who never smoked.

In addition to cigarette smoking, many other causes of lung cancer have been established. Numerous occupational lung carcinogens have been identified; the substances involved include radon (found in underground mines), arsenic, asbestos, chromium, chloromethyl ethers, nickel, and polycyclic aromatic hydrocarbons. Synergism with smoking has been shown for several of these agents, such as asbestos and radon. Many other agents are suspected occupational carcinogens.

Air pollution is associated with increased lung cancer risk. Outdoor air pollution increases lung cancer risk through inhalation of air contaminants from combustion sources that generate polycyclic aromatic hydrocarbons and radionuclides. Carcinogens in indoor air vary with the setting but may include radon, tobacco smoke, smoke from wood or coal burning, and cooking fumes.

The observed familial aggregation of lung cancer suggests that genetic factors may influence susceptibility. Identifying specific genes and the specific genetic and epigenetic alterations associated with susceptibility to lung cancer is actively being researched. Data from genome-wide association studies (GWAS) have provided promising leads. The results of four GWAS have been remarkably consistent in identifying genetic variants within a region on the long arm of chromosome 15 that are associated with lung cancer risk. For example, those with at least one variant allele of a specific SNP in this region (rs8034191) had a 1.3-fold greater risk of lung cancer than those homozygous for the wild type allele.

Lung Cancer Histopathology

As assessed by the clinical approach of light microscopy, primary cancer of the lung occurs as multiple histological types, the most common being squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small cell carcinoma. The pathogenetic bases of these four histological types are

uncertain, and various cells of origin and pathways of differentiation have been hypothesized. Further, multiple sections from the same patient sometimes contain heterogeneous tumors that contain elements of several histological types.

Although knowledge of the etiological and pathological bases of the different lung cancer types is limited, temporal trends have documented an increase in the proportion of adenocarcinoma. This shift is hypothesized to be due to the changes in cigarette manufacturing over time that led to changes in (1) smoking-delivered carcinogens and (2) how cigarettes are smoked, such as depth of inhalation.

Diet

Dietary Hypotheses and Mechanisms

Epidemiological research on diet and lung cancer has been both hypothesis-driven and descriptive, exploring associations between foods, nutrient indexes, or biomarkers and lung cancer risk. Interest in macronutrients has emphasized indices of dietary fat, which has long been known to have the capacity to act as a tumor promoter. Micronutrients have been extensively studied, spurred initially by the pioneering studies of Bjelke and the original vitamin A and β -carotene hypotheses. Bjelke and subsequent researchers originally focused on vitamin A because of its role in cellular differentiation, but this line of inquiry was subsequently expanded to include antioxidant micronutrients, with an emphasis on β -carotene. The more general hypothesis has been advanced that antioxidant micronutrients may protect against oxidative damage to DNA and thereby protect against cancer. Hypotheses concerning specific beverages have also been proposed; for example, animal studies have shown a link between alcohol and changes in lung lipids, including surfactant, and in levels of enzymes that can activate procarcinogens and mutagens.

Another epidemiologic approach, empirical rather than hypothesis-driven, has been to explore the intakes of several

specific foods or food groups for associations with lung cancer risk. The evidence documenting inverse associations between fruit and vegetable consumption and lung cancer stem from this empirical approach.

Certain methodological issues are relevant to the topic of diet and lung cancer. Foremost among these is the major challenge posed by the potential confounding effects of cigarette smoking due to the potent causal role of cigarette smoking, combined with the fact that smokers tend to eat less healthful diets than nonsmokers. This makes it very difficult to disentangle the potential impact of cigarette smoking on any observed diet–lung cancer association. Thus, even when efforts are made to attempt to control for smoking, residual confounding of diet–lung cancer associations may persist. Complicating matters further is that cigarette smoke can directly affect nutritional biomarkers (Figure 1); for example, smokers tend to have lower levels of circulating antioxidant micronutrients even after accounting for differences in dietary intake. Similar associations have even been noted for secondhand smoke exposure.

Characteristics of epidemiological studies in general further limit interpretation of studies that are specific to dietary factors. Approaches to dietary assessment are not fully standardized, so there may be differences between studies in the number of foods queried, the measurement of serving sizes, and the data collection approach employed. There is also uncertainty as to the biologically relevant exposure window for lung cancer, and dietary agents may plausibly act in early or later stages of carcinogenesis. Clinically diagnosed lung cancer reflects a series of complex molecular genetic events that occur over many years, and the relevant windows for dietary exposures are uncertain. Case-control studies usually measure past diet during some reference period, whereas cohort studies tend to focus on current diet. Case-control studies have commonly been employed to study diet and lung cancer, many of these studies focus on diet during the five years preceding diagnosis. These studies provide direct information concerning dietary factors in the later stages of

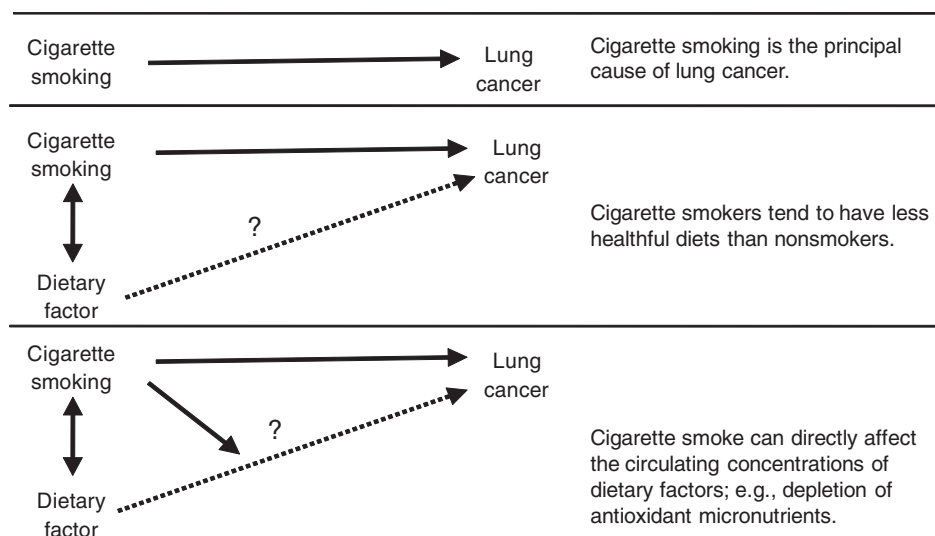


Figure 1 Cigarette smoking complicates the study of diet and lung cancer.

carcinogenesis. To the extent that such measures reflect usual adult (or lifetime) diet, these studies may also be relevant to the earlier stages of carcinogenesis. However, as lung cancer tends to be rapidly fatal, many case-control studies include data collected from deceased subjects' next-of-kin. Data from surrogate respondents are probably less accurate than self-reported data, and therefore introduce substantial misclassification.

Evidence concerning relationships between lung cancer and fruits, vegetables, micronutrients, phytochemicals, fat, body mass index, beverages, and meat intake is described in the section on dietary associations with lung cancer. To provide a guide for assessing the evidence for each dietary factor, evidence ratings from an objective assessment of the world's evidence on these topics, summarized in a seminal 2007 report of the World Cancer Research Fund (WCRF), are used for factors that were assigned evidence ratings. The rating scale used included evidence ratings of 'convincing,' 'probable,' and 'limited-suggestive' for whether a dietary factor was associated with increased or decreased risk of lung cancer.

Dietary Associations with Lung Cancer

Fruit

In total, the evidence points toward greater levels of fruit consumption being inversely associated with lung cancer risk. The WCRF systematic review rated the evidence on this topic as 'probable' that fruit consumption is associated with decreased risk of lung cancer. No clear pattern emerges when studies have examined specific fruits or classes of fruits. For example, apples and citrus fruits are associated with reduced risk of lung cancer in some studies but not in others.

Vegetables

Evidence for an inverse association between vegetable consumption and lung cancer risk parallels the evidence for fruit consumption, but is not quite as strong or consistent as the evidence for fruit. Consequently, the WCRF report rated the overall evidence as 'limited-suggestive' that higher levels of vegetable intake are associated with decreased lung cancer risk, pointing more equivocally toward a protective association. In addition to vegetable intake as a whole, the results for a number of specific vegetables, such as carrots and cruciferous vegetables, have been consistently associated with a reduced risk of lung cancer. The association with cruciferous vegetable intake has tended to remain strong and robust even in studies that have carefully controlled for cigarette smoking. As discussed below, the growing evidence of an inverse association between cruciferous vegetable intake and lung cancer risk has bolstered interest in isothiocyanates as a promising chemopreventive agent.

Micronutrients

Two different strategies have been used to evaluate the relationship of micronutrients to lung cancer. One approach has been to use food-frequency questionnaires to measure micronutrient intake. A second approach is to use biomarkers, assaying the circulating concentrations of micronutrients. The former approach provides a better average measure of

micronutrient 'exposure,' whereas the latter approach has the advantage of measuring micronutrient concentrations closer to cellular level, where the biologic effect is postulated to occur. However, a single assay of circulating micronutrient concentrations may fail to capture the biologically appropriate window of exposure.

The strongest evidence for the biomarker approach is generated from prospective cohort studies, where blood is collected from a population that is initially cancer-free and the population is then followed for the occurrence of lung cancer. The results of such prospective studies bolster the evidence supporting the premise that in general, the higher the circulating concentrations of carotenoids (α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, and total carotenoids), the lower the risk of lung cancer. Circulating concentrations of retinol, tocopherol, and selenium have not been associated with a reduced risk of lung cancer in most studies. In contrast to prospective studies, biomarker studies of micronutrients based on measurements made after a patient is diagnosed with lung cancer are limited because a clinical diagnosis of lung cancer and its treatment and concomitant changes in diet can lead to decreases in circulating micronutrient levels, introducing the potential for reverse causality.

For dietary intake, the evidence is most abundant for vitamins A, C, and E, and carotenoids. The evidence relating measures of retinol intake to lung cancer risk provide 'limited-suggestive' evidence that retinol is actually associated with increased risk of lung cancer. However, studies of dietary intake of carotenoids and vitamin C point more consistently toward an inverse association. For example, in a systematic review of the evidence from prospective studies, both dietary intake and circulating concentrations of total carotenoids were associated with 20–30% lower risk of lung cancer in the highest-versus-lowest exposure categories. Associations of similar magnitude were observed for the provitamin A carotenoids β -carotene, α -carotene, and β -cryptoxanthin and for the nonprovitamin A carotenoids lycopene and lutein. Based on these data, the WCRF concluded that foods containing carotenoids were 'probable' protective factors for lung cancer.

Thus, prospective studies of both biomarkers and of dietary intake favor a protective association between carotenoids and lung cancer. It is uncertain, however, if a generally protective association is specific to these carotenoids or whether carotenoid intake merely serves as a marker of the intake of other protective substances or healthier dietary habits in general. The evidence for vitamin C is suggestive of a protective association, whereas the data on vitamin A, vitamin E, and selenium have yielded null findings.

Phytochemicals

Phytochemicals are low molecular weight molecules produced by plants. Of the many classes of phytochemicals, those studied in relation to lung cancer include phytoestrogens, flavonoids, and glucosinoids.

The tumor promoting effects of steroid hormones can be blocked by phytoestrogens. Soya beans are a primary source of a specific class of phytoestrogens known as isoflavonoids. The relatively few studies to date of isoflavonoids in relation to lung cancer have not provided evidence of a link.

Flavonoids are polyphenolic compounds found in many foods derived from plants; flavonoids often exhibit potent antioxidant activity. Some fruits contain high levels of flavonoids, such as apples (quercetin) and white grapefruit (naringin). Flavonoid intake has been at least weakly associated with reduced risk of lung cancer in many, but not all, of the studies to date.

Isothiocyanates are metabolites of the class of phytochemicals known as glucosinolates. Isothiocyanates could exert anticancer effects by blocking carcinogens *via* induction of phase II detoxification enzymes, such as glutathione S-transferase. Cruciferous vegetables contain high concentrations of glucosinolates, and hence consumption leads to higher endogenous isothiocyanate levels. As with cruciferous vegetables, lung cancer risk is also consistently lower with higher intakes or urinary levels of isothiocyanates.

A postulated biologic relationship between isothiocyanates and a common polymorphism in the *GSTM1* gene provides an example of a potential gene–diet interaction relevant to lung carcinogenesis. A growing focus in cancer epidemiology is to characterize interindividual susceptibility to cancer by studying polymorphisms in genes involved in carcinogenic pathways, including how these genetic markers interact with environmental exposures to contribute to cancer risk. The role of glutathione S-transferase as a phase II detoxification enzyme has made a common polymorphism in the glutathione S-transferase M1 (*GSTM1*) gene of interest in relation to lung cancer. Compared to persons with the *GSTM1* present genotype, those with the *GSTM1* null genotype have a small but statistically significantly higher risk of lung cancer.

When isothiocyanates have been studied in combination with *GSTM1*, the decreased risk of lung cancer associated with isothiocyanates has been especially pronounced in persons with the *GSTM1* null genotype. This association could represent the cancer blocking activity of isothiocyanates being allowed to play an enhanced role in *GSTM1* null individuals because they are not being metabolized as quickly as in those with the *GSTM1* present genotype. This example illustrates the potential interactions between genetic and dietary factors. Integrating genetic and epigenetic markers into the study of nutritional factors provides a mechanistically-based approach that holds promise for advancing understanding of the complex role of diet in the etiology of lung cancer.

Fat

Evidence that dietary fat may facilitate tumor growth was reported as early as 1940. Correlation exists between international or regional dietary fat consumption and lung cancer mortality. In case–control studies, total fat intake is consistently associated with lung cancer risk, with less consistent results for saturated fat, unsaturated fat, and cholesterol intake. The prospective evidence shows a slightly different picture, with both total fat and saturated fat intake strongly associated with lung cancer in men but not women, and unsaturated fat and cholesterol not consistently associated with lung cancer risk in men or women. The results of a large, pooled cohort study found lung cancer risk was not strongly associated with fat (total, saturated, or unsaturated) or cholesterol intake. The evidence is equivocal, but tends to trend in

the direction of increased risk, as reflected in the assessment of the overall evidence rating in the WCRF report that the evidence is ‘limited–suggestive’ that total dietary fat is associated with increased lung cancer risk. With respect to specific foods, the same level of evidence was applied to butter.

Body Mass Index

In contrast to the situation for most types of cancer, prospective cohort studies consistently show a strong inverse relationship between body mass index (BMI) and lung cancer risk. These remarkably strong, consistent findings clearly demonstrate that leanness is statistically associated with lung cancer risk. The key remaining question is whether this association is genuine or whether it is indirect. Confounding by cigarette smoking is a viable explanation for these findings because cigarette smoking is strongly associated both with the risk of lung cancer and with leanness. However, the need to further test the hypothesis that leanness is a susceptibility factor for lung cancer is indicated by the results of studies in which this association persists even after careful control for cigarette smoking.

Beverages

Potential confounding by cigarette smoking recurs for the topic of beverage consumption. Many beverages, including alcohol, coffee, tea, and milk have been studied for a possible link to lung cancer. The majority of studies of alcohol drinking in relation to lung cancer risk that have adjusted for age and cigarette smoking have observed either null or weak associations.

Some studies have observed heavy coffee consumption to be associated with an elevated risk of lung cancer after adjustment for cigarette smoking, but a host of case–control studies have generated findings that fluctuate around the null. The issue of confounding between coffee drinking and other health behaviors, particularly cigarette smoking, has not been addressed adequately, indicating that much stronger evidence is needed for coffee drinking to be considered a risk factor for lung cancer. Despite numerous *in vitro* and *in vivo* studies that have observed potential tumor-inhibitory effects of tea, the epidemiologic evidence does not presently provide support for a link between tea drinking and lung cancer risk.

The associations observed between milk drinking and lung cancer depend on milk fat content. Milk drinking is not strongly associated with lung cancer risk when milk fat content is ignored. The associations between whole milk and lung cancer tend to be either null or in the direction of increased risk, whereas the associations for reduced fat or nonfat milk tend to be either null or in the protective direction. Perhaps milk consumption, including type of milk, is merely serving as a marker of fat intake, which as noted above tends to be associated with increased lung cancer risk. Consistent with the equivocal nature of the evidence and concerns about confounding by cigarette smoking, the WCRF report did not provide evidence ratings for any of these beverages in relation to lung cancer risk.

Drinking water can be a route of exposure to environmental contaminants. This is exemplified by the clear increase in lung cancer risk associated with drinking water that is contaminated with high levels of arsenic. Based on studies

conducted in geographic regions where drinking water is contaminated with high concentrations of arsenic, the WCRF report rated the evidence as 'convincing' that high concentrations of arsenic in drinking water is a risk factor for lung cancer.

Meat and Fish

Increased lung cancer risk has been observed to be associated with greater intakes of red meat and processed meat, but this evidence is counterbalanced by some null studies. The cooking method may play a role, as heterocyclic amines from cooked meat may contribute to an increased lung cancer risk. Based on the slight trending of the results toward increased risk, the WCRF report rated the evidence for both red meat intake and processed meat intake to be 'limited-suggestive' of increased risk. The current evidence does not support a strong link between fish consumption and lung cancer; the WCRF report did not rate this evidence.

Diet and Prevention

Chemoprevention Trials

Three large-scale, randomized, double-blind, placebo-controlled trials were undertaken to test the hypothesis that β -carotene supplementation protects against lung cancer. All three studies indicated that β -carotene supplementation in later adulthood does not protect against lung cancer. On the contrary, β -carotene supplementation was associated with an increased risk of lung cancer among the high-risk populations of heavy smokers in the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study and smokers and asbestos-exposed workers in the Carotene and Retinol Efficacy Trial (CARET) Study. The WCRF thus rated this strong, consistent evidence from two randomized controlled trials as 'convincing' that β -carotene increases lung cancer risk in current smokers. These experimental results thus not only failed to corroborate the evidence from observational studies, but clearly demonstrated that β -carotene supplementation increased risk in groups at the highest risk of lung cancer. The combined results of multiple randomized controlled trials of vitamin E supplements are clearly consistent with no effect on lung cancer risk.

Observation Versus Experiment

The ATBC and CARET studies enrolled older, high-risk individuals who had high cumulative exposure to tobacco smoke or asbestos. The results therefore presumably apply mainly to the latter stages of carcinogenesis. The doses administered were far higher than the normal dietary range, and the dose-response relationship for preventive effects, anticipated from the observational evidence, may not be applicable. Because antioxidant nutrients may exert their protective effect in the earlier stages of carcinogenesis, β -carotene may have been administered too late to halt the evolution of cellular changes that lead to lung cancer. Alternatively, compounds present in fruits and vegetables other than the micronutrients studied in the trials may protect against lung cancer. The protective associations for fruit and vegetable consumption were allied to the micronutrient hypothesis, but the results of the

chemoprevention trials of both β -carotene and vitamin E raise questions about the potential pay-off from large trials designed to test single micronutrients, unless there is a strong mechanistic basis combined with substantial observational evidence pointing to an individual micronutrient as the primary protective agent. Indeed, fruits and vegetables contain an abundance of antioxidants and phytochemicals with diverse anticarcinogenic activities. Then again, fruit and vegetable intake may be acting as a marker of a healthier lifestyle that is associated with lower cancer risk.

Conclusions

Knowledge of the relationship between diet and lung cancer has increased tremendously during past 40 years. Promising leads suggest that nutritional factors could have a substantial impact on lung cancer risk in humans. In general, persons who eat more fruits and vegetables have a lower risk of lung cancer than persons who consume less of these foods. In observational studies, the same holds true for intake of specific micronutrients, such as carotenoids. The specific constituents of fruits and vegetables that may confer protection are unknown. An important unanswered question is whether fruits and vegetables directly confer protection against lung cancer or whether estimates of fruit and vegetable consumption are indicators of differences between individuals who eat healthy and unhealthy diets that are leading to uncontrolled confounding. Nevertheless, the protective association noted for fruit and vegetable consumption has the potential to contribute to prevention. A diet adequate in fruit and vegetables is prudent for preventing chronic diseases in general.

Even for factors such as fruit and vegetable consumption, the highest category of intake is usually associated with at most a halving in the risk of lung cancer. An association of this magnitude could result from residual confounding by cigarette smoking. Future research that provides the strictest possible control for cigarette smoking, such as studies that match cases and controls in the study design or studies limited to never smokers, will help to resolve longstanding questions about dietary factors and lung cancer by addressing head-on the persistent concern about residual confounding by cigarette smoking.

Advances in understanding of the role of diet in lung cancer etiology should not obscure the fact that cigarette smoking is the predominant cause of lung cancer. Many important questions about the complex relationship between diet and lung cancer remain, but the primary way that the lung cancer epidemic will be controlled is to prevent the uptake of cigarette smoking among children and effectively assist addicted smokers to stop smoking cigarettes.

See also: Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Body Composition. Carotenoids: Chemistry, Sources and Physiology. Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Phytochemicals: Classification and Occurrence

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CARBOHYDRATES

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Glossary

Glycosidic bond A covalent bond that joins the hemiacetal group of a saccharide molecule and the hydroxyl group of some organic compound (e.g., an alcohol).

Maillard reaction Chemical reaction between an amino acid and a reducing sugar, which is important in the food industry as a form of non-enzymatic browning.

Stereoisomers Molecules that share the same molecular formula and sequence of bonded atoms, but differ in the

three-dimensional orientation of their constituent atoms in space.

Sugar alcohol Hydrogenated form of a carbohydrate, whose carbonyl group has been reduced to a hydroxyl group, widely used as an artificial sweetener.

Uronic acids Sugar acids with a carbonyl and carboxylic acid function, with important biochemical functions.

Introduction

Carbohydrates are the most abundant constituents of cereals, fruits, vegetables, and legumes, and the most important energy source in human nutrition. They contribute to the texture and flavor of foods and diet variability and palatability.

Carbohydrates are polyhydroxy aldehyde or ketone molecules and their derivatives with the general formula $(CH_2O)_n$. They comprise a group of substances with different structures and varying physical, chemical, and physiological properties. Dietary carbohydrates are important in maintaining glycemic homeostasis and gastrointestinal health. Furthermore, they contain necessary micronutrients, phytochemicals, and antioxidants.

Classification

Chemical Structure

Depending on their chemical structure and according to their degree of polymerization, carbohydrates are classified into four categories: monosaccharides, disaccharides, oligosaccharides, and polysaccharides.

Monosaccharides

Monosaccharides are the simplest form of carbohydrate and cannot be further hydrolyzed to smaller subunits. According to their chain length, monosaccharides fall into several categories, the more nutritionally important being the pentoses (5-carbon atom skeleton), e.g., ribose, and the hexoses (6-carbon atom skeleton), e.g., glucose.

The presence of asymmetrical carbons in monosaccharides with different functional groups attached gives rise to optical activity. Monosaccharides are optically active, which means that if polarized light is passed through a solution of these compounds, the plane of light will be rotated to the left (levorotatory or L-form) or to the right (dextrorotatory or D-form). Consequently, similar structures of the same compound are formed and are called stereoisomers. Monosaccharides of the D-form are nutritionally important because most naturally occurring monosaccharides are D-stereoisomers and metabolic and digestive enzymes are specific for them.

Monosaccharides demonstrate another type of stereoisomerism due to their formation of cyclic structures. The pentoses form furanose (5-carbon ring) and the hexoses form

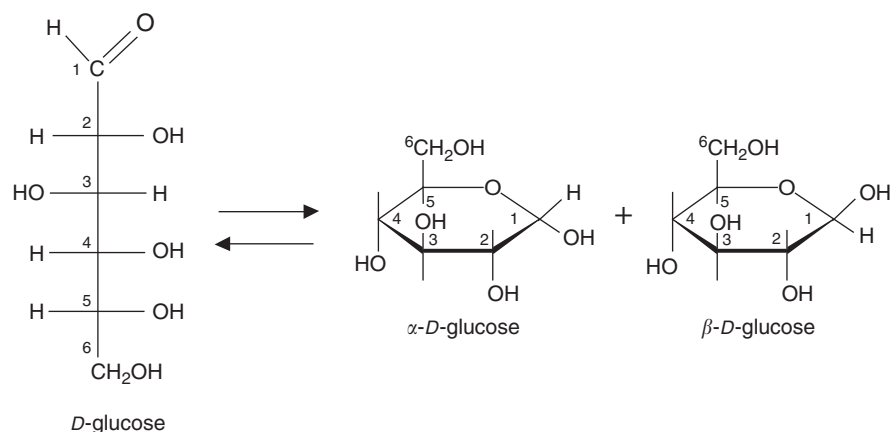


Figure 1 D-Glucose molecule shown as open chain and as a cyclic pyranose ring in the α and β configuration.

Table 1 Some nutritionally important monosaccharides and monosaccharide derivatives

Class	Species	Significance
Hexoses	D-Glucose	Major cell fuel, unbound in body fluids and tissues, building block of several polysaccharides
	D-Fructose	Cell fuel, constituent of sucrose
	D-Galactose	Cell fuel, constituent of galactose
	D-Mannose	Constituent of plant cell wall polysaccharides and gums
Pentoses	L-Arabinose, D-xylose	Constituent of plant cell wall polysaccharides
	D-Ribulose, D-xylulose	Metabolite in pentose pathway
	D-Ribose	RNA constituent
	D-Glucuronic, D-galacturonic	Constituent of plant cell wall polysaccharides
Uronic acids	D-Mannuronic, D-guluronic	Constituent of algal polysaccharides
	Sugar alcohols	
Sugar alcohols	D-Glucitol, D-xylitol	Food ingredient
	D-Galactitol	Metabolite of galactose
	Desoxysugars	
Desoxysugars	D-Desoxyribose	DNA constituent
	D-Desoxygalactose	Constituent of algal polysaccharides
	L-Fucose	Constituent of bacterial polysaccharides
	L-Rhamnose	Constituent of pectic plant polysaccharides
Aminosugars	D-Glucosamine, D-galactosamine	Constituent of aminosaminoglycans, cartilage

pyranose (6-carbon ring). Cyclization can produce two stereoisomers of the α and β configuration, and generally an equilibrium mixture of the straight and the cyclic forms exists in monosaccharide solutions. **Figure 1** illustrates D-glucose in its pyranose form in the α and β configuration. The isomerization produces compounds with different properties and has major metabolic importance because of enzyme specificity for particular stereoisomers.

The most nutritionally important and abundant monosaccharide is glucose, which is used as the major cell fuel in the human body and can be found unbound in body tissues and fluids. Glucose is the building block of several polysaccharides. Galactose and fructose are also used as cell fuel. The most important monosaccharides and monosaccharide derivatives and their significance are outlined in **Table 1**.

Disaccharides

Disaccharides consist of two monosaccharide units, linked together with glycosidic bonds in the α or β orientation. The most important of them are sucrose, lactose, and maltose. Sucrose is the most abundant and consists of a molecule of

α -glucose and β -fructose linked together (**Figure 2(a)**). Lactose is found in milk and dairy products and consists of galactose and glucose linked by a β -1,4-glycosidic bond (**Figure 2(b)**). Maltose is mainly produced by partial hydrolysis of starch and consists of two glucose units linked by an α -1,4-glycosidic bond (**Figure 2(c)**). Some nutritionally important disaccharides and disaccharide derivatives and their significance are outlined in **Table 2**.

Oligosaccharides

Oligosaccharides consist of a chain of three to nine monosaccharide units, covalently linked to form large units and are named trioses, tetroses, etc., denoting the number of carbons in their molecule. Oligosaccharides are distributed widely in plants and, when digested, yield their constituent monosaccharides. The major oligosaccharides consist of the raffinose series, formed by the linkage of galactose, sucrose, and glucose units, and the maltose series, formed by the linkage of glucose units. Some nutritionally important oligosaccharides and their significance are outlined in **Table 3**.

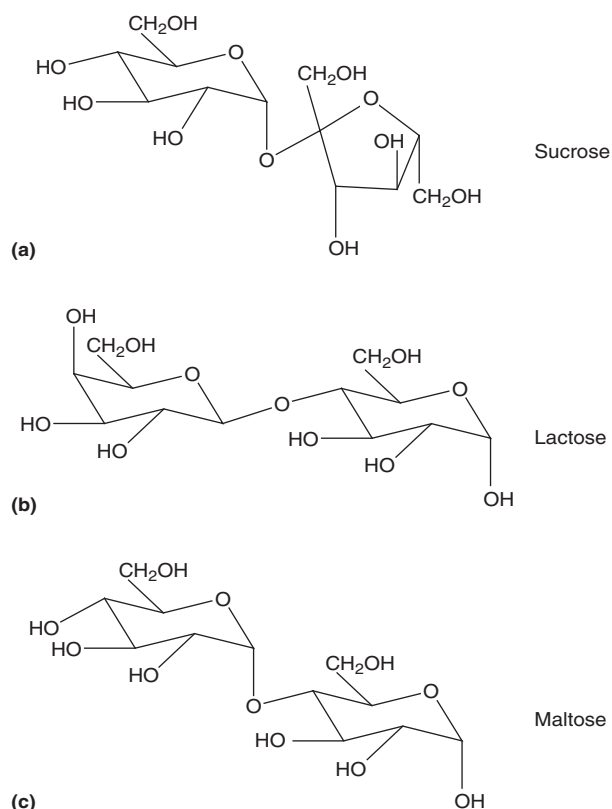


Figure 2 The molecular structures of (a) sucrose, (b) lactose, and (c) maltose.

Table 2 Some nutritionally important disaccharides and disaccharide derivatives

Class	Species	Significance
Disaccharide	Sucrose	Constituent of fruits, vegetables, and sweetener
	Lactose	Milk and dairy products
	Maltose, isomaltose	Constituent of starch
	Trehalose	Food additive and constituent of mushrooms
Disaccharide alcohols	Lactulose	Lactose derivative, laxative
	Maltitol	Constituent of starch, sweetener
	Lactitol	Constituent of lactose, sweetener

Polysaccharides

Polysaccharides consist of long chains of monosaccharide residues (10 or more) linked by glycosidic bonds. These compounds consist of several hundred or even thousands of monosaccharide units. The properties of polysaccharides are determined by the species of monosaccharides in the polymer backbone, the type of linkages between residues, and the extent and type of chain branching.

Glucans are polymers of glucose and the major polysaccharides in the diet. The most important glucans are starch, glycogen, and cellulose. Glycogen is the short-term storage form of glucose in animal tissues. Starch is the most common

Table 3 Some nutritionally important oligosaccharides

Class	Species	Significance
Maltoses	Maltotriose, maltotetraose	Constituent of starch
Raffinoses	Raffinose, stachyose, verbascose	Constituent of vegetables and legumes
Fructoses	Fructotriose	Constituent of cereals, tubers
Lactoses	Fucosyl lactoses	Constituent of human breast milk

digestible storage polysaccharide in plants and cellulose is a major structural component of plant cell walls (**Figure 3**). Some nutritionally important polysaccharides and their significance are outlined in **Tables 4** and **5**.

Polysaccharides with α linkages have a helical shape, e.g., the amylose starch molecule, whereas those with β linkages generally have a linear or flat ribbon-like molecule, e.g., cellulose (**Figure 3**).

Polysaccharide molecules can be linear or branched. Branches can be formed through any unlinked hydroxyl group and vary from alternating and consecutive single-unit branches to multiple-unit branches (ramified structure).

Nutritional Importance

Some carbohydrates that have specific importance in human nutrition are sugars and sugar alcohols, starch, and dietary fiber.

Sugars

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) expert consultation on carbohydrates use the term 'sugar' to describe mono- and disaccharides. Sugars can be separated analytically from the food matrix by gas-liquid chromatography (GLC), high-performance liquid chromatography (HPLC), and enzymatic methods. Sugars are widely utilized in the food industry as sweeteners and preservatives. They improve the texture, body, palatability, and viscosity of foods and beverages.

The UK Department of Health distinguishes between 'intrinsic' and 'extrinsic' sugars. Intrinsic sugars are defined as naturally occurring, which form part of plant cell walls. Extrinsic sugars are defined as added sugars, which are not part of the food matrix. Within the extrinsic sugars, 'milk' and 'nonmilk' are terms used to distinguish between milk and other extrinsic sugars. However, these terms have not been widely accepted.

Sugar Alcohols

Sugar alcohols are monosaccharide and disaccharide derivatives, such as sorbitol and xylitol, which are extensively used as sweeteners in the food industry. They have received increased attention because of their desirable properties: relative sweetness, limited digestion, and absorption.

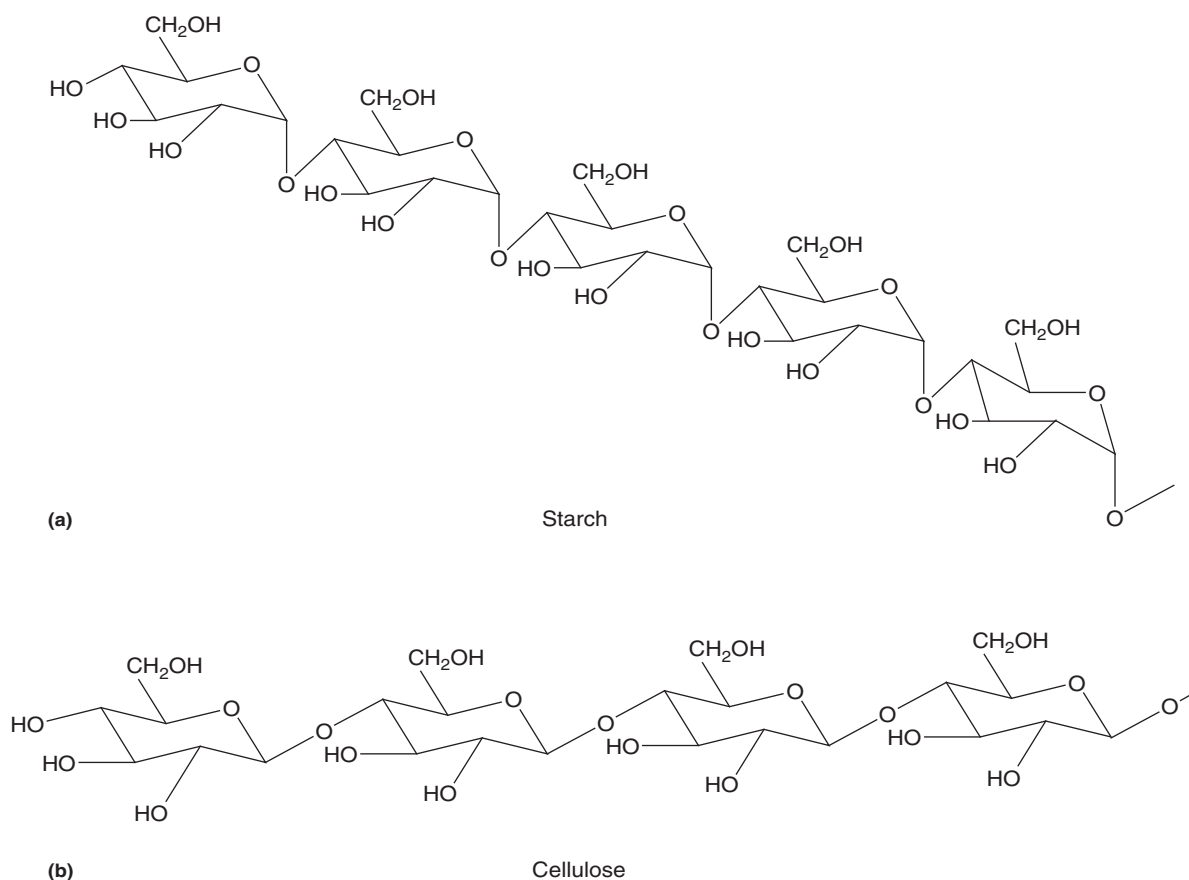


Figure 3 (a) Five units of an α -1,4-D-glucopyranose chain from a starch molecule (amylose). (b) Four units of a β -1,4-D-glucopyranose chain from a cellulose molecule.

Table 4 Some nutritionally important polysaccharides

Class	Species	Significance
Glucans	Starch	Storage polysaccharide in plants
	Glycogen	Short-term storage form of glucose in animal tissues
	Cellulose	Major structural component of plant cell walls
Galactans		Major constituents of noncellulosic matrix of plant cell wall
Xylans		Constituents of mature plant tissues
Mannans		Storage forms in several plants
Uronans	Galacturonans	Major components of water-soluble pectic fraction of plants
	Mannuronans	Components of algal polysaccharides
	Guluronans	Components of algal polysaccharides

Starch

Starch is the most important, abundant, and digestible polysaccharide in human nutrition. Starch consists of large chains of α -linked glucose residues and is found in the form of amylose or amylopectin. Amylose is a linear, unbranched form of starch, which consists of α -1,4-linked glucose units (Figure 3(a)). Amylopectin is a branched-chain polymer, which consists of α -1,6-linked glucose units. Both forms of starch can be found in cereals, potatoes, legumes, and other vegetables, with amylopectin comprising 80–85% and amylose 15–20% of total starch.

Dietary Fiber

The definition of dietary fiber has been debated over decades, together with analytical methods for its determination. In 2001, the Institute of Medicine (IOM) of the National Academies of Science defined *dietary* fiber as intact, nondigestible carbohydrates and lignin from plant sources, and *functional* fiber as nondigestible carbohydrates from plant or animal sources with favorable health outcomes for humans. According to IOM, total fiber consists of both dietary and functional fibers, which cannot be digested by mammalian enzymes and passes almost intact through the small intestine.

Table 5 Some nutritionally important starch and nonstarch polysaccharides

Class	Species	Significance
Starch	Amylose, amylopectin	Most common digestible plant polysaccharides
Nonstarch	Cellulose	Major component of plant cell wall
	Pectin	Constituent of plant cell wall, food additive
	Hemicellulose	Constituent of plant cell wall
	Gums, mucilages	Plant hydrocolloids, food additives
	Algal polysaccharides	Constituents of algae and seaweed, food additives

After several years of research and debate over the definition of dietary fiber, a final agreement was reached on a global definition for the *Codex Alimentarius* (collection of standards, codes of practice, guidelines, and recommendations). At the 2008 meeting of the Codex Committee on Nutrition and Foods for Special Dietary Uses (CCNFSDU), dietary fiber was defined as carbohydrates with 10 or more subunits, which cannot be hydrolyzed by endogenous enzymes of the human small intestine and can belong to the following categories:

1. naturally occurring edible carbohydrates;
2. carbohydrates obtained from food raw material by physical, chemical, or enzymatic methods, and scientifically shown to have a physiological effect or health benefit;
3. synthetic carbohydrates scientifically shown to have a physiological effect or health benefit.

This new definition has generated the need for the development of an integrated analytical method for the determination of total dietary fiber. The AACC International (previously known as American Association of Cereal Chemists) and the AOAC (Association of Official Analytical Chemists) International have previously developed methods for measurement of dietary fiber and dietary fiber components. Under the supervision of these two international organizations, a new method has been developed for determination of total dietary fiber, using HPLC analytical separation. Interlaboratory evaluations of this method are under way.

Dietary fiber consumption has potential important health benefits, including general gastrointestinal health and prevention of several noncommunicable diseases, through blood cholesterol reduction and regulation of blood sugar levels.

Chemistry

Monosaccharides share the same functional groups, but their isomeric forms often exhibit differences in chemical reactions. Disaccharides exhibit a similar range of reactions with monosaccharides due to the presence of similar functional groups. Oligosaccharides generally exhibit properties similar to mono- and disaccharides with similar functional groups, but some oligosaccharides with nine monosaccharide units may exhibit properties similar to polysaccharides. In general, polysaccharides show slower reaction rates due to steric effects.

Solubility

Monosaccharides, disaccharides, and oligosaccharides exhibit similar solubilities. Overall, they are very soluble in water. Sucrose is extremely soluble in water, whereas lactose is soluble to a lesser extent. Furthermore, they are insoluble in nonpolar organic solvents. They exhibit limited solubility in pure alcohols but are very soluble in aqueous alcohol solutions (70–80%, v/v), and therefore these solutions are widely used for extraction and analysis. Oligosaccharides are less soluble in aqueous alcohol solutions than monosaccharides, and their solubility decreases as the number of monosaccharide units increases.

Polysaccharides, in general, form colloidal solutions in water, whereas some other polymers are extremely insoluble in water and require prior treatment with acid, alkali, or organic solvents to get them to dissolve. For example, β -1,4-mannans and glucans (e.g., cellulose) are very insoluble due to hydrogen bonding between parallel chains. However, arabinoxylans are readily soluble in water, because the arabinosyl chains inhibit hydrogen bonding. Galactomannans are also readily soluble in water, producing viscous solutions, and are used as food additive gums. The α -linked glucans (e.g., amylose and amylopectin) have completely different solubilities. The α -1,4-amylose is very soluble in warm water and forms colloidal solutions. When the amylose chains cool down, they form an amylose gel, which subsequently forms an insoluble crystalline material. Amylopectins are also very soluble in hot water but do not form an insoluble crystalline material to the same degree as amylose.

Reducing Properties

Monosaccharides are powerful reducing agents toward a range of metal salts in alkaline solution, due to the presence of aldo- and keto groups and formation of hemiacetal or acetal groups. The extent of reduction differs among different monosaccharides. Important compounds formed in this way are sugar alcohols. For example, sorbitol is produced by reduction of the aldo group of glucose.

Disaccharides and oligosaccharides have the same reducing properties, except sucrose that is nonreducing because it has no free hemiacetal group. Polysaccharides usually contain one reducing group at the terminal end of the polymer chain and as a result have lower reducing properties.

Reactions in Acidic Solutions

Monosaccharides when heated in strong acidic solutions result in dehydration and condensation into a range of furans. The resulting furans condense with several reagents to generate colored products; hence, the presence of monosaccharides and their derivatives is verified.

Reactions in Alkaline Solutions

Monosaccharides when present in weak alkaline solutions result in the isomerization of the aldo-keto group (enolization). In stronger alkaline solutions, they produce a series of degradation compounds, saccharinic acids, which condense

repeatedly to generate a series of highly colored products, in the presence of ammonia, amino acids, and proteins. This reaction, known as Maillard reaction, results in the browning of food products, and it is utilized in the food industry for the production of caramel colors.

Hydrolysis

Acid

Disaccharides and oligosaccharides in mild acidic conditions are hydrolyzed into their constituent monosaccharides. The fructofuranosyl linkages of the fructooligosaccharides are quite susceptible to acid hydrolysis.

Polysaccharides are also hydrolyzed into their constituent monosaccharides by acid hydrolysis, but the conditions necessary for complete hydrolysis depend on the solubility of the polymers. The majority of polysaccharides (e.g., starch) are completely hydrolyzed under weak acid conditions. On the contrary, cellulose requires treatment with strong acid for several hours before hydrolysis, and subsequent heating under weak acidic conditions for the completion of the reaction. The uronans are very resistant to complete acid hydrolysis and generally produce disaccharides of aldobiuronic acids. Acid hydrolysis of polysaccharides results in extensive losses of their monosaccharide constituents.

Enzymatic

Disaccharides are hydrolyzed into specific enzymatic solutions; therefore, enzymatic hydrolysis is a useful method for the analysis of sugar mixtures. Oligosaccharides are also susceptible to enzymatic hydrolysis. The maltooligosaccharides can be rapidly hydrolyzed by glucosidase enzymes.

Polysaccharides are more efficiently hydrolyzed to their monosaccharide constituents by using specific enzymes. Fungal enzymes act specifically for the hydrolysis of different polysaccharides. The α -1,4-glycosidic linkages in starch can be hydrolyzed by various α -amylases (e.g., salivary and pancreatic), producing maltose and isomaltose. The β -1,6-glycosidic linkages in amylopectin are not as easily hydrolyzed and require the presence of the fungal enzyme pullulanase to complete the hydrolysis.

Ester Formation

Monosaccharides contain hydroxyl groups and react with acids to form a variety of esters. The phosphate esters play a major role in carbohydrate metabolism. For example, the first step of glycolysis involves the production of the glucose 6-phosphate ester in a reaction catalyzed by the enzyme glucokinase in the presence of adenosine triphosphate. The uronic acids react with alcohols to form esters. The methyl esters of uronic acids are most important in determining physical properties of the uronans.

The presence of additional hydroxyl groups in di- and oligosaccharides increases the number of sites for esterification reactions. Sucrose reacts with fatty acids to produce nondigestible esters, which have properties similar to the triacylglycerols.

Polysaccharides can also produce ester compounds. For example, galacturonans, which are composed of an α -1,4-galacturonic acid chain with integrated rhamnose units, form salts with cations and may be esterified with methoxyl groups.

Substitution

Monosaccharides undergo substitution reactions with methyl iodide to produce methyl ether derivatives. These compounds have been used to identify the structure of polymers because the site of nonmethyl substituted groups is indicative of the site of the branch points after hydrolysis. Monosaccharides undergo acetylation, which occurs on the free or the reduced molecule, to produce acetylated alditols. These volatile compounds have been used to identify sugar mixtures by GLC. The presence of additional hydroxyl groups in di- and oligosaccharides increases the number of sites for substitution reactions.

See also: Alcohol: Absorption, Metabolism, and Physiological Effects. Carbohydrates: Regulation of Metabolism; Requirements and Dietary Importance. Fiber: Physiological Effects and Effects on Absorption; Resistant Starch and Oligosaccharides. Fructose: Absorption and Metabolism. Glucose: Chemistry and Dietary Sources

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Regulation of Metabolism

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Glossary

Catecholamines “Fight-or-flight” hormones released by the adrenal glands in response to stress.

Diabetes mellitus A group of metabolic disorders, characterized by high levels of blood glucose (impaired glucose tolerance), and results from defects in insulin secretion, insulin action, or both.

Glucagon A hormone secreted by the pancreas in response to low blood glucose levels. Glucagon counteracts

insulin action and stimulates hepatic glucose output, in order to maintain glucose homeostasis.

Glucocorticoids Steroid hormones that derive their name from their role in the regulation of glucose metabolism.

Insulin A hormone secreted by the pancreas, which has a major role in the regulation of carbohydrate and fat metabolism. Insulin is synthesized in response to high blood glucose and promotes glucose storage and utilization.

Introduction

The three basic monosaccharides important in human nutrition are glucose, fructose, and galactose. Glucose is the product of digestion of starch. In human metabolism, all simple sugars are converted into glucose. Glucose is the circulating form of carbohydrates in the bloodstream. Appropriate regulation of glucose metabolism is necessary for cell function and essential for health and survival. Fructose is the sweetest of the simple sugars and it is found in fruits and naturally occurring substances like honey. Fructose consumption has increased greatly in the United States since the 1970s, when high-fructose corn syrup started to be widely used for food processing. High-fructose corn syrup is the major sweetening agent in the food industry. Galactose is produced by the digestion of lactose, the major carbohydrate in milk.

Digestion

Most carbohydrate foods, starches, and sugars have to be converted to glucose in order to be used for energy production. The digestion of carbohydrates starts in the mouth with mastication and the enzymatic action of salivary amylase and their conversion to dextrins and maltose. Successive contractions of the stomach (peristalsis) move the food to the lower part of the stomach, while 20–30% of the carbohydrate is already converted to maltose. Peristalsis facilitates digestion in the small intestine, whereas the chemical digestion of carbohydrates is completed by pancreatic amylase (continues the breakdown of starch to maltose) and intestinal disaccharidases (sucrase, lactase, and maltase for the breakdown of fructose, lactose, and glucose, respectively). The monosaccharide products of carbohydrate digestion are then absorbed into the portal circulation.

Absorption

Glucose consists of the largest quantity of absorbed carbohydrate (~80%), and galactose and fructose account only for

a small amount (~20%). The body quickly absorbs and transports the simple sugars, which enter the portal circulation via the capillaries of the intestinal villi and are transported to the liver. In the liver, fructose and galactose are converted to glucose, which is either used immediately for energy or stored in the form of glycogen. Liver can store approximately 5% of its mass in the form of glycogen, which can be readily converted to glucose for the production of energy.

Transport

Monosaccharides traverse the epithelial lining of the intestine by facilitated diffusion or by active transport. The transport system for the passage of glucose and galactose through the apical membrane of the intestinal villi is called the sodium (Na^+)-dependent glucose transporter (GLUT). Fructose utilizes a different transporter for the same passage, called GLUT5. All monosaccharides are then transported from the enterocyte to the bloodstream through another sugar transporter known as GLUT2. The passage of glucose and galactose across both membranes of the intestine requires the presence of Na^+ , whereas the passage of fructose is not dependent on Na^+ but on fructose concentration.

Carbohydrates and Energy Metabolism

Glucose

The breakdown of glucose can be divided into two major parts: the anaerobic conversion of glucose to pyruvate, known as glycolysis, and the aerobic breakdown of pyruvate to carbon dioxide (CO_2) and water (H_2O), which consists of the tricarboxylic acid cycle and the electron transport chain.

Glycolysis is the series of enzymatic steps, leading to the breakdown of one molecule of glucose for the production of two molecules of pyruvate (Figure 1). Glycolysis occurs in the cytosol of different cells, and all human cells are capable of carrying out the process of glycolysis. However, the largest

part of glycolysis occurs in the liver, muscle, and adipose tissue.

The fate of pyruvate is determined by the cell type and the availability of oxygen. In the absence of oxygen, pyruvate is reduced to lactate in the cytosol. This would occur in the muscle during strenuous exercise, when the demands for energy are high. In cells that do not contain mitochondria, such as the erythrocytes, the glycolytic pathway is the only mechanism of energy production.

In the presence of oxygen, pyruvate is converted to acetyl coenzyme A (acetyl CoA) in the mitochondria and enters the tricarboxylic acid cycle and subsequently the electron transport chain. As a result, pyruvate is fully oxidized to CO_2 and H_2O and large amounts of energy are produced.

Fructose and Galactose

Fructose and galactose enter the glycolytic pathway through their conversion to intermediate compounds (Figure 1). This

occurs primarily in the liver and, as a result, these two monosaccharides are not generally available for uptake by other tissues. The end products of the catalysis of these monosaccharides are similar to glucose; however, when they are absorbed, they do not elicit the same hormonal response as glucose does.

In the liver, fructose breakdown, known as fructolysis, is initiated by the conversion of fructose to fructose 1-phosphate catalyzed by fructokinase, and subsequent hydrolysis to glyceraldehyde and dihydroxyacetone phosphate catalyzed by fructose 1-phosphate aldolase. These products of hydrolysis can be used for further glycolytic conversion. Fructolysis in the liver bypasses the highly regulated step of phosphofructokinase and can produce a large amount of glycolytic metabolites. In the muscle and kidney cells, fructose can enter the glycolytic pathway through its conversion to fructose 6-phosphate, before the highly regulated phosphofructokinase step.

In the liver, galactose enters the glycolytic pathway through its phosphorylation to galactose 1-phosphate and subsequent

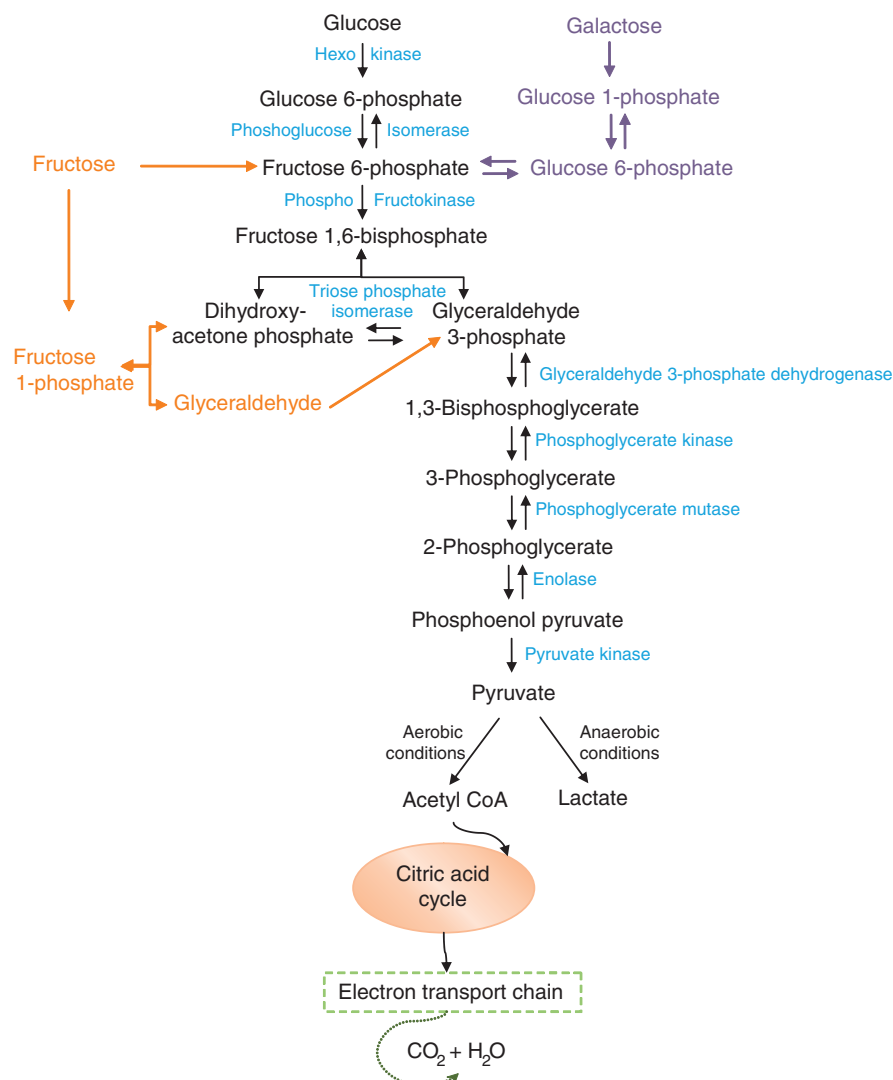


Figure 1 Outline of glucose metabolism, with entry points for fructose and galactose.

epimerization to glucose 1-phosphate. This metabolic intermediate can either enter glycolysis by its conversion to glucose 6-phosphate or can be used in glycogen synthesis, depending on the nutritional state of the organism.

Glucose Production by the Liver and Kidneys (Glycogen)

Gluconeogenesis

The biosynthesis of glucose from pyruvate, lactate, glycerol, or other precursors is known as gluconeogenesis. It is not a direct reversal of glycolysis because several steps of glycolysis are irreversible. Gluconeogenesis mainly occurs in the liver and less in the kidney. These tissues contain all the necessary enzymes for gluconeogenesis and the enzymatic activity of glycerol kinase, which allows glycerol to enter the gluconeogenic pathway at the level of glyceraldehyde 3-phosphate (Figure 2).

It is vital for the organism to synthesize glucose for those tissues that are unable to synthesize glucose. In humans, liver glycogen stores can sustain the organism for 18 h without the ingestion of dietary carbohydrates. After this period, the liver must produce glucose and transport it to other organs. Liver is the main gluconeogenic contributor (~90%), whereas the kidney produces glucose gluconeogenically to a lesser extent (~10%).

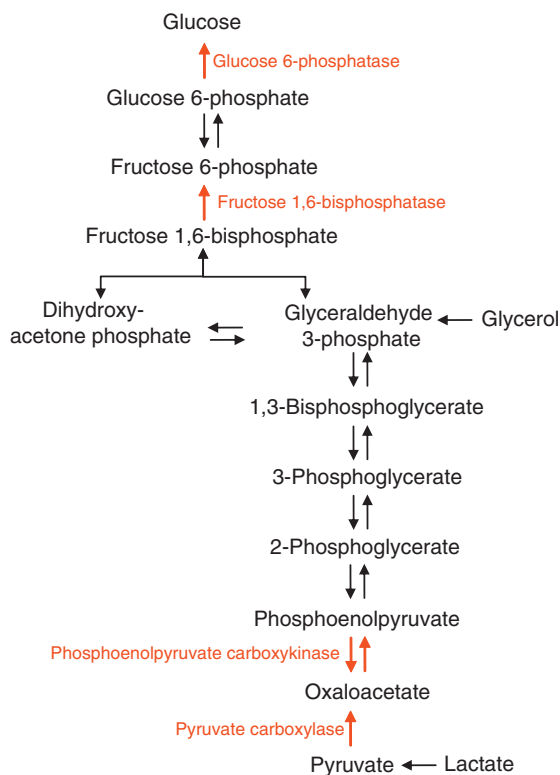


Figure 2 Outline of gluconeogenesis, with entry points for lactate and glycerol. The reactions shown in red are distinctive for gluconeogenesis, whereas the other reactions are common with glycolysis.

Glycogenolysis

Glycogen is a branched polymer of glucose, which contains as many as 100 000 glucose units. The breakdown of glycogen for the production of glucose units is known as glycogenolysis. Glycogen breakdown is initiated at the nonreducing ends of its branches. It consists of phosphorolysis of single glucose units by the cooperating enzymatic action of glycogen phosphorylase and the debranching enzyme. The product of phosphorolysis, glucose 1-phosphate, needs the additional action of phosphoglucomutase to be converted to glucose 6-phosphate. The liver contains the enzyme glucose 6-phosphatase for hydrolysis of glucose 6-phosphate to free glucose and for export from the organ to target tissues. However, the muscle and the brain do not contain such an enzyme and the produced glucose 6-phosphate enters the glycolytic pathway for energy production. Glycogen is a very efficient storage form of glucose, having an overall storage efficiency of approximately 97%.

Control of Carbohydrate Metabolism

Hormonal Regulation

Hormones regulate (activate or inhibit) specific enzymes that catalyze the reactions of metabolic pathways. This is achieved mainly by covalent regulation or conversion of the enzymes into their active or inactive form. Furthermore, hormones can regulate enzymes by induction or regulation of their transcription. Regulation of the expression of specific genes controls the concentration of enzymes and transport proteins necessary for carbohydrate metabolism.

Insulin

When a meal is ingested, glucose is liberated from hydrolysis of dietary carbohydrate in the small intestine and then it is absorbed into the blood. Increased glucose concentrations stimulate the production and secretion of insulin by the β cells of the pancreas. Insulin promotes the transfer of glucose into the target cells (i.e., skeletal muscle, liver, and adipose tissue) for utilization as energy and for storage in the form of glycogen, in the liver, primarily.

Insulin also stimulates glycolysis by increasing the activity of glycogen synthase and the transcription of glycolytic enzymes. Insulin inhibits gluconeogenesis by decreasing the transcription of several gluconeogenic enzymes and by moderating peripheral release of gluconeogenic precursors (Figures 3 and 4).

Fasting results in a decrease in insulin concentration and reduction of glucose uptake by the muscle and adipose tissue, which use alternate forms of energy (e.g., free fatty acids). Glucose then becomes available for uptake by the brain, red blood cells, and renal medulla, which are strongly dependent on glucose for energy.

Glucagon

Glucagon is a hormone secreted in the bloodstream by the α cells of the pancreas in response to low glucose levels. Glucagon counteracts the action of insulin and its main role is to

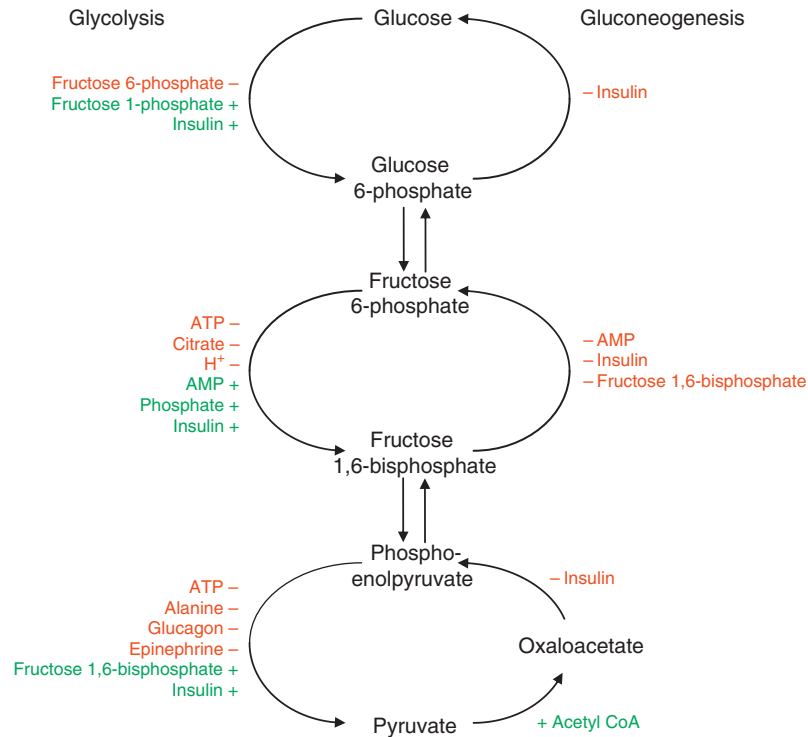


Figure 3 Regulation of glycolysis and gluconeogenesis in the liver.

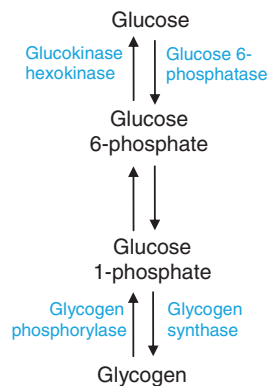


Figure 4 Points of regulation of glycogen synthesis and breakdown.

stimulate hepatic glucose output and to maintain glucose homeostasis. Glucagon stimulates glycogenolysis by activating glycogen phosphorylase and inhibits glycogen synthesis by inactivating glycogen synthase (Figure 4). Furthermore, glucagon stimulates gluconeogenesis by increasing the gene expression of gluconeogenic enzymes and blocking glycolysis. In the liver, glucagon enhances the rate of gluconeogenesis by lipolysis, resulting in increased concentrations of free fatty acids and glycerol.

Catecholamines

Epinephrine and norepinephrine are catecholamines that have a regulatory effect on carbohydrate metabolism. This effect is mainly dependent on the type of receptor present in each cell. Catecholamine receptors are divided into two types: two α and

three β receptors. The β and α_1 receptors stimulate catabolic reactions, whereas the α_2 receptor inhibits them. The presence of catecholamine receptors on different cell types explains the selective breakdown of stores from certain tissues.

During fasting, catecholamines stimulate gluconeogenesis and glycogenolysis in the liver, as a result of increased secretion of glucagon by epinephrine. Catecholamines normally do not play a central role in maintaining glucose homeostasis during fasting, but they prevent hypoglycemia when glucagon secretion is low.

Glucocorticoids

Cortisol, the principal glucocorticoid, stimulates hepatic glucose output and the expression of genes encoding for gluconeogenic enzymes, thus stimulating gluconeogenesis. Cortisol is essential for the action of several hormones and has a much slower effect on hepatic glucose production in comparison with glucagon and the catecholamines.

Growth Hormone

Growth hormone, like cortisol, increases hepatic glucose production by changing substrate availability and promoting the expression of gluconeogenic enzymes. Growth hormone secretion is enhanced by starvation. Like cortisol, the effect of growth hormone on hepatic glucose production is much slower than that of glucagon and the catecholamines.

Allosteric Enzyme Regulation

Allosteric enzymes are activated or inhibited by substances produced in the pathway in which the enzymes function.

These substances are called modulators and can alter the activity of allosteric enzymes by changing their conformation. Adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) are important modulators of allosteric enzymes in carbohydrate metabolism. The effect of ATP is opposed by AMP and ADP. When energy supply is adequate, ATP accumulates and negatively modulates enzymes that catalyze energy-producing or catabolic pathways, e.g., glycolysis. When energy is depleted and ATP concentration is decreased, AMP and ADP accumulate. As a result, there is a need for energy production and a positive modulation of allosteric enzymes in catabolic pathways. An increase in ATP inhibits further energy production and blocks glycolytic enzymes, whereas an increase in AMP or ADP stimulates glycolytic enzymes for energy production (**Figure 3**).

Directional Shifts

The majority of enzymes catalyze reversible reactions and their action is highly dependent on the concentration of the reactants involved. An increase in the concentration of one reactant will drive the reaction in the direction that results in the breakdown of the reactant and the achievement of homeostasis. An example of a directional shift is the interconversion of glucose 1-phosphate and glucose 6-phosphate. During glucogenolysis, the concentration of glucose 1-phosphate increases and the reaction is driven toward the production of glucose 6-phosphate. During glycogen synthesis and gluconeogenesis, the concentration of glucose 6-phosphate increases and the reaction is driven toward the production of glucose 1-phosphate and subsequently toward the formation of glycogen.

Regulation of Gene Expression

Gene expression regulation enables the human body to respond to changes in nutrient concentration. During increased availability of a specific nutrient, there is no need for expression of the genes encoding for enzymes involved in the metabolism of that nutrient. Gene expression is highly regulated by hormones, which respond to the concentration of nutrients in the blood. Selective expression of specific genes plays a major role in regulation of carbohydrate metabolism.

Hormonal and nutrient concentrations affect several regulatory domains of genes that encode for enzymes involved in anabolic and catabolic pathways. Insulin and glucose concentrations increase messenger ribonucleic acid (mRNA) levels and transcription rates of the glycolytic enzymes and decrease those of the gluconeogenic enzymes. On the contrary, glucagon has the opposite effect of insulin.

Glycogen Synthesis and Breakdown

The regulatory mechanism of glycogen synthesis and breakdown involves two counteracting enzymes: glycogen synthase and glycogen phosphorylase (**Figure 4**). Insulin activates glycogen synthase and therefore increases glycogen synthesis in the liver and muscle. When blood glucose levels decrease, glucagon inhibits glycogen synthase and activates glycogen

phosphorylase for the breakdown of glycogen in the liver. Epinephrine also activates glycogen breakdown in both the liver and skeletal muscle.

Peripheral Glucose Uptake of Glucose by Skeletal Muscle and Adipose Tissue

Glucose enters the target tissues by facilitated diffusion through a family of transporters known as glucose transporters. Five different isoforms of glucose transporters, known as GLUT1–GLUT5, are the predominant and most extensively characterized isoforms. GLUT4 is mainly present in skeletal and cardiac muscle and brown adipose tissue and differs significantly from the other isoforms in that it is stimulated by insulin. The other types of glucose transporters do not require insulin's action for glucose transport. GLUT1 and GLUT3 are responsible for glucose transport in most body tissues and are found in the brain, kidney, placenta, red blood cells, and fetal tissue. GLUT2 exists mainly in the liver and the pancreas and GLUT5 is responsible for glucose and fructose transport in the small intestine.

The glucose transporters are encoded by different genes and the regulation of their expression is highly tissue specific. GLUT4 is highly regulated by insulin and its concentration is significantly increased in response to the presence of the hormone. As a result of the increase in GLUT4 concentration, there is increased glucose uptake by the adipose tissue and skeletal muscle.

Diseases of Carbohydrate Metabolism

Carbohydrate Malabsorption

Fructose Intolerance and Essential Fructosuria

Fructose intolerance and essential fructosuria are the two genetic defects of fructose metabolism. Fructose intolerance is an autosomal recessive disease, caused by a genetic defect in fructose 1-phosphate aldolase (aldolase B) in the liver. The symptoms of aldolase B deficiency start when the infant is exposed to fructose. Aldolase B deficiency results in phosphate depletion and fructose 1-phosphate accumulation in the liver. Consequently, gluconeogenesis and glycogenolysis are blocked, resulting in inhibition of protein synthesis and subsequent liver failure.

Essential fructosuria is caused by a defect in the fructokinase gene. This disorder is asymptomatic and results in the excretion of fructose in the urine, as well as the conversion of fructose to fructose 6-phosphate in the muscle and adipose tissue.

Glucose and Galactose Malabsorption

Carbohydrate intolerance is a hereditary disorder that occurs infrequently and poses serious health risks. This disorder is caused by a deficiency in a digestive enzyme (e.g., sucrase- α -dextrinase) and defective glucose–galactose transport. Carbohydrate intolerance can be detected by the development of profuse infant diarrhea immediately after birth.

Glycogen Storage Diseases

A lack of the enzyme glucose 6-phosphatase in the liver, kidney, and intestinal mucosa causes a disease known as Von Gierke disease. This disease results in fasting hypoglycemia, hepatomegaly, and recurrent acidosis. Genetic defects in glucose 6-phosphatase, glucose 6-phosphatase translocase, or the pyrophosphate transporter result in a metabolic imbalance and inability of the liver to maintain glucose homeostasis by either glycogenolysis or gluconeogenesis.

Gene mutations in the liver and muscle glycogen phosphorylases result in rare autosomal recessive disorders. In the liver, the disease results in liver glycogen accumulation and is known as Hers' disease. It is characterized by hypoglycemia, hepatomegaly, and growth delay. In the muscle, the disease results in progressive muscle weakness and liver glycogen accumulation and is known as McArdle disease. It is characterized by exercise intolerance. A mutation in the debranching enzyme also results in glycogen accumulation in liver and muscle and is known as Cori disease. It is characterized by fasting hypoglycemic convulsions, hepatomegaly, and myopathy.

Diabetes Mellitus

Diabetes mellitus is a group of metabolic disorders, characterized by high levels of blood glucose (impaired glucose tolerance), and results from defects in insulin secretion, insulin action, or both. Diabetes mellitus is the seventh leading cause of death in the United States and a major cause of premature mortality, stroke, cardiovascular disease, peripheral vascular disease, congenital malformations, perinatal mortality, and long- and short-term disability. There are four principal types of diabetes mellitus: type 1 (formerly known as insulin-dependent diabetes mellitus or IDDM), type 2 (formerly known as non-insulin-dependent diabetes mellitus or NIDDM), gestational diabetes mellitus (GDM), and maturity-onset diabetes of the young (MODY).

Type 1 Diabetes

Type 1 diabetes is caused by autoimmune pancreatic β -cell exhaustion and loss of insulin secretion. Onset of the disease occurs when most of the pancreatic β cells have been destroyed by the immune system. This form of diabetes is generally diagnosed in children and young adults and accounts for 5–10% of all cases of diabetes mellitus.

Type 2 Diabetes

Type 2 diabetes is a complex heterogeneous disorder caused by interactions of various genetic and environmental factors. It is characterized by insulin resistance, obesity, a sedentary lifestyle, and occasionally by decreased insulin secretion. Because obesity and physical inactivity are increasing in children, the prevalence of pediatric type 2 diabetes has increased dramatically over the past 20 years and has reached epidemic proportions. More than 85% of the cases of diabetes mellitus are type 2.

Gestational Diabetes Mellitus

GDM is a condition characterized by high blood glucose levels in pregnant women without previously diagnosed diabetes. It is usually ameliorated after childbirth, but it increases the future risk of developing type 2 diabetes.

Maturity-onset Diabetes of the Young

MODY is an autosomal dominant trait, which primarily affects insulin secretion and accounts for 2–5% of the cases of diabetes. MODY can be caused by mutations in the glucokinase genes, leading to reduced rate of glycolysis in the pancreas, reduced glycogen synthesis, and increased gluconeogenesis in the liver.

See also: Carbohydrates: Chemistry and Classification; Requirements and Dietary Importance. **Diabetes Mellitus:** Classification and Chemical Pathology; Dietary Management; Etiology and Epidemiology. **Fructose:** Absorption and Metabolism. **Glucose:** Chemistry and Dietary Sources; Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level

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Relevant Websites

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American Diabetes Association.
- <http://www.fao.org/>
Food and Agriculture Organization of the United Nations.
- <http://www.eufic.org/>
The European Food Information Council.
- <http://www.usda.gov/>
United States Department of Agriculture.

Requirements and Dietary Importance

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Glossary

Adequate intake (AI) The observed intake of a particular group of healthy people.

Dietary reference intakes (DRIs) A set of reference values for nutrient intake.

Estimated average requirement (EAR) The average daily intake value of a nutrient that is estimated to fulfill the needs of healthy people in a particular lifestage or group.

Glycemic index (GI) The area under the curve of the blood glucose increase 2 h after carbohydrate ingestion of a

set amount of a particular food (e.g., 50 g) as compared to the blood glucose increase 2 h after the ingestion of the same amount of a reference food (white bread or glucose).

Hexose A monosaccharide with six carbon atoms having the chemical formula $C_6H_{12}O_6$.

Recommended dietary allowance (RDA) The minimum daily intake that fulfills the needs of almost all healthy people in a particular lifestage or group.

Tolerable upper intake level (UL) The maximum daily intake level of a nutrient that is not likely to pose an adverse health effect for almost all people.

Introduction

Carbohydrates are an important energy source in the human diet. They generally supply approximately 45% of energy requirements in developed countries and up to 85% in developing countries. Carbohydrates have been considered a fundamental source of nourishment and an inexpensive and versatile staple of the diet.

The type and composition of dietary carbohydrates vary greatly among different food products. Dietary carbohydrates can be predominantly found in the form of sugar (monosaccharides and disaccharides) and starch or nonstarch polysaccharides. Furthermore, in the food industry, they can be used in the form of hydrolyzed cornstarch, high-fructose corn syrups, modified starches, gums, mucilages, and sugar alcohols.

The current global emphasis for healthy eating is on increasing carbohydrate consumption, particularly in the form of whole grains, fruits, and vegetables. Epidemiological and clinical studies have shown a positive association between carbohydrate consumption and reduced risk of chronic disease and certain types of cancer.

Dietary Sources and Intakes

The major sources of carbohydrates are cereals, consisting of more than 50% of carbohydrates consumed in both developed and developing countries, followed by sweeteners, root crops, pulses, vegetables, fruits, and milk products. Carbohydrate and nutrient intake, in general, can be estimated using data from food production and balance sheets, household surveys, and individual assessments (Table 1). Figure 1 shows the trends in carbohydrate consumption by food groups as a percentage of total carbohydrates in developed and developing countries, obtained from food balance data in 1994.

Sugars

The term 'sugar' includes monosaccharides and disaccharides. The most common monosaccharides are glucose (or dextrose), fructose, and galactose. Glucose is found in fruits, honey, maple syrup, and vegetables. Glucose is also formed from sucrose hydrolysis in honey, maple syrup, and invert sugar. It is also produced from starch hydrolysis in corn syrups. The properties of glucose are important for improving food texture, flavor, and palatability. Glucose is the major cell fuel and the principal energy source for the brain. Fructose is found in honey, maple sugar, fruits, and vegetables. Fructose is also formed from sucrose hydrolysis in honey, maple syrup, and invert sugar. It is commonly used as a sweetener in soft

Table 1 Approaches for determination of trends in nutrient consumption worldwide

Approach	Advantages	Disadvantages
Food production	Figures available for every crop	Affected by agricultural practices, weather conditions, external forces
Food balance sheets	Figures available for every food item	Inadequate to determine food waste and spoilage
Household surveys	Figures close to actual food consumption	Inadequate to determine food consumption outside the home, food waste and spoilage
Individual assessments	Figures close to actual food consumption	Data not available for all countries Diverse methods of assessment

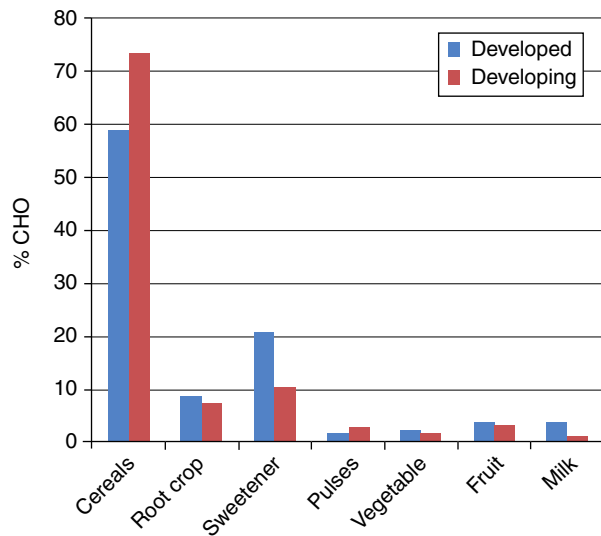


Figure 1 Energy consumed by carbohydrate food groups as a percentage of total carbohydrates in developed and developing countries, obtained from food balance data in 1994. Data obtained from FAO/WHO (1998) Carbohydrates in human nutrition. Report of a Joint FAO/WHO Expert Consultation. *FAO Food and Nutrition Paper* 66: 1–140.

drinks, bakery products, and candy, in the form of high-fructose corn syrups. Galactose is found primarily in milk and dairy products.

The most common disaccharides are sucrose, lactose, and maltose. Sucrose is mostly found in sugarcane and beet, and in lesser amounts in honey, maple sugar, fruits, and vegetables. The properties of sucrose are important in improving viscosity, sweetness, and flavor of baked foods, ice cream, and desserts. Maltose is formed from starch digestion. It is also produced from the germination of grain for malt liquors. Lactose is found in milk and dairy products and is not as sweet as glucose or sucrose.

In the second part of the twentieth century, sugar intake increased markedly in the United States. This is due in particular to increased consumption of added sugars as a result of their greater use in beverages and foods. According to the US Food Supply data, consumption of added sugars has increased from 27 teaspoons per person per day in 1970 to 32 teaspoons per person per day in 1996, which represents a 23% increase. A major fraction of this increase has been in the form of high-fructose corn syrup. Soft drinks are the most frequently used form of added sugars and consist of one-third of the total sugar intake. In Europe, the trend of sugar consumption has been a steady one.

Polysaccharides

Starch

Starch is the most important and abundant food polysaccharide. It is predominantly derived from plant seed, such as wheat, maize, rice, oats, and rye, and from plant roots, such as potatoes. Legumes and vegetables also contribute to the starch content of the diet. Bread and pasta are popular forms of starch, whereas tropical starchy foods, such as plantains,

cassava, sweet potatoes, and yams are increasingly contributing to carbohydrate intake. Starch accounts for 20–50% of total energy intake, depending on the total carbohydrate consumption.

Nonstarch

Nonstarch polysaccharides (NSP), formerly referred to as 'dietary fiber,' can be either soluble or insoluble and are mainly derived from cereals, especially whole grain. Wheat, rice, and maize contain predominantly insoluble NSP, whereas oats, rye, and barley contain predominantly soluble NSP. Vegetables are also a source of NSP and contain equal amounts of insoluble and soluble NSP. Intakes of NSP range from approximately 19 g day^{-1} in Europe and North American countries to 30 g day^{-1} in rural Africa.

Health Effects of Carbohydrates

Carbohydrates are the major energy source in most human diets. Because the majority of amino acids and lipids can be converted to glucose via gluconeogenesis, there is no absolute requirement for carbohydrates. Furthermore, in the absence of dietary carbohydrates, the human body can utilize the products of lipolysis of stored triacylglycerol or ketone bodies for the production of energy. This process, known as ketosis, results in a characteristic breath odor.

Carbohydrates are stored in the human body as glycogen, mainly in the liver and muscle. The human body has a limited storage capacity for carbohydrates compared to that for fat. The total amount of carbohydrates stored in tissues and circulating in the blood as glucose is approximately 7.56 MJ (1800 kcal). Diets high in carbohydrates ensure adequate glycogen storage available for immediate energy utilization. Carbohydrates are the preferred energy source for the human brain and have an important role in reducing protein breakdown when energy intake is inadequate.

Dietary carbohydrates are absorbed in their hexose form (glucose, fructose, and galactose) and provide 15.6 kJ g^{-1} (3.75 kcal g^{-1}) of energy. Although sugars and polysaccharides provide similar amounts of energy, they differ in their physiological and metabolic properties. The effects of carbohydrate-containing foods on blood glucose levels during digestion and absorption are variable, depending on the type of dietary carbohydrate. Postprandial glucose response is reduced when glucose absorption is slow. Glycemic index (GI) has been used for the quantification of blood glucose response after carbohydrate consumption. GI is the area under the curve of the blood glucose increase 2 h after carbohydrate ingestion of a set amount of a particular food (e.g., 50 g) as compared to the blood glucose increase 2 h after the ingestion of the same amount of a reference food (white bread or glucose). GI is influenced significantly by the carbohydrate types and physical determinants of digestion rate (intact versus ground grains, cooked versus uncooked food, and soluble fiber content). Carbohydrate ingestion in the presence of fat and protein reduces the GI of a meal. The GI of carbohydrate-containing meals has been linked to several health outcomes. The role of carbohydrates in health is a growing area of research and has received a great amount of interest in the past decade.

Carbohydrates and Nutrient Density

Increased sugar consumption has generated a concern in the recent years because of the potential to displace the micronutrient content of the diet by increasing 'empty calories' and energy intake. There is some evidence that essential nutrient intake decreases with increasing total sugar intake. However, sugar intake has not been shown to accurately predict micronutrient ingestion. Moderate intakes of sugar coincide with sufficient nutrient intake. The risk of low micronutrient status is increased for individuals with a diet high in sugars and low in total energy intake, as in the case of children or people on restrictive diets. Data analysis on food intake of preschool children suggests that the intake of some micronutrients (calcium, zinc, thiamin, riboflavin, and niacin) is inversely related to sugar intake. However, the dilutional effects of sugars may be somewhat distorted by the fact that some rich sources of added sugars are also fortified with micronutrients, as in the case of breakfast cereals. The Institute of Medicine (IOM) of the National Academies of Science, using national food intake data, reported that a clear dilutional effect on micronutrient intake starts when sugar intake approaches 25% of total calories. The American Heart Association dietary guidelines stress the consumption of fruits, vegetables, grains, and complex carbohydrates so that micronutrient requirements are met by whole rather than supplemented foods.

Several human studies have demonstrated that diets rich in NSP may reduce the bioavailability of minerals, such as iron, calcium, and zinc. Nevertheless, this effect is more likely because of the presence of phytate, which inhibits the absorption of those minerals, than the NSP content of the diet.

Carbohydrates and Obesity

Several studies have been conducted to establish an association between sugar ingestion and total energy intake. There have been consistent reports of a negative association between sugar intake and body mass index in adults and children. However, this observation could be confounded by the correlation of dietary fat and obesity, because high-fat diets are usually low in carbohydrates. Some *ad lib* dietary studies have shown that diets low in sugar are associated with weight loss, may be as a result of reduced calorie intake. Nevertheless, in human metabolic studies, no effect on weight or energy expenditure was observed when carbohydrate was replaced by fat or protein in isocaloric diets.

Foods high in sugars or GI are highly palatable and can create a potential risk for energy overconsumption and weight gain. However, there is insufficient evidence to support this claim or confirm the role of GI on body weight regulation. Foods high in sugar have high energy density, and thus decreasing their consumption can assist in weight reduction. On the contrary, foods rich in NSP are bulky and as a result induce greater satiety when ingested. As a result, diets rich in NSP may be useful for obesity prevention, because they prevent energy overconsumption. However, evidence indicating that increased carbohydrate content of a low-energy diet facilitates weight loss is lacking.

The consumption of sugar-sweetened soft drinks may contribute to weight gain because of the low satiety of liquid

foods. Short-term human studies have shown that sugar-sweetened soft drink consumption does not result in a decrease of total energy intake. Thus, sugar-sweetened soft drinks can significantly increase the total caloric intake and result in weight gain. Consumption of these drinks has been associated with childhood obesity.

Carbohydrates and Cardiovascular Disease

Dietary factors influence obesity, diabetes, and hyperlipidemia, which are risk factors for the development of cardiovascular disease (CVD). A diet rich in carbohydrates, in the form of whole grain cereals, fruits, and vegetables, may assist in the reduction of saturated fat and the increase of the antioxidant content of the diet, therefore reducing the risk of heart disease. On the contrary, a high intake of carbohydrates (>65% of total calories), especially in the form of refined sugars and starch, may increase serum triglyceride levels and adversely affect plasma lipoprotein profile. Short-term studies show a consistent relationship between sugar consumption and elevation of triglyceride levels, as well as a decrease in plasma high-density lipoprotein (HDL) levels, which could result in increased atherosclerosis and heart disease risk. However, longitudinal cohort studies have failed to show a consistent association of sugar consumption and CVD, mainly because of the confounding factors associated with increased heart disease risk.

Certain NSP (e.g., β -glycans) have been shown to reduce low-density lipoprotein (LDL) and total cholesterol levels on a short-term basis. Therefore, a protective effect for CVD has been shown with consumption of foods high in NSP. This protective effect has not been duplicated with NSP supplements. However, no long-term effect has been established.

High-GI diets have been shown to slightly increase hemoglobin A_{1c}, total serum cholesterol, and triglycerides, and decrease HDL cholesterol and urinary C-peptide in diabetic and hyperlipidemic individuals. On the contrary, low-GI diets have been shown to decrease cholesterol and triglyceride levels in dyslipidemic individuals. However, there are insufficient studies performed on healthy individuals and further research on the role of GI on lipid profile and CVD risk factors is warranted.

Carbohydrates and Type 2 Diabetes

Some recent evidence suggests that rapidly digested refined sugars, which have a high GI, may increase the risk of type 2 diabetes. Short-term studies have shown that decreasing the GI of a meal can improve glucose tolerance and insulin sensitivity in healthy people. Furthermore, the substitution of high-GI carbohydrates with low-GI carbohydrates can decrease postprandial glucose and insulin levels. Some epidemiological studies have demonstrated a protective effect of NSP consumption against type 2 diabetes.

Carbohydrates and Dental Caries

The quantity and frequency of sugars in the diet play a significant role in the development of dental caries. Their

digestion by salivary amylase provides an acid environment for the growth of bacteria in the mouth, therefore increasing the rate of plaque formation. Sucrose is the most cariogenic of the sugars, followed by glucose, fructose, and maltose. The milk sugars (lactose and galactose) are considerably less cariogenic. There is no epidemiological evidence to support a cariogenic role of polysaccharide foods with no added sugars.

Dental caries is a multifaceted disease, affected not only by the frequency and type of sugar consumed but also by oral hygiene, fluoride supplementation and use. Despite the increase in sugar consumption, the incidence of dental caries has decreased worldwide because of the increased use of fluoride and improvement of oral hygiene.

Carbohydrates and Cancer

Case-control studies have shown that colorectal cancer risk increases with high intakes of sugar-rich foods, whereas other studies have failed to prove such a relationship. Thus, there is insufficient evidence to support the role of sugar in the risk for colorectal cancer. On the contrary, carbohydrate consumption in the form of fruits, vegetables, and cereals has been shown to be protective against colorectal cancer.

Carbohydrate foods are a good source of phytoestrogens, which may protect against breast cancer. However, studies related to carbohydrate intake and breast cancer have been inconsistent and are insufficient to establish an association between carbohydrates and breast cancer risk.

The Health Professionals Follow-up Study showed a negative association of prostate cancer risk with high-fructose intakes. Additional data on the role of sugar consumption on prostate cancer risk are lacking. Some evidence suggests that increased fiber intakes are related to decreased prostate cancer risk.

Carbohydrates and Gastrointestinal Health

High intakes of NSP, in the range of 4–32 g day⁻¹, have been shown to contribute to the prevention and treatment of constipation. Population studies have linked the prevalence of hemorrhoids, diverticular disease, and appendicitis to NSP intakes, although there are several dietary and lifestyle confounding factors that could directly affect these relationships. High-carbohydrate diets may be related to bacterial growth in the gut and subsequent reduction of acute infective gastrointestinal disease risk.

Low-Carbohydrate Diets

The recent trend of weight loss diets promotes some level of carbohydrate restriction and increased protein consumption. Some examples are Dr Atkins New Diet Revolution, the South Beach Diet, and the Carbohydrate Addict's Diet. This dietary advice is contrary to that proposed by governmental agencies (US Department of Agriculture/Department of Health Services, National Institutes of Health) and nongovernmental organizations (American Dietetic Association, American Heart Association, American Diabetes Association, and American Cancer Society).

There is consistent evidence that weight loss is triggered by negative energy balance resulting from low caloric intake, and that it is not a function of macronutrient composition. There is insufficient scientific evidence to suggest that low-carbohydrate diets are more metabolically efficient than restricted calorie conventional diets. Several studies have shown that low-carbohydrate diets result in weight loss because of reduced caloric intake.

Low-carbohydrate diets may promote ketosis, reduce glucose and insulin levels, and suppress appetite, resulting in increased blood uric acid concentration. Some studies have shown that the consumption of high amounts of nondairy protein results in a decline in kidney functions in individuals with mildly compromised kidney function. However, no such effect has been shown in individuals with normal kidney functions. Furthermore, low-carbohydrate diets can have side effects, like bad taste, constipation, diarrhea, dizziness, headache, nausea, thirst, and fatigue.

Low-carbohydrate diets lack essential vitamins and minerals because of inadequate consumption of fruits, vegetables, and grains, and require supplementation to achieve nutritional adequacy. Controlled trials of low-carbohydrate diets are necessary to establish long-term effectiveness and adverse health effects or benefits.

Requirements and Recommendations

According to the IOM, Dietary Reference Intakes (DRIs) have been defined as a set of reference values for nutrient intake and include the Estimated Average Requirement (EAR), Recommended Dietary Allowance (RDA), Adequate Intake (AI), and Tolerable Upper Intake Level (UL). EAR refers to the average daily intake value of a nutrient that is estimated to fulfill the needs of healthy people in a particular life stage or group. RDA refers to the minimum daily intake that fulfills the needs of almost all healthy people in a particular life stage or group. AI refers to the observed intake of a particular group of healthy people, and it is used when there is lack of scientific experimentation for the determination of the EAR or the RDA. UL refers to the maximum daily intake level of a nutrient that is not likely to pose an adverse health effect for almost all people.

The DRIs for carbohydrate consumption of individual groups and life stages are outlined in [Table 2](#). These values were based on the average minimum amount of glucose needed by the brain. A UL for carbohydrates was not set because no studies have shown that excessive consumption of carbohydrates can be harmful to one's health. Based on the dilutional effect of added sugars on micronutrients, the expert panel of IOM suggests a maximal intake of less than 25% of energy from added sugars. Total sugar intake can be decreased by limiting foods high in added sugars and consuming naturally occurring sugar products, like milk, dairy products, and fruits.

The IOM does not specify dietary requirements or recommendations for NSP consumption but has provided recommended intakes for fiber, which includes NSP. The DRIs for total fiber consumption of individual groups and life stages are outlined in [Table 3](#). It has not been shown that a high-fiber intake can have harmful effects in healthy individuals, and thus a UL for fiber has not been set.

Table 2 Carbohydrate requirements and recommendations (DRIs, IOM 2001)^a

Age group/Lifestage	EAR (g day ⁻¹)		RDA (g day ⁻¹)		AI (g day ⁻¹)
	Males	Females	Males	Females	
Infants (0–6 months)					60
Infants (6–12 months)					95
Children (1–18 years)	100	100	130	130	
Adults (> 18 years)	100	100	130	130	
Pregnancy		135		175	
Lactation		160		210	

^aDRIs, Dietary Reference Intakes; EAR, Estimated Average Requirement; RDA, Recommended Dietary Allowance; AI, Adequate Intake.

Table 3 Total fiber recommendations (DRIs, IOM 2001)^a

Age group/Lifestage	AI (g day ⁻¹)	
	Males	Females
Children (1–3 years)	19	19
Children (4–8 years)	25	25
Children (9–13 years)	31	26
Children (14–18 years)	38	26
Adults (19–50 years)	38	25
Adults (> 51 years)	30	21
Pregnancy		28
Lactation		29

^aDRIs, Dietary Reference Intakes; AI, Adequate Intake.

There is insufficient evidence to support a recommendation by IOM of low-GI foods or the replacement of high-GI foods, like bread and potatoes. Although several studies propose adverse effects of high-GI carbohydrates and beneficial effects of low-GI foods, a recommendation on consumption of low-GI foods is a major dietary change that requires substantial scientific evidence. Therefore, a UL based on GI is not set.

The 1998 report of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) regarding the role of carbohydrates in human nutrition recommends the consumption of at least 55% of total energy in the form of carbohydrates from a variety of sources. The committee proposes that the majority of carbohydrates consumed should originate from NSP, principally from cereals, vegetables, legumes, and fruits. Furthermore, it suggests that free sugars should be restricted to less than 10% of total energy. This report recognizes that there is no direct causal link between sugar consumption and chronic disease but acknowledges that sugars increase the energy density of the human diet significantly and high-sugar drinks have been associated with childhood obesity.

A 2002 report of the American Heart Association suggests the restriction of sugar consumption. This report recognizes that there are no beneficial effects of increased sugar consumption. On the contrary, some studies suggest that it may have adverse health effects. To enhance the nutrient density and reduce the energy density of the diet, increased consumption of high-sugar foods should be avoided.

See also: Carbohydrates: Chemistry and Classification; Regulation of Metabolism. **Dental Disease:** Etiology and Epidemiology. **Diabetes Mellitus:** Classification and Chemical Pathology. **Fiber:** Physiological Effects and Effects on Absorption. **Glucose:** Chemistry and Dietary Sources. **Hypertension:** Dietary Factors. **Nutritional Considerations for the Management of Hypertension.** **Obesity:** Definition, Etiology, and Assessment

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CAROTENOIDS

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Health Effects

Chemistry, Sources and Physiology

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Glossary

Carotene A carotenoid formed only from carbon and hydrogen, such as lycopene, β -carotene, and α -carotene.

Carotenoid A family of pigments with a characteristic conjugated double bond system, widely distributed in nature.

Isoprenoid Compounds formed from the basic five-carbon building block, isoprene.

Polyene An organic compound containing many double bonds, especially one having double bonds in a long aliphatic hydrocarbon chain.

Provitamin A carotenoids The subfamily of carotenoids that have appropriate chemical structures for vitamin A formation. The most important of these carotenoids are β -carotene, α -carotene, and β -cryptoxanthin.

Retinoid A natural or synthetic derivative of vitamin A (retinol, retinal, retinoic acid).

Xanthophyll Oxygenated carotenoids, such as β -cryptoxanthin, lutein, and zeaxanthin.

Chemistry

Structure

Most carotenoids are 40-carbon isoprenoid compounds. Isoprenoids are formed from the basic five-carbon building block, isoprene (**Figure 1**). In nature, carotenoids are synthesized through the stepwise addition of three isopentenyl pyrophosphate (IPP) units to one dimethylallyl diphosphate (DMAPP) to form the 20-carbon precursor geranylgeranyl diphosphate (GGPP). Both IPP and DMAPP are derived from the methylerythritol phosphate (MEP) pathway. Two molecules of GGPP are combined by phytoene synthase to form the first carotenoid in the biosynthetic pathway, phytoene. Phytoene is then desaturated to form lycopene, the red pigment in ripe tomato fruit (**Figure 1**).

Nearly all other carotenoids can be derived from lycopene. Lycopene can be cyclized on either or both ends to form α - or β -carotene, and these in turn can be oxygenated to form xanthophylls such as β -cryptoxanthin, zeaxanthin, and lutein (Figures 1–3). Carotenoids having fewer than 40 carbons can result from loss of carbons within the chain (norcarotenoids) or loss of carbons from the end of the molecule (apocarotenoids). Longer carotenoids, homocarotenoids (C45–C50), are found in some bacterial species. The alternating double bonds along the backbone of carotenoid molecules form a polyene chain, which

imparts unique qualities to this group of compounds. The alternation of single and double bonds also allows a number of geometrical isomers to exist for each carotenoid (**Figure 1**). For lycopene, the theoretical number of isomers is 1056; however, when steric hindrance is considered, that number is reduced to 72. In nature, most carotenoids are found in the all-*trans* form although mutants are known in plants, e.g., *Lycopersicon esculentum* (Mill.) var. Tangerine tomato, and eukaryotic algae that produce poly-*cis* forms of carotenoids. Light can also cause *cis* to *trans* isomerization of these carotenoids depending on the surrounding environment. The isomeric form determines the shape of the molecule and can thus change the properties of the carotenoid affecting solubility and absorbability. *Trans* forms of carotenoids are more rigid and have a greater tendency to crystallize or aggregate than the *cis* forms. Therefore, *cis* forms may be more easily absorbed and transported. End groups such as the β or ϵ rings of α -carotene and β -carotene and the amount of oxygenation will also affect carotenoid properties.

Chemical Properties

In general, carotenoids are hydrophobic molecules and are soluble only in organic solvents, although they are soluble in aqueous environments if they are integrated into liposomes or

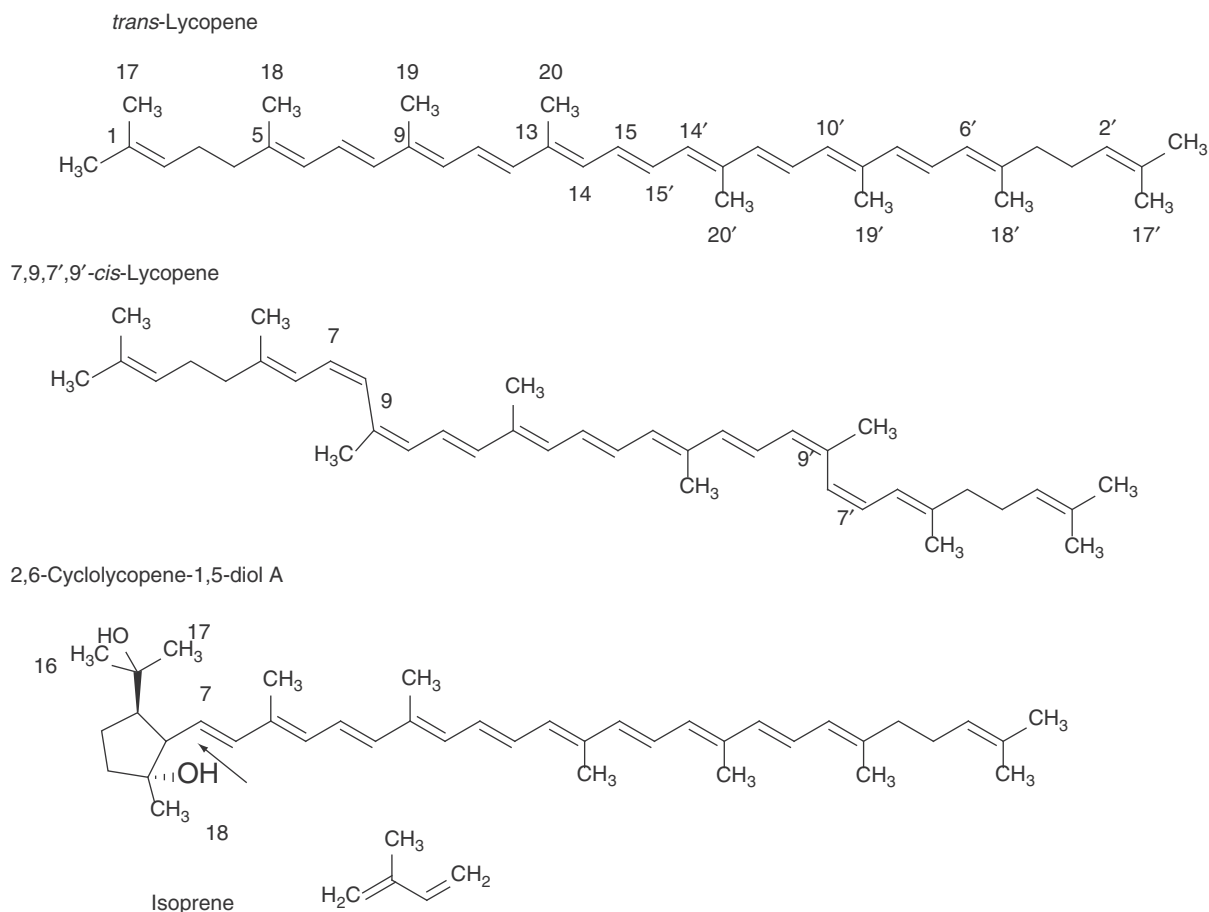


Figure 1 Basic carotenoid structure. *trans*-Lycopene is shown with numbered carbons. The down arrow on 2,6-cyclolycopene-1,5-diol A indicates the only difference from the B isomer.

cyclic oligosaccharides such as cyclodextrins. Addition of hydroxyl groups to the end groups causes the carotenoid to become more polar, affecting solubility. Typically carotenoid molecules are very sensitive to elevated temperatures and to the presence of oxygen, acid, and light when in solution, and are subject to oxidative degradation.

Electrochemical Properties

What sets carotenoids apart from other molecules and gives them their electrochemical properties is their conjugated double bond system. In this alternating double and single bond system, π -electrons are delocalized over the entire polyene chain. This polyene chain imparts characteristic electronic spectra and photophysical and photochemical properties to this group of molecules. The highly delocalized π -electrons require little energy to reach an excited state so that light energy can cause a transition. The length of the conjugated polyene or chromophore affects the amount of energy needed to excite the π -electrons. The longer the conjugated system, the easier it is to excite, so longer wavelengths of light can be absorbed. The result is that phytoene, having three conjugated double bonds is colorless, and phytofluene, having five, is colorless, but fluoresces green under UV light. Zeta-carotene has seven conjugated double bonds, absorbs light at ~ 400 nm

and appears yellow, neurosporene has nine, absorbs light at ~ 451 nm, and appears orange, and lycopene has eleven conjugated double bonds, absorbs at ~ 472 nm, and appears red. The polyene chain also allows transfer of singlet or triplet energy.

Physical Properties

Carotenoids are hydrophobic molecules that are associated with lipophilic sites in cells, such as bilayer membranes. Polar substituents such as hydroxyl groups decrease their hydrophobicity and alter their orientation with respect to membranes. Lycopene and β -carotene are aligned parallel to membrane surfaces to maintain a hydrophobic environment, whereas the more polar xanthophylls such as lutein become oriented perpendicular to membrane surfaces to keep their hydroxyl groups in a more hydrophilic environment. These differences can affect the physical nature of a membrane as well as its function. Carotenoids can form complexes with proteins, which aids them in moving through aqueous environments. They can also interact with hydrophobic regions of lipoproteins. Carotenoproteins have been found mainly in plants and invertebrates, but intracellular β -carotene-binding proteins have been found in bovine liver and intestine and in livers of the rat and ferret. In addition, a xanthophyll-binding protein has been found in human retina and

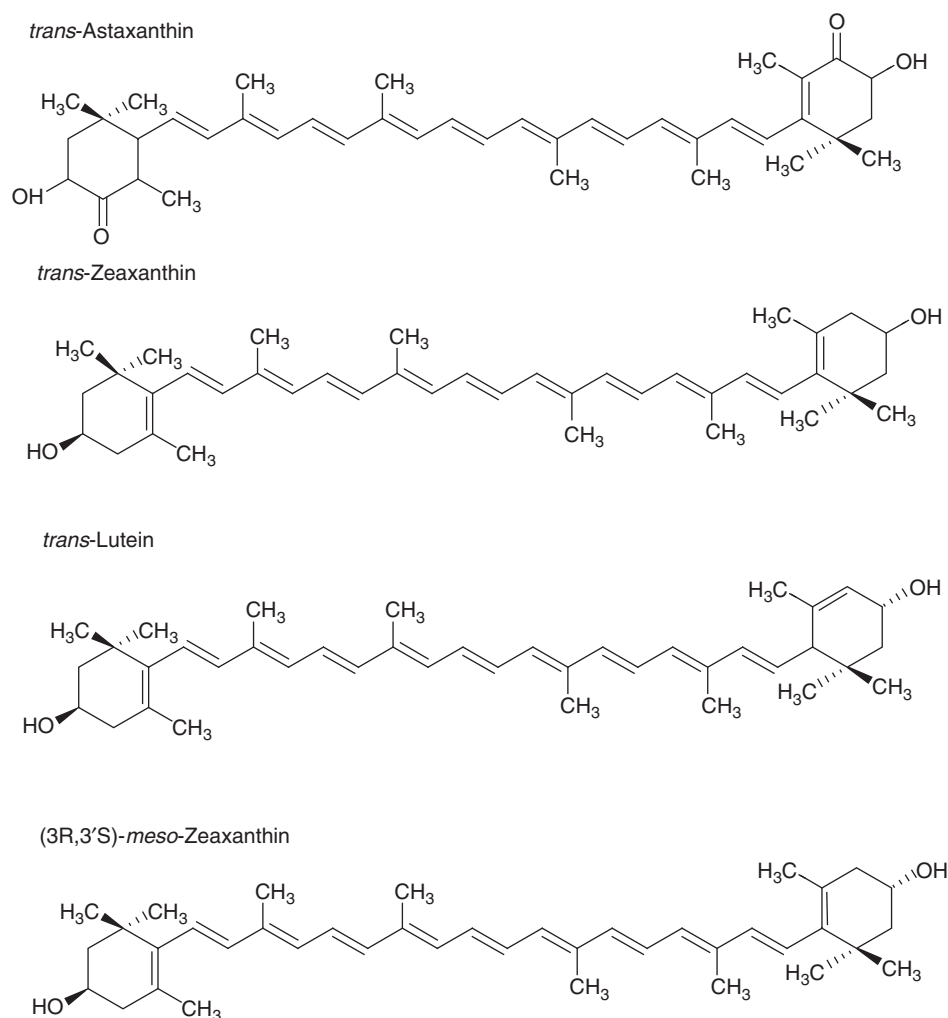


Figure 2 Examples of non-provitamin A carotenoids.

macula. Carotenoids are also present in nature as crystalline aggregates (lycopene in chromoplasts) or as fine dispersions in aqueous media (β -carotene in oranges).

Reactions

Light and Chemical Energy

Carotenoids are excellent absorbers of phototoxic blue light. Basic energy-transfer reactions are assumed to be similar in plants and animals, even though environments differ. Excess light can excite porphyrin molecules (porphyrin triplet state). These triplet-state porphyrin molecules can transfer their energy to oxygen, forming singlet oxygen, $^1\text{O}_2$. Singlet oxygen can damage DNA and cause lipid peroxidation, thereby killing the cell. Carotenoids having nine or more conjugated double bonds can prevent damage by singlet oxygen through: (1) transfer of triplet energy from the excited porphyrin to the carotenoid, forming a carotenoid triplet, which has too low an energy for further transfer and returns to the ground state, dissipating energy as heat; or (2) transfer of singlet oxygen

energy to the carotenoid, also forming a triplet carotenoid, dissipating heat, and returning to the ground state.

Cleavage to Vitamin A

Provitamin A carotenoids are important sources of vitamin A. Provitamin A activity requires at least one unsubstituted β -ionone ring, the correct number and orientation of methyl groups along the polyene backbone, and the correct number of conjugated double bonds, preferably in the *trans*-isomer orientation. Of the ~ 60 carotenoids having provitamin A activity, β -cryptoxanthin, α -carotene, and especially β -carotene are the most important (Figure 3). Vitamin A (retinol and its derivatives retinal and retinoic acid) performs vital functions in the vertebrate body. 11-*cis*-Retinal combined with opsin functions in the visual system in signal transduction of light reception. Retinol and retinoic acid function in genetic regulation, reproduction (spermatogenesis), growth regulation (general development and limb morphogenesis), and cell differentiation. There are two pathways for the formation of retinal from carotenoids. In the central cleavage mechanism β -carotene 15,15'-mono-oxygenase catalyzes β -carotene cleavage

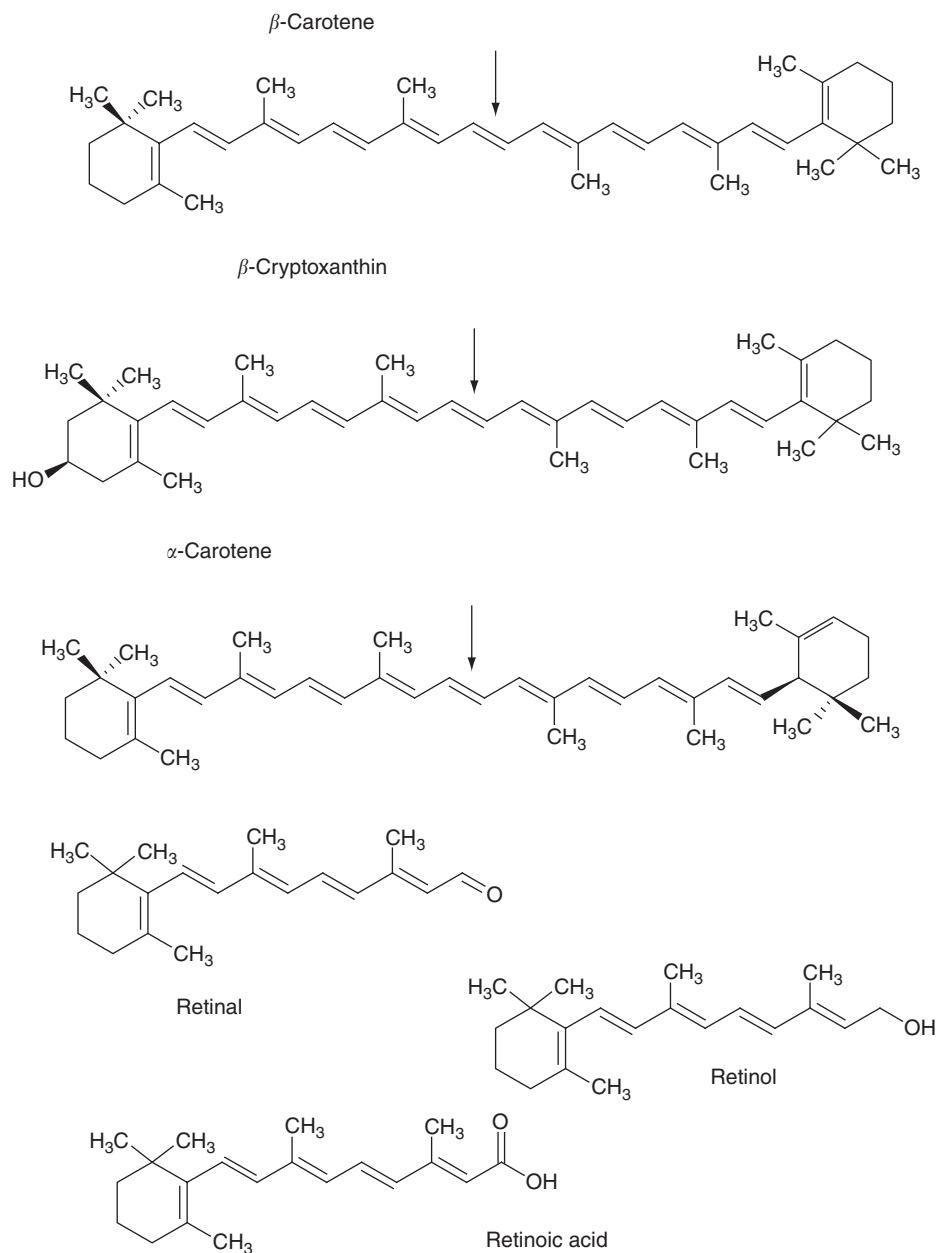


Figure 3 Provitamin A carotenoids. Arrows indicate the bond cleaved to form vitamin A.

to form two molecules of retinal, or one molecule of retinal from other provitamin A carotenoids. Retinal can then be converted to retinol or retinoic acid (Figure 3). Alternatively, carotenoids (including both provitamin A carotenoids and non-provitamin A carotenoids such as lycopene) can be cleaved at other double bonds along the polyene backbone by the eccentric cleavage pathway. Products of these reactions (apocarotenals) are further metabolized to retinoic acid and retinol. For example, β -carotene 9',10'-oxygenase cleaves β -carotene at the 9'-10'-double bond to form β -ionone and β -apo-10'-carotenal. There is some debate on the mechanism of the eccentric cleavage enzyme, but evidence leans toward it being a dioxygenase. Central cleavage is known to predominate under normal physiological conditions because of

the scarcity of eccentric cleavage products detected *in vivo*, but at least some eccentric cleavage occurs in vertebrates.

Radical Reactions

Excess amounts of radicals, molecules having unpaired electrons, e.g., peroxy radicals ($\text{ROO}\cdot$), can be created in tissues exogenously, e.g., by light exposure, or endogenously, e.g., by overexercising. Radicals react with lipids, proteins, and DNA causing damage, which possibly contributes to disease symptoms and aging. The special properties of the polyene chain make carotenoids susceptible to electrophilic attack, resulting in formation of resonance-stabilized radicals that are less reactive.

Three possible reactions can occur with carotenoids: (1) Adduct formation ($\text{CAR}^\bullet + \text{R}\cdot \rightarrow \text{R}-\text{CAR}\cdot$); these products should be stable because of resonance in the polyene structure. If the radical were a lipid peroxy, this reaction ($\text{CAR} + \text{ROO}\cdot \rightarrow \text{ROO}-\text{CAR}\cdot$) would prevent further propagation (chain-breaking). (2) Hydrogen atom abstraction ($\text{CAR}^\bullet + \text{R}\cdot \rightarrow \text{CAR}\cdot + \text{RH}$), where a hydrogen atom is taken from the carotenoid allylic to the polyene chain, leaving a resonance-stabilized carotenoid radical. (3) Electron transfer ($\text{CAR}^\bullet + \text{R}\cdot \rightarrow \text{CAR}\cdot + \text{R}^\bullet$), which has been reported in plant and cyanobacterial photosystems using laser flash photolysis of Photosystem II. In many cases, the products formed are colorless, thus revealing the bleaching effect of many oxidants on carotenoids. Further oxidation of the carotenoid or carotenoid radical can occur. Approximately 50 breakdown products of β -carotene have been detected. This large number of products seems to indicate a random attack along the polyene chain of β -carotene by a linoleoylperoxy radical. Lycopene can form a number of apo-lycopenals and apo-lycopenones, and metabolites of lutein and zeaxanthin have also been detected in human tissues.

Prooxidant Behavior

At higher partial pressures of oxygen ($p\text{O}_2$), β -carotene can become a prooxidant *in vitro*, through autooxidation. Experiments in intact murine normal and tumor thymocytes showed that β -carotene lost its antioxidant potency at higher $p\text{O}_2$, and the effect was more pronounced in tumor cells. One of the hypotheses formulated to explain results from intervention trials that fed pharmacological doses of β -carotene to smokers or individuals suffering from asbestosis – where the incidence of carcinogenesis was higher in individuals given the supplement – was that β -carotene might have acted as a prooxidant. However, whether carotenoids have significant prooxidant behavior *in vivo* is still unclear.

Dietary Sources

Carotenoids cannot be synthesized by humans; therefore, they must be obtained from dietary sources. The richest sources are highly pigmented red, orange, and yellow fruits and vegetables. β -carotene, α -carotene, β -cryptoxanthin, lutein, and zeaxanthin are yellow to orange. Green, leafy vegetables also contain carotenoids, whose colors are masked by the green color of chlorophyll. Smaller amounts are also available from animal sources such as ocean fish and dairy products. The pink color of salmon, for example, is derived from the xanthophylls, astaxanthin and canthaxanthin, which they obtain from eating small crustaceans and krill. Lutein imparts its yellow-orange color to eggs, and butter and orange cheeses contain retinols and β -carotene. Carotenoids, such as lutein from marigolds and bixin (red color) from annatto, are also used widely as colorants in processed foods to make them more attractive. The carotenoid lycopene is red; however, not all red fruits and vegetables contain lycopene. For example, the red in strawberries, apples, and cherries is a result of their anthocyanin content; whereas, tomatoes, watermelon, and

pink grapefruit derive their red color from lycopene. **Table 1** lists carotenoids found in foods. Approximately 50 carotenoids exist in the human diet, but only six of these are common in human blood: α -carotene, β -carotene, β -cryptoxanthin, lutein, lycopene, and zeaxanthin.

Concentrations of carotenoids in fruit and vegetable sources vary, resulting from differences in conditions under which they are grown (temperature, amount of sunlight, degrees of stress from extremes in climate such as drought, heat, and cold), genotype, and maturity or ripeness. The carotenoid content in animal sources depends on amounts contained in animal feeds and seasons of the year, which affect the availability of carotenoid-containing plants eaten by grazing animals.

Human diets and tissues contain six carotenoids in significant amounts (listed in **Table 1**). β -Carotene comes from a wide variety of orange and green fruits and vegetables, whereas lutein comes mainly from a variety of green vegetables, as well as egg yolk. Lycopene is typically the carotenoid consumed in greatest amounts in Western Diets. More than 85% of the lycopene in North American diets comes from tomato products, which also contain significant amounts of other carotenoids as well as vitamins C, A, and E, and potassium and folic acid. The tomato is the most popular fruit in the world. In the US, the annual per capita consumption of tomatoes for 2009 averaged approximately 19.3 pounds of fresh and 72.2 pounds of processed tomatoes.

Effects of Storage and Processing

Carotenoids are susceptible to oxidative degradation and isomerization resulting from storage and processing conditions. These reactions result in both loss of color and biological activity and formation of unpleasant volatile compounds. Degradation occurs on exposure to oxygen, light, heat, and conditions that destroy cell walls and ultrastructural integrity. Degradation is accelerated by the presence of metals, enzymes, unsaturated lipids, and prooxidants. Heating can also promote isomerization of the naturally occurring all-*trans* to various *cis* isomers, increasing bioavailability. Processing affects bioavailability by macerating tissues, destroying or weakening cell ultrastructure, denaturing or weakening complexes with proteins, and cleaving ester linkages, thereby releasing carotenoids from the food matrix. Processed foods are frequently fortified with carotenoids to increase nutritive value or enhance attractiveness. For example, annatto, an extract from the seeds of the *Bixa orella* tree containing the carotenoids bixin and norbixin, is added to butter, margarine, and processed cheese to give a yellow-orange color to these products. Tomato oleoresin is added to processed tomato products, increasing lycopene content while enhancing their attractive red color.

Physiology

Digestion

Digestion of food in the stomach increases accessibility of carotenoids for absorption by maceration in acid and digestive

Table 1 Carotenoid content (mg per 100 g fresh weight) of fresh fruit and vegetables

Carotenoid	Concentration (mg per 100 g fresh weight)	Source
Lycopene	38–305	Gac (<i>Momordica cochinchinensis</i> , Spreng) aril
	2.7–20	Tomato
	2.3–7.2	Watermelon
	5.3	Guava
	1.9–4.0	Papaya
	0.8–3.3	Grapefruit, pink
β -Carotene	10.1–77	Gac aril
	4.9–25.7	Carrot, orange
	1.6–21.6	Cantaloupe
	1.5–9.2	Kale
	trace–93	Sweet potato
	4.7–8.9	Spinach
	4.6	Turnip greens
	2.6–6.4	Apricot
	0.3–7.0	Tomato
	4.2	Squash, butternut
	4.0	Swiss chard
	1.4–3.4	Mango
	3.3	Collards
	0.4–1.0	Grapefruit, pink
Lutein	6.4–15.0	Kale
	0.6–12.9	Mango
	10.8	Parsley
	3.9–9.5	Spinach
	3.3–5.1	Collards
	1.5–2.8	Broccoli
	2.7	Chinese cabbage
	2.6	Watercress
	2.5	Pepper, orange
	2.4	Squash, butternut
	1.7	Egg yolk, chicken
Zeaxanthin	1.6–8.5	Pepper, orange
	2.2	Pepper, red
	1.2	Egg yolk, chicken
	0.7	Watercress
	0.1–0.5	Spinach
	0.5	Parsley
	0.5	Japanese persimmon
	0.1–0.3	Kale
β -Cryptoxanthin	0.3	Squash, butternut
	2.2	Pepper, sweet red
	1.4	Japanese persimmon
	1.1	Starfruit
	0.07–0.9	Pepper, chilli
	0.2–0.8	Pepper, orange
	0.05–2.2	Tangerine
	0.4	Cilantro
	0.14	Papaya
α -Carotene	0.1	Watermelon
	2.0–20.6	Carrot
	0.8	Squash, butternut
	0.2	Collards
	0.1	Tomato

enzymes. The acidic environment of the stomach helps to disrupt cell walls and other cellular ultrastructure of raw fruits and vegetables and causes further breakdown of cooked foods to release carotenoids from food matrices in which they are contained or bound. Carotenoids in green leafy vegetables are found in chloroplasts; those in fruit are located in chromoplasts. Absorption studies comparing plasma levels of β -carotene and retinol after consuming fruit versus green leafy vegetables show that β -carotene is more efficiently absorbed from fruit, indicating that chloroplasts (or the bonds linking chloroplast proteins and carotenoids) are more resistant to disruption in the digestive tract than chromoplasts. Thus, the location of a carotenoid in the cell affects its accessibility.

Numerous factors affect the intestinal absorption of carotenoids. Carotenoid isomerization can occur in the acidic gastric milieu. Lycopene present in fruits and vegetables occurs almost exclusively as the all-*trans* isomer, but is converted to *cis* isomers, which seem to be more bioavailable. Although almost 100% of lycopene in red tomatoes is in the all-*trans* form, plasma and tissue profiles show that *cis* isomers make up more than 50% of the total lycopene present. However, studies show that no *trans/cis* isomerization of β -carotene occurs in the stomach. Evidence has been found for transfer of a significant portion of both β - and α -carotene to the fat phase of the meal in the stomach, which would increase bioavailability of these carotenoids for absorption. In the intestinal lumen (**Figure 4**) where carotenoids are released from the food matrix, cleavage of carotenoproteins and fatty acid esters by carboxylic ester hydrolase (CEH), which is secreted by the pancreas, can occur. Carotenoids are then solubilized into lipid micelles.

Absorption

Carotenoid absorption is saturable and somewhat specific (e.g., all-*trans*- β -carotene is preferentially absorbed to *cis*- β -carotenes and α -carotene). The presence of other carotenoids can affect the absorption of carotenoids into intestinal mucosal cells, because one carotenoid can compete for or facilitate the absorption of another. Human studies show that β -carotene decreases lutein absorption, whereas lutein has either no effect or a lowering effect on β -carotene absorption. The inhibitory effect of lutein on β -carotene absorption might be partly attributed to the inhibition of the β -carotene cleavage enzyme by lutein, shown in rats. β -carotene also seemed to lower absorption of canthaxanthin, whereas canthaxanthin did not inhibit β -carotene absorption. Studies showed that β -carotene increased lycopene absorption, although lycopene had no effect on β -carotene. α -Carotene and β -cryptoxanthin show high serum responses to dietary intake compared to lutein. In addition, *cis* isomers of lycopene seem to be more bioavailable than the all-*trans*, and selective intestinal absorption of all-*trans* β -carotene occurs, as well as conversion of the 9-*cis* isomer to all-*trans* β -carotene. It is clear, then, that selective absorption of carotenoids takes place into the intestinal mucosal cell. These results, as well as cell culture and isotope data suggest that much of carotenoid absorption is controlled by an active transport mechanism (**Figure 5**). A major receptor facilitating this absorption is scavenger

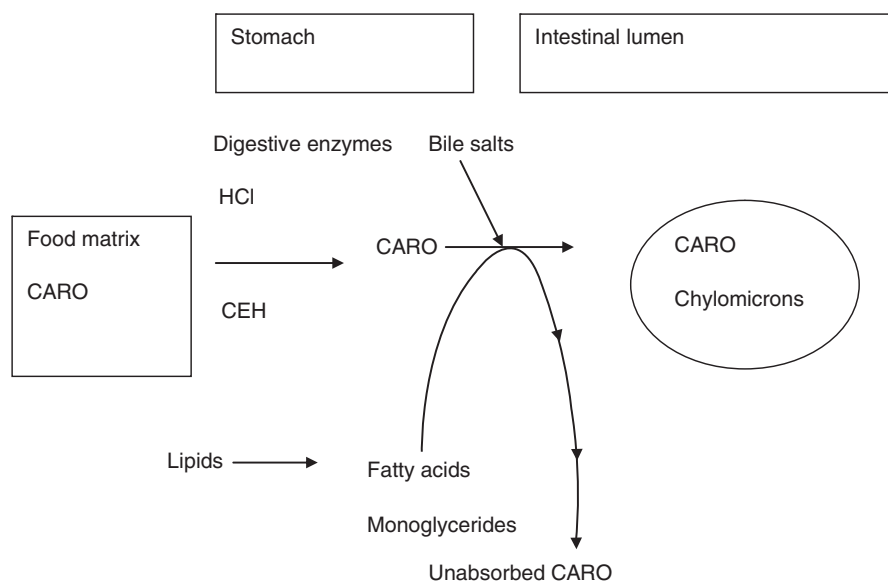


Figure 4 Factors affecting the digestion of carotenoids. CARO, carotenoids; CEH, carboxylic ester hydrolase, secreted by the pancreas.

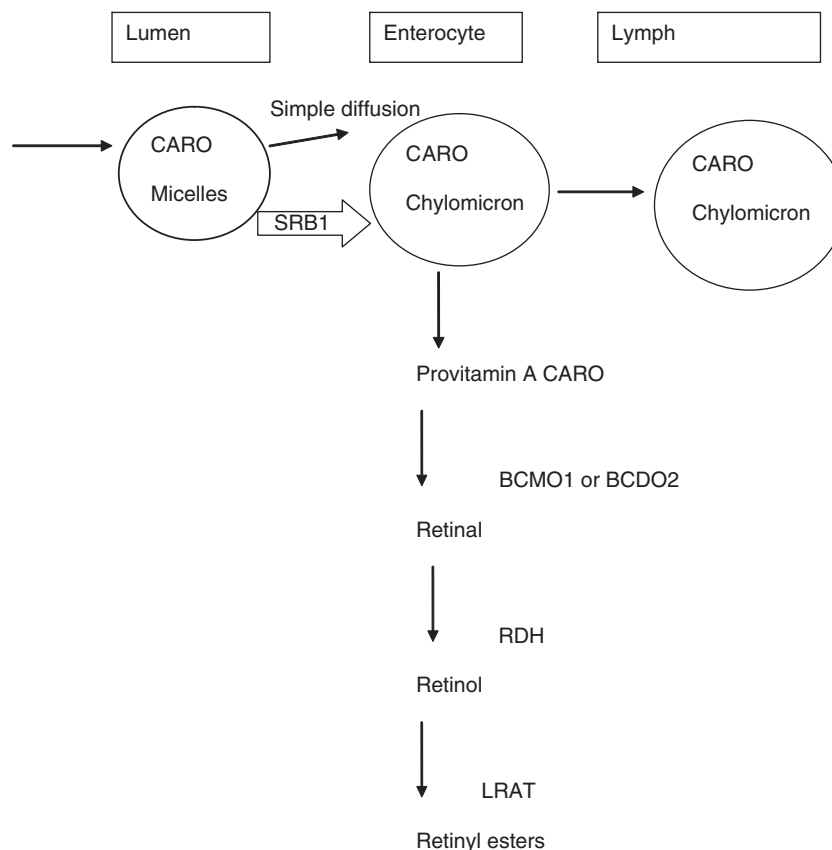


Figure 5 Factors affecting the absorption of carotenoids. CARO, carotenoids; SRB1, scavenger receptor B1; BCMO1, β -carotene 15,15'-monooxygenase; BCDO2, β -carotene 9',10'-oxygenase; RDH, retinol dehydrogenase; LRAT, lecithin retinol acyltransferase.

receptor B1 (SRB1), which also facilitates the absorption of cholesterol and vitamin E. However, abolishing SRB1 activity does not completely abolish carotenoid absorption, which can also occur through passive diffusion. SRB1 may also have a

role in the differential accumulation of carotenoids in some tissues, such as increased carotenoid uptake in the retina.

Carotenoids are more efficiently absorbed when accompanied by at least a small amount of fat. The amount of fat for

optimal carotenoid absorption seems to differ among carotenoids. For example, lutein esters require more fat for optimal absorption than β -carotene. These differences have not been quantified for each carotenoid. In addition, the presence of a nonabsorbable, fat-soluble component decreases carotenoid absorption. Sucrose polyester, a nonabsorbable fat replacer decreased carotenoid levels in plasma by 10–60%. The extent of this inhibition depends on the amount of non-absorbable compound ingested, as well as the particular carotenoid under consideration. The mechanism for this inhibition is apparently similar to the action of fiber, i.e., sequestration. The type of fat that is ingested along with carotenoids will also affect carotenoid absorption. As macerated food passes into the intestinal lumen, carotenoids freed from the food matrix then become incorporated into micelles, consisting of free fatty acids, monoglycerides, phospholipids, and bile acids. Many other factors can affect intestinal absorption such as micelle size, phospholipid composition, and solubilization of carotenoids into mixed micelles, and concentration of available bile salts, among others.

Another complicating factor in the intestinal mucosal cell is the partial conversion of provitamin A carotenoids (β - and α -carotenes and cryptoxanthin) to vitamin A (primarily to retinyl esters). Therefore, in absorption studies these metabolic reactions must be accounted for in measuring intestinal transport. Non-provitamin A carotenoids such as lycopene, lutein, and zeaxanthin are incorporated intact, although some cleavage can occur.

Transport

In the intestinal mucosa, both carotenoids and retinyl esters are incorporated into chylomicrons and secreted into the lymph for transport to blood. In blood, lipoprotein lipase rapidly degrades chylomicrons, and the liver sequesters the resulting carotenoid-containing fragments. The liver then secretes carotenoids back into the bloodstream in association with hepatic very low-density lipoproteins (VLDL). Most carotenoids in fasting plasma are carried by low-density lipoproteins (LDL) and high-density lipoproteins (HDL). Seventy-five per cent of the hydrocarbon carotenoids, e.g., lycopene and β -carotene, are associated with LDL, the rest is associated with HDL and, in smaller amounts, with VLDL. More polar carotenoids such as lutein and zeaxanthin are found equally distributed between HDL and LDL. After ingestion, carotenoids first appear in the bloodstream in chylomicrons, resulting from excretion from intestinal mucosal cells (4–8 h). HDL carotenoid levels peak in the circulation between 16 and 28 h; LDL carotenoid levels peak between 24 and 48 h. The bloodstream then transports carotenoids to different tissues (e.g., liver, prostate gland, fat, ocular macula) where they are sequestered by various mechanisms.

Tissue Distribution

In general, carotenoid concentrations in serum and tissues reflect concentrations contained in the food that is ingested. Carotenoids have been found in various human organs and tissues. These include human liver, lung, breast, cervix, skin,

adipose, and ocular tissues. The major storage organs are adipose tissue (probably because of its volume) and the liver. Tissues containing large amounts of LDL receptors seem to accumulate high levels of carotenoids, probably as a result of nonspecific uptake by lipoprotein carriers. Preferential uptake, however, is indicated in some cases. For example, unusually high concentrations of phytoene in the lung, ζ -carotene and phytofluene in breast tissue, lycopene in the prostate and colon, lycopene, β -carotene, and phytofluene in cervical tissue, and lutein and zeaxanthin in ocular tissues have been found.

Mechanisms Affecting Human Health

Carotenoids are the major source of vitamin A activity for most of the world's people. Thus, the provitamin A carotenoids (especially β -carotene, but also α -carotene and β -cryptoxanthin) may be said to be important for genetic regulation, vision, and other physiological functions associated with vitamin A. The ability of carotenoids to quench sensitized triplets is useful in treating protoporphyria (PP) and congenital erythropoietic porphyria (CEP) in humans. PP and CEP are disorders resulting from a defect in heme biosynthesis. Precursor porphyrins accumulate and can be sensitized to the singlet state and drop to the lower triplet state. The triplet-state is longer-lived and thus more likely to react with other molecules such as oxygen to form singlet oxygen, which can cause cellular damage. Because β -carotene can transfer and dissipate either sensitized triplet or singlet oxygen energy it is used to treat these disorders.

Other mechanisms are supported by *in vitro* studies and epidemiological research, but their relation to human health is not established. Light absorption and possibly scavenging of destructive oxygen species by the xanthophylls lutein and zeaxanthin are important in the macula of the primate eye. Lutein, zeaxanthin, and a unique zeaxanthin stereoisomer 3R, 3'S(=meso)-zeaxanthin form the pigment of the macula lutea, creating a yellow area in the central retina responsible for high visual acuity. The macula is able to concentrate lutein and zeaxanthin, and change concentration ratios to achieve higher zeaxanthin concentrations in the center of the macula lutea. The exact mechanism for this accumulation is not known; however, a specific membrane-associated, xanthophyll-binding protein has been isolated from the human retina. Lutein and zeaxanthin absorb blue light of approximately 450 nm, thus filtering light to the light receptors behind the carotenoid layer in the macula. Filtering blue light can reduce oxidative stress to retinal light receptors and chromatic aberration resulting from the refraction of blue light. A similar filtering effect may occur in the lens, but its concentration of the xanthophylls is much lower. Several studies investigated whether carotenoid supplementation could reduce the risk of age-related macular degeneration (AMD), which affects the central portion of the retina and is the most common cause of irreversible blindness in the Western world. These studies have been disappointing. Similarly, carotenoids are believed to play a significant role in protecting skin from oxidative damage. *In vivo* measurements in humans of lycopene, β -, ζ -, γ -, and α -carotenes, lutein and zeaxanthin, phytoene, and phytofluene have shown that carotenoid concentrations are correlated with

the presence or absence of skin cancer and precancerous lesions. Carotenoids are believed to protect against several other types of cancer, cardiovascular diseases, and aid in immune function and gap-junction communication between cells. However, as in the case of AMD, most intervention trials have not been successful. Because carotenoid intervention trials typically feed high carotenoid doses, this may suggest a therapeutic window for carotenoid action.

Conclusions

Numerous studies indicate that carotenoids and their metabolites play a role in combating oxidative damage and other degradative reactions that are harmful to human health. Most of these functions seem to be related to their antioxidant nature and ability to dissipate energy from light and free radical-generating reactions. Some carotenoids are also important precursors of vitamin A, an essential nutrient. Much research is still required to shed light onto mechanisms involved in these functions. Other fascinating roles in nature are also being discovered, for example, the signaling of apparent good health and consequently good potential parenting in birds by the red coloration of beaks, which seems to serve as an attractant to prospective mates.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Carotenoids: Health Effects

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Health Effects

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Glossary

Antioxidants Compounds that neutralize free radicals in the body formed from normal biological processes.

Bioaccessibility The amount of a nutrient or compound of interest that is released from food and available for absorption.

Bioavailability How much of a compound of interest is absorbed from food and available for physiological function or storage.

Bioconversion The amount of active compound that is formed from an absorbed precursor.

Bioefficacy The amount of active compound that is made by the body from how much was theoretically contained in food.

Carotenoids A group of compounds found in fruits and vegetables, which generally consist of hydrocarbon isoprenoid groups.

Hydrocarbon carotenoids Carotenoids that do not contain oxygen but are composed of hydrogen and carbon chains.

Xanthophylls Oxygen-containing carotenoids.

Introduction

The colors of many fruits and vegetables are due to carotenoids (**Figure 1**). More than 700 carotenoids have been identified in nature. Humans can absorb carotenoids from the foods that they eat whereas many other animals cannot. Thus, carotenoids are considered an important class of phytochemicals, which are compounds derived from plants that may or may not have nutritional value. Many carotenoids circulate in humans, and the most commonly studied include β -carotene, α -carotene, β -cryptoxanthin, lycopene, lutein, and zeaxanthin. Carotenoids are nutritionally significant because approximately 50 of them are used by the body to make vitamin A. These are known as provitamin A carotenoids, of which the three most abundant in foods are β -carotene, α -carotene, and β -cryptoxanthin. Provitamin A carotenoids, especially β -carotene, provide less than one-half of the vitamin A supply in North America, but provide more than one-half in Africa and Asia.

Dietary recommendations for the intake of specific carotenoids have not been established due to lack of an adequate evidence base. To date, carotenoids are not considered as essential nutrients. Dietary recommendations for vitamin A exist: 900 retinol activity equivalents (RAEs) for men and 700 RAEs for women. An RAE is equivalent to 1 μg of retinol, the active and storage form of vitamin A. The recommendations for infants and children are less and range from 300 to 600 RAEs depending on age. If dietary preformed vitamin A is not sufficient, consumers need to eat sufficient amounts of carotenoid-rich fruits and vegetables to meet their daily vitamin A requirement, achieve optimal dietary carotenoid intake, and lower the risk of certain chronic diseases. In 2001, the Institute of Medicine established the amount of carotenoids needed to provide vitamin A from foods as 12 μg β -carotene or 24 μg other provitamin A carotenoids to yield 1 RAE. Currently, high-dose pharmacological supplementation with

carotenoids is not advised. Despite this, a tolerable upper intake level, the maximum daily amount of a nutrient that appears to be safe, has not been established for any individual carotenoid; although, supplemental β -carotene at 20 mg day⁻¹ or more is contraindicated for use in current heavy smokers by the European Commission.

Many factors affect the release and absorption of carotenoids from foods (**Figure 2**). When most sources of vitamin A are from provitamin A carotenoids in the population, bioavailability becomes important. Bioavailability of preformed vitamin A, that is, retinol and retinyl esters, is not a major concern because 80–95% is absorbed. Foods that are high in preformed retinol (e.g., liver, eggs, and fortified milk), however, are not consumed by everybody due in part to cost and availability. When discussing carotenoids from food, there are four terms that need to be defined: bioaccessibility, bioavailability, bioconversion, and bioefficacy (see **Table 1**).

- Bioaccessibility refers to how much carotenoid can be released from the food and is available for absorption.
- Bioavailability is how much carotenoid is absorbed from the food and is available for physiological function.
- Bioconversion relates to the provitamin A carotenoids and is defined as the amount of retinol that is formed from absorbed provitamin A carotenoids.
- Bioefficacy encompasses all of the biological processing of provitamin A carotenoids and is the amount of retinol formed from the amount of carotenoid contained in the food.

The study of provitamin A carotenoid bioefficacy from foods is important in international health because the most frequently consumed sources of vitamin A are fruits and vegetables. A 100% bioefficacy means that 1 μmol of dietary β -carotene provides 2 μmol of retinol in the body; however, 100% bioefficacy does not actually occur in the process of

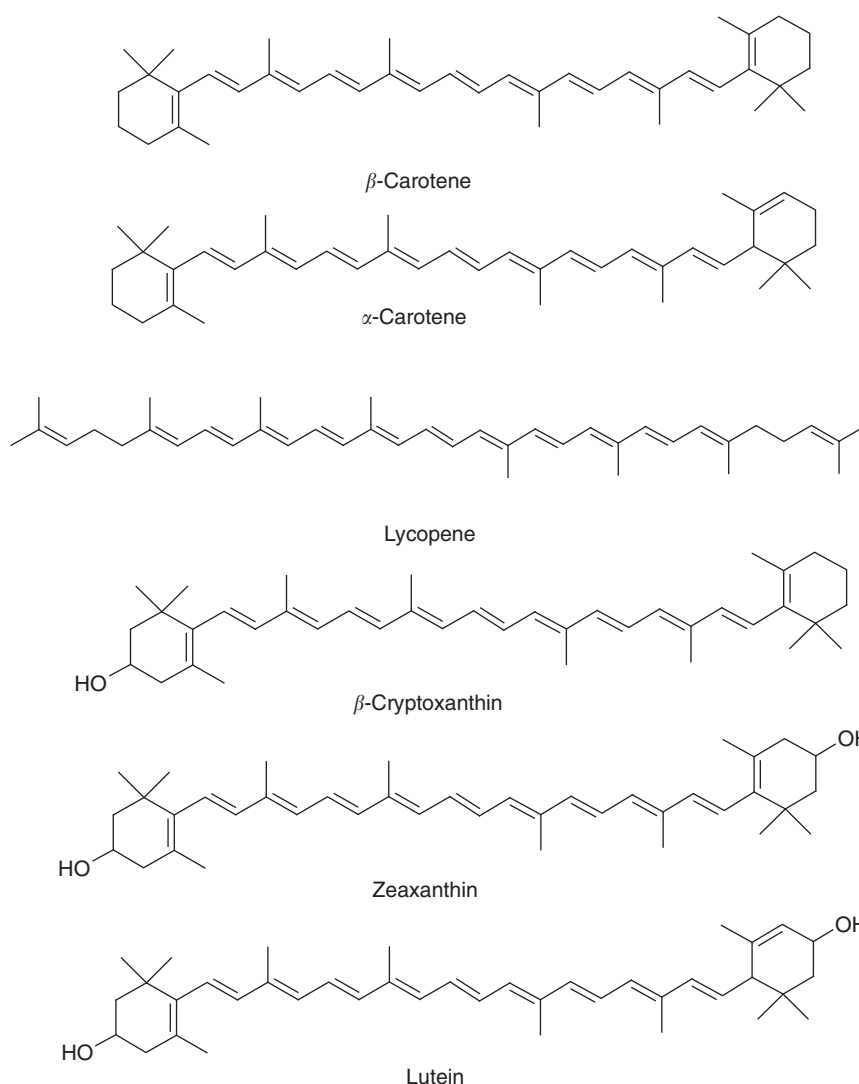


Figure 1 The structures of the most common carotenoids found in the human body. Three of them, β -carotene, α -carotene, and β -cryptoxanthin, can be used by the body for vitamin A. All carotenoids are antioxidants found in fruit and vegetables.

digestion and carotenoid uptake by the body. Currently, bioefficacy is an important research endeavor due to the proposed introduction of provitamin A-enhanced staple crops into countries at risk of vitamin A deficiency. Staple crops currently being targeted for provitamin A enhancement, known as biofortification, include sweet potato, maize, cassava, and rice.

In the body, carotenoids can act as potent antioxidants which are substances that neutralize free radicals formed from the natural metabolic processes of cells. Free radicals damage tissues and cells through oxidative processes. Although free radical formation is a natural process in the body, environmental factors such as smoking and pollution can increase free radical load and thus increase disease risk. Carotenoids may counter these influences by functioning as antioxidants and quenching oxygen-containing free radicals. In high- and low-density lipoproteins and cell membranes, carotenoids may also regenerate the antioxidant form of vitamin E as well as protect vitamin E from oxidation.

At the whole-body level, some population studies have indicated that certain carotenoids from either dietary intake or blood concentration data are associated with better immune response, lower rates of age-related macular degeneration (AMD) and cataract, as well as lower risk for certain cancers, cardiovascular disease, and osteoporosis. β -Carotene may increase immunological functions by enhancing lymphocyte proliferation independent of its provitamin A functions. The associations between specific carotenoids and decreased risk of various diseases are summarized in [Table 2](#). Some of these purported health effects may be due to their function as antioxidants.

Blood levels of specific carotenoids are often used as biomarkers for fruit and vegetable intake to strengthen or replace dietary intake data or show compliance to an intervention. A wide variation in analytical methods exists and standardization between laboratories does not routinely occur. Nonetheless, higher blood concentrations have been favorably correlated with certain disease states. For example, serum

vitamin A and carotenoid concentrations were measured in middle-aged women who later developed breast cancer. Median concentrations of β -carotene, lycopene, lutein, and total carotenoids were significantly lower in women with breast cancer compared with case-control women who had not developed breast cancer. Vitamin A concentrations, in contrast, were either not different or showed a mixed response between

cohorts, suggesting that carotenoids may be protective against or utilized during breast cancer. Furthermore, the Nurses' Health Study, which included a cohort of more than 83 000 women, also showed a significant inverse association between dietary β -carotene intake and breast cancer risk. This was especially strong for premenopausal women with a family history of breast cancer or high alcohol consumption. Other prospective studies, however, have had mixed results.

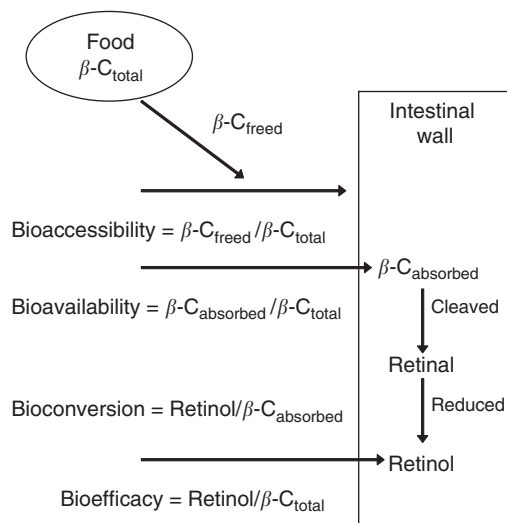


Figure 2 A schematic outlining the path of β -carotene (β -C) as it moves out from the food into the intestinal wall. The definition of terms associated with understanding β -carotene release, absorption, and conversion to retinol are illustrated: bioaccessibility, bioavailability, bioconversion, and bioefficacy.

Table 1 Terms which are associated with the β -carotene vitamin A value of foods and subsequent utilization as retinol

Term	Definition	100%
Bioaccessibility	β -Carotene freed	1 μ mol freed
	β -Carotene in food	1 μ mol in food
Bioavailability	β -Carotene absorbed	1 μ mol absorbed
	β -Carotene in food	1 μ mol in food
Bioconversion	Retinol formed	2 μ mol formed
	β -Carotene absorbed	1 μ mol absorbed
Bioefficacy	Retinol formed	2 μ mol formed
	β -Carotene in food	1 μ mol in food

Table 2 A summary of epidemiologic and clinical studies where carotenoids and a significant association with a specific disease risk has been shown in at least one study^a

Carotenoid	Cardiovascular disease	Cataract	Macular degeneration	Lung cancer	Prostate cancer	Bone health
β -Carotene	Yes	–	–	Yes ^b	–	–
α -Carotene	Yes	–	–	Yes	–	–
β -Cryptoxanthin	–	–	–	Yes	–	Yes
Lycopene	Yes	–	–	Yes	Yes	Yes
Lutein/zeaxanthin	Yes	Yes	Yes	Yes	–	–

^aFor a more complete discussion of the association of specific carotenoids to disease please refer to: Krinsky NI, Mayne SJ, and Sies H (eds.) (2004) *Carotenoids in Health and Disease* New York: Marcel Dekker, Inc.

^bThe opposite finding has been observed in clinical trials.

Hydrocarbon Carotenoid: β -Carotene

β -Carotene is one of the most widely studied carotenoids – for both its vitamin A activity and its abundance in fruits and vegetables. Epidemiological studies have often pointed to the abundance of dietary carotenoids as being protective against many diseases. Diets rich in fruits and vegetables are recommended to reduce the risk of disease and promote optimal health. When removed from the plant matrix and administered as a supplement, however, these benefits sometimes disappear. For example, lung cancer is the leading cause of cancer death in many developed countries and the β -Carotene and Retinol Efficacy Trial (CARET), in the 1990s, set out to test whether β -carotene conferred cancer protection. CARET was based on a number of observational studies that showed high levels of β -carotene from food sources were protective against lung cancer; however, the trial demonstrated an increased risk for lung cancer in the treatment group over the control. Subsequent studies in ferrets showed that the amounts of β -carotene commonly consumed from fruits and vegetables were protective against lung damage but higher amounts, equivalent to those in CARET, increased the formation of abnormal tissue in the lung.

A similar outcome was observed by the α -Tocopherol β -Carotene (ATBC) Study Group. Although evidence clearly exists showing an association between β -carotene and enhanced lung function, as in the CARET study, the ATBC trial also found an increase in lung cancer rates among smokers. It is plausible that the lung cancer had already been initiated in the smokers and supplementation with β -carotene could not prevent development. The ATBC study also showed an increased incidence of angina pectoris, a mild warning sign of heart disease characterized by chest pain, among heavy smokers. This may have been due to low blood levels of vitamin C in the study group leading to the inability of the individuals to quench β -carotene radicals, but this relationship requires more research.

In both the CARET and ATBC intervention trials, much higher doses of β -carotene were used than could be obtained from the typical diet, and the blood levels attained were two to six times higher than the 95th percentile of β -carotene in a survey of a representative sample of the United States population. Thus, it remains unclear whether β -carotene is a pro-carcinogen or an anticarcinogen. The associations for lower disease risk observed in epidemiologic studies may reflect other protective dietary agents or an interaction between dietary components. Furthermore, people with higher intake of fruits and vegetables may have healthier lifestyles that contribute to their lower risk of chronic diseases. The higher disease risk observed in clinical trials may be correlated to high-dose β -carotene with yet unidentified mechanisms, the limited treatment duration, and the timing of the interventions with regard to cancer development due to a history of heavy smoking. More research on β -carotene's biological actions is needed to explore mechanisms. Current consensus is that the beneficial effects of β -carotene are associated with dietary consumption, whereas the harmful effects in some subpopulations are with supplements at pharmacological levels.

Another explanation for a lack of beneficial outcome with β -carotene supplementation may be that not all people respond to β -carotene treatment. Individuals who do not respond to β -carotene supplementation may be better at converting it to vitamin A, which could be driven by vitamin A status. Blood response to β -carotene supplementation may also be inversely related to body mass index (BMI) due to increased sequestration of lipophilic β -carotene by fat stores present in people with larger BMI. However, some individuals with a larger BMI do not necessarily have a high body fat percentage, but rather increased lean muscle mass.

Excellent food sources of β -carotene include carrots, winter squash, red-orange sweet potato, and various types of dark green leafy vegetables. No deficiency or toxicity has been observed from dietary β -carotene intake, although high intakes can be associated with yellow pigmentation of the skin because carotenoids are stored in adipose tissue. Supplements containing β -carotene are common. In the largest observational/intervention study in postmenopausal women to date, the Women's Health Initiative, approximately 50% reported using a supplement containing β -carotene. The Women's Health Initiative included both a clinical trial and an observational study with more than 160,000 women. The Physicians' Health Study II also included β -carotene as an intervention to determine the balance of risks and benefits of this carotenoid with cancer, cardiovascular disease, and eye disease.

Hydrocarbon Carotenoid: α -Carotene

α -Carotene, another carotenoid frequently present in food, also has provitamin A activity. Based on its structure, it is converted to one molecule of biologically active retinol after central cleavage and twice the molar amount is equivalent to β -carotene. Like other carotenoids, it has antioxidant and possibly anti-carcinogenic properties, and may enhance immune function as well. Some, but not all, epidemiological studies observed that higher α -carotene intake was associated with lower risk of cardiovascular disease and cancer, whereas

others did not. Influences of clinical trials to test α -carotene, in humans, have not been conducted to date. This is probably because α -carotene is usually associated with ample amounts of β -carotene when found in fruits and vegetables and singling out α -carotene is difficult.

α -Carotene's concentration is especially high in orange carrots and high serum concentrations are associated with carrot intake. Low or high dietary intake of α -carotene alone has not been associated with any specific disease outcome or health condition.

Xanthophyll: β -Cryptoxanthin

β -Cryptoxanthin has provitamin A activity and may be more bioefficacious than α -carotene even though, theoretically, both carotenoids supply one vitamin A molecule based on structure. This is likely due to the bipolar nature of β -cryptoxanthin. Several epidemiological studies suggest that dietary β -cryptoxanthin is associated with lower rates of lung cancer and improved lung function in humans. A large prospective study on dietary intake and cancer, which included an interview on dietary habits and lifestyle, identified β -cryptoxanthin as protective against lung cancer after correcting for smoking. The beneficial effects for β -cryptoxanthin suggested by these results, however, could be merely an indicator for other antioxidants or a measure of a healthy lifestyle that are more common in people with high dietary intakes of β -cryptoxanthin. In tissue culture, β -cryptoxanthin has a direct stimulatory effect on bone formation and an inhibitory effect on bone resorption. In postmenopausal women, β -cryptoxanthin was lower in women with osteoporosis than those without, perhaps due to increased utilization. Further investigations are needed to elucidate the action of β -cryptoxanthin in bone health.

No deficiency or toxicity has been observed from dietary β -cryptoxanthin intake. The best food sources for β -cryptoxanthin are oranges, papaya, peaches, tangerines, and yellow and orange maize. Tropical fruit intake is directly proportional to β -cryptoxanthin blood concentrations.

Hydrocarbon Carotenoid: Lycopene

Lycopene, although lacking provitamin A activity, is a potent antioxidant with twice the activity of β -carotene for quenching singlet oxygen and 10 times the antioxidant activity of α -tocopherol in some model systems. The antioxidant potential of food chemicals varies widely according to the location in the body and presence of other body chemicals. Epidemiological evidence shows an inverse association between lycopene consumption and the incidence and development of certain cancers. This association is especially strong for prostate cancer, which is the most common cancer among men in western countries and the second leading cause of cancer death in American men. Prostate cancer rates in Asian countries are much lower, but appear to be increasing rapidly. The current consensus is that high consumption of tomatoes or high circulating concentrations of lycopene are associated with a 30–40% risk reduction for prostate cancer, especially the most aggressive forms. Recent studies in rats show that

tomato products are more protective against prostate cancer than isolated lycopene. Epidemiologic studies have also observed lower rates of bladder, cervical, and breast cancers as well as cancers of the gastrointestinal tract among people with high intake of lycopene. The discovery of significant concentrations of lycopene in specific tissues in the body, in addition to prostate, suggests that lycopene may play a role in these tissues, that is, plasma, testes, adrenal glands, liver, and kidney.

Lycopene, as an antioxidant, may be protective against heart disease by slowing down the oxidation of polyunsaturated fats in the low-density lipoprotein particles in the blood. Epidemiological and clinical studies show that higher blood lycopene concentrations are associated with lower risk and incidence of cardiovascular disease. Higher fat stores of lycopene have also been associated with lower risk of myocardial infarction. The most profound protective effect is in nonsmokers. The evidence for protective cardiovascular effects is compelling, as studies have shown a 20–60% improvement in cardiovascular parameters with higher blood concentrations of lycopene.

In addition to links with cancer and cardiovascular effects, lycopene intake was associated with lower risk for hip and nonvertebral fractures in the Framingham study. Serum concentrations of lycopene were also noted to be lower in mid-Western postmenopausal women with osteoporosis, perhaps indicating higher utilization because dietary intake did not differ. Finally, higher intake of fruits and vegetables is associated with better lung function, and specifically, high tomato intake is associated with higher timed expiratory volume.

Although the body of evidence seems strong, several studies have found either no or weak associations between lycopene consumption and disease. Some of these may be explained by the fact that blood lycopene concentrations were much lower in these studies than in those that showed a beneficial effect. Dietary-based studies should include blood sampling to further define the range of blood lycopene concentrations in the population and ideally with method standardization so that studies can be directly compared. The prostate cancer association is usually stronger for cooked tomato products rather than raw tomatoes or total lycopene intake. This supports the idea that whole foods with a broad array of nutrients and non-nutritive bioactive components are important for overall health rather than isolated compounds. The beneficial effects of tomatoes may be increased by processed and concentrated products that enhance the nutrient bioavailability.

The major food source of lycopene globally is tomatoes and tomato products. In the United States, more than 80% of dietary lycopene comes from tomatoes. Other sources include watermelon, pink grapefruit, and red carrots.

Xanthophylls: Lutein and Zeaxanthin

Lutein and zeaxanthin, structural isomers, are non-provitamin A carotenoids that are measurable in human blood and tissues. Lutein and zeaxanthin have been identified as the xanthophylls that constitute the macular pigment of the human retina. The relative concentration of lutein to zeaxanthin in the macula is distinctive. Zeaxanthin is more centralized and lutein predominates toward the outer area of the macula. A putative xanthophyll-binding protein has also been described,

which may explain the high variability of people to accumulate these carotenoids into eye tissues. Increased lutein intake from both food sources and supplements is positively correlated with increased macular pigment density, which is theorized to lower risk for macular degeneration. AMD is the leading cause of irreversible blindness in the elderly in developed countries. AMD adversely affects the central field of vision and the ability to see fine detail. Some, but not all, population studies suggest lower rates of AMD among people with higher levels of lutein and zeaxanthin in the diet or blood. Possible mechanisms of action for these carotenoids include antioxidant protection of the retinal tissue and the macular pigment filtering of damaging blue light.

Free radical damage is also linked to the development of cataracts. Cataracts remain the leading cause of visual disability in the United States and approximately one-half of the 30–50 million cases of blindness throughout the world. Cataract is treatable, but blindness occurs because individuals have either chosen not to correct the disease or do not have access to the appropriate medical treatment. Several epidemiological studies have shown inverse associations between the risk of cataracts and carotenoid intake. However, these studies also present inconsistencies with regard to the different carotenoids and their association with cataract risk. Lutein and zeaxanthin are found in the lens and are thought to protect cells in the eye against oxidative damage, seemingly protecting the eye and preventing cataracts. However, there is no evidence that any carotenoid supplement can protect cataract development. Eating plenty of fruits and vegetables, good sources of many antioxidants including carotenoids, is a preventative measure for many diseases.

Human data on the consumption of lutein and zeaxanthin are important to understand disease prevention. One complicating factor that requires better understanding is the bioavailability of lutein from food sources and supplements. The food matrix is an important factor influencing lutein bioavailability, and the amount and type of food processing generally influences the bioavailability of all carotenoids. For example, the processing of spinach does not affect bioavailability of lutein, but it does enhance that of β -carotene. Bioavailability studies have been conducted with lutein supplements and foods containing lutein. In humans, lutein from vegetables seems to be more bioavailable than that of β -carotene; however, this may be partially explained by bioconversion of β -carotene to vitamin A. Competition between carotenoids, such as lutein and β -carotene, for incorporation into chylomicra has been noted in humans consuming vegetables and supplements. The amount of fat consumed with the lutein source also affects bioavailability, as higher fat increases the bioavailability of lipid-soluble carotenoids. Lutein from egg yolk and oil-based supplements is vastly bioavailable due to the fat matrix.

Lutein may also protect against some forms of cancer and enhance immune function. Lutein may work in concert with other carotenoids such as β -carotene to lower cancer risk due to antimutagenic and antitumor properties. Owing to these potential health benefits, lutein and zeaxanthin supplements are sold commercially and incorporated into some multivitamins. Levels of these xanthophylls in single supplements vary widely, and neither benefit nor safety has been adequately studied.

Major dietary sources of both lutein and zeaxanthin in the diet include corn, green leafy vegetables, and eggs. Lutein tends to be the predominant isomer in foods, although some varieties of maize contain significant amounts of zeaxanthin. Lutein supplements are often derived from marigold flowers.

Summary

Most of the epidemiological evidence suggest that carotenoids are a very important class of phytochemicals. Although some of the effects may be attributable to a diet high in fruits and vegetables, and an overall healthy lifestyle, the presence of specific carotenoids localized in different areas of the human body lend evidence to their overall importance in optimal human health. Noninvasive methods have been developed to assess carotenoid levels in the skin and eye. Large-scale studies that determine carotenoid levels in blood, skin, and the eye may lead to a better understanding of their importance in human health and disease prevention. Additional epidemiologic studies to further strengthen the associations that have been observed in populations are needed.

Study design and statistical analyses vary across published work and no single study can give conclusive evidence. An integrated multidisciplinary approach to study the functions and actions of carotenoids in the body is necessary to fully understand the role of carotenoids in health and disease prevention. This includes comparisons of carotenoids in whole fruits and vegetables and their effect on human health and well-being. High fruit and vegetable intake is associated with a decreased risk of cancer, cardiovascular disease, diabetes, AMD, and osteoporosis. Removing any one class of phytochemical from the intricate matrix of the whole plant may not give the same beneficial outcome in terms of human health. Considering that the average intake of fruit and vegetables is still less than that recommended by health professionals, programs that promote the consumption of more fruits and vegetables may be more effective at preventing disease in the long term than using individual pharmacological carotenoid supplements.

A question that remains is whether carotenoids can be considered nutrients. A variety of phytochemicals contained in fruits and vegetables, including carotenoids, are assumed to be needed for optimal health and reduction of chronic disease risk, but have not been classified as nutrients. Indeed, in 2000, the Institute of Medicine was unable to recommend a daily reference intake for any carotenoid. Factors have been defined that categorize substances as nutrients: (1) they must be obtained from the diet because the body cannot synthesize the active form, and be used in the body for growth, maintenance, and tissue repair; (2) studies must be done to determine the essentiality of the substance and its specific function in the body; (3) the concentration in specific tissues needs to be defined, and consumption or supplementation must result in tissue concentration increases and improved tissue function; and (4) a daily established dosage needs to be defined and a biomarker identified to assess status.

A large body of observational studies suggests that high blood concentrations of carotenoids obtained from food are associated with chronic disease risk reduction. However, there is

little other evidence of their specific role in the body. Lutein and zeaxanthin are the only carotenoids found in a specific tissue, the macular region of the retina, which seem to have a specific function. Providing lutein in the diet increases macular pigment in humans. Animal studies show that a diet low in lutein can deplete macular pigment, but the influence on the health of the eye is not yet well understood. To further our understanding, large randomized prospective intervention trials need to be conducted to explore the essentiality of lutein supplementation for reducing ocular disease risk in humans. Although the evidence is mounting for lutein, to date, no specific carotenoid has been classified as an essential nutrient.

See also: Antioxidants. Bioavailability. Cancer: Dietary Management; Epidemiology and Associations Between Diet and Cancer; Epidemiology of Lung Cancer. Carotenoids: Chemistry, Sources and Physiology. Coronary Heart Disease: Prevention. Lycopenes and Related Compounds. Supplementation: Dietary Supplements

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CELIAC DISEASE

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Introduction

Celiac disease is the end result of a collision between the human immune system and the widespread cultivation of wheat, where the point of contact is the lining of the small intestine. This collision results in inflammatory and architectural changes of the absorptive mucosa in those susceptible to celiac disease. The inflammation leads to the destruction and eventual loss of the absorptive surface (villi), increased net secretion, crypt hyperplasia, and malabsorption, leading to a multitude of consequences. Celiac disease predominantly affects Caucasians in North and South America, Europe, North Africa, the Middle East, and South Asia, and it is relatively rare in peoples from sub-Saharan Africa and Southeast Asia, which may be due to different genetic backgrounds or the absence of wheat from the diet, or skewing of the immune system by high levels of infections. The disease occurs in people who carry the particular tissue types human leukocyte antigen DQ2 (HLA-DQ2) or HLA-DQ8, which appear to play an essential role in the disease pathogenesis. The inflammation usually resolves completely with the exclusion of gluten from the diet, will recur if gluten is reintroduced, and, as such, is regarded as a permanent loss of tolerance. Although once thought to be a rare disease, it is recognized as a common chronic disorder that affects as many as 1% of some Western populations. Indeed, in some populations, it is regarded as the most common genetic disease that affects the gastrointestinal tract. It is now frequently detected by the presence of circulating auto-antibodies against tissue transglutaminase, which is released in the damaged intestine. The final diagnosis of celiac disease

is defined by biopsy evidence of the characteristic inflammatory changes in the small intestine and ultimately a response to the gluten-free diet.

Pathogenesis

Established celiac disease is characterized by an inflammatory response in the proximal small intestine. This inflammation consists of increased numbers of lamina propria lymphocytes, and increased numbers of lymphocytes in the surface layer of the epithelium, called intraepithelial lymphocytes (IELs). The surface enterocytes are shorter and wider than normal and have poorly ordered nuclei. The normally tall thin villi are shortened and flattened. The cryptal layer is increased in depth. These changes may be patchy and affect variable lengths of the proximal small intestine (**Figure 1**).

One type of lamina propria lymphocyte that is crucial to the pathogenesis of celiac disease is the gluten specific cluster of differentiation 4 (CD4) + T cell. Clones of these cells reveal that they are DQ2 or DQ8 restricted and will produce inflammatory cytokines, such as interferon- γ (IFN- γ) in response to *in vitro* stimulation with gluten-derived peptides. These cells will slowly decrease when gluten is removed.

Another crucial lamina propria lymphocyte is the plasma cell. In established celiac disease, it will secrete IgA and IgG, directed against gluten peptides as well as connective tissue autoantigens, particularly tissue transglutaminase. The dynamics of the humoral response seems to parallel the

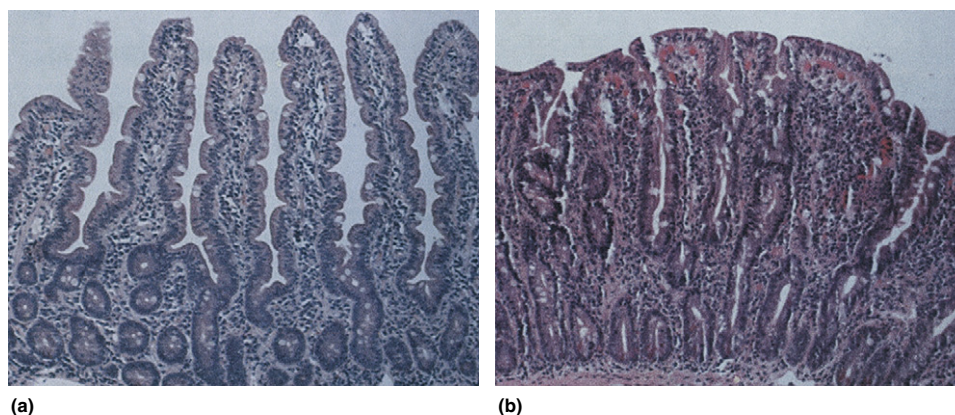


Figure 1 Inflammatory response in proximal small intestine. Proximal small intestinal tissue from a normal control (a) and from a celiac patient (b) was stained with hematoxylin and eosin. Shortened and flattened villi, shorter and wider enterocytes, increased numbers of intraepithelial lymphocytes, and a cryptal layer with increased depth are all present in (b) but not in (a).

dynamics of cellular injury, although antibodies may rise before mucosal relapse and disappear before healing.

There is also an increased permeability of the small intestine to macromolecules. It is not clear if this increased permeability precedes the development of a CD4+ T cell mediated response to gluten. Family members without the disease may have increased permeability, suggesting that the gluten dependent permeability is independent of HLA haplotype and consequently the CD4+ T cell response to gluten. A number of papers have demonstrated that gluten itself can rapidly cause an increase in paracellular permeability due to the uncoupling of intercellular tight junctions.

Adaptive Immune Response to Gluten

Celiac disease is characterized by an immune response to the storage proteins (gluten) of wheat, rye, and barley, with wheat as the most immunogenic protein. Wheat gluten is composed of glutenins and gliadins, and evidence demonstrates that the gliadin fraction causes celiac disease. Information gathered from the CD4+ T cell clones derived from chronic lesions of the small intestines of celiac patients with established disease demonstrate that gliadin peptides are presented by HLA class II molecules to CD4+ T cells. It has since been recognized that the gliadin peptides are made more antigenic by tissue transglutaminase in a process called deamidation. This occurs when tissue transglutaminase binds to gliadin-derived peptides and deamidates specific amino acid residues, increasing the affinity of the peptide especially for DQ2 molecules, as well as DQ8 molecules, on antigen presenting cells. It is in this manner that established celiac disease is perpetuated. However, it is less clear whether the initial response to gluten develops against native epitopes of gluten or whether it is against deamidated peptides. Several studies with pediatric patients have suggested that unaltered native gliadin peptides were primarily antigenic in pediatric cases but less so in more established (older) celiac patients. One study would suggest that aberrant innate immune responses against gluten, such as an increased production of interleukin-15 (IL-15) in the lamina propria of celiac disease patients, are crucial in the initiation phase and in driving the subsequent CD4+ T cell response. Potentially then, the CD4+ T cell response is against a broad repertoire of gliadin-derived peptides at initial gluten exposure and over time focuses upon deamidated gliadin peptides, which would then perpetuate and amplify celiac disease.

It is also thought that in celiac disease tissue transglutaminase is produced by fibroblasts in the setting of intestinal inflammation, because tissue transglutaminase is normally involved in cell-to-cell signaling and extracellular matrix formation. Under these circumstances, tissue transglutaminase would then bind to its preferred substrate, gliadin, and convert (deamidate) the specific glutamine residues in gliadin to glutamic acid, resulting in negatively charged residues and improved binding of the gliadin peptides to specific pockets in the DQ2 or DQ8 molecules. Interestingly, the release of tissue transglutaminase into the inflamed celiac gut also results in a strong autoimmune response to tissue transglutaminase with high levels of circulating IgA specific for tissue transglutaminase (tTG-IgA) present in untreated celiac patients. Thus, the

main characteristics of celiac disease are the DQ2/DQ8 restricted responses to gliadin peptides, the strong intestinal T cell response to deamidated gliadin peptides, and the production of tTG-IgA.

Observations in transgenic mice that express HLA-DQ8 and HLA-DQ2DR3 support many of the above results. These mice can evoke strong T cell responses in a DQ restricted fashion, and in the context of inflammation, increased IELs. One study observed that sensitization of HLA-DQ8 mice with gliadin and Complete Freund's Adjuvant (CFA) with subsequent gavage with gliadin resulted in increased numbers of IELs. Another study found that increased levels of IL-15 in the small intestine of HLA-DQ8 transgenic mice also led to the development of increased numbers of IELs with gavage of gliadin.

Innate Immune Response

Many of the studies on gut responses to gluten have been performed in the established chronic lesion. Recent studies have focused on the effect of gluten upon the permeability of intestinal epithelium. These studies revealed that gluten causes the release of an endogenous modulator of epithelial tight junctions (called zonulin) in (at least) rabbit and human mucosa. Of interest was that zonulin expression was upregulated in celiac patients, leading to a 'leaky' gut with a gluten-containing diet and a greatly increased transfer of gliadin-derived peptides across the epithelial barrier. Gluten also causes the production of the proinflammatory cytokine IL-15 at the surface epithelium in celiac patients. This aberrant production of IL-15 in the small intestine then leads to the activation of natural killer (NK)-like IELs and subsequent killing of epithelial cells that express major histocompatibility complex (MHC) Class I-related Chain A (MIC-A). The NK-like IEL is a key player in both the damage to the surface epithelium as well as a proinflammatory influence on the adaptive response that occurs in the underlying lamina propria. These aberrant responses by the innate immune response to gluten by celiac patients have important consequences. Because the gluten peptides enter into the epithelial compartment and paracellular regions, the introduction of the gluten peptides to the immune system occurs in the lamina propria, as opposed to the Peyer's patches. As such, this bypass of the Peyer's patches may lead to a loss of tolerance and even an induction of sensitization, resulting in an uncontrolled immune response in the intestinal mucosa. Thus, both arms of the immune system, the innate and the adaptive, play a role in the development of celiac disease.

Triggers for Lack of Tolerance to Gliadin

Celiac disease develops only in a minority of DQ2+ individuals. How the consumption of gluten generates an inflammatory state in these individuals can be theorized as follows. First, there may be a trigger of the innate immune response, such as a viral or bacterial gastrointestinal infection or even physical injury (such as surgery) that initiates inflammation, which later results in permeability. With repeated

inflammatory triggers over time, the intestinal immune response and cytokine milieu will be skewed towards an inflammatory response to gliadin and result in the increased production of inflammatory cytokines that include IFN- γ , IFN- α , and IL-17. This process could be applied to the inhibition of tolerance development in infants or the loss of tolerance to gliadin in adults. Determining which factors lead to a lack of tolerance to gluten in DQ2+ or DQ8+ individuals who later develop celiac disease will be crucial in understanding the pathogenesis of celiac disease.

With infants and the development of tolerance to gliadin, a number of studies have demonstrated that, in addition to the above factors, quantity and timing of exposure to gluten during childhood may also affect the development of tolerance to gliadin. Many of these studies were done on the Swedish celiac epidemic in the 1980s and 1990s. This 'epidemic' was a sharp increase in the incidence of celiac disease that was later determined to be a result of increasing the concentration of gluten in infant formula. Other studies have determined that the timing of introduction of gluten into the diet of infants genetically predisposed towards developing celiac disease affects the risk for developing celiac disease. Specifically, infants who are introduced to dietary gluten before 6 months of age are at the greatest risk of developing celiac disease, and those introduced to gluten between 6 and 9 months of age have a decreased risk. This increased risk before 6 months of age is probably due to the lack of a fully developed intestinal immune system that is capable of developing tolerance to dietary proteins.

At the other end of the spectrum is the loss of tolerance to gliadin in adults. More recently, it has become apparent that many celiac patients initially present with disease as adults. Possible factors that may lead to the loss of tolerance in adults are recurring gastrointestinal infections, surgery, or even pregnancy. Interestingly, the aging process has been implicated in the loss of tolerance to many different antigens, resulting in an increased propensity to autoimmunity associated with advanced age. It may be that the loss of tolerance to gluten in adults is associated with this tendency towards autoimmunity later in life.

Overall then, it is thought that inflammatory environmental triggers, the innate responses to gluten, and finally the adaptive responses to gluten combine to result in the enteropathy that characterizes celiac disease. This process can be best described as a 'maelstrom' of immune activity that leads to celiac disease if unchecked (Figure 2). Removal of gluten, the major instigator, will result in the reversal of this process and healing of the intestine. However, reintroduction of gluten will often result in a prompt recurrence of the disease, demonstrating that celiac disease is a permanent intolerance to gluten.

Epidemiology of Celiac Disease

Celiac disease is one of the most common, chronic genetic gastrointestinal conditions affecting just under 1% of Caucasian individuals. Whilst it was initially recognized in Northern Europeans, celiac disease will affect Caucasians wherever they live. It can affect people of mixed ethnic

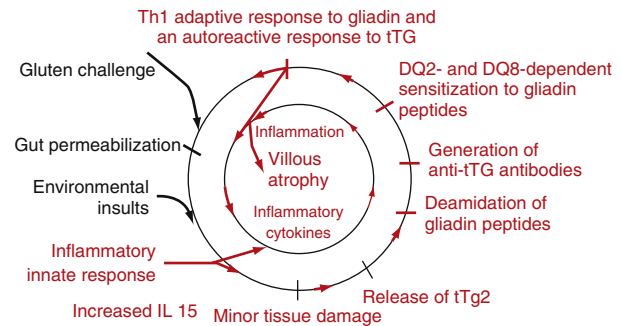


Figure 2 Maelstrom of celiac disease. An initial perturbation of the mucosal immune system would activate the innate immune response, increase epithelial permeability, and inflame the mucosal environment, resulting in overexpression of MHC class II molecules and the presentation of gliadin peptides to CD4+ T cells. tTG produced by fibroblasts in the damaged lining would deamidate gliadin peptides and amplify the CD4+ T cell response to gliadin. The released tTG would subsequently be targeted by the humoral immune response as an autoantigen, further advancing the mucosal immune system down a spiraling pathway towards self-destruction and villous atrophy.

background. It is apparently rare in Southeast Asia and sub-Saharan Africa. Although celiac disease was once considered primarily a childhood disease, in many geographic locations, celiac disease is more commonly diagnosed in adulthood. The median age in one study in the US was 50 years of age. Even in childhood, the age at diagnosis is now increased from early infancy to later in childhood or adolescence. The spectrum of disease has also changed with an increasing proportion of patients being diagnosed with monosymptomatic or less severe celiac disease. Many of these patients would not have been diagnosed in the past, but their symptoms are described as functional disorders. It is not uncommon to diagnose celiac disease in individuals of an advanced age, and even patients in their 80s have been diagnosed for the first time with celiac disease. This may be because some of them have had symptoms that persisted for many years before diagnosis, or because some of them developed the symptoms *de novo* in their 7th and 8th decades of life. The delay time for diagnosis may be anywhere between 8 and 11 years from the time of first clinical presentation to the actual diagnosis being made.

Celiac disease is diagnosed more often in women than men; yet in population-based screening, the prevalence of the disease is equal between genders. The explanation for this is unclear; however, presentation of disease may be more common in women because of the nutritional challenges posed by pregnancy and menstruation, especially iron deficiency anemia. The predisposition of women to autoimmune disease may also increase the likelihood of the occurrence of symptomatic celiac disease.

The incidence of celiac disease and the prevalence of celiac disease have been estimated in a number of geographic locations. These range between 1 and 9 cases per 100 000 person years and prevalence rates of anywhere from 1 in 2000 to 1 in 300. The latter high estimate is based on geographic locations where there have been active case findings such as Tampere in Finland or Olmsted County, MN, in the US. The lower rates are the prevalence based on clinically diagnosed cases.

However, these measures may greatly underestimate the true prevalence of celiac disease in the community. Two specific lines of research would suggest this. One is a systematic follow-up of birth cohorts for the occurrence of celiac disease by using serologic screening. In one geographic location in Denver, Colorado, almost 1% of children became persistently seropositive for markers for celiac disease by 7 years of age. This cumulative prevalence is remarkably similar to population-based studies in both Europe and North America that suggest prevalence in an adult general population of approximately 1 in 133. The estimates of the prevalence of celiac disease in most Caucasian studies have been remarkably similar, varying from 1 in 300 to 1 in 87.

All the studies so far have been carried out in predominantly Caucasian populations. No studies have been carried out that have incorporated substantial numbers of African-Americans, nor have systematic studies been carried out in sub-Saharan Africa. Areas of North Africa are at least as commonly affected as Europe. Individuals from Southeast Asia very rarely develop celiac disease.

The major effect of these screening studies has been to illustrate the wide spectrum of disease that incorporates celiac disease. Many individuals are completely asymptomatic. Others may be severely ill, presenting at an early age. It is possible that some individuals who have developed celiac disease may have no symptoms whatsoever despite the presence of demonstrable serologic and histopathological changes of celiac disease.

Associated Disorders

Dermatitis Herpetiformis

Dermatitis herpetiformis is the skin manifestation of celiac disease and is much less common than celiac disease with an approximate ratio of 3:1 in North America. It is characterized by an extremely pruritic papulovesicular eruption, which usually occurs symmetrically on the elbows, knees, buttocks, and back. Approximately 80% of patients with dermatitis herpetiformis have small intestine histopathology indistinguishable from celiac sprue. The diagnosis is established by demonstrating the presence of granular IgA deposits in biopsies of perilesional skin. The skin lesions, as well as small bowel histology, improve on a gluten-free diet. Dapsone is an effective short-term treatment for dermatitis herpetiformis; however, it does not have any impact on management of small bowel enteropathy. Also, those with dermatitis herpetiformis who are not compliant with the gluten-free diet are at higher risk for malignancy, as are those with celiac disease.

Type 1 Diabetes

Celiac disease has also been associated with other autoimmune as well as nonautoimmune disorders. It has been reported that the longer the exposure to gluten in patients with celiac disease, the greater the occurrence of other autoimmune diseases. There is evidence for a strong association between type 1 diabetes and celiac disease. Approximately 8% of patients with type 1 diabetes have the characteristic features of celiac sprue on small bowel biopsy. When the two diseases coexist, 90% have the

diagnosis of diabetes before celiac disease. Among the symptoms that may be suggestive of coexisting celiac disease, in addition to those considered classical for celiac disease, are delayed puberty, hypertransaminasemia, anemia, iron deficiency, arthralgias, dental enamel defects, hypoglycemia, and unexplained reduction in insulin requirements. Treatment with a gluten-free diet may improve diabetic control and decrease the occurrence of hypoglycemia episodes.

Down's Syndrome and Others

There is a strong association between Down's syndrome and celiac disease. Individuals with Down's syndrome and celiac disease more commonly have gastrointestinal manifestations such as intermittent diarrhea, failure to thrive, anemia, and low serum iron and calcium. The prevalence of celiac disease in patients with Down's syndrome varies between 5% and 12%. An increased prevalence of celiac sprue has also been reported in individuals with Turner's syndrome and William's syndrome. There is also a strong association between selective IgA deficiency and celiac sprue. Studies including adults and children in Ireland and Italy reported the frequency of selective IgA deficiency in celiac sprue to be approximately 2%, and 5–11% of IgA-deficient individuals have celiac disease.

Clinical Presentation

Celiac disease may present in a wide variety of ways (Table 1). In children, the onset of celiac disease is classically described as occurring within the first to seventh year of life with the introduction of cereals to the diet. Symptoms may vary with the age of the child at onset of disease. Young children may develop chronic diarrhea, failure to thrive, muscle wasting, abdominal distension, vomiting, and abdominal pain. Older children may present with anemia, rickets, behavioral disturbances, or poor performance in school. In some children constipation, pseudo-obstruction, and intussusception may be seen. It has been estimated that 2–8% of children with unexplained short stature may have celiac disease. Dental enamel defects involving secondary dentition as well as neurological syndrome and epilepsy with intracranial calcification have also been reported in children with celiac disease.

Table 1 Presentations of celiac disease

Gastrointestinal presentations

- Classic malabsorption syndrome – diarrhea, steatorrhea, weight loss, bloating, failure to thrive, multiple deficiencies
- Monosymptomatic – anemia, diarrhea, lactose intolerance, constipation
- Acute abdomen – abdominal pain, intussusception, vomiting, obstruction perforation, lymphoma

Nongastrointestinal presentations

- Neurological diseases – migraine, ataxia, peripheral neuropathy, dementia, depression, epilepsy
- Autoimmune diseases – dermatitis herpetiformis, pulmonary hemosiderosis
- Diseases associated with nutritional deficiencies – bruising, epistaxis, chronic fatigue, infertility, bone disease, short stature

In adults, celiac disease may be overt in presentation with classic gastrointestinal symptoms of diarrhea, weight loss, and abdominal pain. The presence of diarrhea or steatorrhea, which occurs in approximately 50% of patients, indicates severe disease and malabsorption. Often celiac disease is diagnosed in adults with nongastrointestinal symptoms, including iron deficiency anemia, abnormal liver tests, osteopenic bone disease, neurological symptoms, or menstrual abnormalities. Anemia is common in both children and adults with celiac disease and may be secondary to iron deficiency, folate deficiency, or a combination of the two. Iron deficiency is frequently associated with celiac disease. Six to ten percent of patients with unexplained iron deficiency anemia when evaluated by upper endoscopy with small bowel biopsy were diagnosed with celiac sprue in the absence of any other features suggestive of the disease.

Unexplained elevated serum transaminases (alanine transaminase (ALT), aspartate transaminase (AST)) should also raise the suspicion of undiagnosed celiac disease. Up to 9% of adults with unexplained elevated serum transaminases have been diagnosed with celiac disease based on serological testing or small bowel biopsy. Liver biopsies in these individuals may show reactive hepatitis. In this setting, adherence to a gluten-free diet results in improvement or normalization of the liver enzyme levels. The prevalence of celiac sprue is higher in adults with autoimmune liver disease than in the general population. Volta *et al.* demonstrated a 4% prevalence of celiac sprue in 181 patients with autoimmune hepatitis. Similarly, a high prevalence of celiac disease has been reported in other autoimmune liver disorders such as primary biliary cirrhosis, autoimmune cholangitis, and primary sclerosing cholangitis.

Patients with untreated celiac disease are at increased risk for the development of osteoporosis and low bone mineral density. Celiac disease can result in malabsorption of calcium and vitamin D. Malabsorption of calcium results from impaired transport by the diseased small bowel as well as precipitation of the ingested calcium with unabsorbed intraluminal fats to form insoluble soaps that are then excreted in the stool. Untreated, patients with celiac disease have been observed to have increased bone turnover and elevated levels of 1,25 dihydroxycholecalciferol because of secondary hyperparathyroidism that helps maintain a positive calcium balance. This results in diminished bone densities associated with increased risk of fractures in patients with classical celiac disease. Untreated patients with celiac sprue are at risk for developing low bone mineral density and osteoporosis; it was reported that 34% of the study population with celiac disease had fractures in the peripheral skeleton. For those with classical celiac symptoms, the odds ratio for fracture was 5.2 compared to those without celiac disease. Although the reduced bone mineral density improves on a gluten-free diet, adults with celiac sprue may be at increased risk for the development of peripheral bone fractures.

Infertility and recurrent spontaneous abortions have been reported in women with celiac disease. Male infertility has also been observed in patients with untreated celiac disease. Restoration of fertility both in males and females has been observed following treatment with a strict gluten-free diet and may be unexpected in some couples who have been unable to become pregnant before this time.

Patients with celiac disease may in addition present with neurological symptoms such as ataxia, muscle weakness, paresthesias, weight sensory loss, epilepsy, and bilateral parieto-occipital calcification. Symptoms of depression, epilepsy, and migraine have been reported in 30% of patients with celiac disease.

Diagnosis

Small bowel biopsy remains the gold standard for diagnosis of celiac disease. Over the past two decades the diagnostic criteria for celiac sprue have changed. Based on the 1990 revised criteria of the European Society of Pediatric Gastroenterology and Nutrition, the diagnosis of celiac sprue can be made with a diagnostic small bowel biopsy in a patient with highly suggestive clinical symptoms, followed by an objective clinical response to a gluten-free diet. Endoscopic biopsies from the distal duodenum are preferable because the presence of Brunner glands in the duodenal bulb and proximal second portion of the duodenum may affect histologic interpretation. The original criteria requiring a series of three biopsies, i.e., first to confirm the diagnosis, second for demonstration of response to a gluten-free diet, and the third for deterioration after gluten challenge, are required only in those few patients in which there still remains some diagnostic uncertainty.

Endoscopic features observed in patients with celiac disease include scalloped or fissured folds, absence of folds when the duodenum is inflated, and visible submucosal blood vessels; however, these findings are unreliable in diagnosing celiac disease as only roughly half of the patients will have the findings detected endoscopically. Other causes of atrophy are indistinguishable from celiac disease.

Characteristic histologic changes described are partial or total villous atrophy, elongation of crypts, a decreased villous:crypt ratio, and increased IELs (>25 per 100 enterocytes). Marsh and colleagues proposed a classification for the spectrum of histologic changes ranging from type 0 or preinfiltrative/normal, type 1, or infiltrative lesion (increased IELs), type 2 or hyperplastic lesion (presence of crypt hyperplasia), type 3 or destructive lesion (variable degree of villous atrophy), and type 4 or the hypoplastic lesion (total villous atrophy with crypt hypoplasia).

The role of radiological studies in the initial diagnosis of celiac sprue is limited. The findings of flocculation and segmentation of barium representing excessive fluid secretion in the lumen of the small intestine, thickened mucosal folds, and dilation of the small intestine are nonspecific and insensitive for celiac disease. Reversal of the fold patterns between the jejunum and ileum may also be seen.

Computerized tomography (CT) techniques may be useful in diagnosing the complications of celiac sprue such as development of lymphoma, malignancy, hyposplenism, or cavitating mesenteric lymphadenopathy. CT enterography techniques are currently under investigation and may become an accepted diagnostic test in the future.

Serological Screening Tests

Serological tests are helpful in detecting celiac disease in individuals with nongastrointestinal symptoms, and high-risk

groups who may or may not have signs of disease. Serological results are often used to triage those who need small bowel biopsy. The high-risk groups include first-degree relatives of confirmed cases of celiac disease, those with type 1 diabetes mellitus, Down's syndrome, Turner's syndrome, and unexplained dental enamel deficits, and children with unexplained short stature. Serological tests are also used to monitor progress after diagnosis as well as in prevalence studies in unselected populations. The serological tests utilized in current clinical practice include the endomysial antibody, tissue transglutaminase antibody, and the deamidated gliadin peptide (IgA and IgG).

IgA and IgG subclass of antibodies to gliadin have been used for the diagnosis of celiac disease; however because of poor sensitivity and specificity, they have a limited role in clinical practice. Recently deamidated gliadin antibody tests have replaced the conventional gliadin antibody test. The reported sensitivity and specificity of deamidated gliadin IgA are 74% and 95%, and of deamidated gliadin-IgG are 65% and 98%, respectively. The combined sensitivity and specificity of deamidated gliadin IgA and IgG reaches 75% and 94%, respectively, making this an accurate test for diagnosis, especially in patients with IgA deficiency.

The IgA antiendomysial antibody (EMA) assay is directed against the connective tissue protein found in the collagenous matrix of human and monkey tissue. This antibody is found in association with celiac sprue. The test is based on immunofluorescence techniques using monkey esophagus or human umbilical cord as a substrate. Although quite sensitive (85–98%) and specific (97–100%), the test has several limitations, including false-negative results in 2–3% of patients with celiac disease who have selective IgA deficiency. Other factors that have an impact on the sensitivity and specificity of this test include laboratory variations and disease severity. In a study of 101 patients with untreated celiac disease the sensitivity of the endomysial antibody in those with total villous atrophy was excellent (100%), but decreased remarkably (31%) in patients with partial villous atrophy. The endomysial antibody test as performed by indirect immunofluorescent staining is technically challenging and is being replaced by the tissue transglutaminase antibody test as the primary test for celiac disease.

Tissue transglutaminase (tTG) is a cytosolic protein released by damaged epithelial cells. This is the autoantigen recognized by the endomysial antibody indirect immunofluorescence assay in patients with celiac disease. The advantages of this test are that it is performed using enzyme-linked immunosorbent assay (ELISA) techniques, which makes it easier to perform, is widely available, and less costly. It eliminates the use of monkey esophagus as well as the subjective interpretation of immunofluorescence analysis of the endomysial antibody test. Although the tTG test is comparable to EMA in sensitivity, there is loss of specificity in patients with autoimmune disorders; hence it is important to confirm the diagnosis with small intestine biopsy.

In some patients biopsies are taken during an endoscopy that has been performed for another reason. In these patients serological tests can be useful to help confirm the diagnosis if there is some uncertainty with an equivocal biopsy, although negative serology in this circumstance may indicate another cause. Celiac disease may occur in the absence of antibodies however.

Treatment

Once the presumptive diagnosis of celiac disease is made the treatment may be commenced. It is important that the patients do not start to restrict their diet until each of the steps including the biopsy has been completed. Once confirmed, the responsibility for directing the management of the patient lies with the physician.

The treatment starts with an explanation of the condition and its cause. It is important that the patient understands that this is a chronic inflammatory condition of the gut and not a simple food allergy, that it is permanent even though the intestine will heal, and that the central and indeed only treatment at present is a gluten-free diet for life. The clinician should expect shock and even a fully expressed grief reaction on the part of the patient. Disbelief that something as basic to the Western diet as wheat is responsible is common. Some patients are overwhelmed both by the realization of having a chronic illness and others by relief that an explanation for their suffering has been found. The tone that the physician sets is crucial to the patient's success. A positive and upbeat though serious demeanor on the part of the doctor is appropriate, as most patients will do very well so long as they stick to the diet. Probably the most important thing that the doctor can do beyond diagnosis is to refer the patient for professional dietary advice that is up to date on how to achieve a gluten-free life style.

Patients should be encouraged to join both local and national support groups as an essential adjunct to management. The feeling of isolation so common in newly diagnosed patients in the past can be quickly dispelled by participation in an active support group.

It is important to identify and to correct deficiencies with nutritional supplementation. Deficiencies of the fat-soluble vitamins (D, E, A, and K), iron, folate, B₁₂, and even zinc or selenium are common. Baseline bone mineral density should be measured, as osteoporosis and osteomalacia are common.

Occasionally, intensive nutritional support and fluid replacement may be needed in very ill patients. Coexisting malignancy/autoimmune disease should be considered especially in elderly or ill patients. Followup of patients to ensure response to gluten-free diet and compliance is crucial to ensure long-term compliance as well as detecting potential complications of the disease. Screening of at-risk family members should be considered.

The Gluten-Free Diet

The term 'gluten' as used in the context of celiac disease refers to the storage proteins of wheat (gliadins and glutenin) and of barley (prolamines), and rye (hordeins), and oats (avenins). Gluten is defined in the setting of celiac disease as any protein-containing derivative of the offending grains or their derivatives. Grains that should be avoided are as follows: (1) wheat; (2) barley; (3) rye; (4) spelt; (5) kamut; and (6) triticale.

The role of oats in celiac disease is still controversial. Several recent, well-constructed studies have demonstrated no ill effects when a moderate amount of oat products has been

included in the diet of either newly diagnosed or already treated celiac patients. These recent studies have clearly demonstrated that oats are nontoxic for most patients with celiac disease; however, there is the concern that contamination of oat products by gluten is taking place during the growing, milling, or processing of oat products. When completely gluten-free oats become available then it will probably be safe to recommend them to most celiac patients. A small number of patients may still react to oats, some markedly so. Where patients intend to include oats in their diet they need to have a careful and informed medical followup.

Although foods such as bread, cookies, biscuits, and pasta are obvious sources of gluten, many other seemingly 'safe' foods contain hidden gluten. It is important to enquire if a food has any ingredients that are in any way derived from, or processed with, wheat, barley, or rye. This part of the diet is not self-evident, and the patient needs both expert counseling from a dietitian as well as being versed with and up to date on the gluten-free diet with ongoing support from a local or national support group. Nonfood items, such as medications and communion wafers, may also be unappreciated sources of gluten, as are fat substitutes and food contaminants. Ingredients that should be viewed with suspicion include: (1) malt or malt flavoring; (2) hydrolyzed vegetable protein (HVP); (3) modified food starch (and starch in foreign foods); (4) natural flavorings; (5) vegetable gum; and (6) fat substitutes. It is important that the most up to date instruction manuals are used. Older manuals may contain out of date or even misleading information.

Some ostensibly gluten-free foods may become contaminated with gluten during processing, packaging, transport, handling in the store, or even preparation in the patient's own kitchen.

Home testing kits for gluten are now available but generally are not helpful or practical for most patients. Large listings of the commercially available processed foods that are gluten free are available but must be updated at least yearly and must be tailored to geographic location. Patients should not rely on the self-test of reaction to gluten as a means of detecting gluten in foods as symptoms may be delayed.

Maintaining a life-long gluten-free diet is challenging. Degradation in the intestine of dietary gluten results in peptides with a high affinity for the HLA-DQ2 molecule. The HLA-DQ2 molecule and gluten peptide complex is recognized by the CD4+ T cells and results in damage to the lining of the small intestine. Alternative dietary strategies being investigated include development of a glutenase to destroy the immunogenic gluten peptides after a gluten meal in celiac patients, or blockers that target the HLA-DQ2 molecule and prevent it from binding to gluten peptides. There are also ongoing studies that are developing different strategies to generate or restore tolerance to gluten, such as bioengineering probiotics to produce gluten.

Bone Metabolism

Osteomalacia is a well-recognized, although uncommon, complication of celiac disease, with bone pain and pseudo-fractures as features. It is associated with elevated alkaline

phosphatase and often normal levels of calcium and phosphate. It usually responds well to a gluten-free diet and calcium and vitamin D supplementation.

Osteoporosis, which is common in adults with celiac disease, affects both men and women, and the exact mechanisms are not clear. The prevalence of osteoporosis is even higher in refractory sprue compared to gluten-free-diet-responsive patients. Diagnosis depends on bone mineral density testing with a T-score less than 2.5 standard deviation (SD) below mean peak value in young adults. The primary treatment for the osteoporosis in a celiac is the strict gluten-free diet with adequate calcium (1500 mg day^{-1} and vitamin D). Other measures directed at preserving or building bone density may be necessary if the bone mineral loss has been substantial or does not recover with a gluten-free diet.

Complications

Nonresponsive Celiac Disease

Whilst most patients with celiac disease respond appropriately to a gluten-free diet with the resolution of many symptoms occurring within days to weeks after the initiation of a gluten-free diet, a small proportion of patients (approximately 10%) do not have the expected complete response to a gluten-free diet or they have a relapse of symptoms while apparently on a gluten-free diet. This scenario termed 'nonresponsive celiac disease' is multifactorial in nature.

The single most common cause of continued or relapsing symptoms in patients with celiac disease is that of inadvertent gluten ingestion. There are many ways in which gluten can get into the diet, and in one series the most common source was commercial cereal in which minor ingredients were derived from the offending grains. However, other sources such as communion wafers and environmental contamination with flour, particularly of baked goods, are also possible.

In patients whose serologic tests have returned to normal and where a careful dietary review, including a detailed food record, does not reveal any potential source of gluten contamination, the occurrence of a second associated disease or a complication of celiac disease must be considered. A common cause of diarrhea would be microscopic colitis, either lymphocytic or collagenous. Typically, these patients will have watery diarrhea whereas symptoms related to malabsorption such as weight loss, bloating, and steatorrhea will have resolved. The patient will continue to have watery diarrhea or may, indeed, develop watery diarrhea while on a gluten-free diet. The taking of biopsies from the colon can readily identify this condition. Whilst in some patients adhering to a strict gluten-free diet may improve the colitis, in many circumstances, it does not suffice or it has not sufficed. The use of empiric therapy such as Pepto-Bismol[®], loperamide, or, in some circumstances, delayed release budesonide may be valuable. Another cause of continued diarrhea is disaccharidase deficiency such as lactose intolerance. In most patients with celiac disease, the lactose intolerance that occurs is secondary to the injury and resolves with treatment. In a few unfortunate patients there may be a genetic predisposition to lactose intolerance. Avoidance of lactose or the use of lactase enzyme supplementation may

suffice for correction of symptoms. In patients who have continued steatorrhea but in whom small bowel biopsies are found to have become normal, pancreatic exocrine insufficiency or bacterial overgrowth syndrome might be considered.

Where the small intestine has failed to recover histologically, particularly in patients who have continued symptoms and signs of malabsorption, the diagnosis of refractory sprue is made. This condition is often associated with severe illness, significant bone disease, and hypoalbuminemia. These patients are particularly prone to ulceration in the proximal small intestine, the so-called ulcerative jejunitis. Some have clonal expansion of T cells within their intestine. These

patients are probably entering a prelymphoma state and the mortality in these circumstances is high with a relatively poor response to immunosuppression; many will progress to lymphoma within 5 years. Other patients appear to have refractory sprue but without clonality, and they tend to respond much better to immunosuppression. This probably represents a now self-perpetuating autoimmune process within the intestine. The rare case of collagenous sprue, which has features similar to those of celiac disease but is characterized by a thick layer of collagen in the intestine subepithelial layer in the colon, typically responds poorly to all therapies and often require long-term nutritional support.

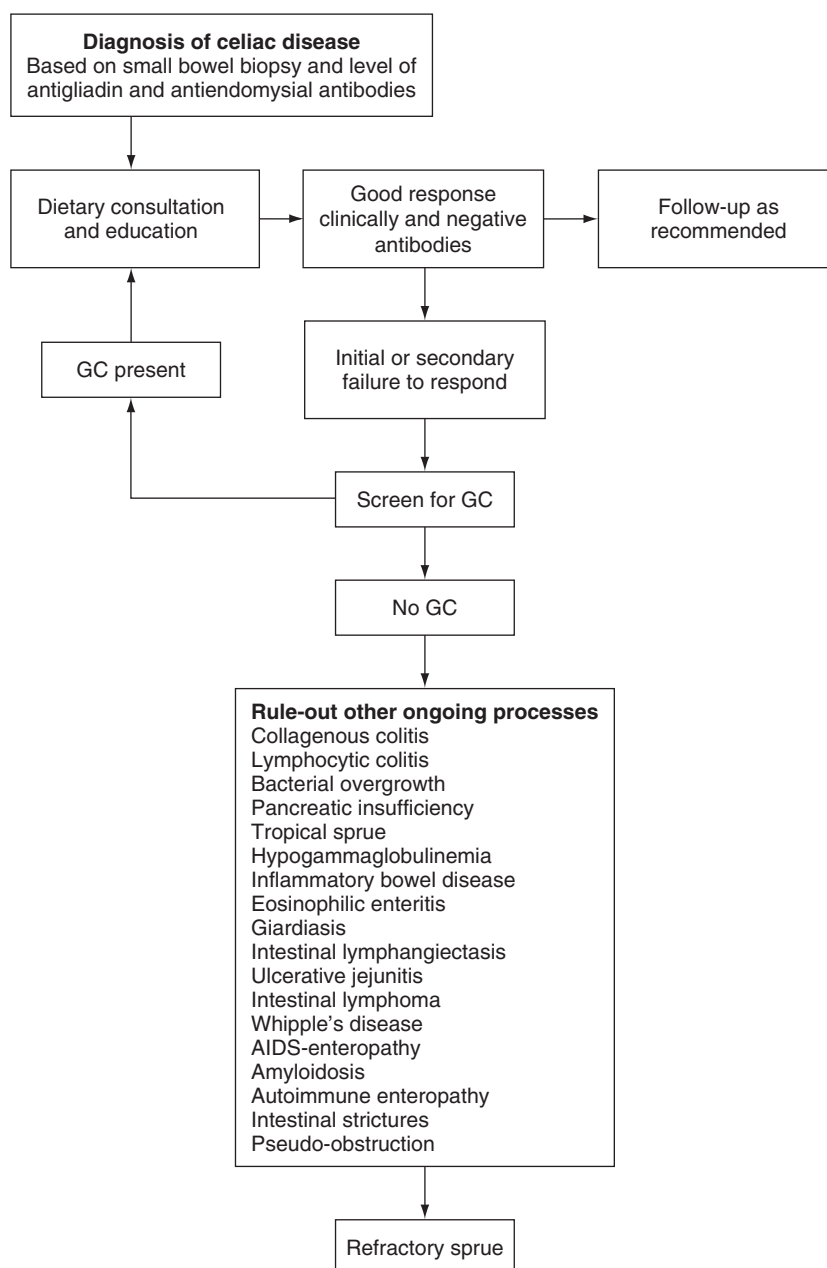


Figure 3 Flow chart for diagnosing and treating celiac patients. The proper procedure for diagnosing a patient who potentially has celiac disease, education, and treatment of that patient, followed by the steps that need to be taken in the event that the patient is not responsive to a gluten-free diet are illustrated in a flow chart. GC, gluten challenge.

The approach to diagnosing and treating nonresponsive celiac disease is outlined in **Figure 3**.

Malignant Complications of Celiac Disease

The complications of celiac disease can be divided into malignant and nonmalignant complications. Celiac patients are at increased risk for developing Non-Hodgkin's lymphoma, especially in patients who have not been compliant with the diet or within 3 years of diagnosis. The risk of lymphoma or other malignancies appears to drop once a gluten-free diet has been instituted. Although the relative risk of malignancy in celiac disease is greatly increased for specific diseases, the actual absolute risk is relatively small. The presentation of lymphomas in the small intestine can be acute with a surgical emergency such as obstruction, perforation, and bleeding, or gradual with insidious return or progression of severe malabsorptive symptoms. It is often associated with hypalbuminemia and severe weight loss and malnutrition. The treatment for lymphoma is often unsuccessful. Those patients presenting acutely and managed surgically appear to do better than those who have a slow insidious onset. Occasional cases of response to stem cell transplantation have been reported with refractory celiac disease type II patients, but not with enteropathy-associated T-cell lymphoma (EATL) patients.

The second most common malignancy occurring in celiac disease is that of adenocarcinoma of the small intestine. This adenocarcinoma seems to occur in the setting of the chronic inflammation of celiac disease. It is associated with defects and mismatch repair and whilst this is an unusual tumor, the survival with aggressive surgical therapy may be better than that for small bowel adenocarcinomas that occur sporadically. Usually, these patients present with iron deficiency anemia, gastrointestinal bleeding, obstruction, or pain. Other malignancies such as esophageal cancer or melanoma are increased in frequency in celiac disease, though again the absolute risk is low. Some recent evidence suggests that risk of breast cancer may be reduced in patients with celiac disease, though this is yet to be confirmed.

Nonmalignant Complications of Celiac Disease

Nonmalignant complications of celiac disease include ulcers and structuring within the intestine that occasionally may present with small bowel obstruction or bleeding, and recurrent acute pancreatitis as the result of inflammation, probably of the sphincter of Oddi. Nongastrointestinal complications

are usually the consequence of malnutrition or specific deficiencies. However, others such as neurological problems, including ataxia, peripheral neuropathy, or dementia, are of uncertain mechanism and perhaps autoimmune in nature. Other consequences of celiac disease have been discussed in the section on atypical or nongastrointestinal presentations (**Table 1**). Many, but not all, of these nonmalignant complications of celiac disease will respond to a gluten-free diet.

See also: Cereal Grains. Cytokines: Nutritional Aspects. Osteoporosis: Nutritional Factors

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CEREAL GRAINS

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Glossary

Aleurone layer The layer of cells beneath the seed coat, that surrounds the endosperm of Poaceae seeds, such as cereal grains; usually a single celled layer, but may be multi celled; part of the bran fraction.

Bran The outer layers of the caryopsis of cereals, consisting of the aleurone layer and other tissues, the bran fraction which is often removed in milling, is darker in color and has greater concentrations of fiber, protein, micronutrients, and phytochemicals than the endosperm.

Caryopsis Botanically, a single, dry, one-seeded fruit, which consists chiefly of the bran layers, endosperm, and embryo. The caryopsis is enclosed in an inedible hull in some grain types, such as rice, but it is synonymous with the grain of other types such as maize or wheat.

Corn Generically, the seeds of cereals or cereal plants. Regionally, and thus ambiguously, it may denote the major

cereal crop grown. For example, maize (US), wheat (England), oats (Ireland and Scotland).

Cultivar A plant variety developed by plant breeding for use in cultivation.

Endosperm The major component of the caryopsis, enclosed in the bran; high in starch and with nutritionally significant amounts of protein; refined flours mainly consist of endosperm.

Germ A minor part of the caryopsis, which is relatively high in oil and some micronutrients. The embryo is the major component of the germ fraction, which is often separated in milling.

Hull Also known as husk; the tough, high-fiber, inedible outer layer that encloses the caryopsis of some cereals, and which is removed by de-hulling.

Poaceae The grass family of flowering plants that includes the cereals, formerly called the Gramineae family.

Introduction

Cereal grains are dietary staples that provide a very substantial proportion of dietary energy, protein, and micronutrients for much of the world's population. The major cereal crops are maize (corn), rice, wheat, barley, sorghum, millets, oats, and rye. Worldwide these cereals are subjected to a very diverse range of traditional and technologically advanced processes before consumption. Thus, cereal-based foods vary enormously in their structural, storage, and sensory characteristics. Cereal-based foodstuffs also vary in nutritional value due to the inherent differences in nutrient content and the changes resulting from processing, which may be beneficial or detrimental. Cereals are also the raw material for the production of alcoholic beverages and food ingredients, including starches, syrups, and protein and fiber isolates. Furthermore, very substantial quantities of cereals enter the food chain as livestock feed.

Types of Cereal and their Role in the Diet

Cereal grains are seeds of cultivated annual species of the grass family (Poaceae). Cultivated cereal species have evolved with humans and include a range of types differing widely in their environmental adaptation, and their utility for food or other uses. Some cereals are adapted to tropical or subtropical

regions, others to temperate climates, whereas some tolerate sub-zero temperatures. The cereal grown is largely determined by climatic and edaphic factors, although economic and cultural factors are also important. The total world cereal production is over 2.5 billion tonnes (**Table 1**). The major cereals produced are maize (corn), rice, wheat, barley, sorghum, millets, oats, and rye. Some of these represent single species; others include a number of species with different agronomic and utilization characteristics. Each species comprises a range of cultivars (varieties; genotypes) which also differ in characteristics. Other cereals include triticale (*Triticosecale*), a wheat–rye hybrid. However, buckwheat (*Fagopyrum esculentum*) and quinoa (*Chenopodium quinoa*) are not Poaceae and are pseudocereals. All cereals are used for human nutrition, but the forms in which they are consumed and their dietary significance vary substantially across cereal types and regions.

Grain Characteristics

The harvested grain of some cereals (wheat, maize, rye, sorghum, and some millets) is, botanically, a caryopsis. In other cereals (barley, oats, rice, and some millets) the harvested grain generally includes the hull (or husk) that encloses the caryopsis. The hull is tough and very high in fiber. It is unsuitable for human nutrition and is removed in primary

Table 1 World production of cereals, 2008; figures are in thousands of tonnes

	<i>Total</i>	<i>Maize</i>	<i>Rice^a</i>	<i>Wheat</i>	<i>Barley</i>	<i>Sorghum</i>	<i>Millet</i> s	<i>Oats</i>	<i>Rye</i>	<i>Other^b</i>
World	2 518 822	826 224	685 875	683 407	155 054	66 848	35 708	25 508	17 701	22 497
Asia (excluding China and the Russian Federation)	699 679	72 061	429 254	162 333	13 766	8 855	12 338	368	358	346
North and Central America	502 077	345 881	11 661	100 656	17 823	19 026	336	5 714	519	461
China	479 804	166 032	193 354	112 463	3 100	2 503	1 551	300	300	201
Europe (excluding the Russian Federation)	397 450	86 544	2 728	184 328	82 183	763	300	11 025	11 903	17 676
Africa	152 110	55 279	23 618	19 783	3 939	25 787	20 420	125	51	3 108
South America	145 832	93 140	24 315	18 314	2 688	6 045	15	955	46	314
Russian Federation	105 467	6 682	738	63 765	23 148	76	711	5 835	4 505	7
Oceania	36 400	606	205	21 764	8 405	3 794	37	1 185	20	384

^aPaddy (rough) rice.^bIncludes triticale, mixed grain, canary seed, and other cereals not accounted for elsewhere.**Table 2** Food utilization of cereals, 2007; figures are per capita supply in grams per day

	<i>Total</i>	<i>Maize</i>	<i>Rice^a</i>	<i>Wheat</i>	<i>Barley</i>	<i>Sorghum</i>	<i>Millet</i> s	<i>Oats</i>	<i>Rye</i>	<i>Other^b</i>
World	402	46	145	181	2.5	10.9	10.8	1.5	2.3	2.4
Asia (excluding China and the Russian Federation)	584	26	210	165	1.3	7.4	12.3	0.2	0.2	0.8
North and Central America	339	108	31	185	1.1	2.6	0.0	6.7	0.5	4.9
China	418	18	210	185	0.5	1.7	1.2	0.1	0.1	0.4
Europe (excluding the Russian Federation)	348	24	13	279	4.0	0.0	0.2	5.5	17.6	2.2
Africa	396	112	54	125	9.2	50.4	37.0	0.3	0.0	8.6
South America	316	74	80	154	1.3	0.0	0.0	2.7	0.1	2.3
Russian Federation	418	2	13	367	2.7	0.0	3.4	4.8	25.7	0.1
Oceania	250	12	35	194	0.2	0.0	0.0	6.3	0.7	1.8

^aMilled rice.^bIncludes triticale, mixed grain, canary seed, and other cereals not accounted for elsewhere.

processing. The caryopsis represents the edible part of the cereal grain. Cereal caryopses have the same basic structure. The major part is the endosperm (63–91% of the total). The endosperm is high in starch and contains nutritionally significant amounts of protein. Enclosing the endosperm are cell layers, amounting to 5–20% of the caryopsis. These represent the bran that is often separated from the endosperm in milling processes. The embryo, which is found at one end of the caryopsis, accounts for 2.5–12% of its weight. The embryo is the major component of the germ fraction separated in some milling processes.

At harvest, cereal grains are low in moisture (12–16%) and are hard and inedible without processing. Some cereals may undergo simple milling procedures and be made into palatable unleavened products; others are subjected to more complex milling procedures and further processed into leavened, extruded, or fermented products using technologically advanced processes.

The utilization of cereals for food (**Table 2**) shows that wheat and rice are the major world food cereals, and that maize is important in some regions. However, the data in **Table 2** represent food usage of the crops. Since varying proportions of these crops are lost in milling, the actual consumption levels may be substantially less.

Maize

World production of maize (corn; *Zea mays*) is over 800 million tonnes annually (**Table 1**). However, only approximately 14% is used directly for human food and approximately 10% is industrially processed to yield oil and other products for human consumption. Maize is grown in tropical and warm temperate regions. Maize is a dietary staple in parts of Africa. Per capita supply is highest in Lesotho (430 g day⁻¹) and is 300–400 g day⁻¹ in other African countries (Malawi, Zambia, Zimbabwe) and in Mexico, where it has a pre-Colombian

tradition. Harvested maize is a caryopsis. There is a wide range of types varying in grain size, color, and endosperm characteristics, including white, yellow, and red types, and endosperms that range from soft floury to hard flinty textures. Sweet corn is generally regarded as a vegetable; it is harvested before the grain is mature and has a high water content (70–90%).

Rice

World rice production is nearly 700 million tonnes annually (Table 1) and approximately 80% of the crop is used for human nutrition. Approximately 90% of the crop is produced in Asia and China, and the per capita supply of milled rice exceeds 400 g day⁻¹ in six countries (Bangladesh, Brunei Darussalam, Cambodia, Lao People's Democratic Republic, Myanmar, and Viet Nam). Rice makes a modest contribution to diets in most industrialized countries, except Japan, where rice is the major dietary cereal. Rice (*Oryza sativa*) can be grown in a wide range of environments, under water or on dry land. The sub-species *indica* is grown in the tropics, and the sub-species *japonica* is grown mainly in warm temperate regions. Harvested rice, known as paddy (or rough) rice, has a hull whose removal yields brown rice, which is edible after boiling. However, the type of rice preferred in most regions is white, polished rice, where the bran layers are removed by milling. There are variations in grain morphology and in cooking characteristics. When boiled, short-grain *indica* types generally become soft and sticky, and aggregate, whereas long-grain *japonica* types remain relatively firm and separated.

Wheat

World wheat production is nearly 700 million tonnes annually (Table 1). Approximately 70% of the world's supply is used for food, and it is the major dietary cereal in all regions except Asia and China (Table 2). Per capita supply exceeds 500 g day⁻¹ in Algeria, Azerbaijan, Tunisia, Turkey, Turkmenistan, and the United Arab Emirates. Other species are grown, but the major species are common or bread wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*), and the former accounts for approximately 95% of production. Common wheat can be grown across widely diverse geographical regions including subtropical, warm temperate, and cool temperate climates, where it can withstand frosts. There is an extensive range of genotypes, varying in agronomic adaptability and grain quality. Quality is usually assessed by suitability for milling and baking. Whole grain comprises approximately 82% endosperm and 18% bran. Bran is used in foodstuffs including ready-to-eat cereals. Approximately 50% of the bran consists of the metabolically active aleurone layer, which contains high concentrations of minerals and vitamins. The aim of milling white flour is to separate the starchy endosperm from the darker, coarser bran. The yield of white flour depends on milling efficiency, and extraction rates of 75–81% are achieved. Wheat grains are classified as hard or soft and as strong or weak. The terms hard and soft indicate milling quality, and hard wheats have superior milling characteristics. The terms strong and weak refer to bread baking

quality. Strong flours yield bread with a large loaf volume and good crumb structure. The quantity and quality of the viscoelastic gluten proteins are important for bread quality. Weak flours produce poor bread products but can be blended with strong flours or used for other products. Durum wheat is hard and vitreous. Durum is used almost exclusively to produce pasta in most regions, but in the Middle East and North Africa, approximately 85% of durum wheat is used to produce breads, couscous, and other nonpasta products.

Barley

World barley production is over 155 million tonnes annually (Table 1). Barley (*Hordeum vulgare*) is grown mainly in temperate regions and can withstand sub-zero temperatures. Only approximately 5% of the total is used directly for food. The highest per capita supply is in Morocco (107 g day⁻¹), and in the Republic of Moldova, Latvia, and Algeria where per capita supplies are, respectively, 56, 54, and 52 g day⁻¹. Most barley grain has a fibrous hull that adheres to the caryopsis. However, naked or hull-less types are grown in some regions. Barley is processed for human use by removing the hull and polishing to yield pearl barley that is utilized in soups and other foods. Pearl barley is also ground to a coarse meal that is cooked as a gruel or ground to barley flour to make flat breads.

Sorghum

World production of sorghum (*Sorghum bicolor*) is approximately 67 million tonnes per year (Table 1) and approximately 42% of this is used for food. Sorghum is grown in semiarid zones and is important in tropical and subtropical regions. Food usage of sorghum is the highest in Africa. The per capita supply per day in the major consuming countries is Burkina Faso, 242 g, Eritrea, 211 g, Sudan, 198 g, Chad, 133 g, Mali, 122 g, Nigeria, 117 g, and Niger, 106 g. Both sorghum and millets are generally milled by traditional methods to yield grits and flours that are used in a variety of traditional foodstuffs including porridges, steamed products, breads, and pancakes.

Millets

The annual production of millets is approximately 36 million tonnes (Table 1). Approximately 77% of production is used for food, and millets are very important food crops in semiarid regions of Africa. There are at least nine species of millet. Pearl or bulrush millet (*Pennisetum glaucum*) accounts for approximately 50% of production. Foxtail millet (*Setaria italica*), proso millet (*Panicum miliaceum*), and finger millet (*Eleusine coracana*) each contribute over 10% to world production. Other species include Japanese or barnyard millet (*Echinochloa crus-galli*), kodo millet (*Paspalum scrobiculatum*), teff (*Eragrostis tef*), fonio (*Digitaria exilis* and *D. iburua*), and little millet (*Panicum sumatrense*). Millet is a dietary staple in Niger, where the per capita supply is 380 g day⁻¹, and the per capita supply is relatively high (100–200 g day⁻¹) in Burkina Faso, Mali, Gambia, Nigeria, and Chad.

Oats

World oat production is over 25 million tonnes annually (Table 1). Oats (*Avena sativa*) require a temperate climate and thrive in cool, wet conditions, but are less cold tolerant than rye, wheat, or barley. Approximately 14% of world production is used for food. Formerly a dietary staple in Northern and Western Europe, oats now contribute very modestly to diets worldwide (Table 2). Food usage of oats is the highest in Belarus, Denmark, and Latvia where the per capita food supplies are, respectively, 26, 21, and 20 g day⁻¹. Oat grain has 18–36% of hull that is removed in milling to yield caryopses (groats). Naked or hull-less types exist. Groats are processed by cutting, rolling, or grinding to give oatmeal, oat flakes, and oat flour products. These are wholemeal products with a composition similar to groats. Oat bran, which is less structurally distinct than wheat bran, is made by sieving coarse-milled groats. Oat mill products are used for traditional porridge and oatcakes, as ingredients in baby foods, and in breakfast cereals.

Rye

Rye (*Secale cereale*) is grown in temperate regions and is the most cold tolerant of the cereals. World rye production is approximately 18 million tonnes annually (Table 1). Approximately 38% of rye is used for food. Per capita supplies are the highest in Belarus (90 g day⁻¹), Poland (83 g day⁻¹), Latvia (54 g day⁻¹), Lithuania (53 g day⁻¹), Finland (47 g day⁻¹), and Austria (37 g day⁻¹). Harvested rye is a caryopsis, which is milled to extractions ranging from 65% to wholemeal (100%). Rye flour is used to make crispbreads, and for other breads, where it is often mixed with wheat flour.

Energy, Macronutrient, and Fiber Content

Tables 3–6 show the water, energy, macronutrient, and fiber contents of cereals and cereal products. The macronutrients (carbohydrate; protein; fat) and dietary fiber comprise the bulk of the dry matter of cereals. Carbohydrates are the major constituent, and there is a nutritionally significant amount of protein. Cereals can also be an important source of dietary fiber. However, most cereals are low in fat.

Dietary Energy

Dietary energy values are higher when fat is higher, and lower when water or fiber contents are higher (Tables 3–6). Higher fiber contents are found in whole grain and bran-rich products, whereas water and fat contents may be changed during processing.

Carbohydrates

Digestible carbohydrate, in the form of starch, is the major dry matter component of all cereals (Tables 3–6). Sugars, which usually account for much less than 1% of cereal grain, may be added in processing; cornflakes, for example, have approximately 7% sugars. Cornflour is a milling product with very high starch (Table 3). Most cereal starches have 20–30% amylose, the rest being amylopectin. However, there are types of rice, maize, and barley with up to 80% amylose or with up to 100% amylopectin (waxy types). In some cereal products a proportion of the starch is present as resistant starch, which resists enzymic digestion.

Table 3 Water, energy, macronutrient, and fiber contents of maize (corn) and rice products; representative values per 100 g

	Maize meal	Cornflour	Cornflakes ^a	Brown rice, uncooked	White rice, uncooked	Brown rice, boiled	White rice, boiled	White rice, flour
Water (g)	12.2	12.5	3.0	13.9	11.7	56.0	69.9	11.9
Energy (kJ)	1517	1508	1515	1518	1536	599	522	1531
Energy (kcal)	357	354	355	357	361	141	123	366
Carbohydrate (g)	77.2	92.0	84.9	81.3	86.8	32.1	29.6	80.1
Protein (g)	9.4	0.6	7.9	6.7	6.5	2.6	2.2	6.0
Fat (g)	3.3	0.7	0.6	2.8	1.0	1.1	0.3	1.4
Dietary fiber (g)	2.2	0.1	0.9	1.9	0.5	0.8	0.2	2.4

^aReady-to-eat cereal.

Table 4 Water, energy, macronutrient, and fiber content of wheat products; representative values per 100 g

	Wholemeal flour	White bread-making flour	Bran	White bread	Wholemeal bread	Uncooked pasta ^a	Boiled pasta ^a
Water (g)	14.0	14.0	8.3	37.3	38.3	9.7	75.9
Energy (kJ)	1320	1452	872	1002	915	1473	411
Energy (kcal)	310	341	206	236	215	346	97
Carbohydrate (g)	63.9	75.3	26.8	49.3	41.6	74.9	20.9
Protein (g)	12.7	11.5	14.1	8.4	9.2	12.0	3.2
Fat (g)	2.2	1.4	5.5	1.9	2.5	1.9	0.6
Dietary fiber (g)	9.0	3.1	36.4	1.5	5.8	3.0	1.0

^aVarious forms.

Table 5 Water, energy, macronutrient, and fiber contents of barley, oat, and rye products; representative values per 100 g

	<i>Pearl barley, uncooked</i>	<i>Pearl barley, boiled</i>	<i>Oatmeal</i>	<i>Oatmeal porridge^a</i>	<i>Oat bran</i>	<i>Wholemeal rye flour</i>	<i>Rye crispbread</i>	<i>Rye bread</i>
Water (g)	10.6	69.6	8.5	87.4	9.5	15.0	6.4	37.4
Energy (kJ)	1535	510	1644	210	1478	1268	1367	937
Energy (kcal)	360	120	388	50	349	298	321	220
Carbohydrate (g)	83.6	27.6	69.4	9.0	53.5	65.9	70.6	45.8
Protein (g)	7.9	2.7	11.8	1.5	19.8	8.2	9.4	8.3
Fat (g)	1.7	0.6	9.0	1.1	7.7	2.0	2.1	1.7
Dietary fiber (g)	5.9	2.0	7.0	0.8	15.1	11.7	11.7	4.4

^aMade with water and salt.**Table 6** Water, energy, macronutrient, and fiber contents of sorghum and millets; representative values per 100 g

	<i>Sorghum</i>	<i>Millets</i>					
		<i>Pearl</i>	<i>Foxtail</i>	<i>Proso</i>	<i>Finger</i>	<i>Japanese</i>	<i>Fonio</i>
Water (g)	12.0	11.0	11.3	13.5	11.7	11.1	10.0
Energy (kJ)	1422	1468	1364	1491	1377	1382	1541
Energy (kcal)	335	346	321	351	323	326	363
Carbohydrate (g)	69.9	68.1	67.8	70.7	76.0	65.4	75.8
Protein (g)	10.7	11.8	9.9	11.6	6.4	10.4	8.5
Fat (g)	3.3	4.8	3.0	4.4	1.4	4.3	3.5
Dietary fiber (g)	7.5	6.9	na	8.5	na	na	8.5

na, No data available.

Protein

Protein is the major nitrogen-containing component of cereal grains, and most protein data are based on nitrogen determination, followed by multiplication by nitrogen-to-protein conversion factors, which range from 5.7 to 6.31 for cereal products. The values in Tables 3–6 indicate that protein is the lowest in rice, barley, and finger millet, and the highest in wheat, oats, pearl millet, and proso millet. However, the protein content of cereals can vary substantially, and greater than two-fold ranges in protein content are found between crops of the same species. This variation is due partly to genetic differences, but agronomic factors are of greater importance. This variation may be of little significance with bulk crops encountered in industrialized operations, but may be important in less developed regions. Although not usually considered as a good protein source, many cereals provide an adequate amount, relative to energy, for adults. However, protein quality must also be considered, since cereal diets tend to be deficient in one or more essential amino acids (see the Section on Protein Quality).

Protein Quality

Cereal protein is predominantly endosperm storage proteins, which are low in dietary essential (indispensable) amino acids. These amino acids are required in differing amounts, and thus quality needs to be related to requirements. For example, the young have higher requirements for both protein and essential amino acids than adults. The first limiting essential amino acid in cereals is generally lysine. However, there are variations between cereals. In oats, rice, and finger millet

the deficiency in lysine may only be marginal, whereas in sorghum, maize, and other millets it is more pronounced (Tables 7 and 8). Tryptophan is also limiting in maize and some millets, whereas threonine and methionine may also be limiting in some cereals. Protein quality must be considered in relation to the total protein contents. Furthermore, as the protein content is increased, for example, with the use of nitrogenous fertilizers, the relative amounts of the indispensable amino acids tend to decline as a percentage of the protein. High lysine types of many cereals have been bred using conventional and genetic modification techniques, but lower grain yields preclude their wide use.

Fat

Cereals are generally very low in fat, and most contain only 2–4% (Tables 3–6). However, some types of maize and oats have more than 10% fat. The distribution of fat within the grain is variable. In oats, fat is distributed throughout the endosperm, whereas in maize fat is concentrated in the germ from which it can be extracted after separation. Fat is often added in processing, for example, in baked products.

Fatty Acid Composition

Cereal fat is liquid at room temperature; it is high in unsaturates and is more correctly described as oil. The major fatty acids in cereal oils are oleic (monounsaturated), linoleic (polyunsaturated), and palmitic (saturated) and representative values for the fatty acid composition of cereals are given in Table 9. Stearic and linolenic acids are present in small but

Table 7 Amino acid composition of maize, rice, wheat, barley, oats, and rye; representative values in grams per 100 g protein

Amino acid	Maize	Rice	Wheat	Barley	Oats	Rye
<i>Indispensable</i>						
Histidine	2.6	2.4	2.3	2.1	2.1	2.2
Isoleucine	3.6	3.8	3.5	3.5	3.8	3.5
Leucine	11.1	8.2	6.7	6.7	7.2	6.2
Lysine	2.3	3.7	2.7	2.6	3.7	3.4
Methionine	1.6	2.1	1.2	1.6	1.8	1.4
Cysteine	2.0	1.6	2.5	2.2	2.7	1.9
Phenylalanine	4.4	4.8	4.6	5.1	5.0	4.5
Tyrosine	3.5	4.0	1.7	3.0	3.4	1.9
Threonine	3.3	3.4	2.8	3.4	3.4	3.4
Tryptophan	0.7	1.3	1.5	1.6	1.3	1.1
Valine	4.0	5.8	4.3	5.0	5.1	4.8
<i>Dispensable</i>						
Alanine	8.2	5.8	3.5	4.2	4.5	4.3
Arginine	4.4	7.5	4.3	4.8	6.2	4.6
Aspartic acid	7.2	9.6	4.9	5.6	7.7	7.2
Glutamic acid	18.6	19.2	32.1	23.5	21.0	24.2
Glycine	3.9	4.3	4.0	3.8	4.6	4.3
Proline	8.8	4.6	10.7	10.9	5.1	9.4
Serine	4.6	4.6	4.5	4.0	4.6	3.8

Table 8 Amino acid composition of sorghum and millets; representative values in grams per 100 g protein

Amino acid	Sorghum Millets						
	Pearl	Foxtail	Proso	Finger	Japanese	Fonio	
<i>Indispensable</i>							
Histidine	2.2	2.2	2.3	2.2	2.6	1.9	2.2
Isoleucine	4.1	4.4	5.0	4.5	5.1	4.5	4.1
Leucine	14.6	12.2	13.3	12.9	13.5	11.5	10.8
Lysine	2.2	3.3	2.1	2.2	3.7	1.7	2.2
Methionine	1.4	2.2	2.6	2.0	2.6	1.8	4.3
Cysteine	1.7	1.5	1.4	1.7	1.6	1.5	2.5
Phenylalanine	5.0	5.2	5.3	5.2	6.2	5.9	5.9
Tyrosine	3.2	3.2	2.7	3.9	3.6	2.7	3.7
Threonine	3.3	3.9	3.9	3.4	5.1	2.7	3.7
Tryptophan	1.1	1.6	1.5	0.9	1.3	1.0	1.6
Valine	5.4	5.7	5.2	5.1	7.9	6.1	5.5
<i>Dispensable</i>							
Alanine	9.1	8.5	8.9	9.3	8.0	9.2	9.4
Arginine	4.3	4.8	6.1	4.4	5.2	3.2	3.6
Aspartic acid	6.4	8.7	6.9	5.5	7.9	6.3	9.0
Glutamic acid	22.6	21.2	18.8	20.5	27.1	20.7	22.3
Glycine	3.2	3.6	2.9	2.2	4.8	2.7	3.0
Proline	7.6	7.2	10.6	7.2	6.7	10.3	7.2
Serine	4.2	4.9	5.8	6.3	6.9	5.8	5.4

significant amounts (**Table 9**) and a range of other fatty acids are present in trace amounts.

Dietary Fiber

Although not strictly a nutrient, the importance of dietary fiber in the prevention or alleviation of disease is increasingly being

Table 9 Fatty acid composition of cereals; representative values in grams per 100 g total fatty acids (total includes 2–3% other minor fatty acids)

	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)	Linolenic (18:3)
Maize	12	2	32	50	2
Rice	22	2	34	38	2
Wheat	18	2	18	56	3
Barley	22	1	13	56	5
Sorghum	13	2	34	46	2
Pearl millet	20	4	26	44	3
Foxtail millet	10	3	17	64	3
Proso millet	9	2	21	64	2
Finger millet	24	2	46	24	1
Kodo millet	18	2	36	40	2
Oats	19	2	36	38	2
Rye	15	1	17	58	7

recognized. Fiber can also yield some dietary energy in the form of short-chain fatty acids produced by fermentation in the large intestine. Fiber is concentrated in the outer bran layers of cereals, and thus levels are higher in bran and whole grain products and lower in refined milling products (Tables 3–5). Dietary fiber is mainly composed of nondigestible polysaccharides (cellulose, hemicelluloses, pectins, and gums). However, the definition of dietary fiber has recently been extended to include resistant starch and nondigestible oligosaccharides. Thus, the data in Tables 3–6 will need to be reviewed as new analyses become available. A significant amount of soluble fiber (3–5 g per 100 g) occurs as β -glucan gum in oats and barley, and oat bran contains at least 5.5% β -glucan. This gum is the major factor responsible for the reductions in serum cholesterol resulting from diets high in these cereals. Wheat bran, which improves gut function, is high in total fiber (~40%) but contains only 3–4% soluble fiber.

Micronutrient Content

Micronutrients comprise the inorganic mineral elements and the vitamins. Ash (inorganic mineral matter) comprises 1–3% of grain dry matter. Major minerals elements (K, Na, Ca, P, Mg), and minor or trace elements (Fe, Zn, Cu, Mn, etc.) are found in all cereals. However, there are significant variations due to processing and other factors (Tables 10–13). There can also be substantial variations in the levels of trace minerals between crops due primarily to differences in their availability from the soil.

All cereals provide vitamin E (tocopherols and tocotrienols; tocols), thiamin, riboflavin, niacin, vitamin B₆, pantothenate, folate, biotin, and choline (Tables 10–13). Choline is not a vitamin *per se*, but is as an essential nutrient involved, *inter alia*, in methyl metabolism. Folate and betaine which occur in high concentrations in wheat bran and wheat aleurone, also play roles in methyl metabolism. Vitamin A (retinol) is not found in cereals. However, carotenes and cryptoxanthins, which yield retinol and thus have provitamin A activity, are found in maize, pearl millet, and

Table 10 Mineral and vitamin contents of maize (corn) and rice products; representative values per 100 g fresh weight (water contents as in **Table 3**)

	<i>Maize meal</i>	<i>Cornflakes^a</i>	<i>Brown rice, uncooked</i>	<i>White rice, uncooked</i>	<i>Brown rice, boiled^b</i>	<i>White rice, boiled^b</i>	<i>White rice, flour</i>
Sodium (mg)	40	1110	3	6	1	2	5
Potassium (mg)	350	100	250	110	99	38	76
Calcium (mg)	20	15	10	4	4	1	10
Magnesium (mg)	140	14	110	13	43	4	35
Phosphorus (mg)	290	38	310	100	120	34	98
Iron (mg)	3	6.7	1.4	0.5	0.5	0.2	0.4
Zinc (mg)	2	0.3	1.8	1.3	0.7	0.5	0.8
Copper (mg)	0.40	0.03	0.85	0.18	0.33	0.06	0.13
Manganese (mg)	0.60	0.08	2.30	0.87	0.90	0.30	1.20
Vitamin E (mg)	0.50	0.40	0.80	0.10	0.30	0.02	0.13
Thiamin (mg)	0.40	1.00	0.59	0.08	0.14	0.01	0.14
Riboflavin (mg)	0.11	1.50	0.07	0.02	0.02	0.01	0.02
Niacin (mg)	2.2	16.0	5.3	1.5	1.3	0.3	2.6
Vitamin B ₆ (mg)	0.53	1.80	0.70	0.30	0.30	0.10	0.44
Pantothenate (mg)	0.6	0.3	1.2	0.6	0.4	0.2	0.8
Folate (μg)	30	250	40	20	10	3	4
Biotin (μg)	10	2	7	3	2	1	1
Choline (mg)	22	3	31	6	4	2	6

^aReady-to-eat cereal, fortified.^bUnsalted water.**Table 11** Mineral and vitamin contents of wheat products; representative values per 100 g fresh weight (water contents as in **Table 4**)

	<i>Wholemeal flour^a</i>	<i>White flour^a</i>	<i>Bran</i>	<i>White bread^b</i>	<i>Wholemeal bread^b</i>	<i>Uncooked pasta^c</i>	<i>Boiled pasta^{c,d}</i>
Sodium (mg)	4	3	28	520	550	8	1
Potassium (mg)	370	130	1180	110	230	237	24
Calcium (mg)	36	15	110	110	54	24	6
Magnesium (mg)	130	31	520	24	76	52	14
Phosphorus (mg)	320	120	1200	91	200	190	45
Iron (mg)	3.9	1.5	12.9	1.6	2.7	1.8	0.5
Zinc (mg)	2.9	0.9	16.2	0.6	1.8	1.5	0.5
Copper (mg)	0.45	0.18	1.34	0.20	0.26	0.30	0.09
Manganese (mg)	3.50	0.68	9.00	0.45	1.90	0.87	0.25
Vitamin E (mg)	1.40	0.30	2.60	trace	0.20	trace	trace
Thiamin (mg)	0.50	0.10	0.90	0.21	0.34	0.30	0.03
Riboflavin (mg)	0.15	0.03	0.36	0.06	0.09	0.05	0.01
Niacin (mg)	5.7	0.7	14.0	1.7	4.1	2.8	0.5
Vitamin B ₆ (mg)	0.50	0.15	1.38	0.07	0.12	0.13	0.01
Pantothenate (mg)	0.8	0.3	2.4	0.3	0.6	0.3	trace
Folate (μg)	57	22	79	30	40	30	4
Biotin (μg)	7	1	45	1	6	1	trace
Choline (mg)	31	19	88	15	27	15	6

^aUnfortified.^bMade from UK fortified white flour containing 140 mg calcium, 2.1 mg iron, 0.32 mg thiamin, and 2.0 mg niacin per 100 g.^cVarious forms.^dUnsalted water.

sorghum. Provitamin A levels are variable with the highest amounts in yellow endosperm types and negligible amounts in white endosperm types. Typical values for retinol equivalents in maize, pearl millet, and sorghum are 44, 42, and 8 μg per 100 g, respectively. Brown rice contains a trace (0–11 μg per 100 g), but most of this is lost on milling. Vitamin A deficiency can be a major problem where rice is a dietary staple. To attempt to combat this, genetically modified ‘golden

rice’ has been bred with provitamin A levels of approximately 3 mg per 100 g. Vitamins B₁₂, C, and D are not found in unfortified cereals.

Effects of Processing

Vitamin and mineral contents may be profoundly influenced by processing. Vitamins and minerals are found at

Table 12 Mineral and vitamin contents of barley, oat, and rye products; representative values per 100 g fresh weight (water contents as in **Table 5**)

	<i>Pearl barley, uncooked</i>	<i>Pearl barley, boiled^a</i>	<i>Oatmeal</i>	<i>Oatmeal porridge^b</i>	<i>Oat bran</i>	<i>Wholemeal rye flour</i>	<i>Rye crispbread</i>	<i>Rye bread</i>
Sodium (mg)	3	1	21	560	4	1	220	580
Potassium (mg)	270	92	360	46	586	410	500	190
Calcium (mg)	20	7	54	7	79	32	45	80
Magnesium (mg)	65	22	110	18	241	92	100	48
Phosphorus (mg)	210	71	380	47	723	360	310	160
Iron (mg)	3.0	1.0	4.0	0.5	6.1	2.7	3.5	2.5
Zinc (mg)	2.1	0.7	3.3	0.4	4.2	3.0	3.0	1.3
Copper (mg)	0.40	0.14	0.36	0.03	0.31	0.42	0.38	0.18
Manganese (mg)	1.30	0.44	3.80	0.46	5.80	0.68	3.50	1.00
Vitamin E (mg)	0.40	0.10	1.60	0.21	3.30	1.60	0.50	1.20
Thiamin (mg)	0.12	0.02	0.70	0.06	1.10	0.40	0.28	0.29
Riboflavin (mg)	0.05	0.01	0.10	0.01	0.18	0.22	0.14	0.05
Niacin (mg)	2.5	0.5	0.9	0.1	0.9	1.0	1.1	2.3
Vitamin B ₆ (mg)	0.22	0.04	0.23	0.01	0.15	0.35	0.29	0.09
Pantothenate (mg)	0.5	0.1	1.1	0.1	1.0	1.0	1.1	0.5
Folate (μg)	20	3	60	4	37	78	35	24
Biotin (μg)	na	trace	21	2	38	6	7	4
Choline (mg)	38	13	30	7	32	30	20	3

^aUnsalted water.^bMade with water and salt.

na, No data available.

Table 13 Mineral and vitamin contents of sorghum and millets: representative values per 100 g fresh weight (water contents as in **Table 6**)

	<i>Sorghum</i>	<i>Millets</i>					
		<i>Pearl</i>	<i>Foxtail</i>	<i>Proso</i>	<i>Finger</i>	<i>Japanese</i>	<i>Fonio</i>
Sodium (mg)	20.5	7.4	6.8	9.4	15.9	na	15.0
Potassium (mg)	285	432	243	215	367	na	160
Calcium (mg)	28	39	22	14	321	32	30
Magnesium (mg)	156	125	116	104	129	na	40
Phosphorus (mg)	291	335	268	220	251	330	175
Iron (mg)	5.1	8.4	5.3	4.7	4.6	4.3	6.0
Zinc (mg)	2.2	3.2	1.9	1.6	1.3	na	3.0
Copper (mg)	0.98	0.50	0.71	1.15	0.53	na	1.6
Manganese (mg)	1.84	1.45	1.87	1.64	1.19	na	3.0
Vitamin E (mg)	1.13	1.69	2.75	1.94	na	na	na
Thiamin (mg)	0.35	0.34	0.51	0.39	0.35	0.33	0.47
Riboflavin (mg)	0.15	0.17	0.10	0.19	0.10	0.10	0.10
Niacin (mg)	3.8	2.0	3.1	1.3	4.0	na	1.9
Vitamin B ₆ (mg)	0.50	na	na	0.40	na	na	na
Pantothenate (mg)	1.2	1.1	0.7	1.0	na	na	na
Folate (μg)	19	63	18	85	na	na	na
Biotin (μg)	42	na	na	na	na	na	na

na, No data available; there are no data available for choline in sorghum and millets.

the highest concentrations in the outer bran layers. Comparison of the various whole grain and milled products in Tables 10–12 shows that bran is higher in vitamins and minerals, whereas flour fractions are depleted (Tables 10–12). Sodium may be substantially increased by the addition of salt, leavening agents, or other additives. Other minerals and vitamins are often added in fortification to replace, standardize, or augment the natural levels present.

Although white bread made from fortified flour contains less minerals and vitamins than wholemeal bread (**Table 11**), wholemeal bread contains higher levels of phytic acid, which will influence availability (see the Section on Availability). Cornflakes are fortified with a number of minerals and vitamins, including vitamins B₁₂ and D (**Table 10**). The fortification of breads and other cereal foodstuffs with folic acid is increasingly common.

Availability

The presence of micronutrients does not ensure availability for metabolic processes. Mineral availability is decreased by phytic acid and phytates. A substantial proportion of the total phosphorus in cereals (usually >50%) occurs as phytic acid (myoinositol 1,2,3,4,5,6-hexakis dihydrogen phosphate) and is not fully available for absorption. Phytic acid also forms phytates with calcium, magnesium, iron, zinc, and copper, rendering these minerals unavailable for absorption. There is approximately 1% phytic acid in whole grain cereals, concentrated in the bran and germ fractions. Wheat bran and germ contains 3–4% phytic acid, whereas white endosperm flour contains 0.1–0.2%.

Vitamin B₆ and niacin in cereals are also of limited availability. This is particularly significant in pellagra, the niacin deficiency disease, which develops when maize grits are the dietary staple. However, niacin, which is present in a bound form, becomes available on alkali treatment in traditional maize tortilla production. Niacin can also be synthesized in the body from tryptophan; 60 g of tryptophan yields 1 g of niacin.

Dietary Contribution

Data on the relative amounts of minerals and vitamins present in cereals are useful for comparative purposes. However, data on availability and requirements are needed to provide a fuller evaluation. Relative to energy contents, whole grain cereals have the potential to contribute significantly to intakes of potassium, magnesium, phosphorus, iron, copper, zinc, vitamin E, thiamin, riboflavin, niacin, vitamin B₆, and folic acid. Cereals can also provide significant amounts of dietary selenium, but the levels depend on its availability in the soil.

Nonnutrients of Potential Benefit

Cereals also contain a number of minor nonnutrient components, which have the potential to exert beneficial physiological effects. Some of these phytochemicals, which include phytic acid, sterols, phenolics, and flavonoids, have *in vitro* antioxidant and estrogen-like activities. Thus, these components may play an important role in the protective effects against heart disease and certain cancers that are conferred by diets rich in whole grains.

Potential Adverse Effects

Cereals do not have any intrinsic nonspecific toxins. Acrylamide, a potential carcinogen, occurs in baked and fried foods including breads and processed cereals, but there does not appear to be an association between dietary acrylamide intake and increased cancer risk. Detrimental effects may be caused by antinutrients in cereals and, in susceptible individuals, by adverse immune responses (celiac disease; food allergies). Cereals may also be a source of toxins of fungal origin (mycotoxins) or of toxic environmental, agricultural, or industrial contaminants.

Antinutrients

Phytic acid and phytates are antinutrients found in all cereals, and that reduce mineral availability (see the Section on Availability). Most cereals contain polyphenolic tannins, which can bind to proteins and enzymes and reduce protein digestibility. Cereals also contain specific protease inhibitors but the levels are low in comparison with some seed legumes. Tannins and protease inhibitors are unlikely to have any significant adverse effects in human nutrition. However, pearl millet contains phenolic flavonoids, which may be implicated in the onset of goiter, a symptom of iodine deficiency.

Adverse Immune Responses

Many natural products, including cereals and other common foodstuffs, induce allergic responses in susceptible individuals. In such cases, after appropriate diagnosis, individuals should avoid the foodstuff responsible. Celiac disease (gluten enteropathy) is a condition characterized by a severe, adverse, immunological gastrointestinal reaction to gliadin, which is a component of gluten, the viscoelastic protein found in wheat and other cereals. Celiac disease is found in all regions where wheat is commonly consumed. Celiac patients must exclude gluten from their diets. Thus, products containing wheat, rye, barley, and triticale are not permitted. Although originally proscribed, oats appear to be safe for most celiac patients.

Mycotoxins

Mycotoxins are produced by fungi, which may infect crops and stored grain. Ergot (*Claviceps purpurea*) infects rye and other temperate cereals and produces alkaloid toxins. If ingested in sufficient amounts these alkaloids induce mental derangement, gangrene, and other symptoms. Aflatoxin is a toxin and potent carcinogen, produced by the fungus *Aspergillus flavus* that may occur in maize crops and in stored grains. Other fungal toxins include ochratoxin produced by *Penicillium* species and trichothecenes produced by *Fusarium* species. Mycotoxins are a lesser problem in cereals than in seed legumes and nuts. In addition to controlling mycotoxin levels by correct agronomy and storage, grains are monitored to ensure that safe levels are not exceeded.

Contaminants

Cereals may become contaminated with toxic environmental, agricultural, or industrial chemicals during production, storage, and processing. However, the use of these chemicals is controlled, and incidents of hazardous contamination are rare.

See also: Bioavailability. Carbohydrates: Chemistry and Classification. Celiac Disease. Cholesterol: Factors Determining Blood Levels. Fats and Oils. Fiber: Resistant Starch and Oligosaccharides. Food Safety: Mycotoxins – Occurrence and Toxic Effects. Legumes. Niacin and Pellagra. Nuts and Seeds. Phytochemicals: Classification and Occurrence. Protein: Quality and Sources. Whole Grains

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CEREBRAL PALSY

Nutritional Aspects

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Glossary

Bisphosphonates A class of drugs that prevent the loss of bone mass, used to treat osteoporosis and similar diseases. They are called bisphosphonates because they have two phosphonate (PO₃) groups.

Cervical auscultation Cervical auscultation with stethoscope or with transducers can be incorporated into the clinical examination to enhance the clinical examiner's ability to detect aspiration and to determine specialized diet management.

Gross Motor Function Classification Scales (GMFCS) The Gross Motor Function Classification

System (GMFCS) is a five-level classification system that describes the gross motor function of children and youth with cerebral palsy on the basis of their self-initiated movement with particular emphasis on sitting, walking, and wheeled mobility. Distinctions between levels are based on functional abilities, the need for assistive technology, including hand-held mobility devices (walkers, crutches, or canes) or wheeled mobility, and to a much lesser extent, quality of movement.

Muscle tone Muscle tone refers to the amount of tension or resistance to movement in a muscle.

Nutritional issues (e.g., diet, body composition, growth) are integral aspects of medical care for persons with cerebral palsy (CP). This article focuses on CP and its nutritional implications. The first section defines CP and describes its causes, prevalence, and classification types. Associated deficits related to CP are also explored. The topic of nutritional assessment of children with CP includes discussions on growth, body composition, and energy, and protein, fluid, and nutrient needs. Feeding and swallowing problems and the influence of muscle tone on the ability to eat safely are discussed in depth, as are alternative feeding routes. An interdisciplinary approach is emphasized throughout as the ideal model to provide services to people with CP to ensure quality of life in the community.

Definition and Etiology

CP refers to a number of nonprogressive disorders of movement and posture that result from an injury to the central nervous system during early brain development (Table 1).

Classification

There are several different classifications of CP. The three most common types are pyramidal, extrapyramidal, and mixed-type. The type of CP and the degree of involvement play an important part in nutritional assessment and treatment.

Pyramidal (Spastic) Cerebral Palsy

Children with spastic CP have increased muscle tone with a clasped-knife quality. In spastic quadriplegia (30% of cases

of pyramidal CP), all four extremities are involved. In spastic diplegia (25%), both lower extremities are spastic with minimal upper extremity involvement. Hemiplegia (45%) implies involvement on only one side of the body, with the upper extremity usually more affected than the lower extremity.

Table 1 Causes of cerebral palsy

Cause	Percentage of cases
Perinatal	44
First trimester	
Teratogens	
Genetic syndromes	
Chromosomal abnormalities	
Brain malformations	
Second and third trimesters	
Intrauterine infections	
Problems in fetal/placental functioning	
Labor and delivery	19
Preeclampsia	
Complications of labor and delivery	
Perinatal	8
Sepsis/central nervous system infection	
Asphyxia	
Prematurity	
Childhood	5
Meningitis	
Traumatic brain injury	
Toxins	
Not obvious	24

Extrapyramidal Cerebral Palsy

Choreoathetosis involves the presence of abrupt, involuntary movements of the upper and lower extremities. This condition can greatly increase energy expenditure and is further discussed in the section on energy needs.

Mixed-type Cerebral Palsy

Mixed-type CP includes characteristics of both the pyramidal and the extrapyramidal types. For example, a child may have rigidity in the upper extremities and spasticity in the lower extremities.

Associated Disabilities/Deficits

Associated deficits of CP are important to note because they affect nutritional status. Cognitive impairments are quite common. Intellectual disability occurs in 60% of CP cases, with the remainder at high risk for some type of learning disability. Sensory deficits are prevalent, including those in the visual and auditory modalities. Seizures occur in 20–30% of cases, with the highest proportion in the spastic type. In addition to medical management, the ketogenic diet is often prescribed to treat seizures. It is a high-fat diet with limited nutrients and fluid and requires implementation and monitoring by both a neurologist and a nutritionist trained in the diet. Feeding, behavioral, or emotional problems are also frequently noted. Complementary and alternative therapies should be evidence-based, effective, and practical.

Nutritional Assessment

The goal for nutritional assessment and intervention is to have healthy, alert, interactive individuals who are able to take advantage of all that the environment has to offer. Each person must be able to participate to his or her capacity in the learning and therapeutic rehabilitative processes and in social, community, and leisure activities.

Growth

The literature describes children with CP who are shorter and lighter than the reference standard. This may be the result of several factors. Individuals with CP have alterations in muscle tone affecting their limbs and torso, depending on the level of severity and topography. They often exhibit muscle contractures, depending on the type of CP; muscle spasticity may retard bone growth. Limited physical activity may impede growth. Immobilization may be required after orthopedic surgery. Immobilization inhibits bone formation and longitudinal growth and results in suppression of certain growth-stimulating hormones. It has been suggested that dysregulation of growth hormone secretion may be another factor affecting growth.

A growth reference for children with spastic quadriplegia has been developed to facilitate uniformity in clinical assessment as well as to simplify comparative interpretation of growth data. These growth curves can be seen in Figures 1–6. It is important to always evaluate growth velocity from

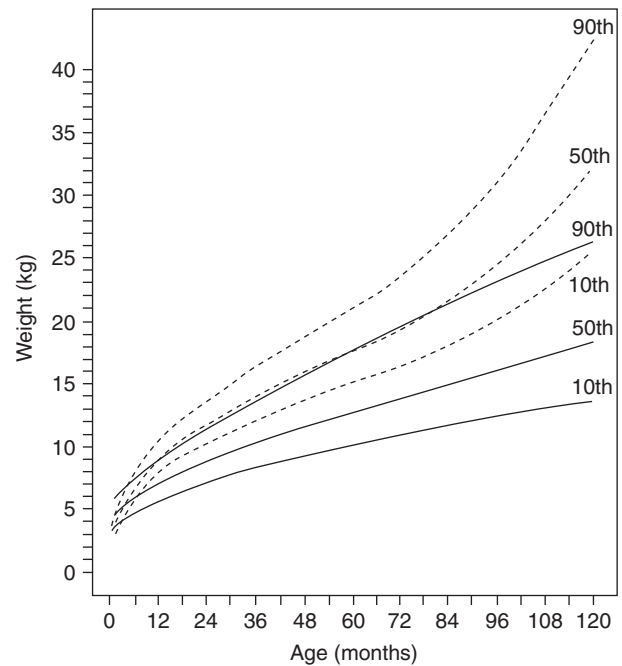


Figure 1 Weight-for-age for girls aged 0–120 months. The solid lines represent girls with quadriplegic cerebral palsy and the dotted lines represent the National Center for Health Statistics standard curves for 10th, 50th, and 90th percentiles.

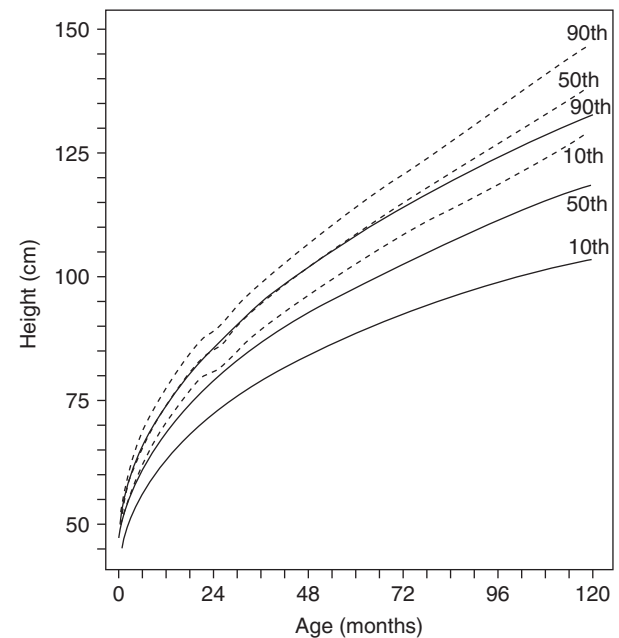


Figure 2 Length-for-age for girls aged 0–120 months. The solid lines represent girls with quadriplegic cerebral palsy and the dotted lines represent the National Center for Health Statistics standard curves for 10th, 50th, and 90th percentiles.

one measurement to another, to aid clinical management. The rate of growth in children with CP is slower so that as they get older, the difference from the standard becomes greater.

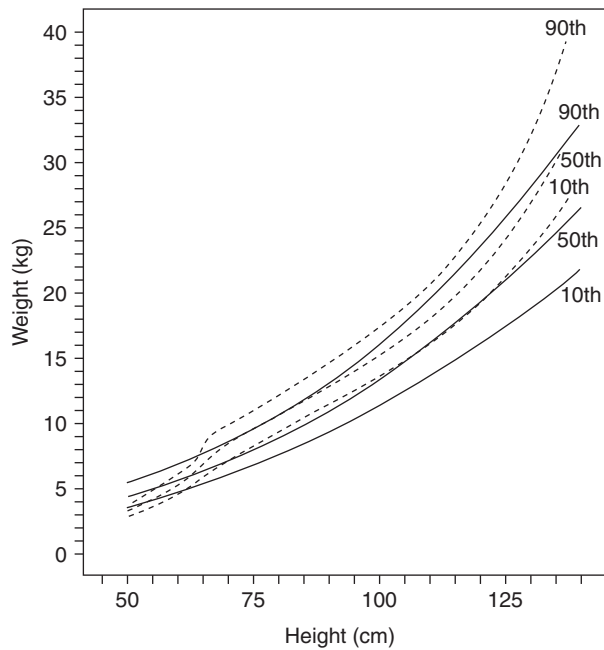


Figure 3 Weight-for-length for girls aged 0–120 months. The solid lines represent girls with quadriplegic cerebral palsy and the dotted lines represent the National Center for Health Statistics standard curves for 10th, 50th, and 90th percentiles.

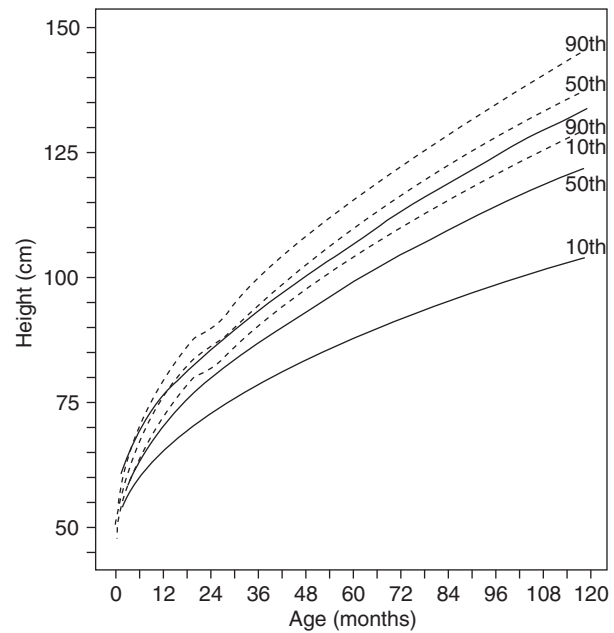


Figure 5 Length-for-age for boys aged 0–120 months. The solid lines represent boys with quadriplegic cerebral palsy and the dotted lines represent the National Center for Health Statistics standard curves for 10th, 50th, and 90th percentiles.

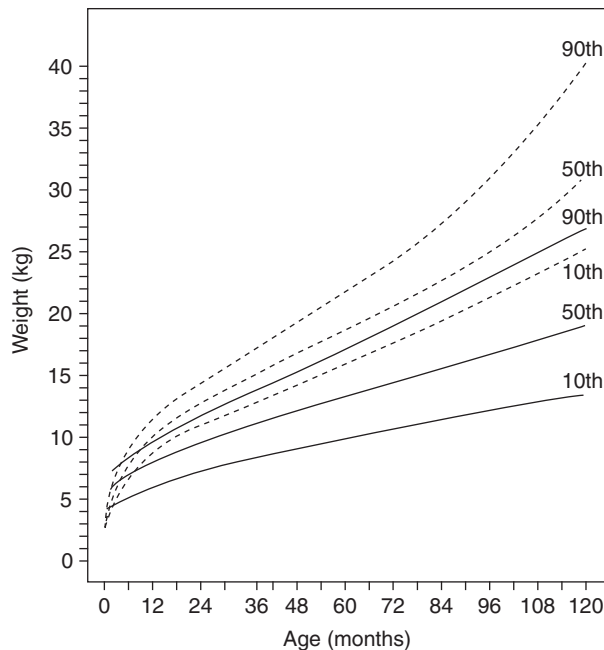


Figure 4 Weight-for-age for boys aged 0–120 months. The solid lines represent boys with quadriplegic cerebral palsy and the dotted lines represent the National Center for Health Statistics standard curves for 10th, 50th, and 90th percentiles.

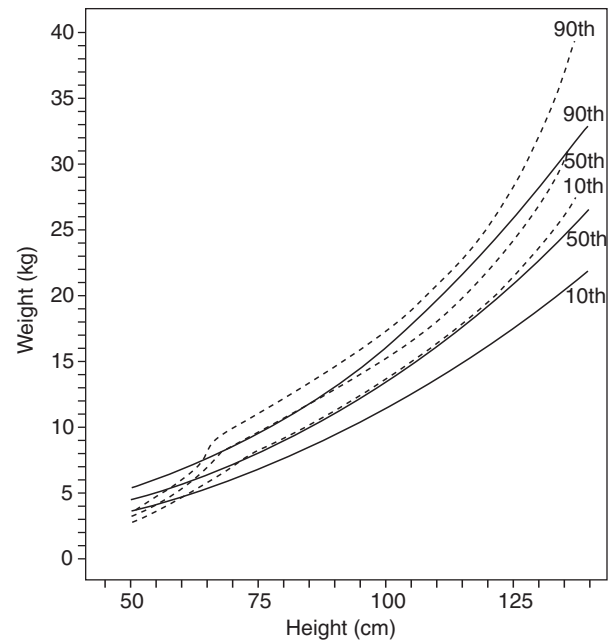


Figure 6 Weight-for-length for boys aged 0–120 months. The solid lines represent boys with quadriplegic cerebral palsy and the dotted lines represent the National Center for Health Statistics standard curves for 10th, 50th, and 90th percentiles.

Steven Day published the largest retrospective study in 2007 describing growth according to the five distinct levels of motor function using the Gross Motor Function Classification Scales and feeding dysfunction. It reviewed almost

25 000 children aged 0–20 years and growth charts were developed.

Both nutritional and nonnutritional factors influence growth in children with CP. Nonnutritional influences that

have been suggested to impact growth include weight-bearing opportunities and, by extension, interventions using aggressive physical therapy, growth hormones, and electrical stimulation of muscle. In 1995, Stevenson reviewed growth in hemiplegics and noted that there is diminished growth, decreased muscle mass, and decreased fat stores on the affected side, and that the magnitude of the differences increases with age and functional severity. Gender, age, cognitive impairment, and ambulatory status have also been noted to contribute to the slow growth seen in this population.

Measurement of length or height for individuals with CP may require techniques and standards using arm span, lower leg length, or segmental measurements because of the difficulties encountered with joint contractures and scoliosis. The use of height age, rather than chronological age, is a common technique and is defined as the projected age at which the current child's height crosses the 50th percentile on the National Center for Health Statistics chart.

The use of *z* scores for length-for-age, weight-for-age, and weight-for-length promotes an accurate evaluation of discrete changes from one measurement date to another. Percentile tables describe ranges, and consequently detection of movement within the range is difficult to describe. The *z* score denotes standard deviation units from the median and allows the practicing clinician and investigator to pinpoint precisely any given measurement.

For screening purposes, conventional length/height and weight measures can be completed and compared to the Centers for Disease Control and Prevention growth charts. Reference standards for body mass index for children with CP do not exist; therefore, one must use body mass index data in conjunction with body composition data to determine adequacy of growth. Researchers from the multicenter North American Growth in Cerebral Palsy Project suggest that a practical method to assess nutritional status in a child with CP is to measure body fat. This can be done in the form of either the triceps skinfold (TSF) or both the TSF and the subscapular skinfold. However, patient cooperation with the measuring techniques, required for accuracy and safety, may be difficult to obtain or maintain. For some individuals with CP, the process may be difficult, and training is needed to learn the technique for body fat measures and segmental measures mentioned previously.

When trying to obtain growth measurements, joint contractures, muscle spasms, and poor cooperation will impact accuracy. Upper extremity (arm) length, tibial length, and knee height are often noted in the literature as valid proxies for length in children with CP up to the age of 18. (See [Table 2](#) for estimation of height using segmental measures.)

Ideal Body Weight

The estimate of ideal body weight (IBW) is also in part determined by the severity of the CP. The IBW should be aimed at maintaining adequate fat and muscle stores to endure repeated surgeries or a common virus while facilitating daily physical care and management. Weight-for-length is an indicator of nutritional status, which obscures the issue of chronological age and addresses whether the individual is

Table 2 Estimation of height from segmental measures

Age 0–12 years
$(4.35 \times \text{UAL}) + 21.8$
$(3.26 \times \text{TL}) + 30.8$
$(2.68 \times \text{KH}) + 24.2$
Age 6–18 years
White male $(2.22 \times \text{KH}) + 40.54$
Black male $(2.18 \times \text{KH}) + 39.60$
White female $(2.15 \times \text{KH}) + 43.21$
Black female $(2.02 \times \text{KH}) + 46.59$

UAL, upper arm length; TL, tibia length; KH, knee height.

proportionate. IBW can be expressed as this ratio. Those with CP should attain and maintain an IBW that takes into account their age, level of physical ability, and their independence. Measurement of arm anthropometry will provide a description of body composition and support clinical judgments related to IBW. For example, children with spastic quadriplegia are the most dependent and the 10th percentile weight-for-length would be designated as the IBW. However, this assignment is done in tandem with assessment and monitoring of body composition, and if either the arm fat or the arm muscle area were less than the 5th percentile, then the IBW would be adjusted upward.

Body Composition

Since the 1970s, researchers reviewing body composition have noted reduced lean body mass in children with CP. Recent work examining adults with CP and their age-matched controls found no difference in lean body mass or percentage of body fat.

Bone Mineral Density

Bone mineral density (BMD) is markedly reduced in non-ambulatory children with CP, placing them at risk for non-traumatic fractures. Osteopenia defined as <2 standard deviations below the mean BMD was found in the femur of most nonambulatory children by the age of 10. Decreased BMD results from a combination of factors, including immobilization, antiepileptic therapy, and nutritional deficiencies. Serum levels of calcium, phosphate, alkaline phosphatase, and osteocalcin were not found to be reliable indicators of low BMD when studied by Henderson. The same author noted that fracture rate is fourfold higher following spica casting and more than threefold higher following an initial fracture.

Many nonambulatory children require, and are given, fewer calories than recommended for their non-CP counterparts; therefore, the clinician is obliged to review the adequacy of the micronutrients, specifically calcium. Serum vitamin D levels should be evaluated and the nutrition should be supplemented based on the findings. Most likely, their diets will require supplementation to meet 100% of the dietary reference intake (DRI) standards for age and gender.

Methods to increase BMD include weight-bearing activities, dietary adequacy, and the use of bisphosphonates. In several

studies, bisphosphonate use has demonstrated increased bone density by 20–89% with no obvious adverse effects.

Energy Needs

Equations that are frequently used to predict energy requirements were developed using healthy children and adults in usual environmental and physical activity conditions and do not provide an accurate assessment of the needs of those with CP. From a nutritional perspective, wide-ranging studies demonstrate underreporting of energy needs on food records, which at best provide a qualitative measure of intake. Therefore, clinicians have turned to the use of more sophisticated technology, such as doubly labeled water and indirect calorimetry, to assess the energy needs of this population. Additionally, the energy cost of movement, whether it be wheelchair propulsion, crutch ambulation, or the involuntary movements of the individual with athetosis, must be considered. Those with CP may undergo repeated orthopedic surgery that may impair nutritional status, due to increased nutrient and energy demands. It has also been hypothesized that whole body metabolic rate may be related to differences in skeletal muscle fiber proportions and differences in enzymatic activity. People with CP have abnormal variation in the size of muscle fibers and altered distribution of fiber types.

Altered energy needs are common among those with CP and differ widely from the norm. Clinicians use a variety of approaches to estimate energy needs, such as the DRIs for chronological age, the recommended daily allowances for height age, and the World Health Organization equation. When estimating energy needs, information related to muscle tone, activity level, and needs for growth or catch-up growth must be added to the estimate for resting energy expenditure (REE).

The equation designed specifically for this population is

$$\text{REE} \times \text{muscle tone factor} \times \text{activity factor} + \text{growth factor(s)} \\ = \text{kilocalories per day}$$

The REE can be determined using indirect calorimetry or can be derived from estimating body surface area standard metabolic rate for 24 h. Body surface area (m^2) is calculated from length and weight using the nomogram derived from the formula of DuBois and DuBois, and the standard metabolic rate ($\text{kcal m}^2 \text{h}^{-1}$) is identified using height, age, and sex by applying Fleisch data. The modifying factors applied are as follows:

- Muscle tone factors: Multiply by 10% for high tone (hypertonicity) and decrease by 10% for low tone (hypotonicity); no adjustment for normal tone.
- Activity factors: Multiply by 15% for bedridden state, 20% for wheelchair, and 30% for ambulation.
- Growth factors: Add 5 kcal (20.92 kJ) per gram of desired growth, expected growth, and catch-up.

Energy needs must be viewed on an individual basis as simulating the concepts noted previously. The use of any approach is regarded as a guidepost and requires careful monitoring of body weight. Modifications to the diet should be based on clinical observation and measurement. There is a

subset of individuals with CP who require significantly less kilocalories than anticipated (as few as perhaps 15 kcal kg^{-1}). The use of intrathecal Baclofen to treat spasticity can also reduce muscle tone and, therefore, energy needs.

Nutrient and Fluid Needs

Nutrient and protein needs are based on DRIs similar to those of the population without CP. Height age is often used in these determinations.

Fluid needs are based on body size rather than calorie intake. Table 3 demonstrates how to calculate fluid needs. Constipation is a chronic problem for most children with CP and is related to muscle tone, loss of sensation, limited physical activity, medication side effects, and inadequate dietary fiber intake or fluid intake. Oral motor dysfunction results in diminished intake as well as in food and fluid loss. Modified food and fluid textures result in less free water and fiber in the diet. Discomfort associated with constipation may decrease appetite and increase gastroesophageal reflux (GER). Dietary intervention may therefore be limited and medical management may be necessary.

Assessment of Feeding Skills and Safety

Eating skills are acquired in a sequential pattern so that a developmental history will be helpful in evaluating current function and planning treatment options. Factors affecting feeding performance are shown in Table 4.

Oral Motor Evaluation

Feeding and swallowing problems are common in the child with CP, depending on the type of muscle tone, the presence of primitive reflexes, movement patterns, and the integrity of the sensory system. Clinical indicators of feeding and swallowing dysfunction are shown in Table 5. Problems often include poor intake, inefficient and lengthy mealtimes, abnormal oral motor patterns, inappropriate progression of feeding skills, and physiological compromise with feeding. Sensory, cognitive, and language deficits may also complicate the feeding process. An interdisciplinary team evaluation is essential for the assessment and development of appropriate

Table 3 Fluid needs based on body weight^a

Body weight (kg)	Fluid need ($\text{cm}^3 \text{kg}^{-1}$)
≤ 10	100
11–20	+ 50
≥ 21	+ 25

^aSuggest monitoring urine-specific gravities when available and quantity, color, and odor of urine and adjust for periods of stress and temperature. Example: 28-kg child.

$100 \text{ ml} \times 10 \text{ kg} = 1000 \text{ ml}$

$50 \text{ ml} \times 10 \text{ kg} = 500 \text{ ml}$

$25 \text{ ml} \times 8 \text{ kg} = 200 \text{ ml}$

Total need = 1700 ml.

Table 4 General factors affecting feeding performance

Neuromotor performance	Constipation
Perceptual deficits	Amount of physical and verbal assistance required
Cognition and communication skills	Physiological support
Vision and hearing	Oral motor skills and swallowing status
Behavior/interaction	Medications
Growth	Dental and gum disease
Dietary adequacy	Multiple orthopedic procedures
GER and other gastrointestinal-related issues	Family/psychosocial stressors

Table 5 Clinical indicators of feeding and swallowing dysfunction

Congestion	Difficulty managing secretions
Noisy 'wet' sounds	History of upper respiratory infections
Multiple swallows to clear bolus	Apnea during feeding
Unexplained fevers, unexplained irritability	Failure to thrive, failure to maintain weight
Coughing/choking/gagging before, during, or after swallow	
Food refusal	

goals and facilitation of a treatment plan that respects the developmental progression. A clinical assessment of the feeding process should include observance of facial muscle tone, oral reflex activity, functional oral motor skills, structural abnormalities, sensory responses, behavior and interaction during feeding, respiratory and phonatory status, and posture and positioning.

Radiographic and ultrasound studies can provide more detailed information about the oral structures and the competency of the oral, pharyngeal, and esophageal phases, including the detection of aspiration. Cervical auscultation can also be helpful in evaluating the pharyngeal phase of swallowing. In addition, these techniques can assist in determining the suitable solid and liquid texture and appropriate head and neck positioning. Hypertonicity leads to abnormal movements of the tongue, lip, and jaw. These abnormal movements can be manifested as tongue retraction, tongue tip elevation, tongue thrust, tonic biting, jaw thrust, jaw instability, lip retraction, and lip/cheek instability. An abnormally strong gag reflex, tactile hypersensitivity in the oral area, and drooling can also complicate feeding.

Dental

Increased incidence of cavities and erosion frequently occur due to poor oral hygiene and teeth grinding. Hypersensitivity in the oral area and hyperplasia of the gums from long-term use of phenytoin may also be seen. Malocclusion is a common musculoskeletal problem and contributes to drooling, which can negatively affect daily oral care. Injury to the mouth can be seen following falls, other accidents, or physical abuse.

Aspiration and Gastroesophageal Reflux

Clinical signs of aspiration may include coughing, choking, gagging, inability to handle oral secretions, wet upper airway sounds with poor vocal quality, apnea, food refusal, frequent upper respiratory infections, and aspiration pneumonia. Aspiration of food may occur without physical evidence if the protective cough or gag is not functioning, sensory deficits exist, and the swallowing mechanism is dysfunctional. This results in what is termed silent aspiration. Although aspiration from solid food can be detected, the possibility of aspiration from GER may also need to be considered. The regurgitation of gastric contents from the stomach into the esophagus can lead to irritability during or after feeding, arching, esophagitis, and ultimately food refusal. Other symptoms of GER include respiratory compromise, apnea, and drooling. Treatment for GER includes the use of antacids (H_2 blockers or proton pump inhibitors), medications to increase gastrointestinal motility, reduction in feeding rate, positioning, thickening of foods or liquids, or surgical intervention. Small, frequent feedings help to decrease the volume in the stomach at one time.

Fatigue may occur in the child who is not able to sustain the work involved with feeding and may be expressed by an increase in respiratory rate, diaphoresis, or increased work of breathing. The causes may be muscular, respiratory, or cardiac, and they may increase the risk of aspiration or hypoxia. The work required to eat a meal is accomplished at a higher physiological cost to the child, thereby increasing caloric needs.

Muscle Tone and Positioning

It is important to understand the influences of muscle tone and proper positioning on the ability to eat safely and efficiently in this population. Increased or decreased muscle tone contributes to difficulty preserving a patent airway, compromised self-feeding skills, poor rib cage expansion and esophageal motility, and difficulty maintaining a stable supported base for seating. Fluctuating muscle tone leads to involuntary movements and limited postural stability. Despite the type of muscle tone, optimal positioning is crucial for feeding and swallowing. The proper feeding position includes neutral alignment of head and neck, midline orientation, symmetrical trunk position, 90° pelvic/femoral alignment, and symmetrical arm position with neutral shoulders. An example of proper positioning can be seen in [Figure 7](#). Consultations with orthopedists and rehabilitation physicians to address current and potential musculoskeletal problems, physical and occupational therapists for functional assessment, orthotists for deformity management, and durable medical equipment specialists to customize standard wheelchair components are valuable.

Underweight and Overweight

Overweight

Most children with CP who are overweight or obese have low muscle tone. Their nutritional status impacts sleeping and breathing patterns, mobility, physical care, and peer

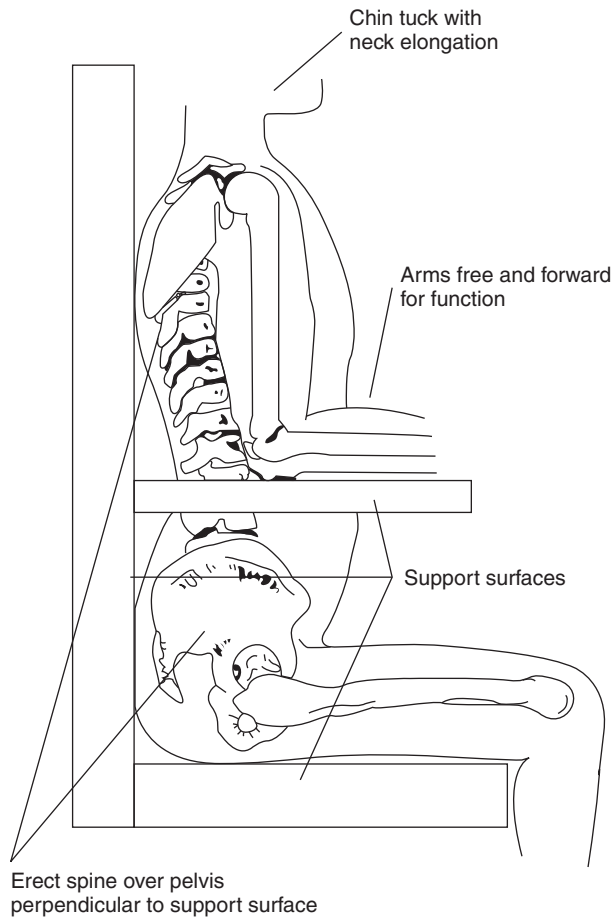


Figure 7 Proper seating position.

relationships. It is difficult to attain an IBW because energy needs are significantly reduced and the options for exercise are limited.

Underweight

Typically, children with athetosis struggle to maintain weight given their excessive involuntary movements, which significantly increase energy needs. As these children age, the problem becomes more apparent, and many of these children will require enteral supplementation. One evaluation of this population noted that the basal energy requirement was 40% higher than expected.

Superior Mesenteric Artery Syndrome

Superior mesenteric artery (SMA) syndrome is a condition in which the third portion of the duodenum is intermittently compressed by the overlying SMA, resulting in gastrointestinal obstruction. Symptoms include recurrent vomiting, abdominal distension, weight loss, and postprandial distress. People with CP are at high risk for several of the reported causes of SMA syndrome, including body cast compression, severe weight loss, prolonged supine positioning, and scoliosis surgery. Consequently, it is important to recognize the symptoms and know the appropriate treatments for this syndrome.

Most people can be treated nonsurgically with gastric aspiration and nasojejunal or gastrojejunal feedings distal to the obstruction. One study also found that turning to the left from a supine position displaces the SMA from the right to the left side of the aorta in scoliosis cases. Thus, positioning can help alleviate symptoms and special considerations may be indicated in light of the limitations imposed by the CP.

Behaviors at Mealtimes

Parent-child interactions can also influence feedings. Ineffective communication, lack of bonding, the absence of social interaction or poor interactive skills, family dysfunction, and decreased environmental stimuli can exacerbate feeding difficulties or lead to frustration and anxiety with subsequent food refusal or parental withdrawal. Aversion to oral feeds can also be an outcome of medical complications, such as esophagitis and GER, or lack of feeding experience at critical milestones secondary to prolonged tube feedings. Behavioral treatment should only be undertaken after thorough medical, nutritional, and neurodevelopmental assessments are completed.

Feeding Issues

The feeding plan should be safe, promote growth or weight maintenance without excessive energy expenditure to obtain the required calories, and meet the needs of the family. It should reflect their resources in time and skill, and it should address their concerns and expectations. The goals for treatment once feeding and swallowing problems are identified are: to prevent aspiration and thereby respiratory compromise; to provide adequate calories, protein, vitamins, minerals, and fluid; and to educate caregivers regarding nutritional requirements.

Oral Motor Considerations

Management strategies for daily mealtime feeding include positioning, modification of the sensory properties of the food, oral motor facilitation techniques, and equipment adaptations. For individuals with increased energy needs, the nutrient density of their meals may need to be maximized. **Table 6** lists commonly used calorie boosters. It is important to acknowledge the inability to change the underlying feeding problem while providing a method of circumventing the problem to allow adequate nutrition and growth. For example, facilitative techniques to minimize excessive jaw movement may entail the feeder providing physical jaw control/support; a change in the food consistency, texture, temperature, or taste to improve the ability to propel a bolus through the oropharynx; the careful selection of adaptive feeding equipment to assist with self-feeding or increased intake; and an appropriate seating system. Proper positioning also allows the feeder to use both hands.

Alternative Feeding Routes

Many children with CP are not able to meet some or all of their calorie needs by mouth due to one or more of the

Table 6 Calorie boosters

Instant breakfast	Margarine, butter, oils, gravy
Powdered, evaporated milk	Sugar, honey, syrup
Whole milk cheeses	Cream cheese
Peanut butter	Sour cream
Wheat germ	Concentrate juices
Yogurt, pudding, custards	Breading or cracker meal
Milkshakes, eggnog	Fruit canned in heavy syrup
Commercial supplements	

following conditions: oral motor dysfunction, excessive energy needs, recurrent infections, illnesses, and orthopedic surgical interventions. Consequently, if the gastrointestinal tract is functioning, supplemental or total tube feedings may be indicated. Early intervention with enteral nutrition may prevent protein–energy malnutrition and its complications. Studies have shown improvements in weight gain (fat mass as opposed to fat-free mass) with supplemental tube feedings, which better enables individuals to endure short-term medical insults.

Enteral nutrition may be delivered by nasogastric, nasojejunal, gastrostomy, gastrostomy–jejunal, and jejunostomy tubes. The degree of GER and risk of aspiration determine where the tube is placed, whereas the length of time needed for tube feedings determines whether a nasoenteral or surgically placed tube is required. The decision regarding continuous, intermittent, or combination tube feeds is dependent on the individual needs of the patient.

Tube feedings should be considered a tool to improve nutritional status rather than failure of the child's ability to eat. Based on the medical diagnosis and developmental stage of the child, the prognosis for return to oral feeding varies, and the length of time to achieve this goal is extremely variable. For some children, the goal of returning to full or partial oral feeding is not realistic. In a study evaluating the health of children with CP, Liptak describes those who were tube fed as having the lowest mental age, requiring the most health-care resources, using the most medications, and having the most respiratory problems. These children were characterized as especially frail and required numerous health-related resources and treatments. Oral motor therapy should focus on maintaining existing oral motor skills, encouraging pleasurable oral experiences, and tolerance of oral hygiene practices. Nonnutritive oral stimulation must be performed when tube feedings are employed as the route of nutrition. The benefits of nonnutritive oral stimulation are listed in **Table 7**. Improvement in nutritional status can result in positive changes in oral feeding.

Parenteral nutrition should only be used when the gastrointestinal tract is dysfunctional. When initiating feedings in patients with major weight loss or failure to thrive, whether enteral or parenteral nutrition is used, it is important to be aware of the 'refeeding syndrome.' This syndrome refers to phosphorus depletion and alterations in potassium, magnesium, and glucose metabolism, resulting in severe metabolic and physiological complications. It is imperative to increase calorie delivery slowly with close laboratory monitoring.

Table 7 Benefits of nonnutritive oral stimulation

Maintains oral sensation and tolerance
Facilitates saliva production, swallowing, and other oral motor patterns
Maintains or develops coordination of respiration and swallowing
Facilitates parent–child interactions

Medications

Drug–nutrient interactions should be considered for all children receiving long-term medications for seizure disorders, alterations in muscle tone, attentional deficits, gastrointestinal disorders, and other chronic conditions. One drug or the combination of multiple drugs may affect nutrition in many ways, such as causing decreased appetite, interference with absorption of specific nutrients, nausea, and vomiting.

Medication treatment options offer challenges to nutrition. For instance, diazepam, often used to decrease spasticity, increases the potential for drooling. This raises concerns of fluid loss/balance as well as loss of the protective effect of saliva on esophageal mucosa. Additionally, attention must be paid to tone reduction in the trunk and oral structures that would compromise safety of feeding skills.

Tone-lowering drugs potentially reduce energy expenditure and, as a result, require increased vigilance to avert excessive weight gain.

Repeated Orthopedic Surgeries

These are common in children with CP, and each surgery must be preceded by an evaluation of nutritional status and assessment of the child's ability to physically heal and recover quickly from the trauma. Many children who are marginal oral feeders will decompensate, lose weight, and have a difficult time healing because of a cascade of events including pain, poor positioning for safe feeding, worsening constipation, minimal intake, lethargy, and increased medications for pain that may have a sedative effect. They may require supplemental feedings before surgery or during the post-operative period.

Coordinated Services

The provision of nutrition services and prevention of further disabling conditions can be done in a variety of health care, school, vocational, home, and community settings. Participation in physical fitness activities targeting muscle strength and cardiorespiratory fitness should be encouraged to prevent secondary conditions such as chronic pain, fatigue, and osteoporosis. It is the responsibility of the family in concert with the health-care team to promote nutrition care planning in these settings. More than 90% of children with CP live to adulthood; however, their life expectancy is less than that of the general population. Similar to the general population, individuals with CP are susceptible to developing obesity, hypertension, diabetes, and heart disease and, therefore, require awareness and adherence to prudent dietary guidelines. The chronicity of nutrition problems for

individuals with CP is recognized and has in part created a need for care coordination and integrated service planning to provide meaningful and cost-effective services.

See also: Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Nutritional Support: Adults, Enteral

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Nutritional Requirements

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Abbreviations

μg	microgram(s).
E%	percent of total energy coming from the energy-giving nutrient.
g	gram(s).

kcal	kilocalorie, 1 kcal=4.184 kJ.
kg	kilogram.
MJ	megajoule, 1 MJ=1000 kJ=239 kcal.
PUFA(s)	polyunsaturated fatty acid(s).

Introduction

This article will describe nutrient requirements for children. It includes the methodology for determining nutrient requirements and recommended intakes and reviews the physiological importance of select nutrients, including the risks of inadequate and excessive intakes. **Table 1** shows the nomenclature used by countries and institutes regarding nutrient reference values. Note that several different names exist for similar definitions. The main emphasis in this article will be nutrient intake after 1 year of age.

Proper nutrition in early life is very important. Nutritional status is known to affect the rate of maturation, learning ability, and neurological development. It also can affect health in adulthood, such as risk of cardiovascular disease, obesity, and type 2 diabetes. Poor nutrient intake may affect the immune system, making one more susceptible to food allergies and intolerances, as well as infections.

Nutrient needs vary throughout the lifespan. In toddlers and in young school children, growth velocity is slower than in infancy; however, at adolescence, growth rate increases again before stabilizing. Owing to differences in growth trajectories, many nutritionists prefer to develop energy and nutrient recommendations based on body weight in children, measured in kilograms.

The requirement for a particular nutrient is defined by the physiological role that it plays in the body and its bioavailability from the diet. If the requirement is not met, symptoms of nutrient deficiency may appear, and severe deficiency can lead to disease. The recommended dietary intake set for a nutrient meets the needs of 97.5% of individuals. **Tables 2–6** show the daily recommended dietary intake for minerals, trace elements, water-soluble vitamins, and fat-soluble vitamins. **Table 4** shows the 2004 Nordic nutrition recommendations.

Methodology for Definition of Nutrient Requirements and Recommended Intakes

Studies on specific energy levels and nutrient requirements for children are scarce. Variations between countries may be due to distinct dietary habits and nutrient bioavailability, differences in rate of childhood growth and average body size, different climates, or how the nutrient requirement is categorized. For example, countries categorize the needs for children by using different age ranges.

The estimated amounts of required nutrients for children are determined by several methods. Extrapolation from infant and adult data is the usual approach to estimate the required amount of nutrients in childhood. The factorial approach defines the requirement in a two-step equation that includes maintenance and growth. The amount required for maintenance is calculated from clinical trials, which estimate unavoidable losses during a period of negligible intake. The amount required for growth is a calculated accretion of the nutrient in the body. Another method of determining requirements is through balance studies, where nutrient intake is manipulated to equal losses. The required amount of a nutrient also can be established through avoiding deficiency symptoms that represent a biological nutrient inadequacy, such as iron deficiency anemia. The methodology behind the development of nutritional recommendations and reference values for children is discussed further in an article by Prentice *et al.* (2004). A general framework for improving nutrient recommendations was recently proposed by the European Micronutrient Recommendations Aligned project, which emphasizes collaboration among various countries.

The World Health Organization has suggested using the grading of recommendations, assessment, development, and evaluation (GRADE) system for judging the quality of evidence for nutritional recommendations. The highest grades

Table 1 Terminology for nutritional recommendations

Authority	Mean – 2SD	Mean	Mean + 2SD	Less evidence-based data	Upper limit of intake
NNR2004	Lower level of intake	Estimated average requirement (EAR)	Recommended intake		Upper intake levels (UL)
US/Canada		EAR	Recommended dietary allowance	Adequate intake	Tolerable upper intake level (TUL)
UK		EAR	Reference nutrient intake	Estimated safe + adequate dietary intake (ESADI)	
Australia		EAR	Recommended dietary intake	Adequate intake	UL
DACH ^a	Lower reference nutrient intake		Recommended nutrient intake	Estimated value for adequate intake	
FAO/WHO		EAR	Recommended nutrient intake	Acceptable intake	Upper tolerable nutrient intake
EU ^b		Average requirement	Population reference intake	Acceptable ranges	
UNU ^c term ^a		Average nutrient requirement	Individual nutrient level	AI acceptable range	

^aThe European countries with German-speaking majorities: Germany (D for Deutschland), Austria (A for Austria), Switzerland (CH for Confoederatio Helvetica).

^bEuropean Union.

^cThe United Nations University (UNU).

are on high-quality randomized clinical trials. The World Cancer Research Fund has developed recommendations based on relationships between nutrition and health or disease (e.g., cancer), which are evaluated as convincing or probable. Such an approach is especially valuable for recommendations regarding the energy-providing nutrients and development of food-based dietary guidelines.

Energy

Reference values for children are based on energy required per kilogram of body weight. The stage of childhood growth dictates specific energy requirements. Other components in calculating energy needs are the same as for adults, i.e., basal metabolic rate, diet-induced thermogenesis, and physical activity. Basal metabolic rate comprises the majority of energy requirements and is largely determined by fat-free mass, more so by the vital organs than skeletal muscles. Young children have twice or more the energy requirement of adults, depending on body composition and growth stage. Higher fat mass lowers the energy requirement, an important factor to consider when determining reference values. After the first year of life, the fraction of energy intake used for growth decreases rapidly. By 1–3 years of age, approximately 3% of energy intake is used for growth, and thereafter less than 2%. Although still important, the energy needed for growth is a relatively small proportion of the total energy intake for children after 1 year of age. However, the energy cost of growth still must be taken into consideration.

Basal metabolic rate may be estimated using equations based on age, weight, height, and gender. Total energy expenditure is estimated by adding the energy required for physical activity, diet-induced thermogenesis (approximately 10%), and growth (1–3%).

As energy requirements depend on several different factors, variation in reference values exists among countries. Growth standards have been used to estimate the long-term adequacy of energy intake. Feeding practices in infancy and childhood, genetic variations, and other possible determinants of growth also will contribute to differences in growth standards.

Carbohydrate

Daily requirements of carbohydrate intake (g per day or g per kilogram body weight per day) have not been defined as they have for total energy and essential nutrients. Very low carbohydrate intake causes ketosis and can be avoided by ingesting only a small amount of carbohydrates. It is generally advised that carbohydrates should comprise more than 50% of total energy intake (50E%). This is partly to avoid high fat and protein intake, but high-quality carbohydrates are of key importance to obtain the beneficial health effects of the recommended high-carbohydrate diet. Early introduction of high-quality carbohydrates is believed to have an impact on dietary habits throughout life. The quality is based on where the carbohydrates originate and the nutrient density of the food.

Intake of sugar may be unfavorable for young children, as they are especially vulnerable to developing dental caries. In addition, a high-sugar diet generally includes foods with low nutrient value, and sugar-sweetened beverages have been associated with increased risk of overweight and obesity.

Some health authorities recommend fiber intake to equal the age of the child plus five (i.e., 7-year-old child = 12 g fiber day⁻¹). This concept has been criticized when determining fiber intake for older children. School children can easily adopt the same recommendation as adults, 3 g fiber MJ⁻¹ or 25–35 g day⁻¹. Fiber intake should be increased gradually in early childhood to reach the level of 3 g MJ⁻¹ slowly.

Table 2 Recommendations for minerals for children (mg day⁻¹)

Nutrient	Age	USA/Canada F/M ^a	UK F/M ^a	Australia F/M ^a	DACH F/M ^a
Sodium	1–3	1000	500 ^b	200–400	300
	4–6	1200	700	300–600	410
	7–8	1200	1200	300–600	460
	9	1500	1200	400–800	460
	10	1500	1200	400–800	510
	11–12	1500	1600	400–800	510
	13	1500	1600	400–800	550
	14–18	1500	1600	460–920	550
Potassium	1–3	3000		2000	1000
	4–6	3800		2300	1400
	7–8	3800		2300	1600
	9	4500		2500/3000	1600
	10–13	4500		2500/3000	1700
	14–15	4700		2600/3600	1900
	16–18	4700		2600/3600	2000
Phosphorous	1–3	460	270	460	500
	4–6	500	350	500	600
	7–8	500	200	500	800
	9	1250	450	1250	800
	10	1250	450	1250	1250
	11–18	1250	625/775	1250	1250
Calcium	1–3	700	350	500	600
	4–6	1000	450	700	700
	7–8	1000	550	700	900
	9–10	1300	550	1000	900
	11	1300	800/1000	1000	1100
	12	1300	800/1000	1300	1100
	13–14	1300	800/1000	1300	1200
	15–18	1300	800/1000	1300	1200
Magnesium	1–3	80	85	2 (AI)	80
	4–6	130	120	2.5	120
	7–8	130	200	2.5	170
	9–10	240	200	2.5/3	170
	11–13	240	280	2.5/3	250–230
	14–15	360/410	280	3/3.5	310
	16–18	360/410	300	3/3.5	350/400

^aF = female; M = male.^bSalt and health. Scientific Advisory Committee on Nutrition 2003.

Fat

Dietary requirements have been defined for the essential polyunsaturated fatty acids (PUFAs): linoleic acid (C18:2, *n*-6) and α -linolenic acid (C18:3, *n*-3). Fat gives energy in a concentrated form, and essential fatty acids are involved in important physiological functions in the body. They are required for regulation of renal function, blood coagulation, inflammatory and immunological reactions, and blood pressure control. Human physiology does not have the enzymes necessary to introduce double bonds in the *n*-3 and *n*-6 positions; therefore, these fatty acids must be obtained from the diet. Studies strongly suggest that the *n*-3 fatty acids are important for normal brain and vision development in children. Although the *n*-3 and *n*-6 fatty acids are essential, excess intake is possible.

Adequate intake of fat is necessary to meet the needs for essential fat-soluble vitamins and fatty acids. Dietary fat intake, providing less than 15% of total energy intake, is considered to be inadequate. High intake of saturated fat is associated with cardiovascular disease. Decreases in intake of

saturated and *trans*-fatty acids in the Nordic countries after 1970 and 1980 have been followed by a reduction in prevalence and mortality rate of ischemic heart disease.

The recommended combination of dietary fat in most countries is similar for children as for adults, but for young children, the recommended total fat intake is sometimes higher. The Nordic Nutrition Recommendations advise a total fat intake of 30–35E% for 1–2-year-olds, but 25–35E% after the age of 2 years. In addition, a maximum of 10E% from saturated plus *trans*-fatty acids to a maximum of 10E% of PUFAs with at least 1E% *n*-3 fatty acids, and 10–15E% of monounsaturated fatty acids.

Proteins

Dietary protein requirements include the essential amino acids and protein needs to maintain a positive nitrogen balance necessary for growth. Nitrogen is a part of amino acids, which are the building blocks of proteins. Proteins are

Table 3 Recommendations for trace minerals for children

Nutrient	Age	US/Canada F/M ^a	UK F/M ^a	Australia F/M ^a	DACH F/M ^a	FAO/WHO F/M ^a
Iron (mg day ⁻¹)	1–3	7	6.9 ^b	9	8	5.8
	4–6	10	6.1	10	8	6.3
	7	10	8.7	10	8	8.9
	8	10	8.7	10	10	8.9
	9–10	8	8.7	8	10	8.9
	11–13	8	14.8/11.3	8	15/12	14/14.6
	14–18	15/11	14.8/11.3	15/11	15/12	14/14.6
Zinc (mg day ⁻¹)	1–3	3	5	3	3	5.5
	4–6	5	6.5	4	5	6.5
	7–8	5	7	4	7	7.5
	9–10	8	7	6	7	7.5
	11–13	8	11	6	7/9	10.3/12.1
	14–18	9/11	11	7/13	7/9.5	10.3/12.1
Iodine (µg day ⁻¹)	1–3	70	70	90	100	90
	4–6	90	100	90	120	90
	7–8	120	110	90	140	120
	9–10	120	110	120	140	120
	11	150	130	120	180	120
	12–13	150	130	120	180	150
	14	150	130	150	200	150
	15–18	150	140	150	200	150
Selenium (µg day ⁻¹)	1–3	20	15	25	10–40	20
	4–6	30	20	30	15–45	24
	7–10	30	30	30	20–50	25
	11–13	40	45	50	25–60	30/36
	14	55	45	60/70	25–60	30/36
	15	55	60/70	60/70	25–60	30/40
	16–18	55	60/70	60/70	30–70	30/40
Copper (mg day ⁻¹)	1–3	0.34	0.4	0.7	0.5–1	0.56
	4–6	0.44	0.6	1.0	0.5–1	0.57
	7–8	0.44	0.7	1.0	1–1.5	0.75
	9–10	0.7	0.7	1.1/1.3	1–1.5	0.75
	11–13	0.7	0.8	1.1/1.3	1–1.5	1.0
	14	0.89	0.8	1.1/1.5	1–1.5	1.0
	15–18	0.89	1	1.1/1.5	1–1.5	1.15/1.33

^aF = female, M = male.^bReproduced from Scientific Advisory Committee on Nutrition (2010) *Iron and Health*. London: The Stationery Office. ISBN 978 0 11 706992 3.

important for the transport of various substances in the body, antibody action, enzyme functions, repairing processes, and building cellular structures. The quality of proteins depends on the combination of essential amino acids it contains. A deficiency of protein can result in protein energy malnutrition, a serious condition causing muscle weakness, changes in hair and skin, and edema. Malnourished or wasted children have higher protein requirements than a child of normal body composition and normal growth. Early protein malnutrition may lead to permanent impairment of cognitive functions. Conversely, a high protein intake early in life has been associated with the onset of overweight and obesity.

Infants, children, and even adolescents who have requirements for catch-up growth due to an earlier malnutrition or stunting need a larger portion of total energy intake from protein than the ordinary healthy child. Adequate protein intake from 1 year of age is generally equivalent to 1 g kg⁻¹ body weight, and from 2 years of age approximately 0.9 g kg⁻¹ body weight. After 2 years of age, the total proportion of energy from protein can be the same as for adults, e.g., 10–15% in the Nordic Nutrition Recommendations.

Physical Activity

Physical activity comprises part of the energy balance and contributes to the prevention of many noncommunicable diseases, such as cardiovascular disease, osteoporosis, and certain types of cancer and mental illness. For children and adolescents, a minimum of 1 h day⁻¹ of physical activity has been advised.

Water

Water is an essential nutrient, but very few health authorities have defined the daily requirements and recommended intake of water. A general estimated requirement for children is 1 ml water kcal⁻¹ energy intake, or approximately 1.5–2 l of fluid day⁻¹. The requirement for water is quite variable, and needs change based on climate, physical activity, and diet.

Sodium

Sodium is part of salt (sodium chloride), which is a common food ingredient. Sodium ions are essential for many

Table 4 Nordic Nutrition Recommendations 2004

Nutrient	Unit	12–23 months	2–5 years	Age 6–9 years	10–13 years F/M ^a	14–17 years F/M ^a
Vitamin A	RE ^b	300	350	400	600	700/900
Vitamin D	Micrograms	10	7.5	7.5	7.5	7.5
Vitamin E	α -TE ^c	4	5	6	7/8	8/10
Thiamine	Milligrams	0.5	0.6	0.9	1.0/1.2	1.2/1.5
Riboflavin	Milligrams	0.6	0.7	1.1	1.2/1.4	1.3/1.7
Niacin	NE ^d	7	9	12	14/16	15/20
Vitamin B ₆	Milligrams	0.5	0.7	1.0	1.1/1.3	1.3/1.6
Folate	Micrograms	60	80	130	200	300
Vitamin B ₁₂	Micrograms	0.6	0.8	1.3	2.0	2.0
Vitamin C	Milligrams	25	30	40	50	75
Calcium	Milligrams	600	600	700	900	900
Phosphorus	Milligrams	470	470	540	700	700
Potassium	Grams	1.4	1.8	2.0	2.9/3.3	3.1/3.5
Magnesium	Milligrams	85	120	200	280	280
Iron	Milligrams	8	8	9	11	15 ^e /11
Zinc	Milligrams	5	6	7	8/11	9/12
Copper	Milligrams	0.3	0.4	0.5	0.7	0.9
Iodine	Micrograms	70	90	120	150	150
Selenium	Micrograms	20	25	30	40	40/50

^aF = female, M = male.^bRetinol equivalents (RE); 1 RE = 1 μ g retinol = 12 μ g β -carotene.^c α -Tocopherol equivalents (α -TE) = 1 mg RRR- α -tocopherol.^dNiacin equivalent (NE) = 1 mg niacin = 60 mg tryptophan.^eAt an availability of 15%, 15 mg/d will cover the requirement of 15% of menstruating adolescents.

Recommended intake of nutrients for 1–17-year-olds, expressed as average daily intake over time, for use in planning diets for groups. The requirements are lower for almost all individuals.

metabolic processes. There is no recommendation for sodium, and the required amount of sodium intake for children is not well known. Excess water loss increases the risk for hyponatremia and dehydration. Most Western countries have health problems related to high intake of sodium. Clinical trials show that even in childhood, salt intake is associated with high blood pressure. Based on these findings, it is beneficial to limit sodium intake in children to avoid the preference for a high-salt diet and to prevent later hypertension due to high sodium intake.

Potassium

Potassium is the major intracellular cation. Deficiency is very unlikely, as the average diet provides adequate amounts of potassium. Prolonged diarrhea, vomiting, or abnormal use of laxatives can cause excessive loss of potassium. There is evidence that potassium supplementation can decrease blood pressure, and a balance between potassium and sodium is important for blood pressure regulation. Reference values for children and adolescents are taken from adult recommendations.

Bone Minerals: Phosphorous, Calcium, and Magnesium

Phosphorous

Phosphorous is essential for bone health and an important nutrient during growth. It is widespread in the diet, and deficiency is very seldom observed. Phosphorous requirement is closely related to calcium, as it is a major part of the skeleton

in the form of hydroxyapatite, which contains phosphorous and calcium in the ratio 1:2.

Calcium

Calcium is stored in the bone as hydroxyapatite. The bone continuously undergoes remodeling. Bone formation exceeds bone resorption in children, and their rate of remodeling is higher than in adults. A small but important role of calcium is in the bloodstream, extracellular fluids, and all cells in the body. The absorption of calcium is dependent on 1,25-dihydroxyvitamin D₃, the hormonal form of vitamin D. Calcium is absorbed more efficiently in the body during periods of increased physiological need, i.e., infancy, early childhood, puberty, and when dietary intake is low. The absorption can be diminished by certain factors, such as phytic acid, oxalic acid, or phosphates. Physical inactivity also increases bone resorption and loss of calcium. Risk of osteoporosis has been related to high sodium, high protein, and a lack of physical activity. Very high calcium intake can result in hypercalcemia, kidney stones, and kidney damage.

Magnesium

Relatively little is known about magnesium. Magnesium is involved in many metabolic reactions and depends on vitamin D for its absorption. Deficiency is rare, but the symptoms are hypokalemia, hypercalcemia, neuromuscular hyperexcitability, and cardiac arrhythmias. Epidemiological studies have suggested the importance of magnesium to protect against

Table 5 Recommendations for water-soluble vitamins for children

<i>Nutrient</i>	<i>Age</i>	<i>USA/Canada F/M^a</i>	<i>UK F/M^a</i>	<i>Australia F/M^a</i>	<i>DACH F/M^a</i>	<i>FAO/WHO F/M^a</i>
Thiamine (mg day ⁻¹)	1–3	0.5	0.5	0.5	0.6	0.5
	4–6	0.6	0.7	0.6	0.8	0.6
	7–8	0.6	0.7	0.6	0.8	0.9
	9–10	0.9	0.7	0.9	1.0	0.9
	11–13	0.9	0.7/0.9	0.9	1/1.2	1.1/1.2
	14	1/1.2	0.7/0.9	1.1/1.2	1.1/1.4	1.1/1.2
	15–18	1/1.2	0.8/1.1	1.1/1.2	1.0/1.3	1.1/1.2
Riboflavin (mg day ⁻¹)	1–3	0.5	0.6	0.5	0.7	0.5
	4–6	0.6	0.8	0.6	0.9	0.6
	7–8	0.6	1.0	0.6	0.9	0.9
	9–10	0.9	1.0	0.9	1.1	0.9
	11–13	0.9	1.1/1.3	0.9	1.2/1.3	1.0/1.3
	14	1/1.3	1.1/1.3	1.1/1.3	1.3/1.6	1.0/1.3
	15–18	1/1.3	1.1/1.3	1.1/1.3	1.2/1.5	1.0/1.3
Niacin (mg day ⁻¹)	1–3	6	8	6	7	6
	4–6	8	11	8	10	8
	7–8	8	12	8	10	12
	9–10	12	12	12	12	12
	11–13	12	12/15	12	13/15	16
	14	16/14	12/15	14/16	15/18	16
	15–18	16/14	14/18	14/16	13/17	16
Vitamin B ₆ (mg day ⁻¹)	1–3	0.5	0.7	0.5	0.4	0.5
	4–6	0.6	0.9	0.6	0.5	0.6
	7–8	0.6	1.0	0.6	0.5	1.0
	9–10	1	1.0	1.0	0.7	1.0
	11–13	1	1.2	1.0	1.0	1.2/1.3
	14	1.2/1.3	1.2	12/13	1.4	1.2/1.3
	15–18	1.2/1.3	1.5	12/13	1.2/1.6	1.2/1.3
Folate (μg day ⁻¹)	1–3	150	70	150	200	100
	4–6	200	100	200	300	130
	7–8	200	150	200	300	160
	9–10	300	150	300	300	160
	11–13	300	200	300	400	180
	14	400	200	400	400	180
	15–18	400	200	400	400	200
Vitamin B ₁₂ (μg day ⁻¹)	1–3	0.9	0.5	0.9	1.0	0.09
	4–6	1.2	0.8	1.2	1.5	1.2
	7–8	1.2	1.0	1.2	1.5	1.8–2.4
	9–10	1.8	1.0	1.8	1.8	1.8–2.4
	11–13	1.8	1.2	1.8	2.0	2.4
	14	2.4	1.2	2.4	3.0	2.4
	15–18	2.4	1.5	2.4	3.0	2.4
Vitamin C (mg day ⁻¹)	1–3	15	30	35	60	30
	4–6	25	30	35	70	30
	7–8	25	30	35	70	35
	9–10	45	30	40	80	35
	11–13	45	35	40	90	40
	14	65/75	35	40	100	40
	15–18	65/75	40/45	40	100	40

^aF = female, M = male.

noncommunicable diseases, but the evidence is weak. Excess magnesium intake can cause diarrhea. However, if kidney function is normal, it is highly unlikely.

Other essential minerals include molybdenum, manganese, chromium, and fluorine, but there is limited information available about the requirements for these.

Essential Trace Elements

Recommended daily intake values exist for the essential trace minerals iron, zinc, iodine, selenium, and copper.

Iron

Iron has many essential functions in the body. An important function of iron is to alternate between two oxidation states, ferrous iron (Fe²⁺) and ferric iron (Fe³⁺). Iron forms the

Table 6 Recommendations for fat-soluble vitamins for children

Nutrient	Age	US/Canada F/M ^a	Australia F/M ^a	DACH F/M ^a	FAO/WHO
Vitamin A ($\mu\text{g day}^{-1}$) ^b	1–3	300	300	0.6 ^c	400
	4–6	400	400	0.7 ^c	450
	7–8	400	400	0.8 ^c	500
	9–10	600	600	0.8 ^c	500
	11–13	600	600	0.9 ^c	600
	14	700/900	700/900	1/1.1 ^c	600
	15–18	700/900	700/900	0.9/1.1 ^c	600
Vitamin D ($\mu\text{g day}^{-1}$)	1–3	15	5	5	5
	4–6	15	5	5	5
	7–8	15	5	5	5
	9–10	15	5	5	5
	11–13	15	5	5	5
	14	15	5	5	5
	15–18	15	5	5	5
Vitamin E (mg day ⁻¹) ^d	1–3	6	5	6	—
	4–6	7	6	8	—
	7–8	7	6	10	—
	9–10	11	8/9	10	—
	11–13	11	8/9	13	—
	14	15	8/10	14	—
	15–18	15	8/10	15	—

^aF = female, M = male.^bRE = retinol equivalents.^cg-retinol equivalent per day. 1 mg retinol-equivalent = 6 mg all-trans- β -Carotin = 12 mg other provitamin A-carotinoide = 1 mg retinol = 1.15 mg all-trans-retinylacetat = 1.83 mg. all-trans-retinylpalmitat; 1 IE = 0.3 μg retinol.^d1 mg α -tocopherol equivalence = α -TE = D- α -tocopherol = RRR- α -tocopherol.

oxygen-binding part of hemoglobin, which transports oxygen from the lungs to the tissues, and myoglobin, which transports oxygen within the muscle. Absorption of nonheme iron is increased in diets with meat or fish, as well as in diets including vitamin C. Factors that inhibit iron absorption are phytic acids, tannins, and calcium. Iron-deficiency anemia reduces work capacity and impairs cell-mediated immunological defenses. Prolonged iron deficiency in children slows mental development and permanently affects cognitive function. Iron overload is also possible, particularly in a hereditary condition of nonselective high absorption.

Infants can utilize the iron stores that they are born with for several months. After 6 months of age, they require more iron than can be obtained solely from breast milk, and this is the reason for iron fortification of infant formula and food. It is estimated that more than 40% of infants and children worldwide suffer from iron deficiency, and this problem is most common between the ages of 6 months and 2 years. The recommendation for iron in childhood is relatively high and is based on the need for iron and proportional absorption.

Zinc

The largest portion of zinc is located in the body cells. It is an essential part of many enzymes involved in metabolism, comprises part of the cell nucleus, and participates in gene expression. Mild deficiency symptoms are skin lesions and hair loss. Severe zinc deficiency leads to growth retardation and delayed sexual maturation. Excess zinc intake can occur with dietary supplements, and high intakes can reduce the absorption of copper, another essential mineral.

Iodine

Iodine is an essential component of the thyroid hormones, tetraiodothyronine and triiodothyronine, necessary for normal growth, development, and metabolism. Iodine deficiency is one of the most common nutrition disorders in the world and the leading cause of preventable brain damage. When physiological requirements for iodine are not met, a series of functional and developmental abnormalities occur as a result of thyroid dysfunction. The main deficiency symptom is goiter, which is characterized by enlargement of the thyroid gland.

When iodine deficiency is severe, cretinism can occur, resulting in impaired growth, mental disorders, and disturbances in speech. The recommended intake for children is based on the amount needed to prevent goiter, urinary iodine excretion, and data from adults. Iodine fortification of salt has decreased the incidence of deficiency worldwide. Iodine toxicity may occur when the intake of iodine-fortified foods is increased drastically in a short time period.

Selenium

Selenium is a trace mineral that has antioxidant properties. The main biological function of selenium is thought to be mediated through glutathione peroxidase and other selenoproteins. Low intake of selenium has been associated with cardiomyopathy, affecting children in particular. The recommended intake is based on the levels needed to maximize glutathione peroxidase activity or derived from studies on selenium-deficient children and adolescents. Toxicity is rare; however, excess intake has been associated with nausea, nail

and hair deformities, and in very severe cases, peripheral nerve and liver damage.

Copper

Copper plays a role in the formation of connective tissue, defense against free radicals, and is a part of several enzymes involved in energy metabolism. Copper deficiency is uncommon but has been seen in premature and malnourished infants and children with chronic diarrhea. The symptoms of copper deficiency are leukopenia, anemia, and abnormal hair and skin pigmentation. Some organ dysfunctions have also been observed. Risk of copper deficiency may increase after breastfeeding is discontinued, especially when coupled with low dietary intake. Acute copper toxicity causes gastric pain, nausea, vomiting, and diarrhea. Copper requirements have been calculated from adult reference values.

Water-Soluble Vitamins

Most data on physiological requirements and recommendations for water-soluble vitamins in childhood are extrapolated from adult data. Deficiency of water-soluble vitamins is rare. The vitamins are widely found in food and easily absorbed, as well as excreted. Therefore, the risk of inadequate or excess intake is minimal. However, diseases associated with water-soluble vitamin deficiencies have been well described and will be discussed in this section.

Thiamine (Vitamin B₁)

Thiamine is converted to its biologically active form, thiamine pyrophosphate, in the liver. It is involved in nerve and muscle function, and is essential for the utilization of carbohydrates and branched-chain amino acids. Beriberi is caused by thiamine deficiency. Symptoms include nervous system dysfunction, heart failure, muscle weakness, anorexia, and weight loss. These are generally more severe in children than in adults. Risk of thiamine toxicity is low.

Riboflavin (Vitamin B₂)

Riboflavin is important for the coenzymes flavine mononucleotide and flavine adenine dinucleotide, which are oxidizing agents. Recommended values are set by calculating the amount of riboflavin per energy or protein unit. Deficiency has been associated with poor iron status, and symptoms include skin changes, glossitis, anemia, and mental disturbances. Toxicity from excess riboflavin intake is rare.

Niacin (Vitamin B₃)

Niacin can be formed in the body from tryptophan. It functions as the coenzymes nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, which are involved in a number of redox reactions in the metabolism of all the energy-given nutrients. A deficiency of niacin causes pellagra, which has mainly been observed in populations with

a corn (maize)-based diet. There is no known toxic effect of niacin from food. However, high doses of niacin in the form of nicotinic acid may induce liver damage.

Cobalamin (Vitamin B₆)

The main role of cobalamin is in protein metabolism, where it acts as a coenzyme in the metabolism of amino acids. Dietary deficiency of vitamin B₆ alone is not common, and it is usually seen with other B-vitamin deficiencies. Symptoms of deficiency in infants and children include epileptic convulsions, weight loss, gastrointestinal problems, and anemia. The recommended amount is based on protein intake (0.015 mg g^{-1} protein) and is the same for adults. Toxicity is not common but has been seen with prolonged intake at 15 mg day^{-1} .

Folate

Folate is indirectly involved in the metabolism of amino acids and the synthesis of nucleic acids required for normal cell division. Signs of deficiency are observed first in rapidly replicating tissues, such as blood cells and bone marrow. Folate deficiency is the most common cause of megaloblastic anemia in childhood. The Nordic recommendation is based on body weight at $5 \text{ } \mu\text{g}$ per kg per day. Folate toxicity cannot occur from dietary intake alone. Excess use of folate supplements may mask vitamin B₁₂ deficiency.

Vitamin B₁₂

Vitamin B₁₂ is necessary for normal red blood cell formation and neurological functions. Vitamin B₁₂ must be bound to a protein called intrinsic factor to be absorbed properly. Deficiency results in macrocytic megaloblastic anemia and in severe cases may cause neurological changes. There is no known risk of vitamin B₁₂ toxicity. Children who are breastfed by vegan mothers may be at risk for vitamin B₁₂ deficiency if the mother is not taking supplements. In general, B₁₂ deficiency in childhood is rare. The recommended intake for children is approximately $0.05 \text{ } \mu\text{g kg}^{-1}$ body weight.

Vitamin C

Vitamin C (ascorbic acid) is a potent antioxidant with multiple roles in the body. Deficiency of vitamin C causes scurvy. Signs of mild deficiency include reduced antioxidant capacity, fatigue, and irritability. The requirement for children is extrapolated from adult values and a growth factor. Toxicity is rare, but high intakes can cause diarrhea and gastrointestinal disturbances. Excess intake over a long period increases oxalate formation and risk of kidney stones. Vitamin C is important in childhood due to its ability to enhance the absorption of nonheme iron from porridge and cereal foods commonly given to young children. The iron level in cow's milk is very low and infantile scurvy is regularly reported; therefore, formulas must be fortified with vitamin C. Some studies indicate that low vitamin C intake may be associated with asthma in childhood.

Fat-Soluble Vitamins

Most countries have set recommendations for vitamin A, D, and E, and some have developed recommendations for vitamin K, an important factor in blood clotting. This section discusses the requirements for vitamin A, D, and E.

Vitamin A

Vitamin A is important for vision, maintenance of epithelial surface, immune competence, growth, development, and reproduction. Vitamin A deficiency is one of the most common nutrient deficiencies in the world. Epidemiological and intervention studies on children found low intake of vitamin A and poor vitamin A status to be associated with an increased rate and severity of infections, and increased mortality from infectious diseases, e.g., measles. Retinol is the active form of vitamin A and is found in foods of animal origin. Inactive forms, e.g., β -carotene, are found in plant sources and can be activated in the body. Conditions that can affect the bioavailability and bioconversion of retinol and carotenoids are protein energy malnutrition, zinc deficiency, infections, and degree of food processing. There are no direct studies on vitamin A requirements in childhood; thus, the recommended intakes are extrapolated from studies on adults. High intake of vitamin A can cause hepatotoxicity, and during pregnancy, it increases the risk of infant malformations.

Vitamin D

Vitamin D is a prohormone converted to 1,25-dihydroxy-vitamin D₃ in the body. Active vitamin D is a steroid-like substance that can be synthesized in the skin from ultraviolet B light exposure. In areas where exposure to sunlight is limited, vitamin D must be obtained from diet. A sufficient level of vitamin D in the body ensures that the concentrations of calcium and phosphate in plasma are regulated. Vitamin D enhances the absorption of calcium from the intestine and, together with parathyroid hormone, stimulates the release of calcium from bone, resulting in increased concentration of calcium in plasma. Vitamin D is essential through this mechanism for normal bone mineralization.

Vitamin D deficiency in childhood causes rickets. Insufficient intake also has been associated with cancer, autoimmune diseases, infections, and decreased muscle strength. Supplemental vitamin D early in life has been recommended. The recommended daily intake has been increasing slowly for some decades, and recently, the US recommended dietary

allowance was highly elevated. Vitamin D can be toxic in large amounts; possible adverse effects are hypocalcaemia, nephrocalcinosis, and possible kidney failure.

Vitamin E

Vitamin E comprises two groups of components: tocopherols and tocotrienols. It is a fat-soluble antioxidant essential for neurological function. Vitamin E deficiency in children can occur with high intake of iron and PUFAs, or if they suffer from protein energy malnutrition. The recommendations for children are generally based on dietary intake of PUFAs. Vitamin E is less toxic than the other fat-soluble vitamins. However, high intakes may interfere with blood coagulation.

See also: Nutritional Requirements of Infants

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CHOLESTEROL

Contents

Factors Determining Blood Levels

Sources, Absorption, Function, and Metabolism

Factors Determining Blood Levels

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Introduction

A high blood (serum) cholesterol level is a major risk factor for atherosclerotic coronary heart disease. Consequently, there has been much interest in the causes of elevated serum cholesterol concentrations. Cholesterol is transported in the bloodstream by several specific carriers called lipoproteins. Each lipoprotein has its own characteristics, and each is responsive to a number of factors, among which are diet constituents such as cholesterol, certain fatty acids, and total energy. Other factors affecting lipoprotein metabolism include age, menopause, and genetics. Consideration of each of the factors regulating serum cholesterol concentrations first requires a description of the different lipoprotein species.

Serum Lipoproteins

Lipoproteins are macromolecular complexes that consist of discrete particles and are composed of both lipids and proteins. The lipids include cholesterol, phospholipids, and triacylglycerols (TAG). A portion of serum cholesterol is esterified with a fatty acid; the remainder is unesterified. The protein components go by the name of apolipoproteins. The major forms of apolipoproteins and their functions are listed in **Table 1**. Four categories of lipoproteins that carry cholesterol in the serum are chylomicrons, very low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). The characteristics and metabolism of each lipoprotein will be reviewed briefly.

Chylomicrons

Dietary cholesterol enters the intestine together with fat, which is predominantly TAG. The latter undergoes hydrolysis by pancreatic lipase and releases fatty acids and monoacylglycerols. In the intestine, these mix with bile acids, phospholipids, and cholesterol from the bile. The mixture of hydrolyzed lipids associates with phospholipids and bile acids to form mixed micelles. Fatty acids, monoacylglycerols, and cholesterol are taken up by the intestinal mucosa. In the mucosal cells, the fatty acids and monoacylglycerols are recombined by enzymatic action to form TAG, which are incorporated with the cholesterol into lipoprotein particles called chylomicrons. Most of the cholesterol in chylomicrons is esterified with a fatty acid. The major apolipoprotein of chylomicrons is apo B-48; other apolipoproteins – apo Cs, apo Es, and apo As – attach to the surface coat of chylomicrons and aid in metabolic processing. In the mucosal cells, microsomal lipid transfer protein (MTP) facilitates the transfer of TAG and cholesterol ester into chylomicron particles. The presence of MTP is required for the secretion of chylomicrons from mucosal cells.

Table 1 Apolipoproteins of serum lipoproteins

Apolipoprotein	Function
A-I	Major apolipoprotein of HDL Activator of LCAT
A-II	Structural apolipoprotein of HDL (other functions unknown)
A-IV	Apolipoprotein of chylomicrons (other functions unknown)
B-48	Chylomicron assembly and secretion
B-100	VLDL assembly and secretion Ligand for the LDL receptor unknown
C-I	Unknown
C-II	Activator of LPL
C-III	Inhibitor of LPL
E	Apolipoprotein of remnant lipoproteins Ligand for LDL receptor Promotes hepatic uptake of remnants

HDL, high-density lipoproteins; LDL, low-density lipoproteins; VLDL, very low-density lipoproteins; LCAT, lecithin cholesterol acyl transferase; LPL, lipoprotein lipase.

Chylomicrons are secreted by intestinal mucosal cells into the lymphatic system; from here they pass through the thoracic duct into the systemic circulation. When chylomicrons enter the peripheral circulation they come into contact with an enzyme, lipoprotein lipase (LPL), which is located on the endothelial surface of capillaries. LPL is activated by apo C-II on chylomicrons; this process is modulated by apo C-III, an inhibitor of LPL activity. Nonetheless, most chylomicron TAG is hydrolyzed by LPL; a residual lipoprotein particle, named chylomicron remnant, is released into the circulation and is rapidly removed by the liver. Hepatic uptake of chylomicron remnants is believed to be mediated by binding of remnants with a glycoprotein on the surface of liver cells. Almost all newly absorbed cholesterol thus enters the liver in association with chylomicron remnants.

Very-Low-Density Lipoproteins

The liver also secretes a TAG-rich lipoprotein called VLDL. Fatty acids used in the synthesis of TAG in the liver are normally derived from circulating nonesterified fatty acids (NEFA); even so, the liver has the capacity to synthesize fatty acids when the diet contains mainly carbohydrate. MTP inserts TAG into newly forming VLDL particles. The surface coat of VLDL contains unesterified cholesterol, phospholipids, and apolipoproteins. The major apolipoprotein of VLDL is apo B-100. Other apolipoproteins, notably apo Cs and apo Es, are also present. As VLDL circulate they acquire cholesterol esters from HDL. Circulating VLDL particles lose TAG through interaction with LPL in the peripheral circulation; in this process, VLDL are transformed into VLDL remnants. The latter can have two fates: Hepatic uptake or conversion to LDL. Hepatic uptake of VLDL remnants may occur *via* two mechanisms: Interaction with glycoproteins or interaction with LDL receptors. Both glycoproteins and LDL receptors are located on the surface of liver cells.

Low-Density Lipoproteins

Conversion of VLDL remnants into LDL appears to be largely the result of hydrolysis of remaining TAG by hepatic triacylglycerol lipase (HTGL). Normally, approximately two-thirds of cholesterol is carried by LDL, most of this LDL cholesterol existing in the form of esters. The only apolipoprotein in LDL is apo B-100. LDL is removed from the circulation largely by hepatic LDL receptors. The level of expression of LDL receptors is a major determinant of serum LDL cholesterol concentrations. The synthesis of LDL receptors is regulated in large part by the liver's content of cholesterol. An increase in the hepatic cholesterol content suppresses LDL receptor synthesis and increases serum LDL cholesterol; conversely, a decrease in hepatic cholesterol stimulates receptor synthesis and lowers serum LDL cholesterol. The mechanism by which hepatic cholesterol controls LDL receptor synthesis is through a regulatory protein called sterol regulatory element-binding protein (SREBP). When the hepatic cholesterol content declines, SREBP is activated and stimulates the synthesis of LDL receptors.

The regulatory form of cholesterol in the liver cell is unesterified cholesterol, not cholesterol ester. The hepatic content of unesterified cholesterol depends on several factors including the amounts of cholesterol derived from chylomicrons and

other lipoproteins, hepatic synthesis of cholesterol, secretion of cholesterol into bile, conversion of cholesterol into bile acids, esterification of cholesterol, and secretion of cholesterol into serum with VLDL. Factors that influence each of these processes can alter serum LDL cholesterol concentrations by modifying the hepatic content of unesterified cholesterol and thereby the expression of LDL receptors.

High-Density Lipoproteins

HDL consist of a series of lipoprotein particles of relatively high density, all of which contain apo A-I. A proportion of HDL particles also contain apo A-II. Some HDL species (HDL₃) are denser than others (HDL₂). HDL particles are composed largely of by-products of catabolism of TAG-rich lipoproteins. The surface coats of HDL particles contain phospholipids and unesterified cholesterol, apo A-I with or without apo A-II, and other apolipoproteins (apo Cs and apo Es). Their particle cores consist largely of cholesterol esters, although small amounts of TAG are also present. The cholesterol esters of HDL are formed by esterification with a fatty acid through the action of an enzyme, lecithin cholesterol acyl transferase; the substrates for this reaction derive either from unesterified cholesterol released during lipolysis of TAG-rich lipoproteins or from the surface of peripheral cells. After esterification of cholesterol, the cholesterol esters of HDL are transferred back to TAG-rich lipoproteins and are eventually removed by the liver through direct uptake of remnant lipoproteins or LDL. Whether whole HDL particles can be directly removed from the circulation is uncertain. Some investigators believe that the HDL components are dismantled and removed piecemeal.

Dietary Regulation of Serum Lipoproteins

A large body of research has shown that diet has a major impact on the concentrations and composition of serum lipoproteins, and hence on serum cholesterol concentrations. Three major factors affect cholesterol and lipoprotein concentrations: (1) dietary cholesterol, (2) the macronutrient composition of the diet, particularly dietary fatty acids, and (3) energy balance, as reflected by body weight. The influence of each of these factors can be considered.

Dietary Cholesterol

All dietary cholesterol is derived from animal products. The major sources of cholesterol in the diet are egg yolks, products containing milk fat, animal fats, and animal meats. Many studies have shown that high intakes of cholesterol will increase the serum cholesterol concentration. Most of this increase occurs in the LDL cholesterol fraction. When cholesterol is ingested, it is incorporated into chylomicrons and makes its way to the liver with chylomicron remnants. There, it increases the hepatic cholesterol content and suppresses LDL receptor expression. The result is an increase in serum LDL cholesterol concentrations. Excess cholesterol entering the liver is removed from the liver either by direct secretion into bile or by conversion into bile acids; also, dietary cholesterol suppresses hepatic cholesterol

synthesis. There is considerable variability in each of these steps in hepatic cholesterol metabolism; for this reason, the quantitative effects of dietary cholesterol on serum LDL cholesterol levels vary from one person to another. For every 200 mg of cholesterol per day in the diet, serum LDL cholesterol is increased on average by approximately 6 mg dl^{-1} ($0.155 \text{ mmol l}^{-1}$).

Macronutrient Composition of the Diet

Dietary Fat and Fatty Acids

Most of the fat in the diet consists of TAG that are composed of three fatty-acid molecules bonded to glycerol. The contribution of TAG to the total energy intake varies among individuals and populations, ranging from 15% to 40% of the total nutrient energy. The fatty acids of TAGs are of several types: Saturated, *cis*-monounsaturated, *trans*-monounsaturated, and polyunsaturated fatty acids. All fatty acids affect lipoprotein levels in one way or another. **Table 2** lists the major fatty acids of the diet and shows their effects on serum lipoproteins. The effects of carbohydrates, which also influence serum lipoprotein metabolism are also shown. It should be noted that all lipoprotein responses are compared with and related to those of *cis*-monounsaturated fatty acids, which are widely accepted to be neutral or baseline.

Saturated Fatty Acids

The saturated fatty acids are derived from both animal fats and plant oils. Rich sources of dietary saturated fatty acids include butter fat, meat fat, and tropical oils (palm oil, coconut oil, and palm kernel oil). Saturated fatty acids are straight-chain organic acids with an even number of carbon atoms (**Table 2**). All saturated fatty acids that have from eight to 16 carbon atoms increase the serum LDL cholesterol concentration when they are consumed in the diet. In the USA and much of Europe, saturated fatty acids make up 12–15% of the total nutrient energy intake.

The mechanisms by which saturated fatty acids increase LDL cholesterol levels are not known, although available data suggest that they suppress the expression of LDL receptors. The predominant saturated fatty acid in most diets is palmitic acid ($\text{C}_{16:0}$); it is cholesterol-increasing when compared with *cis*-monounsaturated fatty acids, specifically oleic acid ($\text{C}_{18:\text{cis}1, \text{n}-9}$), which is considered to be 'neutral' with respect to serum cholesterol concentrations. In other words, oleic acid is considered by most investigators to have no effect on serum cholesterol or lipoproteins. Another saturated fatty acid, myristic acid ($\text{C}_{14:0}$), apparently increases LDL cholesterol concentrations somewhat more than does palmitic acid, whereas other saturates – lauric ($\text{C}_{12:0}$), caproic ($\text{C}_{10:0}$), and caprylic ($\text{C}_{8:0}$) acids – have a somewhat lesser cholesterol-increasing effect. On average, for every 1% of total energy consumed as cholesterol-increasing saturated fatty acids, compared with oleic acid, the serum LDL cholesterol level is increased approximately 2 mg dl^{-1} ($0.025 \text{ mmol l}^{-1}$).

One saturated fatty acid, stearic acid ($\text{C}_{18:0}$), does not increase serum LDL cholesterol concentrations. The main sources of this fatty acid are beef tallow and cocoa butter. The reason for its failure to increase LDL cholesterol concentrations is uncertain, but may be the result of its rapid conversion into oleic acid in the body.

Trans-Monounsaturated Fatty Acids

These fatty acids are produced by industrial hydrogenation of vegetable oils or by biohydrogenation in the rumen of cows and sheep. The largest source of *trans*-monounsaturates is processed vegetable oils, with small contributions from animal sources. In many countries they contribute between 2% and 4% of the total nutrient energy intake. A series of *trans* acids are produced by hydrogenation: Most are monounsaturated. The *trans*-monounsaturated fatty acids increase LDL cholesterol concentrations to a level similar to that of palmitic acid when substituted for dietary oleic acid. In addition, they cause a small reduction in serum HDL cholesterol concentrations.

Table 2 Macronutrient effects on serum lipoprotein cholesterol

Nutrient	Symbol ^a	VLDL cholesterol	LDL cholesterol	HDL cholesterol
<i>Fatty acids Saturated</i>				
Palmitic	$\text{C}_{16:0}$	– ^b	↑↑	–
Myristic	$\text{C}_{14:0}$	–	↑↑↑	↓
Lauric	$\text{C}_{12:0}$	–	↑	–
Caproic	$\text{C}_{10:0}$	–	↑	–
Caprylic	$\text{C}_{8:0}$	–	↑	–
Stearic	$\text{C}_{18:0}$	–	–	or ↓
<i>trans</i> -Monounsaturated	<i>trans</i> $\text{C}_{18:1, \text{n}-9}$	–	↑ or ↑↑	↓
<i>cis</i> -Monounsaturated	<i>cis</i> $\text{C}_{18:1, \text{n}-9}$	–	–	–
<i>Polyunsaturated</i>				
<i>n</i> -6 ^c	$\text{C}_{18:2 \text{ n}-6}$	– or ↓	– or ↓	– or ↓
<i>n</i> -3 ^d	DHA, EPA ^e	↓↓↓	– or ↓	–
Carbohydrate		↑↑↑	–	↓↓

^aThe first number denotes the number of carbon atoms; the second number denotes the number of double bonds.

^bThe dash (–) indicates that there is no change in level compared with that produced by *cis*-monosaturated fatty acids (oleic acid) ($\text{C}_{18:1, \text{n}-9}$). All the lipoprotein responses to oleic acid are considered neutral, i.e., no effect.

^cThe letter 'n' and number indicates at which carbon atom, numbered from the terminal methyl group, the first double bond appears.

VLDL, very low-density lipoproteins; LDL, low-density lipoproteins; HDL, high-density lipoproteins; DHA, docosahexanoic acid ($\text{C}_{22:6, \text{n}-3}$); EPA, eicosapentanoic acid ($\text{C}_{20:5, \text{n}-3}$).

There has been some debate on whether plant- and animal-derived *trans* fatty acids are equivalent in terms of their effects on blood cholesterol levels. Although additional studies are expected to yield a definitive answer, a recent review of the literature found no consistent evidence of a differential effect of animal *trans* fatty acids.

Cis-Monounsaturated Fatty Acids

The major fatty acid in this category is oleic acid ($C_{18:1\text{cis}}$, n-9). It is found in both animal and vegetable fats, and typically is the major fatty acid in diet. Intakes commonly vary between 10% and 20% of the total energy. Oleic acid intake is particularly high in the Mediterranean region where large amounts of olive oil are consumed. Other sources rich in oleic acid are rapeseed oil (canola oil) and high-oleic forms of safflower and sunflower oils. Peanuts and pecans are also high in oleic acid. Animal fats likewise contain a relatively high percentage of oleic acid among all their fatty acids; even so, these fats also tend to be rich in saturated fatty acids. When high-carbohydrate diets are consumed, the human body can synthesize fatty acids; among these, oleic acid is the predominant fatty acid produced.

As indicated before, oleic acid is generally considered to be the baseline fatty acid with respect to serum lipoprotein levels, i.e., it does not increase (or lower) LDL cholesterol or VLDL cholesterol concentrations, nor does it lower (or increase) HDL cholesterol concentrations. It is against this neutral fatty acid that the responses of other fatty acids are defined (Table 2). For example, if oleic acid is substituted for cholesterol-increasing fatty acids, the serum LDL cholesterol concentration will decline. Nonetheless, oleic acid is not considered a cholesterol-lowering fatty acid, but instead, this response defines the cholesterol-increasing potential of saturated fatty acids.

Polyunsaturated Fatty Acids

There are two categories of polyunsaturated fatty acids: n-6 and n-3. The major n-6 fatty acid is linoleic acid ($C_{18:2}$, n-6). It is the predominant fatty acid in many vegetable oils, for example, corn oil, soya bean oil, and high linoleic forms of safflower and sunflower seed oils. Intakes of linoleic acid typically vary from 4% to 10% of nutrient energy, depending on how much vegetable oil is consumed in the diet. The n-3 fatty acids include linolenic acid ($C_{18:3}$, n-3), docosahexanoic acid (DHA) ($C_{22:6}$, n-3), and eicosapentanoic acid (EPA) ($C_{20:5}$, n-3). Linolenic acid is high in linseed oil and present in smaller amounts in other vegetable oils. DHA and EPA are enriched in fish oils.

For many years, linoleic acid was thought to be a unique LDL cholesterol-lowering fatty acid. Recent investigations suggest that earlier findings overestimated the LDL-lowering potential of linoleic acid. Even though substitution of linoleic acid for oleic acid in the diet may reduce LDL cholesterol levels in some individuals, a difference in response is not consistent. Only when intakes of linoleic acid become quite high do any differences become apparent. At high intakes, however, linoleic acid also lowers serum HDL cholesterol concentrations. Moreover, compared with oleic acid, it may reduce VLDL cholesterol levels in some individuals. Earlier

enthusiasm for high intakes of linoleic acid to reduce LDL cholesterol levels has been dampened for several reasons: For example, its LDL-lowering ability does not offset the potential disadvantages of HDL lowering, and other concerns include possible untoward side effects such as promoting oxidation of LDL and suppressing cellular immunity to cancer.

The n-3 fatty acids in fish oils (DHA and EPA) have a powerful action to reduce serum VLDL levels. Diets rich in n-3 fatty acids also reduce peripheral LDL delivery and specific uptake, and affect LPL expression in the arterial wall.

Carbohydrate

When carbohydrates are substituted for oleic acid in the diet, serum LDL cholesterol levels remain unchanged. However, VLDL cholesterol concentrations usually increase and HDL cholesterol concentrations decline on high-carbohydrate diets. Thus, a lack of difference in the total serum cholesterol concentrations during the exchange of carbohydrate and oleic acid is misleading. The two categories of nutrients have different actions on lipoprotein metabolism. The differences in response to dietary carbohydrate and oleic acid provide a good example of how measurements of serum total cholesterol fail to reveal all of the changes that are occurring in the lipoprotein fractions.

Other Diet Constituents

Phytosterols are naturally occurring plant sterols and may reduce serum cholesterol levels by reducing intestinal cholesterol absorption. Proposed mechanisms include an inhibition of luminal cholesterol uptake or esterification, or increasing re-excretion of cholesterol into the lumen by enterocytes. Carotenoids, such as capsanthin, have been shown to increase HDL and possibly cholesterol flux into HDL particles. Peanuts, wheat antioxidants, black tea, garlic, and ginseng are among the several diet compounds showing cholesterol-lowering effects under defined experimental conditions.

Energy Balance

Obesity

When energy intake exceeds energy expenditure, the balance of energy is stored in adipose tissue in the form of TAG. When the TAG content of adipose tissue becomes excessive (body mass index 30 or above), a state of obesity is said to exist. In some obese persons, excessive accumulations of TAG occur in tissues other than adipose tissue. Two such tissues are skeletal muscle and liver. High contents of TAG in muscle and liver arise primarily because of continuous leakage of excessive quantities of NEFA from adipose tissue. In the presence of a desirable body weight, normal insulin levels are sufficient to suppress hydrolysis of TAG in adipose tissue, and NEFA release is low. On the other hand, in obese persons NEFA release is excessive, and skeletal muscle and liver are flooded with high serum NEFA concentrations. The result is engorgement of these organs with TAG. When skeletal muscle is overloaded with TAG, insulin-mediated glucose uptake is impaired. This

condition is called insulin resistance. When the liver is packed with TAG, hepatic metabolism is altered and insulin action on the liver is deranged. As a result, there is an overproduction of VLDL; this leads to high VLDL cholesterol concentrations and, because LDL is a product of VLDL, to higher LDL cholesterol levels. In addition, obesity is accompanied by a reduction in HDL cholesterol concentrations. Thus obesity is responsible for multiple alterations in lipoprotein metabolism; it has significant effects on three major lipoprotein species – VLDL, LDL, and HDL. These changes appear to be the result of a combination of excessive hepatic TAG as a substrate for VLDL formation and failure of insulin to exert its usual action to curtail VLDL secretion.

That being said, the epidemiological association between obesity and high cholesterol or TAG blood levels remains elusive to demonstrate. In many countries, cholesterol levels continue to decrease, in spite of the ongoing obesity epidemic.

Exercise

Many of the adverse metabolic effects of obesity are reversed by exercise. Increased energy expenditure through regular and sustained exercise helps to prevent the accumulation of excessive quantities of TAG in adipose tissue. In addition, increased muscle metabolism produced by exercise burns off NEFA and prevents TAG accumulation in the liver. Hence, increased and sustained energy expenditure favorably modifies the lipoproteins, particularly by lowering VLDL cholesterol concentrations and increasing serum HDL cholesterol. The effects of exercise on LDL cholesterol concentrations are more modest, but in some persons, exercise produces a reduction.

Other Factors Affecting Serum Lipoproteins

Aging

Between the ages of 20 and 50 years, there is a gradual increase in serum cholesterol concentrations. In the USA, for example, the serum cholesterol increases on average approximately 50 mg dl^{-1} ($1.295 \text{ mmol l}^{-1}$). This change may be related in part to increasing obesity, according to the mechanisms described above. However, even in persons who do not gain weight with advancing age, serum cholesterol concentrations usually increase to some extent. Available evidence indicates that this increase results from a decrease in the expression of LDL receptors. The reasons for a decline in receptor synthesis with aging are not known, but may reflect metabolic aging. Recent studies have reported an association between high serum cholesterol levels and reduced brain glucose utilization in aging men.

Postmenopausal State

In women, there is a further increase in serum cholesterol concentrations after the age of 50 years. This rise is believed to be due largely to loss of estrogens after the menopause. Estrogens are known to stimulate the synthesis of LDL receptors, and, consequently, receptor expression declines after

menopause. This increment in cholesterol levels can be largely reversed by estrogen replacement therapy.

Genetics

Family studies and research in twins indicate that approximately 50% of the variation of serum cholesterol concentrations in the general population can be explained by genetic polymorphisms. Presumably this variation is related to factors that regulate lipoprotein concentrations. In some cases, specific genetic defects are severe, resulting in marked changes in lipoprotein concentrations. When this occurs, the affected individual is said to have a monogenic disorder. In other cases, multiple genetic modifications are present that combine to change lipoprotein concentrations. When a few modifications are present, the condition is called oligogenic, but when many modifications combine to change lipoprotein concentrations, the condition is named polygenic. Several monogenic disorders have been identified; a few oligogenic conditions have been described, but there are very few instances in which complex polygenic traits have been unraveled. Genome-wide analyses have identified chromosome loci associated with LDL levels. Similarly, genotype analyses suggest that a group of identifiable genetic variants in key genes responsible for cholesterol metabolism could help identify individual risk. Some of these variants have been described (e.g., PCSK9 in the Italian population).

A question of great interest is whether nutritional and genetic factors ever interact synergistically to alter lipoprotein concentrations. Undoubtedly, dietary factors and genetic changes can be additive in their effects on serum lipoproteins, but a synergistic interaction has been difficult to prove. In what follows, consideration will be given to the impact of modification of some of the key gene products regulating lipoprotein metabolism.

LDL Receptors

The most severe elevations in LDL cholesterol levels occur in patients who have mutations in the gene encoding for LDL receptors. Approximately 1 in 500 persons are heterozygous for these mutations. Their condition is called heterozygous familial hypercholesterolemia. LDL cholesterol concentrations are essentially twice the normal level in this condition. Very rarely patients are homozygous for mutation in the LDL receptor gene and thus have homozygous familial hypercholesterolemia. Their LDL cholesterol levels are approximately four times the normal level. Individuals with this condition develop severe premature atherosclerosis.

Many other individuals appear to have a reduction in LDL receptor expression on a genetic basis, but they do not have as severe elevations of serum LDL cholesterol as patients with familial hypercholesterolemia. Presumably, these individuals have genetic modifications in factors that regulate transcription of the LDL receptor gene. Although such genetic modifications may be relatively common, they are poorly defined. Again, an important but unanswered question is whether some persons are genetically susceptible to the cholesterol-increasing effects of dietary cholesterol and saturated fatty

acids. If so, they may possess modifications in the genetic control of LDL receptor expression.

Apolipoprotein B-100 Structure

Approximately 1 in 500 persons also have a mutation in the primary structure of apo B that interferes with its binding to LDL receptors. This mutation gives rise to the disorder called familial defective apolipoprotein B-100. The consequence is an elevation of LDL cholesterol concentrations, and the clinical pattern resembles that of familial hypercholesterolemia.

Apolipoprotein B Synthesis

Rare patients have mutations in the gene encoding for apo B that impair the synthesis of this apolipoprotein. Such patients usually have very low LDL cholesterol concentrations. These individuals are said to have familial hypobetalipoproteinemia. In other rare cases, the intracellular TAG transport protein called MCT is genetically absent; when this occurs, no lipoprotein particles containing apo B can be formed. LDL cholesterol is absent from serum, and the disorder is called familial abetalipoproteinemia.

Some researchers speculate that serum elevations in VLDL cholesterol and LDL cholesterol can result from excessive synthesis and secretion of apo B-containing lipoproteins by the liver. When this occurs on a genetic basis, the disorder is designated familial combined hyperlipidemia. However, a monogenic basis of this clinical phenotype has not yet been identified. Therefore, most investigators have concluded that familial combined hyperlipidemia probably represents an oligogenic or a polygenic disorder. In this disorder, lipoprotein elevations appear to be worsened by nutritional factors – particularly by obesity.

Apolipoprotein E

This apolipoprotein is present on TAG-rich lipoproteins and it facilitates the removal of remnant lipoproteins by LDL receptors in the liver. When apo E is affected by mutation, this enabling action is curtailed and hepatic uptake of remnant lipoproteins is impaired. The result is an accumulation of chylomicron remnants and VLDL remnants in the circulation. The accumulation is accentuated by the coexistence of other disorders of metabolism of TAG-rich lipoproteins. When remnant accumulation occurs on a genetic basis, the disorder is called familial dysbetalipoproteinemia.

Apolipoprotein C

There are two forms of apo C – apo C-II and apo C-III. Apo C-II is required for the activation of LPL; when it is genetically absent, affected patients develop severe elevations of TAG-rich lipoproteins. Apo C-III inhibits the activity of LPL. In certain metabolic disorders, notably insulin resistance, the synthesis of apo C-III is increased; an elevated apo C-III can lead to impaired function of LPL and increases in serum concentrations of TAG-rich lipoproteins.

Apolipoprotein A-I

This is the major apolipoprotein of HDL. Rare patients have mutations in apo A-I that result in very low concentrations of HDL cholesterol. However, most individuals in whom HDL cholesterol concentrations are moderately reduced show increased catabolism of apo A-I. The mechanism for this change has not been fully determined, but one important cause may be an overexpression of HTGL.

LPL

This enzyme is required for lipolysis of TAG in TAG-rich lipoproteins. Rare patients are homozygous for mutations in LPL that impair its function. In such patients, serum concentrations of chylomicrons are markedly increased. The accumulation of chylomicrons in serum is greatly accentuated by the presence of fat in the diet. Only by severe dietary fat restriction is it possible to prevent severe TAG elevations in serum.

See also: Eggs. Fatty acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Metabolism. Lipoproteins. Meat, Poultry and Meat Products. Obesity: Definition, Etiology, and Assessment. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Physical Activity: Beneficial Effects. Trans-Fatty Acids: Health Effects, Recommendations, and Regulations

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Sources, Absorption, Function, and Metabolism

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Absorption, Transport, and Storage

Cholesterol absorption

Cholesterol in the intestinal lumen typically consists of one-third dietary cholesterol and two-thirds biliary cholesterol. The average daily diet contains 300–500 mg of cholesterol obtained from animal products. The bile provides an additional 800–1200 mg of cholesterol throughout each day as gall-bladder contractions provide a flow of bile acids, cholesterol, and phospholipids to facilitate lipid digestion and absorption. Dietary cholesterol is a mixture of free and esterified cholesterol whereas biliary cholesterol is nonesterified and is introduced into the small intestine as a cholesterol–bile salt–phospholipid water-soluble complex. The only other source of intraluminal cholesterol is mucosal cell cholesterol, derived from either sloughed mucosal cells or cholesterol secreted by the mucosal cells into the intestinal lumen. Measurements of exogenous and endogenous cholesterol absorption in humans indicate that there is probably very little direct secretion of newly synthesized cholesterol from mucosal cells into the intraluminal contents.

Cholesterol absorption occurs primarily in the duodenum and proximal jejunum of the small intestine and is dependent on the presence of bile salts. In the absence of bile secretion, or in the presence of bile acid-binding resins, there is virtually no intestinal absorption of cholesterol. On average, humans absorb 50–60% of the intestinal contents of cholesterol, but there is a large inter-individual variance in absorption, with values ranging from as low as 20% to as high as 80%. Intestinal transit time is related to cholesterol absorption with slower transit times resulting in higher fractional absorption rates. Dietary factors that affect the relative percent absorption of cholesterol include the total mass of dietary cholesterol, the concentration of plant sterols in the diet, and the type and amount of dietary fiber. Studies suggest that the ratio of polyunsaturated to saturated fat (P:S) in the diet has little effect on cholesterol absorption rates in humans, nor does the amount of dietary fat.

Two interesting, and as yet undefined, aspects of cholesterol absorption are that it decreases as the mass of cholesterol increases above an intake of 1500 mg per day, and that the fractional absorption below this level is relatively constant for an individual. For example, at a daily cholesterol intake of 800 mg a subject might absorb 60% or 480 mg a day, whereas at a daily intake of 400 mg the absorption remains at 60%, equaling 240 mg a day absorbed. The quandary is, if the system can accommodate absorption of 480 mg at the high cholesterol intake, then why is the amount absorbed 240 mg at the low intake? Clearly the upper value of cholesterol absorption is achievable, yet at the lower intake level the absorption rate stays at a fixed fractional value. The mechanisms controlling this aspect of cholesterol absorption have not been defined.

Experimental evidence indicates that biliary cholesterol and dietary cholesterol are absorbed equally; however, the pattern of exogenous and endogenous cholesterol absorption differs along the length of the intestinal lumen. Dietary cholesterol enters the small intestine solubilized in the oil phase of the stomach digest, whereas the biliary cholesterol enters in the micelle phase of the bile. This differential distribution results in a greater absorption of biliary cholesterol in the upper portion of the small intestine with dietary cholesterol absorption increasing as the oil phase of the intestinal contents are hydrolyzed. As the oil phase is reduced, dietary cholesterol moves from the oil phase to the aqueous micelle phase and becomes available for absorption. In the case of cholesteryl esters in the diet, it is necessary that the esters are hydrolyzed by pancreatic cholesterol esterase (CEase) before the cholesterol is available for absorption. Pancreatic CEase requires the presence of bile salts for activity and may play a key role in the actual absorption process.

The process, and selectivity, of sterol absorption involves a complex interplay of regulated transporters, transporting sterols into and out of the enterocyte, and the assembly and secretion of chylomicrons into the lymph. The enterocyte takes up both cholesterol and phytosterols from the intestinal lumen by what appears to be a common sterol transporter or permease in the brush border membrane. Preliminary studies suggest that the Neiman–Pick C1 Like 1 (NPC1L1) protein is involved in this process. Once the sterols enter the enterocyte, the ATP-binding cassette (ABC) hemitransporters ABCG5 and ABCG8 function in the apical excretion of sterols back into the intestinal lumen. The selectivity of this process accounts for the higher absorption rates of cholesterol (50–60%) compared to the phytosterols, which are very poorly absorbed. Loss of ABCG5/G8 function results in excessive absorption of both cholesterol and phytosterols. Studies in mice have shown that ABCG5/G8 are expressed primarily in the liver and intestine, are coordinately up-regulated at the transcriptional level by dietary cholesterol intake, and require the liver X receptor α (LXR α), a nuclear receptor that regulates the expression of a number of key genes involved in lipid metabolism.

Evidence is accumulating that the fractional cholesterol absorption rates are regulated by one or more genetic determinants. The apolipoprotein (apo) E phenotype has a significant effect on fractional cholesterol absorption and appears to play a major role in determining the plasma lipoprotein response to changes in dietary cholesterol intake. Men with the apoE4 allele have a high cholesterol absorption rate whereas those with the apoE2 allele have a low cholesterol absorption efficiency. The absorption values for the more common apoE3/3 fall between the apoE2 and apoE4 patterns. Polymorphisms of the apolipoprotein A-IV and of the low-density lipoprotein (LDL) receptor gene have also been related to differences in fractional cholesterol absorption. These genetic variants affecting cholesterol absorption no doubt play

a significant role in determining an individual's fractional absorption of cholesterol as well as accounting for much of the heterogeneity of plasma lipid responses to changes in dietary cholesterol intakes (see the Section on Dietary Cholesterol and Plasma Cholesterol below).

Exogenous Cholesterol Transport

Cholesterol is absorbed in the unesterified state, whereas the cholesterol secreted into the lymph is 70–80% esterified. This esterification process generates a concentration gradient of free cholesterol within the mucosal cell, which could facilitate absorption rates. Cholesterol is esterified in intestinal mucosal cells by acyl-coenzyme A: cholesterol acyltransferase-2 (ACAT-2) to form cholesteryl esters, which are secreted from the basolateral surface of the enterocyte as part of the chylomicrons. At this stage it is assumed that cholesterol molecules from exogenous and endogenous sources are indistinguishable, and have similar effects on endogenous cholesterol and lipoprotein metabolism. Chylomicrons are large particles (>70 nm in diameter) composed mainly of triacylglycerols (95% by weight) and containing 3–7% cholesterol by weight, the esterified cholesterol localized in the hydrophobic core and the free cholesterol primarily in the hydrophilic outer layer. The data indicate that the amount of dietary cholesterol consumed has little effect on the cholesterol content of chylomicrons. The chylomicrons are released from the intestinal cells, enter the lymphatic system and are transported *via* the lymphatics (thoracic duct) to the bloodstream. Because chylomicrons are too large to pass through the capillaries, this is the only mechanism by which they can enter the bloodstream.

In the plasma compartment the chylomicrons pick up a number of apolipoproteins, which are required for intravascular metabolism of the particles. The initial metabolism of chylomicrons involves hydrolysis of the associated triacylglycerols by endothelial cell lipoprotein lipase (LPL) located in adipose, muscle, and heart tissues which results in production of chylomicron remnants. The chylomicron remnants, depleted of triacylglycerol and enriched with cholesteryl ester, are taken up by the liver *via* the LDL receptor-related protein (LRP). The ligand for hepatic uptake of the chylomicron remnant appears from various transgenic mouse studies to be the apo-E moiety of the particle. The clearance of chylomicrons from the bloodstream is rapid, with particles having a half-life of less than an hour. The liver cannot take up native chylomicrons but rather takes up the chylomicron remnant, which has lost approximately 90% of its triacylglycerol content and become relatively enriched in free and esterified cholesterol through the actions of the plasma cholesteryl ester transfer protein (CETP), which transfers cholesteryl ester from HDL to the apo-B-containing lipoproteins.

The chylomicron remnants taken up by the liver are subjected to lysosomal hydrolysis resulting in the release of the absorbed dietary and biliary cholesterol into the hepatocyte as free cholesterol. The influx of cholesterol contained in the chylomicron remnant has the ability to affect a number of regulatory sites of hepatic cholesterol metabolism, which function to maintain cholesterol homeostasis in the liver. The liver has four primary fates for the newly delivered cholesterol:

catabolism to bile acids; secretion as biliary cholesterol; storage in lipid droplets as cholesteryl ester; or incorporation into very low-density lipoprotein (VLDL) for secretion from the liver.

Tissue Uptake and Storage

The body pool of cholesterol is approximately 145 g with one-third of this mass localized in the central nervous system. The remainder of the metabolically active cholesterol pool exists in the plasma compartment (7.5–9 g) and as constituents of body tissues. In humans, tissue cholesterol levels are relatively low, averaging 2–3 mg gm⁻¹ wet weight. Little information exists regarding changes in hepatic and extrahepatic tissue cholesterol concentrations with changes in dietary cholesterol intake. Animal studies, which are usually carried out using very high levels of dietary cholesterol, have shown that hepatic cholesterol can increase from 2-fold up to 10-fold, depending on the species and other dietary constituents, when dietary cholesterol is increased.

Biosynthesis

Tissue Cholesterol Synthesis

Cholesterol biosynthesis occurs in every nucleated cell in the body. Although it is often thought that the majority of cholesterol synthesis occurs in the liver, studies have shown that the bulk tissues of the body account for the overwhelming majority of endogenous cholesterol production. Hepatic cholesterol synthesis in humans is thought to contribute 10–20% of the total daily synthesis rate. Because the majority of cholesterol synthesis in the body occurs in extrahepatic tissues, and the only quantitatively significant site for excretion and catabolism of cholesterol is the liver, some 600–800 mg of cholesterol each day must be transported from peripheral tissues through the plasma compartment to the liver to account for daily cholesterol catabolism and biliary secretion. Approximately 9 mg cholesterol per kilogram body weight is synthesized by peripheral tissues every day and must be moved to the liver for catabolism *via* a process termed 'reverse cholesterol transport' (RCT).

RCT describes the metabolism, and important antiatherogenic function, of the HDL-mediated efflux of cholesterol from nonhepatic cells and its subsequent delivery to the liver and steroidogenic organs for use in the synthesis of lipoproteins, bile acids, vitamin D, and steroid hormones. A cellular ABC transporter (ABCA1) mediates the first step of RCT involving the transfer of cellular cholesterol and phospholipids to lipid-poor apolipoproteins. Lecithin:cholesterol acyltransferase (LCAT) mediated esterification of cholesterol generates spherical particles that continue to expand with ongoing cholesterol esterification and phospholipid transfer protein (PLTP) mediated particle fusion and surface remnant transfer. Larger HDL₂ particles are converted into smaller HDL₃ particles when CETP facilitates the transfer of cholesteryl esters from HDL onto apo-B-containing lipoproteins. The scavenger receptor B1 (SR-B1) promotes selective uptake of cholesteryl esters into liver and steroidogenic organs whereas hepatic lipase (HL) and LPL mediated hydrolysis of phospholipids and

triglycerides. SR-BI mediates the selective uptake of cholesteryl esters from HDL and also LDL into hepatocytes and steroid hormone-producing cells without internalizing HDL proteins, which can recycle through the RCT sequence moving cholesterol from peripheral tissues to the liver.

Regulation of Synthesis

The rate-limiting enzyme in cholesterol biosynthesis is 3-hydroxy-3-methylglutaryl coenzyme A (HMG- CoA) reductase, a microsomal enzyme which converts HMG-CoA to mevalonic acid in the polyisoprenoid synthetic pathway. Peripheral tissue cholesterol synthesis is much less responsive to regulatory factors compared with the liver, which is controlled by a variety of dietary, hormonal, and physiological variables. Studies indicate that endogenous cholesterol synthesis is significantly increased in obesity and in patients with the metabolic syndrome. Obesity, insulin resistance, and diabetes have pronounced effects on both cholesterol absorption and synthesis. Findings in type I diabetes appear related to low expression of ABCG5/G8 genes resulting in high absorption and low synthesis of cholesterol. Cholesterol absorption efficiency is lower and cholesterol synthesis higher in obese subjects with type II diabetes compared to obese subjects without diabetes, suggesting that diabetes modulates cholesterol metabolism to a greater extent than obesity alone. In a similar manner, low cholesterol absorption and high synthesis appears to be part of the insulin resistance (metabolic) syndrome.

Research shows that in most individuals, dietary cholesterol alters endogenous cholesterol synthesis and that this feedback regulation can effectively compensate for increased cholesterol input from dietary sources. The precision of these regulatory responses depends on a number of genetic factors, and data suggest that multiple genetic loci are involved. For example, family studies have shown that in siblings of low cholesterol absorption families, cholesterol absorption percentages are significantly lower, and cholesterol and bile acid synthesis, cholesterol turnover, and fecal steroids significantly higher than in siblings of high absorption families.

Metabolism and Excretion

The body's metabolic processes cannot break the sterol rings of cholesterol and therefore must either catabolize cholesterol to other products, which can be excreted in the urine or feces, or directly excrete cholesterol in the bile, with a fraction of the biliary cholesterol lost daily as fecal neutral sterols. In humans, the major route of excretion is as biliary cholesterol (two-thirds of the total lost each day), with catabolism to bile acids and bile acid excretion being the second most important route, accounting for approximately one-third of the daily turnover.

For all practical purposes, the body must excrete daily an amount of neutral and acidic sterols equivalent to the combined inputs of total dietary and newly synthesized cholesterol. Given an average fecal excretion of 1020 mg a day with 250 mg as acidic sterols, it can be calculated that the 770 mg per day excreted as neutral steroids comes from unabsorbed biliary (650 mg) and unabsorbed dietary (120 mg) cholesterol (Table 1). It is easy to see that even small changes in the daily

Table 1 Average cholesterol metabolism values for a 70 kg adult

<i>Cholesterol pools and flux</i>	<i>Mass</i>
Cholesterol pool (70 kg adult)	160 g
Plasma cholesterol pool	8 g
Dietary cholesterol intake	300 mg day ⁻¹
Absorption (average 60%)	180 mg day ⁻¹
Synthesis (12 mg kg-day ⁻¹)	840 mg day ⁻¹
Total cholesterol input	1020 mg day ⁻¹
Bile acid synthesis (= fecal excretion)	250 mg day ⁻¹
Neutral steroid excretion	770 mg day ⁻¹

balance between a cholesterol input and output value of 1020 mg per day could, over years, result in significant tissue cholesterol accumulation.

Bile Acid Synthesis

The results from numerous sterol balance studies carried out in subjects fed diets low and high in cholesterol indicate that in humans dietary cholesterol has little effect on fecal bile acid excretion rates. This finding is in striking contrast to results from studies in some rodent models, which show that intake of pharmacological doses of dietary cholesterol can result in several-fold increases in bile acid synthesis and excretion. In contrast, some rodent species and nonhuman primates have little if any increase in bile acid excretion with increased intakes of cholesterol. Although there have been a few reports of enhanced bile acid excretion on a high-cholesterol diet in some patients, this does not appear to be a major regulatory response in humans.

Biliary Cholesterol Secretion

The majority of cholesterol entering the intestinal tract is biliary cholesterol. Biliary cholesterol secretion averages 1000 mg per day as part of the bile system and enters as free cholesterol already solubilized with bile acids and phospholipids. Both cholesterol absorption by enterocyte and biliary cholesterol secretion by hepatic cells are regulated by expression of the half-transporters ABCG5 and ABCG8. Studies in animals have shown that treatment with a LXR agonist decreases cholesterol absorption, increases biliary cholesterol secretion, and increases fecal neutral sterol excretion. Studies in transgenic mouse models demonstrate that increased expression of ABCG5 and ABCG8 increases biliary neutral sterol secretion and reduces intestinal cholesterol absorption, leading to increased neutral sterol excretion and cholesterol synthesis.

Fecal Excretion

The only route of significant cholesterol excretion is through fecal excretion of neutral sterols. The combination of unabsorbed biliary and dietary cholesterol accounts for the total neutral sterol output, and under most conditions equals 750–850 mg a day. Dietary patterns or drugs that interfere with intestinal cholesterol absorption result in increased fecal neutral steroid excretion. In the colon, intestinal bacteria are able to metabolize cholesterol to a variety of neutral steroids

as well as to nonsteroid end products. There have been some studies suggesting that the intestinal metabolism of cholesterol by bacteria, which can be altered by diet and drugs, can influence endogenous cholesterol metabolism as well as plasma cholesterol levels. What these relationships might be and the mechanisms involved have not been defined.

Metabolic Function

Steroid Hormones

Daily production of steroid hormones is quantitatively a very small fraction of the daily turnover of dietary and newly synthesized cholesterol in the body. For men, the average daily excretion of steroid hormones is approximately 50 mg per day, whereas in women the value can be substantially higher depending on the menstrual phase.

Bile Acid Synthesis

The enterohepatic circulation of bile acids is essential for fat and cholesterol digestion and absorption. Each day the bile acid pool (approximately 3–5 g) cycles through the intestine 6–10 times. The absorption of bile acids by the ileum is very effective and 98–99% of bile acids secreted in the bile are returned to the liver *via* the portal vein. The small amount of bile acids lost each day as fecal acidic steroids are replaced through the conversion of hepatic cholesterol to the primary bile acids, cholic acid, and chenodeoxycholic acid. This catabolism of cholesterol can be as little as 250 mg per day up to 500 mg per day depending on the diet. The bile acids represent the only major catabolic product of cholesterol metabolism and in humans account for some 25–30% of the daily loss of cholesterol from the body.

Very Low-Density Lipoprotein Synthesis

The endogenous pathway for cholesterol transport focuses on the liver with the synthesis and secretion of VLDL particles. Cholesterol in these triacylglycerol-rich particles comes from multiple sources: endogenous synthesis, diet, and plasma lipoproteins. Catabolism of VLDL by LPL leads to formation of intermediate-density lipoproteins (IDL), which can either be taken up by the liver or undergo further metabolism to form LDL. Low-density lipoproteins contain apo-B₁₀₀ and account for 60–80% of the plasma cholesterol in most individuals. During lipolysis of VLDL triacylglycerol, the lipoproteins containing apo-B becomes enriched with cholesteryl ester through the plasma CETP-catalyzed net transfer of cholesteryl ester from HDL. This process, termed reverse cholesterol transport, moves cholesterol from extrahepatic tissues to HDL to VLDL-IDL-LDL and eventual uptake by the liver. Some 70% of the LDL degraded each day is degraded by the hepatic LDL receptor pathway.

Dietary Cholesterol and Plasma Cholesterol

The effect of dietary cholesterol on plasma cholesterol levels has been an area of considerable debate. In 1972 the American

Heart Association recommended that dietary cholesterol intake should average less than 300 mg per day as part of a 'heart-healthy', plasma cholesterol-lowering diet. Since that initial recommendation, a number of other public health dietary recommendations in the USA have endorsed the 300 mg daily limit. Interestingly, few dietary recommendations from other countries contain a dietary cholesterol limitation. The evidence for a relationship between dietary cholesterol and plasma cholesterol indicates that the effect is relatively small, and that on average a change of 100 mg per day in dietary cholesterol intake results in a $0.057 \text{ mmol l}^{-1}$ (2.2 mg dl^{-1}) change in plasma cholesterol concentrations. Studies have also shown that the majority of individuals are resistant to the plasma cholesterol-raising effects of dietary cholesterol (non-responders) and have less than the predicted response. In contrast, a segment of the population (estimated to be between 10% and 20%) are sensitive to dietary cholesterol (responders) and exhibit a greater than expected plasma cholesterol response to a change in dietary cholesterol intake. To date there are no defined physiological or clinical characteristics to differentiate responders from nonresponders, but studies suggest that the apo-E phenotype plays a role, as does the clinical condition of combined hyperlipidemia. Data also suggest that sensitivity to dietary cholesterol is associated with sensitivity to dietary fat, and that overall adiposity may also play a role. Although on a population basis the plasma cholesterol response to dietary cholesterol is relatively small, and in most epidemiological analyses not related to hypercholesterolemia, some individuals are sensitive to dietary cholesterol changes and, if hypercholesterolemic, would experience plasma cholesterol reduction with dietary cholesterol restrictions. For the majority, however, dietary cholesterol restrictions have little effect on plasma cholesterol levels.

Dietary Sources

Dietary Cholesterol Intake Patterns

Dietary cholesterol intakes in the USA have been declining, from an average of 500 mg a day in men and 320 mg a day in women in 1972 to levels in 1990 of 360 mg a day in men and 240 mg a day in women. This decline arises in part from dietary recommendations to the American public to reduce total and saturated fat intake and to reduce dietary cholesterol daily intake to less than 300 mg, and in part from the increased availability of products with reduced fat and cholesterol content. Major efforts in the early 1970s by public health agencies and advertising emphasized reducing dietary cholesterol as a means to lower plasma cholesterol levels, leading to a high degree of consumer concern regarding cholesterol-containing foods and demand for low-cholesterol products. Today, practically all foods sold in the USA are labeled for their cholesterol content and their percentage contribution to the daily value of 300 mg for cholesterol.

Major Dietary Sources

The major sources of cholesterol in the diet are eggs, meat, and dairy products. A large egg contains approximately 185 mg of

cholesterol and contributes some 30–35% of the total dietary cholesterol intake in the USA. Meat, poultry, and fish contribute 45–50%, dairy products 12–15% and fats and oils 4–6%. In the USA, the range of dietary cholesterol intakes is 300–400 mg per day for men and 200–250 mg per day for women; thus for much of the population the national goal of a dietary cholesterol intake of less than 300 mg a day has already been met.

See also: Coronary Heart Disease: Lipid Theory; Prevention. Fats and Oils: An Overview. Fatty Acids: Metabolism. Hyperlipidemia: Overview. Nutritional Considerations for the Management of Hypertension

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CHOLINE AND PHOSPHATIDYLCHOLINE

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Glossary

CpG island A DNA sequence with a higher than expected frequency of CpG sites.

CpG site Cytosine–guanine linear sequence within the DNA, in which the cytosine is the preferential site for methylation.

DNA methylation Chemical modification to the carbon 5 position of the cytosine nucleotide consisting of the substitution of a hydrogen atom with a methyl group.

Epigenetic change Heritable change in gene expression that is not caused by changes in the DNA sequence.

Introduction

Choline (IUPAC name 2-hydroxyethyl(trimethyl)azanium hydroxide) is a water soluble, quaternary saturated amine (Figure 1). It is present in many foods of animal and plant origin, and it is an essential nutrient for humans. The majority of choline is found incorporated into several phospholipids, most abundantly in phosphatidylcholine. The established physiological roles for choline and phosphatidylcholine involve acetylcholine synthesis (a neurotransmitter present in both the peripheral and central nervous systems), cellular membrane structure and function, and the maintenance of the s-adenosylmethionine pools (SAM) required for various methylation reactions (DNA, RNA, protein, and lipid methylation). In addition, its metabolite betaine contributes to the maintenance of the water balance by kidneys.

Although choline is synthesized in humans (*de novo* synthesis), dietary intake is necessary in order to maintain the normal function of various tissues and organs. Dietary choline deficiency may occur if the dietary intakes do not meet specific choline requirements, which can vary among individuals as discussed in the following. Men and postmenopausal women are at higher risk than premenopausal women of becoming choline deficient because endogenous choline synthesis is estrogen dependent. Another group of people at risk of becoming choline deficient are those who carry mutations in genes involved in choline synthesis or its oxidation to betaine.

In rodent models choline deficiency induces fatty liver, followed by liver cancer. In the adult human, diet-induced choline deficiency is associated with fatty liver and muscle damage, over

a period of weeks to months, but no human studies are available to determine alterations induced by prolonged deficiency (years). Of a special importance are the roles that choline has during fetal development. In rodents, gestational deficiency altered fetal brain development, with long-lasting consequences on memory and learning, while gestational supplementation partially prevented the cognitive decline characteristic of brain aging in the offspring. Limited epidemiological studies have associated maternal choline deficiency with neural tube defects (NTDs) and cleft palate in children.

Metabolism and Biochemistry

Intestinal Absorption

Free choline and choline-containing phospholipids are absorbed in the intestine via several mechanisms. Part of free choline is metabolized to betaine by the gut bacteria, which is then absorbed using active Na^+ and Cl^- coupled transport systems, and a passive Na^+ -independent transport system. The remaining free choline is absorbed in the enterocytes using a carrier-mediated transport. Choline-containing phospholipids (including phosphatidylcholine) are first hydrolyzed by pancreatic enzymes and by lipases from intestinal mucosal cells, releasing the choline molecule from the phospholipid backbone. The bioavailability of choline from phospholipids is different in children than in adults because of differences in the physiology of their digestive systems. Because neonates rely almost exclusively on maternal milk or formula, the choline content and its bioavailability in milk are essential factors for achieving the recommended choline intake during the first 6 months of life (Table 1).

Transport

From the intestinal cells choline is transported either to the liver as phosphatidylcholine via portal circulation, or

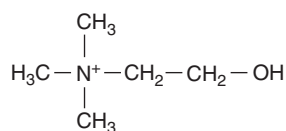


Figure 1 Secondary chemical structure of choline molecule.

Table 1 Dietary choline adequate intakes, upper limits, and adverse effects of oversupplementation

Group	Age	AI (mg day ⁻¹)	UL (g day ⁻¹)	Adverse effects of oversupplementation
Infants and children	0–6 months	125	Not established	Fishy body odor, nausea, and diarrhea (150 mg kg ⁻¹ day ⁻¹ and up)
	7–12 months	150	Not established	
	1–3 years	200	1	Gastrointestinal irritation, vomiting, lacrimation, and anorexia (8 g day ⁻¹ and up)
	4–8 years	250	1	
	9–13 years	375	2	Blurred vision (9 g day ⁻¹ and up)
Boys	14–18 years	550	3	
Girls	14–18 years	400	3	
Pregnant women	All ages	450	14–18 years: 3 g 19 years and older: 3.5 g	
Lactating women	All ages	550	14–18 years: 3 g 19 years and older: 3.5 g	
Other men		550	3.5	
Other women		425	3.5	

incorporated into chylomicrons and released into the systemic circulation via the lymphatic system. All tissues incorporate choline. The intracellular incorporation is mediated by three distinct transport systems, with different affinities for choline. One mechanism is intracellular incorporation by a low-affinity facilitated diffusion process. The other two mechanisms are a Na⁺-dependent and another Na⁺-independent transport system, respectively. Phosphatidylcholine is incorporated via ATP-binding cassette transporters. Free choline can cross the blood–brain barrier via a high-affinity transport system using choline transporters (CHT). Free choline is excreted by the kidneys by glomerular filtration, but then it is actively reabsorbed mainly in the proximal tubules of the nephrons, such that only approximately 2% of the filtered choline is found in the final urine.

During pregnancy maternal choline is delivered across placenta to the fetal circulation by the active transport mechanisms against concentration gradients.

Intracellular Metabolism

Although choline is present in all tissues, its accumulation and metabolism in liver, kidney, mammary gland, brain, and placenta are especially important. A general and simplified overview of choline metabolism is indicated in [Figure 2](#). Three major pathways use choline as substrate. Acetylcholine synthesis is catalyzed by choline acetyltransferase. This process occurs in certain neurons within the central and the peripheral nervous systems, where acetylcholine can act as either a neurotransmitter or as a neuromodulator.

Choline is also used for phosphatidylcholine synthesis via two independent pathways. Choline is phosphorylated by choline kinase to phosphocholine that is subsequently converted to cytidine diphosphocholine (CDP-choline), followed by phosphatidylcholine synthesis. In an alternate pathway (*de novo* choline synthesis) phosphatidylcholine is synthesized from phosphatidylethanolamine using SAM as methyl donor. This reaction is catalyzed by the enzyme phosphatidylethanolamine

N-methyltransferase (PEMT), which is encoded by a gene that in humans presents many identified mutations. In animal and human models this gene is overexpressed in the presence of estrogen, and limited clinical studies in humans suggested that endogenous choline synthesis may be enhanced in the premenopausal women.

A third pathway uses choline for methionine synthesis. Choline is first oxidized to betaine which, in turn, is used as a methyl-donor for the methylation of homocysteine to methionine (subsequently esterified to SAM). It is noteworthy that folate metabolism is also involved in SAM synthesis, pointing toward the relationship between choline and folate metabolism and their common roles in substrate methylation. The importance of choline in SAM metabolism was considerably strengthened lately by studies indicating that choline availability alters SAM's ability to methylate both DNA and specific nuclear proteins (histones), which together act as a modulator for gene expression (chromatin).

Phosphatidylcholine is a major contributor to the structure of cellular membranes, conferring the plasticity required for normal cellular function, allowing for the proper transport across the membranes, and for cellular signaling. Therefore, alterations of either the phosphatidylcholine amount or in the quality of fatty acids incorporated may have profound effects on the cell function.

Dietary Requirements and Food Sources

Many foods consumed by humans contain choline, choline esters, and betaine in various amounts. In current infant formulas choline and betaine are added during preparation. The ratio between the total choline content and betaine varies greatly in different foods, according to their animal or plant origin ([Figure 3](#)). Although best sources of betaine are present in plants, choline is more abundant in foods of animal origin, reflecting the differences in metabolic processes between plants and animals. From the human nutrition standpoint, although dietary recommendations have been established

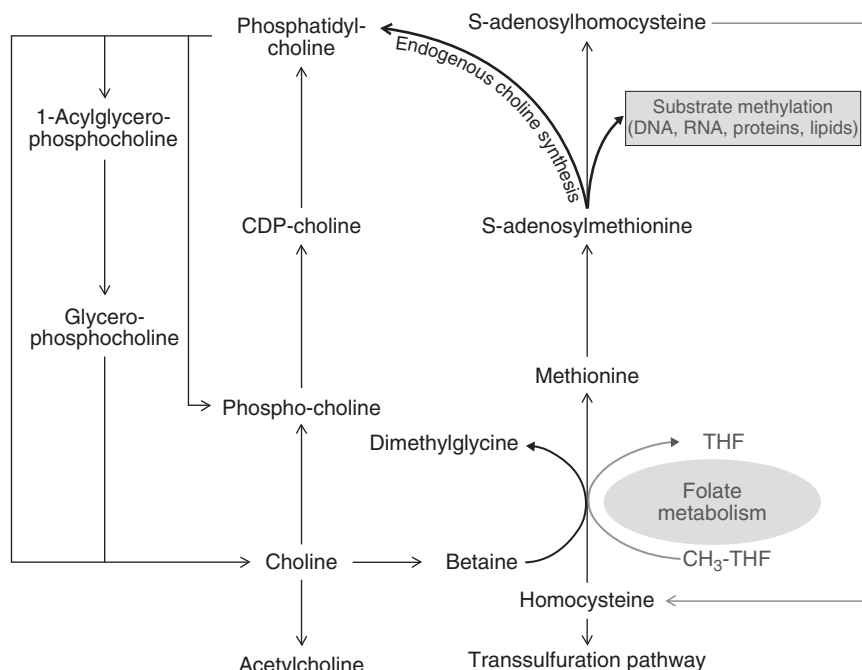


Figure 2 Simplified overview of choline metabolism. Pathways shaded in gray do not pertain to choline metabolism but are presented because of the relationship with SAM synthesis and its usage as a universal methyl donor.

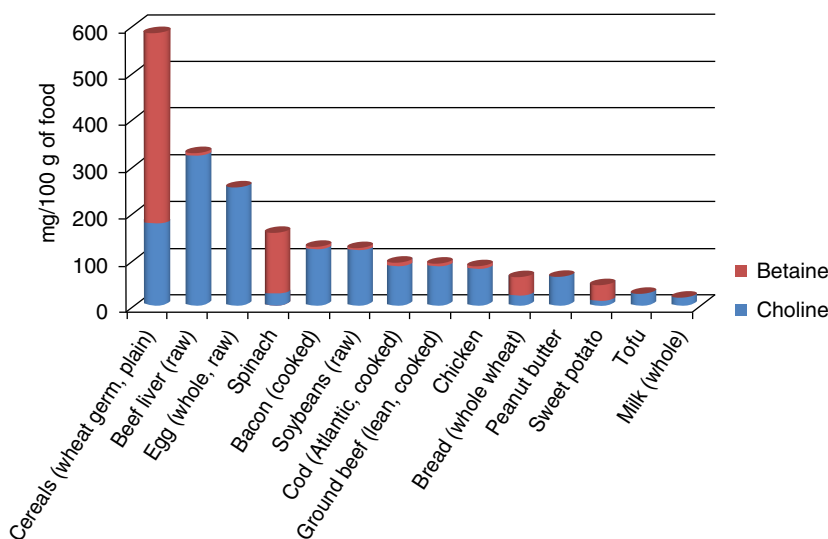


Figure 3 Total choline and betaine content in selected foods, raw or cooked. The source for the data was the USDA Database for the Choline Content of Common Foods (Release Two) available at <http://www.ars.usda.gov>

only for choline (Table 1), it is important to note that betaine from foods also contributes to the homocysteine methylation capacity and, therefore, more research is needed for establishing whether or not betaine intakes should be included in dietary guidelines.

Adequate intakes (AI) have been established by the Institutes of Medicine in the US, and many other countries have established their own guidelines. According to the World Health Organization's survey from 2003, 25 had government-endorsed guidelines. However, the US guidelines

are the most highly documented dietary recommendations and, therefore, other countries have adopted these guidelines.

Table 1 summarizes the AI values for different age and sex categories, along with upper limit values (UL), and known adverse effects for choline oversupplementation. Pregnant women and lactating mothers have increased choline requirements because of its use during fetal development (tissue and organ formation, and growth), which continues at even greater pace after birth.

Functional Consequences of Dietary Choline Availability

Both animal and human studies indicated that variations in choline availability may induce profound changes on the metabolism of liver, brain, muscle tissues, and kidneys. Of special importance are the animal studies indicating that choline is a nutrient required for normal brain development during perinatal periods, with long-lasting consequences on cognition and memory. More recently, choline received greater attention due to studies indicating that, as a source for methyl donation, its availability can modify the methylation of DNA and histones (epigenetic mechanisms) that, in turn, induce changes in gene expression. A second aspect is linked with numerous single nucleotide polymorphisms (SNP, mutations of a single nucleotide within a gene) that are present in several key-genes involved in choline metabolism and synthesis. The presence of such mutations, as limited human studies indicated, may reduce the endogenous choline synthesis and, therefore, may shift the dietary choline intakes toward higher values than those specified in [Table 1](#). Presently, specific requirements for humans who carry such mutations have not been established.

Maternal Choline Availability and Fetal Development

Choline is required for normal formation of the embryo and for organ development during fetal life. Most of what is known about choline comes from studies on rodents; therefore, it is not clear whether or not such findings apply to humans, although few epidemiological studies suggest that this might be the case. When mouse embryos were grown *in vitro*, their treatment with an inhibitor of choline uptake (2-dimethyl-aminoethanol) induced craniofacial hypoplasia and incomplete neural tube closure of the forebrain, mid-brain, and hindbrain areas. The treatment of mouse embryos with an inhibitor of phosphatidylcholine synthesis (1-O-octadecyl-2-O-methyl-rac-glycero-3-phosphocholine) also induced similar defects. These findings suggested that choline and folate deficiencies during gestation induce similar outcomes, a hypothesis supported by their common involvement in methionine synthesis ([Figure 2](#)).

Epidemiological studies relating maternal choline intakes before and during pregnancy to the incidence of NTD in children have also found an increased risk for NTDs and other cranial malformations associated with lower maternal choline intakes. However, one recent study published in 2010 failed to identify a decreased risk of NTDs with choline supplementation.

A second developmental stage when choline plays an important role is during late gestation, when the brain develops at an accelerated pace, and when its specific areas are forming. No such studies have been performed in humans, but animal models revealed that maternal choline availability alters brain development especially in the areas that are responsible for memory formation and learning (hippocampus and septum). Maternal choline deficiency reduced the proliferation of neuronal precursors (cell that have the ability to divide, which then differentiate into mature neurons or glial cells), and

increased the apoptosis (programmed cell death) within these brain areas. Moreover, throughout the whole brain, choline deficiency increased the expression of genes involved in neuronal differentiation, while decreasing the expression of genes involved in cell proliferation. Although not completely elucidated, these changes in gene expression suggested that choline deficiency could affect other brain areas as well. More studies should address this hypothesis.

The mechanisms by which choline deficiency alters cell proliferation in the fetal brain are not completely understood. In both fetal brain and cell culture experiments, choline deficiency induced the overexpression of genes and their protein products that inhibit cell proliferation (cyclin-dependent kinase inhibitors) like p27Kip1, p15Ink4b, and Cdkn3, all of them inducing cell-cycle arrest at the G1 phase. Alterations in other regulatory proteins (retinoblastoma and TGF β) suggested a model in which cell-cycle inhibitors interact with such proteins, the net outcome being the inhibition of cell division.

Long-lasting Effects of Maternal Choline Availability

The functional effects of altering choline availability during gestation are not confined only to this specific period. Studies in rats exposed to various choline levels *in utero* revealed that, even when the offspring receives a choline-sufficient diet for the rest of its life, changes are still present in the postnatal brain, with functional consequences in learning, memory, and brain aging. In the brains of juvenile rats cell signaling was influenced by prenatal choline exposure levels. Choline supplementation increased, while choline deficiency decreased the activation of mitogen-activated protein kinase (MAPK) signaling in the hippocampus. The exposure to maternal choline supplementation also increased levels of nerve growth factor (NGF) in the adult hippocampus and the adjacent cortical areas, whereas opposite changes were reported in choline deficiency for other brain areas (septal nucleus, nucleus of the diagonal band, and the nucleus basalis of Meynert). These findings support the hypothesis that choline is required prenatally, and that permanent changes are initiated during fetal brain development, with long-lasting consequences on the brain function.

The most extensive functional consequences associated with prenatal choline availability were those related with cognitive and memory alterations. Rats supplemented with choline during late gestation and in the first days after birth performed better at water maze tests (indicating improved learning and short memory), and had enhanced long-term potentiation within the hippocampus (LTP, a major cellular mechanism related to synaptic plasticity, learning, and memory formation), whereas choline-deficient rats presented opposite effects.

Choline Availability during Adult Life and Health Outcomes

Liver Damage

Most of the knowledge involving the effects of choline deficiency on the liver comes from animal studies. Both fatty liver and cancer were reported to occur in rats subjected to either choline-deficient or choline-devoid diets. The accumulation of lipids in the liver cells (hepatocytes) begins within

hours after rats are exposed to a choline-deficient diet, and peaks within 6 months of continuous exposure to choline deficiency. Concomitantly, liver cell death (apoptosis) occurs. The mechanism responsible for the accumulation of lipid droplets within the hepatocytes are related to the decrease in phosphatidylcholine levels within the liver. The lipids that are either synthesized or accumulated in the liver (especially triacylglycerol) are exported in the systemic circulation via their incorporation into very-low-density lipoproteins (VLDL) whose formation requires phosphatidylcholine. Therefore, phosphatidylcholine functional deficiency (induced by choline deficiency) will inhibit the physiological export of VLDL from the liver, resulting in the local accumulation of triacylglycerol. It is plausible that the same mechanism applies to humans. When fed a choline-deficient diet under controlled conditions and permanent medical surveillance, human subjects also develop fatty liver (that reverses to normal when the same subjects are then fed a choline-supplemented diet). Moreover, choline deficiency decreases the concentration of VLDL in plasma, suggesting that, as animal studies indicated, VLDL-associated export of lipids from the liver might be altered.

A second outcome associated with choline deficiency is the development of hepatocarcinomas. The occurrence of liver cancer was reported exclusively in animal studies (rodents), and it is unknown whether prolonged choline-deficiency could cause cancer in humans. The mechanism responsible for the choline-induced liver cancer is not clearly understood. Several hypotheses involve either the activation of protein kinase C signaling pathway, a defect of the mechanisms responsible for apoptosis, or the involvement of epigenetic mechanisms (discussed in the Section Roles in the Epigenetic Regulation of Gene Expression).

Other Effects

Liver is not the only organ affected by choline deficiency. Choline-deficient adults exhibit damage of the skeletal muscle tissues as indicated by elevated serum concentrations of phosphocreatine kinase (CPK). Although it has not been directly investigated, the most plausible hypothesis is that the decrease in phosphatidylcholine content of the muscle membranes would render the muscle cells more sensitive to damage during the moderate physical activity. Other alterations described during exposure to a choline-deficient diet include increased apoptosis of the lymphocytes. Interestingly, the assessment of gene expression in lymphocytes indicated measurable differences between individuals who presented with clinical signs of choline deficiency (liver and muscle damage, see the Section Liver Damage), and those who did not have such signs. These studies indicated that the extent of the potential damage induced by choline deficiency may include more than only liver and skeletal muscle. All these alterations were reversible under a choline regimen designed to replenish the subjects with choline at physiological levels.

Genetic Variations Influence Dietary Requirements

As indicated in [Figure 2](#), the endogenous *de novo* choline synthesis contributes to the maintenance of choline pools

at physiological levels. These levels vary based on gender, age, and are increased during pregnancy and lactation. The *PEMT* gene controls choline *de novo* synthesis. Premenopausal women are protected from dietary choline deficiency in a greater degree than men and postmenopausal women because this gene is estrogen responsive. Other genes are also involved in various metabolic reactions that use choline as substrate.

During the past decade our understanding regarding the role of interindividual genetic variations has greatly improved, and the interaction between the genome and the environment (including nutrition) became an important field of study. The understanding that nutrient requirements are individual-specific came from the realization that small but numerous genetic differences, when in interaction with factors from the environment (nutrition, climate, physical activity, etc.) shape a specific phenotype that has specific metabolic requirements. Therefore, individualized nutrition, rather than general dietary recommendations, is a concept that receives increased acceptance among scientists.

Hundreds of genetic variations are present within the genes involved in choline metabolism and in associated folic acid pathways. SNPs within *PEMT*, *CHDH*, *BHMT*, and *MTHFR* (the later pertaining to folate metabolism) were reported to not only alter dietary choline requirements, but also to confer either sensitivity or resistance against induced choline deficiency. The best studied gene is *PEMT*, responsible for *de novo* choline synthesis, and estrogen responsive. Several *PEMT* single nucleotide mutations have been associated with increased risk for choline deficiency in both animal and human studies. In mice, genetically engineered with a deletion within the murine *Chdh* gene, sperm motility is reduced, and homozygous males carrying the deletion cannot reproduce. Mutations within other genes (*BHMT* and *BHMT2*, and *MTHFR*) have been recently reported to have a significant influence on the homeostasis of either betaine, homocysteine, or to be related to the risk of birth defects, but more studies should confirm these recent findings.

Roles in the Epigenetic Regulation of Gene Expression

One important role for choline, as discussed in the Section Intracellular Metabolism, above, is its implication in methionine synthesis ([Figure 2](#)). Choline is oxidized to betaine by choline-dehydrogenase (CHDH). Betaine donates one methyl group to homocysteine (reaction catalyzed by betaine-homocysteine methyl transferase, BHMT), forming methionine (and further SAM). By contributing to the maintenance of the SAM pool, choline has an important role in the methylation reactions. Methylation of DNA and of histones is the basis for the epigenetic regulation of gene expression (heritable changes of gene expression that are not caused by changes in the DNA sequence). Gene-specific DNA and histone methylation profiles are responsible for the establishment of various chromatin structures, which are either permissive or inhibitory for the expression of a certain gene. While the methylation profile is inherited throughout subsequent cell divisions, maintaining the cellular phenotype, the epigenetic profile of some genes is

also inherited by the offspring from parents (imprinted genes), in a parent-of-origin manner.

During the past decade it has become clear that choline, acting as a methyl-donor for homocysteine methylation, can alter the epigenetic status of the genome and, therefore, induce stable modifications in gene expression that are carried out by the offspring throughout its life. Animal studies focused on alterations induced by maternal choline availability on the epigenetic status of genes in the liver and brain of the offspring. In the brains of fetuses exposed to maternal choline deficiency, both DNA and histone methylation changes occurred for genes involved in neuronal differentiation and cell proliferation. Moreover, choline availability altered the DNA methylation and the expression of genes that are responsible for the DNA methylation process itself (DNA methyltransferases, *Dnmt*). Other studies indicated that choline availability during the adult life of rodents can modify the epigenetic status of genes involved in liver carcinogenesis, with subsequent alterations in their expression (RNA levels). The availability of other methyl donors (folate, methionine, and betaine) was also reported to induce similar epigenetic alterations. These findings reinforce the importance of choline as a required nutrient not only during embryonic and fetal development, but also during postnatal development and adulthood.

Detection Methods

Choline can be measured using radioisotopic, high-pressure liquid chromatography (HPLC), or gas chromatography/isotope dilution mass spectrometry (GC/IDMS). These methods are cumbersome and cannot detect all of the choline derivatives. Specific choline-containing compounds can be measured in virtually any type of tissue or food using liquid chromatography/electrospray ionization-isotope dilution mass spectrometry (LC/ESI-IDMS). In addition, choline metabolites can be also measured *in vivo* using the

administration of choline isotopes followed by their detection by magnetic resonance spectroscopy (MRS) and spectroscopic imaging (MRSI), which are noninvasive imaging methods.

See also: Early Origins of Disease: Fetal; Non-Fetal. Folic Acid. Nutrient–Gene Interactions: Health Implications; Molecular Aspects. Pregnancy: Prevention of Neural Tube Defects. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements. Vitamin B₆: Physiology

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Glossary

Hemoglobin A1c Form of hemoglobin that varies with blood glucose and is formed by the nonenzymatic binding of glucose to hemoglobin. It is a measure of the average circulating glucose over the previous two to three months rather than a single point value.

Impaired glucose tolerance Two-hour glucose levels of 140–199 mg dl⁻¹ (7.8–11.0 mmol) during a 75-g oral glucose tolerance test. Values greater than these would qualify for type 2 diabetes mellitus. The fasting glucose may be either normal or mildly elevated.

Insulin resistance Condition where insulin has reduced efficiency and becomes less effective at lowering blood sugars. The body compensates initially with higher insulin output to help control blood glucose. During later stages of insulin resistance, there is not only elevated blood insulin but also elevated glucose leading to adverse health effects including the onset of type 2 diabetes.

Polycystic ovary syndrome (PCOS) Common female endocrine disorder affecting approximately 5–10% of women of reproductive age. The principal features are obesity, irregular menstruation or amenorrhea, acne, and excessive amounts or effects of masculinizing hormones. Although the causes are unknown, insulin resistance, diabetes, and obesity are all strongly correlated with PCOS.

Reactive hypoglycemia Recurrent symptoms occurring 1–2 h after a high carbohydrate meal or oral glucose load. Symptoms range from light headedness and fatigue to confusion, panic attacks, and coma. Absolute level of low blood glucose may or may not be related to severity of symptoms.

Total parenteral nutrition Receiving all forms of nutrition intravenously by bypassing the usual process of eating and digestion. No food or nutrients are consumed by mouth.

Chromium in the trivalent form is an essential nutrient that functions primarily in sugar and fat metabolism. Dietary intake of Cr by humans and farm animals is often suboptimal. Insufficient dietary intake of Cr is associated with increased risk factors associated with type 2 diabetes mellitus (type 2 DM) and cardiovascular and related diseases. Chromium functions in glucose and insulin metabolism primarily via its role in the improvement of insulin activity. Improved insulin function is also associated with an improved lipid profile. People with type 2 DM have a more than twofold increase in the incidence of cardiovascular diseases (CVDs) compared with control subjects. Improved insulin function also leads to a decrease in diseases associated with inflammation, antioxidants, and even some forms of cancer and brain function including Alzheimer disease.

Chromium in foods and dietary supplements is trivalent, whereas Cr often found in paints, welding fumes, and other industrial settings is hexavalent and is severalfold more toxic than the trivalent nutritional Cr. Trivalent Cr is one of the safest nutrient supplements based on the ratio of the amount that is needed relative to the amount that can be consumed over a lifetime with no adverse effects. An expert panel of the US Food and Nutrition Board was unable to set an upper level of safe intake because none of the levels of intake tested showed any signs of toxicity. Toxicity is alleviated by the low levels of absorption, usually less than 2%. This review will be focused on the effects of trivalent Cr on human subjects.

Essentiality and Metabolic Functions of Chromium

The essentiality of trivalent Cr in human nutrition was documented in 1977 when diabetic signs and symptoms of a patient on total parenteral nutrition (TPN) were reversed by supplemental Cr. Diabetic symptoms, including elevated blood glucose, weight loss, impaired nerve conduction, brain disorders, and abnormal respiratory quotient, that were refractory to exogenous insulin were reversed following increased intake of the essential nutrient, Cr. On daily addition of supplemental Cr to the TPN solution for 2 weeks, diabetic symptoms of the patient were alleviated and exogenous insulin requirement dropped from 45 units day⁻¹ to 0 unit day⁻¹. These findings have been repeated and documented in the scientific literature on several occasions.

Signs and symptoms of Cr deficiency listed in **Table 1** are not limited to subjects on TPN. Improvements in glucose and lipid concentrations have been reported in children with protein calorie malnutrition, the elderly, and people with type 2 DM, hypoglycemia, marginally impaired glucose tolerance, and for numerous animal species.

The hallmark sign of marginal Cr deficiency is impaired glucose tolerance. The effects of Cr on people with high, low, and normal glucose tolerance, as well as diabetes, are illustrated in **Figure 1**. Chromium leads to a decrease in blood glucose level in people with elevated blood sugar and an increase in those with low blood sugar due to its role in normalizing

Table 1 Signs and symptoms of Cr deficiency

Function	Animals
Impaired glucose tolerance	Human, rat, mouse, squirrel monkey, guinea pig, cattle
Elevated circulating insulin	Human, rat, pig, cattle
Glycosuria	Human, rat
Fasting hyperglycemia	Human, rat, mouse
Impaired growth	Human, rat, mouse, turkey
Hypoglycemia	Human
Elevated serum cholesterol and triglycerides	Human, rat, mouse, cattle, pig
Increased incidence of aortic plaques	Rabbit, rat, mouse
Increased aortic intimal plaque area	Rabbit
Nerve disorders	Human
Brain disorders	Human
Corneal lesions	Rat, squirrel monkey
Ocular eye pressure	Human
Decreased fertility and sperm count	Rat
Decreased longevity	Rat, mouse
Decreased insulin binding	Human
Decreased insulin receptor number	Human
Decreased lean body mass	Human, pig, rat
Elevated percent body fat	Human, pig
Impaired humoral immune response	Cattle
Increased morbidity	Cattle
Gestational diabetes	Humans
Steroid-induced diabetes	Humans
Atypical depression	Humans

Source: Adapted with permission from Anderson RA (1998) Chromium, glucose intolerance and diabetes. *Journal of American College of Nutrition* 17: 548–555.

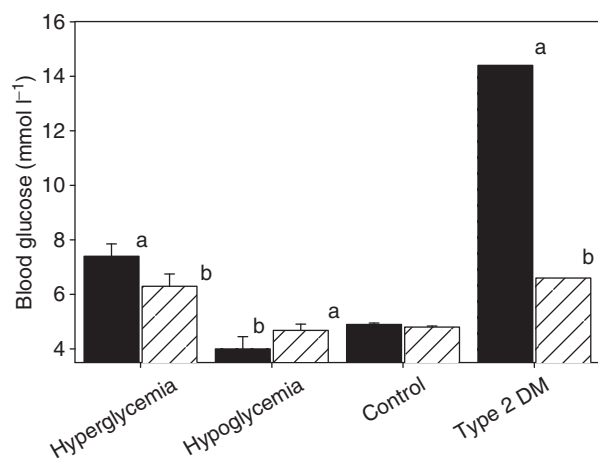


Figure 1 Response to supplemental Cr of people with hyperglycemia, hypoglycemia, optimal glycemia (control), and type 2 DM. The minimal amount of Cr usually showing beneficial effects in people with high or low blood sugar is 200 $\mu\text{g day}^{-1}$. People with diabetes require 400–600 $\mu\text{g day}^{-1}$ or more. Bars with different superscripts denote differences at $p < 0.05$.

insulin. In the presence of Cr in a physiologically active form, insulin is more efficient and much lower levels of insulin are required. During periods of elevated blood glucose, more efficient insulin leads to a decrease in blood glucose. In people

with low blood sugar, reactive hypoglycemia, more efficient insulin leads to a rapid rise in response to a glucose challenge and a more rapid return to baseline values. This leads to less of a drop or a lessening of the hypoglycemic glucose response.

Mechanism of Action

Mechanistically, supplemental Cr leads to increased insulin binding and increased insulin receptor number, and it may be involved in the phosphorylation–dephosphorylation of the insulin receptor proteins. Chromium activates insulin receptor kinase, the enzyme that phosphorylates the insulin receptor, leading to activation of insulin function and appears to inhibit the phosphatase enzyme that deactivates insulin function. Chromium also improves translocation of the glucose transporter 4 found primarily in the striated and cardiac muscle and in adipose tissue leading to improved glucose uptake. Trivalent Cr function may be related to changes in cholesterol membrane concentrations in muscle and fat cells.

Recent Advances

Recent advances in Cr nutrition research include the demonstration that Cr improves cognitive–cerebral function in older adults. Insulin resistance is associated with impaired memory function associated with Alzheimer disease, and factors that help overcome insulin resistance are also associated with improved memory in subjects with mild cognitive impairment and early Alzheimer disease. In a double-blind placebo controlled study, subjects receiving Cr displayed improved learning trials, recall trials, and recognition memory. Functional magnetic resonance imaging also documented the beneficial effects in the brain of the subjects receiving supplemental Cr. These findings demonstrate that supplemental Cr can enhance cognitive inhibitory control and cerebral function in older adults who are at risk for neurodegeneration.

Recent studies also support the beneficial effects of supplemental Cr on body weight and satiety. Although there are several studies reporting beneficial effects of Cr on body weight and composition, there are others reporting no effects. Adult women consuming Cr have reduced food intake, hunger levels, and fat cravings and tend to have decreased body weight. A reduction in body weight was documented in a separate study in which subjects with type 2 DM were all put on sulfonylurea drugs for 3 months and then received either Cr or a placebo for 6 months. Subjects randomized to receive Cr had improvements in insulin sensitivity, hemoglobin A_{1c} (HbA_{1c}), and free fatty acids. In addition, supplemental Cr led to significantly attenuated weight gain and visceral fat accumulation compared with the subjects in the placebo group, further documenting effects of Cr on body weight and composition.

Supplemental Cr was also shown to be effective in the treatment of depression. Preliminary studies suggest that the effects of Cr are greater than those of any drugs currently used in the treatment of atypical depression. Supplemental Cr is also free of side effects associated with drugs, which are often quite serious in the treatment of depression.

Studies also show that Cr is beneficial in the reversal of polycystic ovarian syndrome (PCOS), gestational diabetes, and steroid-induced diabetes associated with the administration of steroids such as prednisone given as anti-inflammatory agents in the treatment of arthritis, asthma, allergies, and related diseases.

There is also an inverse relationship between toenail Cr and the incidence of CVD in studies from the United States and Europe supporting related studies indicating that people with CVD tend to have lower levels of serum and tissue Cr and also substantiating the beneficial effects of supplemental Cr on blood cholesterol, triglycerides, and HDL cholesterol. Chromium concentrations in the hair, blood, and sweat decline with age as insulin sensitivity and the incidences of diabetes, cardiovascular, and related diseases increase.

Not all People Respond to Supplemental Chromium

Response to Cr is due to not only the Cr status of the subjects but also the forms and amount of Cr consumed. Subjects with diabetes or glucose intolerance who consume 200 μg or less of supplemental Cr daily often do not respond to supplemental Cr but may respond to 400–600 μg daily or more. A dose response to Cr for subjects with type 2 DM is shown in Figure 2. Subjects diagnosed with diabetes for approximately 5 years had taken no Cr supplements. There was a progressive decline in the $\text{HbA}_{1\text{C}}$ after 2 and 4 months of consuming 200 or 1000 μg daily of Cr as Cr

picolinate. There were also dose-dependent improvements in glucose, insulin, and cholesterol. These results have been confirmed in separate double-blind placebo-controlled studies.

The people who respond to Cr are difficult to predict and the phenotypic characteristics of the individual may be important. Phenotype is also important in insulin signaling and may explain, in part, the wide range of individual responses to Cr supplementation. Chromium has beneficial effects in insulin-resistant obese but not lean JCR:LA corpulent rats, which are used as a model for insulin resistance. In humans, a response to Cr is more likely in insulin-resistant individuals who have elevated fasting glucose and $\text{HbA}_{1\text{C}}$ levels.

Chromium and Stress

Stresses that have been shown to alter Cr metabolism in humans include glucose loading, high simple sugar diets, lactation, infection, acute exercise, chronic exercise, and physical trauma. Urinary losses can be used as a measure of the response to stress, because once Cr is mobilized it is not reabsorbed by the kidney but is lost in the urine. The degree of stress as measured by the stress hormone, cortisol, is correlated with the amount of Cr lost in the urine.

The administration of glucocorticoids also leads to increased urinary Cr losses as well as insulin resistance. These steroids are often employed as anti-inflammatory agents in the treatment of common chronic diseases such as asthma,

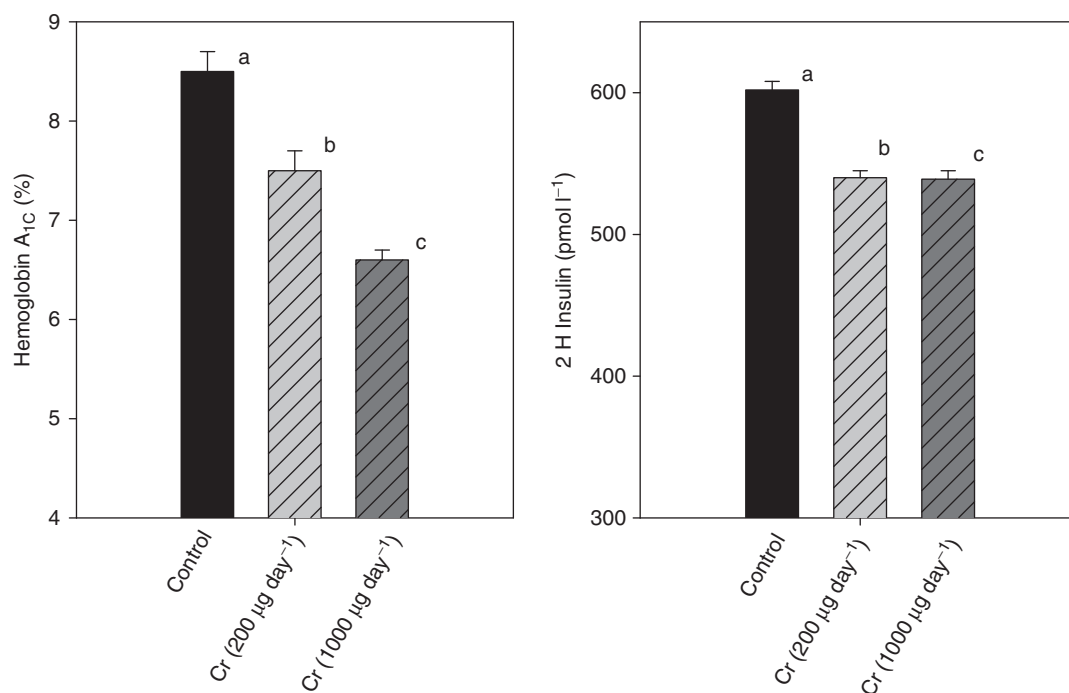


Figure 2 The effects of chromium on hemoglobin $\text{A}_{1\text{C}}$ and 2-hour insulin. In this study, 180 people with type 2 DM were provided with chromium supplementation (200 or 1000 $\mu\text{g day}^{-1}$) for 4 months. Bars with different superscripts denote differences at $p < 0.05$. Adapted with permission from Anderson RA, Cheng N, Bryden NA, Polansky MM, Chi J, and Feng J (1997) Elevated intakes of supplemental chromium improve glucose and insulin variables in individuals with type 2 diabetes. *Diabetes* 46: 1786–1791.

allergies, and arthritis, and are also administered following organ transplantation, but one of the side effects of the glucocorticoids is steroid-induced diabetes. The mechanisms responsible for steroid-induced diabetes are unknown, but decreased insulin sensitivity is an overlying cause. Impaired Cr metabolism also appears to be a related cause because supplementation of 50 people with uncontrolled steroid-induced diabetes with Cr for 10 days resulted in the reversal of the steroid-induced diabetes in 47 of the 50 patients, with no adverse side effects. There was also a decrease in at least 50% of the medication needed before the supplementation of Cr.

Dietary Intake and Requirements of Chromium

A panel on micronutrients convened by the Institute of Medicine has defined an adequate intake (AI) of Cr as 25 μg for women and 35 μg for men 19–50 years, and 20 μg for women and 30 μg for men older than 50 years. Adequate intake “is the recommended average daily intake based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate-used when an RDA cannot be determined.” The AI for Cr is nearly identical to the average Cr intake but much lower than earlier committee recommendations.

It is unclear why the AI for Cr should be lower for people older than 50 years when the primary function of Cr is to combat problems associated with insulin and glucose metabolism that tend to increase with age. Indices of Cr status such as the Cr content of hair, sweat, and urine were shown to decrease with age in a study involving more than 40 000 people. Because Cr losses are increased by high intake of simple sugars such as glucose, sucrose, and fructose, modern diets high in these sugars appear to be increasing the requirements for Cr.

More than 40 studies have reported beneficial effects of supplemental Cr on people with blood glucose values ranging from hypoglycemia to diabetes when consuming diets of average Cr content. In a controlled diet study, consumption of diets in the lowest quartile of normal Cr intakes, but near the new AIs, led to detrimental effects on glucose and insulin in subjects with marginally impaired glucose tolerance (90-minute glucose between 5.6 and 11.1 mmol l^{-1} or 100–200 mg dl^{-1}) following an oral glucose load of 1 g kg^{-1} body weight. The average person older than 25 years has blood glucose in this range. Consumption of these same diets by people with good glucose tolerance (90-minute glucose less than 5.6 mmol l^{-1}) did not lead to changes in glucose and insulin variables. This is consistent with previous studies demonstrating that the requirement for Cr is related to the degree of glucose intolerance and demonstrates that an intake of 20 $\mu\text{g day}^{-1}$ of Cr is not adequate for people with decreased insulin sensitivity such as people with marginally impaired glucose tolerance and certainly not for those with impaired glucose tolerance or diabetes.

Absorption, Transport, Storage, and Excretion

Absorbed Cr is excreted primarily in the urine and only small amounts of Cr are lost in the hair, perspiration, and bile.

Therefore, urinary Cr excretion can be used as an accurate estimation of absorbed Cr. At normal dietary Cr intakes (10–40 $\mu\text{g day}^{-1}$), Cr absorption is inversely related to dietary intake. Chromium absorption is approximately 0.5% at a daily intake of 40 μg and increases to 2% when intake drops to 10 μg . Therefore, the amount of absorbed Cr over this range is approximately 0.2 μg and is reflected in the urinary Cr losses of approximately 0.2 $\mu\text{g day}^{-1}$. This inverse relationship of Cr intake and absorption appears to be a basal control mechanism to maintain a minimal level of absorbed Cr. Intakes above 40 μg result in corresponding increases in total Cr absorbed. There is no direct evidence that Cr absorption involves active transport.

Chromium absorption in young and old normal subjects is similar, but people with type 1 DM absorb two- to fourfold more Cr than other groups of subjects tested. People with diabetes appear to have an impaired ability to convert inorganic Cr to a usable form. Diabetic mice also lose the ability to convert Cr to a usable form. People with diabetes require additional Cr and the body responds with increased absorption, but the absorbed Cr cannot be utilized effectively and is excreted in the urine. Chromium content of tissues of people with diabetes is also lower.

Chromium absorption and incorporation into tissues are also dependent on the form of Cr ingested. An accurate estimation of Cr absorption and utilization in animal studies can be achieved by measuring Cr incorporation into tissues. The tissue with the greatest Cr concentration is the kidney followed by the spleen, liver, lungs, heart, and skeletal muscle.

Tissue Cr is an accurate method of assessing Cr absorption and utilization and is also a measure of Cr storage. The kidney, which is one of the primary sites of tissue Cr storage, is also one of the best sources of insulin-potentiating forms of Cr. Chromium is transported to the tissues primarily bound to transferrin, the same protein that transports iron. There are two metal-binding sites on transferrin: one primarily for iron and a second involved in Cr transport. During conditions of high iron excess or iron overload such as in iron storage diseases (hemosiderosis, hemochromatosis), all the metal transport sites on transferrin are occupied by iron. This may explain the high incidence of diabetes in hemochromatosis patients that may be due in part to Cr deficiency.

Dietary Sources

Dietary Cr content of foods varies widely and there are no comprehensive databases to calculate dietary Cr intake. Chromium content of foods is often erroneously high due to Cr contamination during collection and analyses. For example, stainless steel blender blades are often used in the homogenization of foods, but stainless steel is approximately 18% Cr. In the presence of acidic foods, more Cr may leach from the blender blades than is present originally in the foods.

Chromium is present in several food groups but at low levels. The distribution is similar among fruits, vegetables, dairy products, beverages, and meats with lesser amounts from cereal products and small amounts from fish and seafood. Measured chromium content of foods is a combination of the endogenous Cr present in the foods and the Cr introduced

during the various stages of growing and processing. For example, fruit juices are often high in Cr because it may leach from containers during processing and storage under acidic conditions.

Safety of Chromium

Trivalent Cr, the form of Cr found in foods and in nutrient supplements, is one of the least toxic nutrients. The reference dose established by an expert panel of the US Environmental Protection Agency (EPA) is 350 times the upper limit of the estimated safe and adequate daily dietary intake. The newer AIs established by committees of the Institute of Medicine are lower and would have a safety ratio of more than 2000! The reference dose is defined as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population, including sensitive subgroups, that is likely to be without an appreciable risk of deleterious effects over a lifetime.” This conservative estimate of safe intake has a much larger safety factor for trivalent Cr than almost any other nutrients. The ratio of the reference dose to the requirements is approximately 2 to 5 for other trace elements such as zinc and manganese and 5 to 7 for selenium. Chromium in the form of both Cr chloride and Cr picolinate fed to rats at several thousand times the current AI (based on body weight) resulted in no detectable signs of toxicity.

Summary

In summary, dietary intake of Cr may be suboptimal for most humans. Increased intake of trivalent Cr often leads to improved glucose and lipid metabolism. The physiological role of Cr appears to be primarily through the improved function of insulin. Increased intake of Cr leads to increased insulin binding, increased insulin receptor number, and increased phosphorylation of the insulin receptor proteins leading to increased insulin sensitivity and function and lower glucose and lipids. Cognitive–cerebral function in people with mild cognitive impairment is also improved. Chromium is a nutrient and not a therapeutic agent and only those subjects whose impaired glucose and insulin function are related to suboptimal intake of Cr will benefit from additional Cr. Although a significant number of subjects often respond to supplemental Cr, there are also a significant number of subjects that do not respond to improved Cr nutrition. This is

likely due to the amount and form of Cr consumed and glucose tolerance and Cr status of the subjects. There have been no documented negative effects of supplemental Cr in any of the Cr supplementation studies involving daily Cr intakes of up to 1000 $\mu\text{g day}^{-1}$. Reported negative effects of supplemental Cr have not been documented.

See also: Diabetes Mellitus: Classification and Chemical Pathology; Dietary Management; Etiology and Epidemiology. Ultratrace Elements

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COFACTORS

Contents
Inorganic
Organic

Inorganic

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Glossary

Carbonium ion A carbon ion with a positive charge.

Chloroplasts Light energy sequestering organelles in photosynthetic plants.

Cytosol The internal milieu of a cell.

Endoplasmic reticulum An internal cell compartment enclosed by membranes.

Hemoglobin The major iron protein in the blood.

Noncarbonaceous Not having the element carbon in the structure.

Peroxisomes An internal compartment in a cell.

Ruminants Cattle and such where a four-chambered rumen replaces the stomach.

Surrogate metal A metal that may act as a substitute.

Transferrin A protein that transfers iron to the tissues.

Transition series Belonging to a certain division of the Periodic Table of Elements.

Introduction

The word 'inorganic' implies a noncarbon component. Such is true of inorganic cofactors, which consist mainly of metal ions and noncarbonaceous components. More importantly, one-third of all the enzymes require an inorganic cofactor for function. Their role is by no means minor. Enzymes that exchange electrons between substrates or quench dangerous free radicals or reactive oxygen species use metal ion cofactors to lessen the risk of irreversibly modifying the structure of the enzyme. Some cofactors perform mainly structural roles; for example, stabilizing the overall shape of the enzyme so it is poised to act on a substrate. Its more common, however, for the cofactor to engage the substrate directly and assist the catalysis of the ensuing reaction. More recently attention has turned to metal ions as modulators of genetic expression. These understandings have promoted the view that cofactors may be more than just passive components in biological systems. Here, we define a cofactor as any non-enzyme component that promotes the catalytic prowess of an enzyme. The definition emphasizes function rather than structure. This article will expand the discussion on enzyme cofactors by focusing on the properties of their 'inorganic' counterparts.

History

The nutritional history of the mineral elements, unlike the vitamins, had an early focus on domestic livestock foraging on mineral-poor soils. Typical symptoms were the crimping of wool in sheep, aortic rupture in pigs and cattle, and decrease in myelin in brains of newborn lambs. Symptoms were lessened sharply by supplementing the feed with salts of metal ions such as CuSO_4 , $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2$, ZnCl_2 . Reversing symptoms and reestablishing optimal growth to livestock provided the first solid evidence for the essentiality of metals in nutrition. Thus, no longer were metal ions in the blood and tissues of animals and humans considered a sign of toxic exposure. With this view instead came the realization that metals can be indispensable elements to the animal's health. An early study by Hart *et al.* showed that rebuilding hemoglobin to normal levels in an anemic rat required both iron and copper. Others showed a similar copper-iron interaction in humans. Coupled with the advent of semipurified diets in that same era, the science of nutrition made major steps forward in defining the importance of metal ions in biological systems and many of the studies tended to focus on their role with enzymes. The past decades have witnessed a tremendous interest in metal ions as cofactors and as regulators of enzyme activity.

in tissues and organs. These studies have focused not only what they do but also on how the system is able to adroitly handle potentially dangerous nutrients present in macro and micro quantities and ensure that their functionality and the organism's safety are realized.

Macro- Versus Microminerals

Of the 27 nutritionally essential elements in the Periodic Table, nearly half are minerals, more specifically metal ions. Because they vary in quantity both in the diet and within the organism, minerals are divided into two major classes, the macro- and microminerals. A list of some of the more prominent ones in each class is shown in **Table 1**. Macrominerals, which include sodium, potassium, magnesium, and calcium tend to be present in larger quantities both in the diet and the tissues. As such they perform functions that are attuned to their bulk; functions such as regulating osmotic balance, or forming the matrix of the skeleton, or providing high energy gradients to drive membrane transport. In contrast, the microminerals as their name implies are present in very small amounts in the tissues and have a much lower requirement for adequacy. Despite their scarcity, as a class the microminerals make up the bulk of inorganic cofactors. The

reason for this will be made clear below (*see* Metal-activated Versus Metalloenzymes). This class, which is also referred to as trace elements or trace metals, includes iron, zinc, copper, manganese, etc. (**Table 1**). All of those mentioned in the table are known to perform cofactor functions with enzymes.

In determining their role with enzymes, it is important to realize that most of the microminerals make up the 3d transition series of elements in the Periodic Table of Elements. As transition metals, they can exist in multiple valence states and are capable of forming specific geometric complexes with proteins. More importantly, perhaps, the multivalency common with many of the micro metal ions permits them to exchange electrons between substrate and cofactor. Nonmetal microminerals such as selenium also has this property. In contrast nearly all metal ions in the macromineral category (K^+ , Ca^{2+} , Mg^{2+}) are monovalent and are not capable of donating or accepting electrons (**Table 1**).

Another important property of transition metals is the ability to bind firmly to the structure of the enzyme. Binding is generally through coordinate covalent bonds and exhibits a specific geometric pattern (**Figure 1**). The precision of the angles and distances in the binding pocket are a basis for pairing a particular metal with the enzyme. Macrominerals have more of a tendency to engage an enzyme by ionic attraction and thus their binding to the enzyme surface is much weaker. The differences in these two properties carries over to classifying inorganic cofactors on the basis of activation by the metal or having the metal be an integral part of the enzyme's structure.

Table 1 Examples of inorganic cofactors

	<i>Stable biological form or valence</i>
Macrominerals	
Sodium	Na^+
Potassium	K^+
Calcium	Ca^{2+}
Magnesium	Mg^{2+}
Chloride	Cl^-
Phosphate	HPO_4^{2-}
Microminerals	
Iron	Fe^{2+} , Fe^{3+}
Zinc	Zn^{2+}
Copper	Cu^+ , Cu^{2+}
Manganese	Mn^{2+} , Mn^{4+} , Mn^{5+}
Cobalt	Co^+ , Co^{2+} , Co^{3+}
Molybdenum	MoO_2^{2+} , MoO_4^{2-}
Nickel	Ni^+ , Ni^{2+}

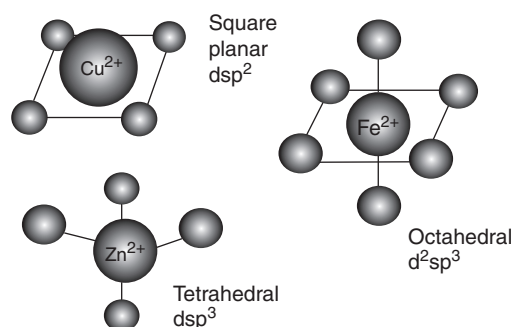


Figure 1 Some common geometries of metal complexes.

Metal-activated Versus Metalloenzymes

Freedom of movement carries over to the strength of binding when the mineral (as a biometal) engages the structure of an enzyme. Because biometals in the macromineral class tend to form bonds that are easily broken, the biometal exists in a state of equilibrium with the enzyme. Enzymes that use weakly bound cofactors are referred to as metal-activated; a term that signifies the enzyme can be primed to greater activity by adding its metal ion cofactor. Because the metal cannot be bound in a more permanent way, metal-activated enzymes typically lose activity during purification. An example is pyruvate kinase, which has a specific requirement for K^+ and is inactivated by dialysis (diffusion through a semiporous membrane). Another example is the class of enzymes referred to as kinases. These enzyme transfer the terminal phosphate group from ATP to the substrate, a reaction that requires Mg^{2+} to activate the substrate (Mg^{2+} -ATP).

In contrast, enzymes that have the metal ion bound firmly are referred to as metalloenzymes. Such enzymes have the metal ion bound mainly through coordinate covalent bonds. Because of the tight binding the metal ion is basically a firm fixture of the protein's structure. With few exceptions, metals in the micromineral class fit into the picture as cofactors for metalloenzymes. Tight binding precludes loss of the metal ion by dialysis or loss to weakly dissociating agents. Metal ion chelators with a strong affinity for the metal, however, can out-compete the enzyme for the metal ion and render the enzyme inactive. As prosthetic groups, metals in metalloenzymes have a stoichiometric relationship (metal ion-enzyme protein ratio

Table 2 Metal-activated enzymes and metalloenzymes

Metal or metal cofactor	Enzyme	Function
Metal-activated enzymes		
K ⁺	Pyruvate kinase	Synthesize pyruvate
Mg ²⁺	Hexokinase	Phosphorylate glucose
–	DNAase	Cleave DNA
–	RNAase	Cleave RNA
–	ATPase	Cleave ATP
Metalloenzymes		
Cu ²⁺ , Zn ²⁺	Superoxide dismutase	Destroy superoxide anion
Fe	Catalase	Destroy H ₂ O ₂
Zn	Alcohol dehydrogenase	Metabolize alcohol
–	DNA polymerase	Synthesize DNA
Mn	Pyruvate carboxylase	Synthesize oxaloacetate
–	Arginase	Synthesize urea
Ca	Alpha amylase	Cleave glycogen, starch

expressed as a whole integer) with the enzyme and are seldom primed to greater activity by adding more metal ion to the enzyme. Spatial geometry is also a concern. Examples of the more common geometrical arrangements are shown in **Figure 1**. For metals in the first transition series one takes note of the 3d orbitals. Examples of both classes of enzymes are shown in **Table 2**.

An exception to note in the table is selenium. Because selenium has properties similar to sulfur, selenium can replace sulfur in the structure of amino acids that make up the enzyme's structure. Thus, when functioning as a cofactor, selenium is present as selenocysteine and selenomethionine and not as elemental selenium coordinated to the protein structure. Another exception to note is in the enzyme *Cu₂, Zn₂ superoxide dismutase*. On rare occasions an enzyme may require more than one metal ion to perform catalysis. Other than those with Zn, enzymes and proteins with first transition series metal ions tend to be highly colorful. Consider, for example the beautiful red color of hemoglobin (iron), the green of chlorophyll or the blue color of ceruloplasmin (whose name means heavenly blue) associated with copper. **Table 2** gives some examples of metalloenzymes and the specific metal each has bound to the structure.

Individual Metal Cofactors

Macrominerals

Although their presence in the diet and within the system far exceeds that of the microminerals, the macrominerals as a whole are not the category of abundance when considering enzyme cofactors. Examples of some of the more familiar are:

Sodium

As a monovalent ion, Na⁺ is generally not considered a cofactor because one has yet to demonstrate an enzyme whose catalysis depends strictly on sodium ions. Sodium-activated

enzymes often respond to surrogate metal cofactors such as Li⁺ or even divalent cations. Sodium ions, however, form a major class as cotransporters for a series of transport proteins referred to as solute-linked carriers. Working with transporters for amino acids and sugars, sodium ion gradients across the membrane provide the driving energy for movement of amino acids, monosaccharides, etc. into cells.

Potassium

The potassium ion (K⁺) makes a rare appearance as a specific cofactor for *pyruvate kinase* in the glycolysis pathway. Both potassium and magnesium form no permanent bonds with their respective enzymes and hence act more as activators.

Magnesium

Magnesium ions (Mg²⁺) are required by a large number of enzymes referred to as *kinases*. Kinases transfer the terminal phosphate group of ATP to substrates. They figure prominently in many biochemical pathways such as glycolysis (*hexokinase*, *fructose-6-phosphate kinase*, *pyruvate kinase*), hormone responses mediated by cyclic AMP, cell signaling, and regulation of cell division. Mg²⁺ also modulates muscle contraction by competing with Ca²⁺ on proteins that trigger muscle contraction.

Calcium

As a group IIa metal ion, Ca²⁺ is limited to a +2 valence state and serves primarily as a divalent cation in its interactions with enzymes. The role of Ca²⁺ is limited mainly to structure stability although it is a cofactor for a limited number of important enzymes apart from the more familiar actin–myosin complex in muscle. *Alpha amylase* and *thermolysin* are two of the most familiar. As a free ion or working through calmodulin, calcium is better understood as an activator of enzymes in hormone-dependent cell signaling pathways. Enzymes, which have been referred to as Ca-ATPases and H⁺/Ca-ATPases are not to be mistaken as calcium-dependent. This is a misnomer in that the Ca²⁺ is the object of the enzyme's action rather than the cofactor for activity. The ATPases comprise a large group of membrane-bound enzymes that either pump Ca²⁺ from the cytosol into the endoplasmic reticulum or expel Ca from the cell through membrane channels.

Micro (Trace) Minerals

Iron

Most iron enzymes engage iron either as heme or as a special arrangement of iron with sulfur groups referred to as iron–sulfur centers (Fe_nS_n) (**Table 3**). Iron in heme bears a striking resemblance to magnesium ion in chlorophyll (**Figure 2**). Heme, basically a porphyrin ring system with iron positioned in the center, is the most common form of iron in biological proteins. In cytochrome *c*, a common heme protein in the mitochondria, the axial ligands to the iron are occupied by histidine and methionine from the protein. Heme enzymes include *calalase* and *peroxidase*. As components of iron–sulfur centers, iron enters into multiple cluster arrangements with cysteine residues on enzymes that offer a more direct contact with the protein. These centers differ in their complexity from the simple 2Fe–2S to the more elaborate 4Fe–4S (**Figure 3**).

Iron in these centers binds substrates as well as transfer electrons and takes part in reactions involving dehydrations and rearrangements. Enzymes with iron–sulfur centers include *xanthine oxidase*, *succinate dehydrogenase*, *aconitase*, and *nitrogenase*. A third class, represented by *ribonucleotide reductase* has an FeO_2 cluster with a dioxygen as a peroxide-anion O_2^{2-} straddled between two iron centers (Figure 4). This arrangement allows the enzyme to remove a hydrogen atom from a very stable C–H bond. No metal can replace iron in these complexes. Enzymes with a heme group generally are reddish-brown in color (depending on the oxidation state of the iron). The color led to early interest in these proteins and was the motivating factor behind naming heme proteins in the mitochondria ‘cytochromes’. Although only a relatively few soluble enzymes have iron as a cofactor, iron is especially prominent in membrane-bound proteins that comprise electron transport pathways. Examples of the latter include the cytochromes in the mitochondria, endoplasmic reticulum, and photosystems I and II in chloroplasts. Perhaps the most unusual iron protein is ferritin, a huge multisubunit iron storage protein that has the capacity to bind more than 2500–5000 iron atoms in its structure.

Table 3 Important iron enzymes

Enzyme	Source	Function	Form of Fe
Cytochrome <i>c</i> oxidase	Mitochondria	Electron Transport	Heme
Aconitase	Mitochondria	Krebs Cycle	Fe_4S_4
Succinate dehydrogenase	Mitochondria	Krebs Cycle	Fe_4S_4
Catalase	Peroxisomes	H_2O_2 destruction	Heme
Peroxidase	Peroxisomes	Peroxide destruction	Heme
Prolyl hydroxylase	Cytosol	Collagen Synthesis	Fe^{2+}
Ribonucleotide reductase	Cytosol	DNA Synthesis	Fe-O-Fe
Cytochrome P450	Microsomes	Sterol Synthesis	Heme

Reactivity

The redox property of iron carries over to much of its chemistry as a cofactor. Iron is nearly always involved with the transfer of electrons and many times donates the electrons to a molecule of oxygen. Two important properties that fit that role are: (1) an iron atom can readily undergo reversible valence changes from Fe^{2+} to Fe^{3+} , which allows facile exchange of

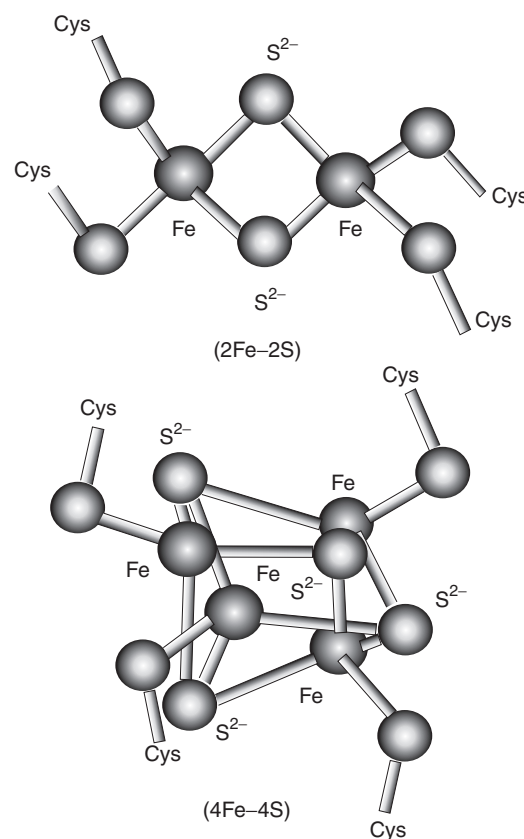


Figure 3 Iron–sulfur clusters. Both the Fe_2S_2 and Fe_4S_4 clusters are bound to the protein via cysteine residues. The iron in these complexes either engages a substrate or holds and passes electrons.

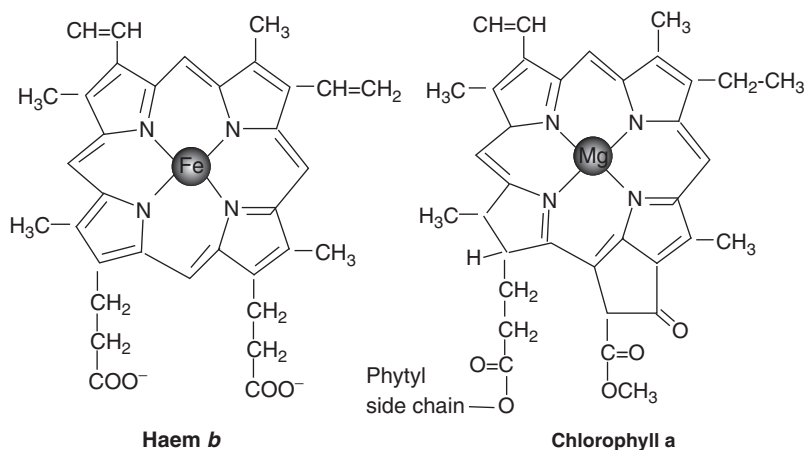
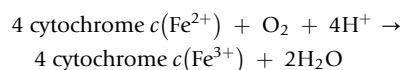


Figure 2 Heme iron in hemoglobin. Heme is a porphyrin ring with iron in the center. Four heme b groups are present in hemoglobin, the iron protein in erythrocytes. A similar structural arrangement is seen with magnesium in chlorophyll a from plants.

electrons, and (2) the ferrous–ferric ion pair has a relatively low electrochemical potential (-0.1 V), which allows iron to be on the high (reducing) end of an electron transport chain. In cytochrome P450 a single oxygen atom is transferred to the substrate after O_2 binds to $Fe(II)$. In the mechanism the $Fe(II)-O_2$ complex is converted into FeO , which features an $Fe(V)$ species that attacks the substrate and incorporates the single oxygen atom into its structure. Although higher valence states such as $Fe(IV)$ and $Fe(VI)$ are formed by the loss of additional $3d$ electrons, only rarely are these higher valences of Fe seen in biological systems. As noted above, *catalase* and *peroxidase*, two heme enzymes, use iron to engage dangerous oxidants. Both enzymes are located in the cytosol and in peroxisomes where harmful oxidation reactions occur during the course of normal metabolic events. Perhaps the most familiar iron-containing enzyme is *cytochrome c oxidase*, the terminal electron acceptor in the mitochondrial electron transport chain and the enzyme capable of splitting a molecule of oxygen to form water.



Zinc

Zinc is perhaps the most ubiquitous and versatile of all metal cofactors. Sequencing the human genome exposed more than 900 proteins that have zinc-binding domains in their structure. Not all of these proteins function as enzymes, however. Hence, it is wrong to say zinc is a cofactor for 900 enzymes. Some of the better characterized zinc enzymes are shown in Table 4.

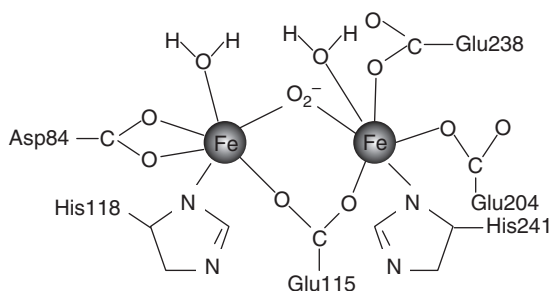


Figure 4 Fe–O–Fe center in ribonucleotide reductase. The two iron atoms are in close juxtaposition to bind dioxygen as the peroxide-anion O_2^{2-} . Side chains of aspartic and glutamic acid residues as well as two histidine residues assist in linking the center to the protein. The center assists in the formation of a free radical that forms on a neighboring tyrosine residue (after Fraústo da Silva and Williams).

Zinc-binding proteins that engage DNA, the so-called zinc finger proteins, are examples of nonenzyme protein but nonetheless proteins whose function in a noncatalytic way is dependent on zinc. Approximately 3% of the genome of mammals codes for zinc finger protein. As a cofactor, zinc can perform both structural and catalytic functions. In *carbonic anhydrase*, for example, Zn enters into a coordinate bond with the CO_2 substrate (Figure 5). In *carboxypeptidase*, zinc takes an active part in the cleavage of the peptide bond (Figure 6). Multisubunit enzymes such as *aspartate transcarbamylase* use Zn to coordinate the positions of the catalytic and regulatory

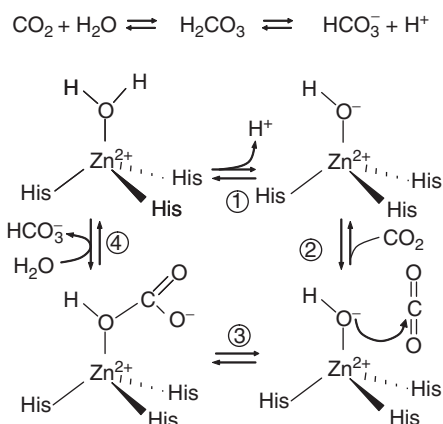


Figure 5 Zinc in carbonic anhydrase. Zinc in the enzyme ‘activates’ a water molecule (1) creating a better nucleophile to attack the CO_2 (2). Once formed (3) the hydrated CO_2 as HCO_3^- is displaced from the enzyme via a second water molecule (4) regenerating the active enzyme.

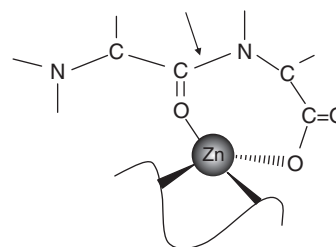


Figure 6 Zinc in carboxypeptidase. In carboxypeptidase, zinc atom forms a binary complex with groups on the C-terminal end of the protein. Arrow shows that bond that will be cleaved with water. Only the C-terminus residue is released from the protein.

Table 4 Important zinc enzymes

Enzyme	Source	Function	Zn/protein
Alcohol dehydrogenase	Liver	Alcohol metabolism	4
Alkaline phosphatase	Placenta	Unknown	4
Carbonic anhydrase	Erythrocyte	CO_2 hydration	1
Carboxypeptidase	Pancreas	Protein catabolism	1
Glutamate dehydrogenase	Liver	Glutamate synthesis	2–6
Leucine aminopeptidase	Intestine	Peptide catabolism	4–6

Table 5 Important copper enzymes

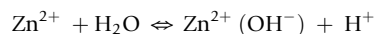
Enzyme	Source	Function	Cu/protein
Ascorbate oxidase	Squash	Ascorbate catabolism	8
Ceruloplasmin	Plasma	Iron oxidation	6–7
Cytochrome <i>c</i> oxidase	Mitochondria	Electron transport	2
Dopamine- β -monooxygenase	Adrenal	Noradrenaline synthesis	8
Lysyl oxidase	Aorta	Collagen, elastin synthesis	1
Superoxide dismutase	Erythrocyte	Superoxide radical destruction	2

subunits, a structural role. Cu_2 , Zn_2 superoxide dismutase requires zinc to position the copper atom in the channel accessed by the substrate HO_2^- , another structural role. In zinc finger proteins, Zn^{2+} contributes to the stability of the loop structure that contacts the major and minor grooves of DNA. These examples illustrate why zinc is an important companion to enzymes and proteins.

One of the lesser known, and perhaps less appreciated functions of zinc is that of a neurotransmitter that modulates neural activity in the brain. Although not to be considered a cofactor role because no enzyme appears to be involved in the action, zinc ions amass in synaptic vesicles and are released into the synaptic junction of glutamatergic neurons. Releasing zinc tends to modulate the activity of the neurons and thus control the impact of the glutamate.

Reactivity

Zinc is considered a bland metal because it behaves as a divalent cation with weak geometric preference. It is perhaps this blandness that allows zinc to adapt to so many different enzyme environments. Zn exists in one valence state, Zn^{2+} , and hence cannot donate or accept electrons. Zn^{2+} ion is configured as a $3d^{10}$, which denotes a filled $3d$ orbital. For that reason, zinc complexes lack color and zinc itself behaves mostly as a cation. The Zn^{2+} ion is capable of recognizing electron pairs (typical of a Lewis acid) and thus can enter into a coordinate bonding arrangement that polarizes groups to which it binds. This property allows zinc to increase the susceptibility of a chemical bond to attack. For example, Zn^{2+} polarizes water, which makes the water behave more like hydroxide ion and be more effective in attacking the CO_2 to form HCO_3^- :



in the reaction catalyzed by *carbonic anhydrase*. Another example is the use of zinc to polarize the ester or amide bonds thus promoting nucleophilic attack of water on the bond as in reactions catalyzed by *carboxypeptidase* and *aminopeptidase*.

Copper

Copper, like iron, can donate and accept electrons. Thus like iron, Cu exists in multiple valence states; Cu^+ and Cu^{2+} (cuprous and cupric) are the most stable. Copper enzymes, although not nearly as numerous as zinc, fill a variety of important biological functions mostly with membrane-bound enzyme (Table 5). A common denominator to these, however, is the donor–acceptor electron fit the category of oxidoreductases, or more specifically ‘oxidases’, meaning they

catalyze reactions in which electrons from the substrate are transferred to O_2 . Copper enzymes can be simple or complex, depending on the number of Cu atoms in the enzyme. Simple enzymes generally contain one Cu per subunit. The more complex include the multicopper oxidases, which may have as few as four, e.g., *laccase*, or as many as eight copper atoms per enzyme e.g., *dopamine- β -monooxygenase*. Copper in these enzymes exists in three different chemical environments referred to as type 1, type 2, and type 3 copper sites. *Ceruloplasmin*, for example, contains 6–7 Cu atoms in three distinct sites. The type 1 copper site gives a blue color to ceruloplasmin and other blue Cu proteins. The copper-binding sites in a multicopper oxidase form a triad consisting of one type 2 and two type 3 coppers arranged as an isosceles triangle. Oxygen binds to the two type 3 coppers at the base of the triangle. Examples of copper enzymes include *cytochrome *c* oxidase*, *lysyl oxidase*, and *ascorbate oxidase*.

Reactivity

Because it is prone to accept electrons, Cu is a powerful oxidant in biological systems. The Cu sites in ceruloplasmin have the capacity to oxidize Fe^{2+} to Fe^{3+} , which prepares ferric ions to bind to transferrin and delivers iron to the organs and tissues. This reaction links iron with copper metabolism and could explain how an absence of copper in the diet impairs the transport of iron and causes anemia in humans. In Cu_2 , Zn_2 superoxide dismutase, the Cu^{2+} at the active site removes the single nonbonding electron from one superoxide anion (O_2^-) and transfers it to another:



Seldom is copper destined to perform only a structural role and many enzymes that possess copper as a cofactor use the metal at the active site. More recent studies have linked Cu ions with the formation of blood vessels, or angiogenesis. One of the more exciting discoveries yet to be fully understood is that depriving an animal or human of Cu delays or even inhibits the growth of cancerous tumors. From a nutritional perspective, this could mean that Cu is essential for the development of the microvascular system.

Manganese

Whereas zinc may be the most common transition metal in enzymes, manganese is perhaps the least common. Part of the reason is that complexes of manganese with proteins tend to be weakly stable with a tendency to dissociate. Notable manganese metalloenzymes include *pyruvate carboxylase* and *manganese superoxide dismutase* in the mitochondria and *arginase* in the

urea cycle. Manganese can also function as a metal-activating cofactor for many enzymes that require magnesium.

Reactivity

Although manganese is not considered a redox metal based on reactivity, it nonetheless can exist in six oxidation states (Mn^{2+} to Mn^{7+}) three of which (Mn^{5+} to Mn^{7+}) are not seen in biological systems. The most common form of manganese is Mn^{2+} . The highest number of multiple valences of manganese occur in the *water splitting enzyme* that is found in chloroplasts of plants as part of photosystem II.

Cobalt

The role of cobalt as a cofactor is limited to its presence in vitamin B_{12} . Cobalt can exist in three valence states, Co^+ , Co^{2+} and Co^{3+} with Co^{2+} being the most common in 5'-deoxyadenosylcobalamin, the familiar form of vitamin B_{12} coenzyme. Cobalt is bound by a planar ring system analogous to heme but with very special features. Cobalt (and nickel) are ions that may have figured more prominently in primitive systems when the atmosphere contained H_2 and CH_4 as common environmental gases. The argument has been made that as biological system gradually adapted to O_2 the necessity for these two metals became less.

Reactivity

Cobalt in the structure of vitamin B_{12} resembles iron in heme by being bound in a square planar arrangement to a ring (corrin). Unlike heme, however, cobalt has two axial ligands that are free from the protein, which allows nonprotein groups to access the central metal from above and below the plane. In the octahedral complex, one axial position (the fifth coordinate) is normally occupied by a benzimidazole and the other by a methyl group (as in methyl cobalamin). The arrangement is unique and allows cobalt to form carbon-metal bonds with the potential for two different reactivities. The methyl group, for example, may be removed as a carbonium ion retaining both electrons on the cobalt, which then reverts to a less stable Co(I) . This is typical of the reaction in which B_{12} acts as a methyl donor. In positional rearrangements, cobalt retains only one electron and forms a stable Co(II) or d^7 ion with the release of a free radical. Free radicals are highly reactive and overcome energy barriers that would stymie other reactants. Thus, cobalt's chemical properties transfer groups as carbonium ions or highly reactive carbon-centered radicals. Both products are possible and hence explains the necessity for Co as a cofactor for a reaction that precede via a free radical mechanism. An example of the latter is the intramolecular rearrangement of methylmalonyl-CoA to succinyl-CoA as catalyzed by *methylmalonyl-CoA mutase*.

Vanadium

A well defined biochemical function for vanadium in higher animals and humans is yet to be described. Recent reports of vanadium in bacteria and algae have provided clues as to the functional necessity of this metal in enzyme catalysis. Approximately 10 years ago, vanadium was found to be essential for the activity of *bromoperoxidase*, an enzyme found in brown and red algae. Shortly thereafter, a vanadium-dependent *iodoperoxidase* was characterized. Vanadium was also found in

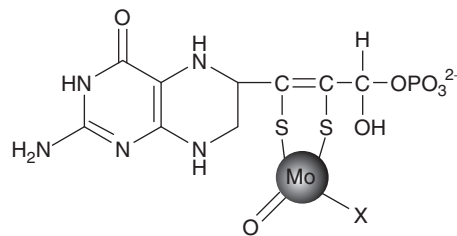


Figure 7 Proposed structure for the molybdenum cofactor in nitrogenase. This center consists of a special pterin cofactor, a relative of tetrahydrofolate. The molybdenum engages two sulfur atoms as a dithiolate complex.

high concentrations in mushrooms and was shown to accumulate in large quantities in ascidians, specifically the blood cells (vanocytes) of these organisms. Speculation as to function of vanadium in microorganisms range from antimicrobial action to electron transfer and the trapping of oxygen. In higher animals, however, vanadium has been shown to have insulin-mimetic properties and to stimulate cell proliferation and differentiation. It is also believed to regulate phosphorylation and dephosphorylation reactions through control of ATPases, phosphatases, and adenylylase that have wide spread effects on cell functions. These are the most plausible understandings to date. However, it should be emphasized that vanadium has not been shown to be a specific activator (or inhibitor) of any enzyme in humans.

Reactivity

Vanadium is like molybdenum in being able to form both oxyanions and oxycations, VO_4^{2-} (MoO_4^{2-}), VO_2^+ (MoO_2^+ , MoO_2^+ , and MoO_2^{2+}) and sulfur centers as well, e.g., VS_4^{3-} (MoS_4^{2-}). Vanadate differs from molybdate in being a rather strong oxidizing agent, $E \sim +.5 \text{ V}$ at pH 7, which may relate to its electron transfer function in lower life forms but has questionable significance in humans.

Molybdenum

Molybdenum is widely distributed in plants and animals. The metal exists in three valence states, Mo^{4+} , Mo^{5+} , and Mo^{6+} . A limited number of redox reactions exploit the multivalence states. Molybdenum-dependent enzymes are found in pathways that metabolize purines, pyrimidines, pterins, aldehydes, and sulfites. A cofactor structure for molybdenum has been proposed (Figure 7) and referred to as molybdopterin. Enzymes that use the cofactor include *xanthine oxidase*, *sulfite oxidase*, and *aldehyde oxidase*. In microorganisms, molybdenum is a key metal for the fixation of nitrogen. *Xanthine oxidase* is the enzyme with importance relevance to a mammalian system.

Reactivity

A major nutritional concern of molybdenum is its ability to antagonize copper. Indiscriminant spraying of soils with molybdenum has been shown to affect the growth and productivity of ruminants. The effect relates to the formation of thiomolybdates in the rumen. The thiomolybdates interact and bind copper preventing its absorption from the rumen. Thiomolybdates have a very high affinity for copper almost to

the exclusion of other metal ions. Lately, thiomolybdates have been used to control copper toxicity in Wilson's disease, a genetic disease of copper poisoning in humans.

Nickel

As a cofactor, nickel occurs infrequently. About the only known occurrence of nickel is in microbial and plant enzymes such as *urease* from jack bean, soybean, rice, and tomatoes. There are roughly two gram-atoms of nickel per mole of the 96 000 dalton subunits of the enzyme. Other metalloenzymes containing nickel include Factor F430 found in the membrane of methanogenic bacteria, *carbon monoxide dehydrogenase* and *hydrogenases I and II*. Nickel has drawn the attention of nutritionists because of the observation that nickel concentrations in serum of women rise sharply immediately after parturition.

Reactivity

Some consider nickel the 'metal that was'. As biosystems evolved and moved from an atmosphere of no oxygen to one rich in oxygen, where methane and H₂ have tended to be minimized as energy substrates, metals that formed a major cofactor in the anaerobic environment and used by the more primitive organisms such as archaebacteria have been replaced in favor of a metal or cofactor more suitable to the present day environment. Thus, nickel, like cobalt may have had its greatest era in enzymes that catabolized CH₄ or H₂.

Other

Although metals such as Cr, Sn, As, and Sr are known to be essential for optimum growth and health of organisms as well as having a major influence on biological systems, cofactor functions for these metal ions have not been assigned because specific enzymes which may require them for activity have not been found.

Nonmetal Mineral Cofactors

Selenium

Selenium belongs to the category of redox nonmetals. Selenium is included in the same class with sulfur (sometimes referred to as metalloids), which implies that selenium should be able to substitute for sulfur in biological complexes. As a congener of sulfur, selenium becomes part of a protein's structure as selenocysteine and selenomethionine, not as a selenium atom ligated directly to the protein as a prosthetic group. The former are the active cofactors in selenium enzymes.

Reactivity

Although a selenium ion is clearly capable of redox reactions, there is still little information available as to how selenium functions as a cofactor. Enzymes such as *glutathione peroxidase* are soluble enzymes that transfer electrons to and from substrates. Replacing the selenium with sulfur in the enzyme negates the activity. With only a few selenoenzymes available, there is little information as to the precise catalytic role of selenium. *Glutathione peroxidase* in the reduced (resting) form is believed to contain an ionized selenol that can react with

either organic peroxides or H₂O₂ according to the reaction (a) shown below:

a selenol enzyme is



also believed to be an intermediate in the reaction (b) catalyzed by *5'-deiodinase*, the enzyme that catalyzes the removal of a single iodine atom from thyroxine, the major thyroid hormone. This T₄ to T₃ transition gives rise to the more active form of the hormone. Because the *5'-deiodinase* activity is suppressed in an iodine deficiency, there have reports of goiter-like conditions being manifest in people with low levels of selenium in the diet.

Silicon

There is still some question as to whether silicon is a cofactor. It is included here because of the importance of silicon in a number of biochemical reactions leading to the synthesis of glycoproteins and polysaccharides in the extracellular matrix of connective tissue ground substance. Silicon as Si(OH)₄ is very abundant in soils and minerals and is as common in human tissues as magnesium. In plants, especially grasses, silicon is a major component of a mineral skeleton and has a metabolic turnover nearly on a par with carbon. In humans, the highest concentrations of silicon occur in connective tissues such as aorta, trachea, tendon, bone, and skin. Lesser amounts are found in liver, heart, and muscle. The epidermis and hair are significantly high in silicon.

Reactivity

Silicon, as silicic acid, has been shown to be required for maximal activity of *prolyl hydroxylase*, the enzyme that converts proline residues to hydroxyproline in collagen. High levels (0.2–2.0 mM) are needed to stimulate the enzyme, which catalyzes a rate-determining factor in collagen biosynthesis.

Boron

Manipulating the boron content of a diet leads to a wide number of metabolic responses, which is testament to the potential importance of boron in human nutrition. Early studies reported increased levels of steroid hormones, testosterone, and estradiol in animals supplemented with boron. Further studies suggest that boron has a regulatory role in the metabolism of other minerals such as calcium and may affect bone metabolism. In a comparative way the role of boron is well established in vascular plants, diatoms, and marine algal flagellates. Zebra fish deprived of boron tend to suffer developmental defects. These data have impelled investigations into the biological functions of boron in higher vertebrates. To date, however, few studies have supported boron's essential role in vertebrates. In a comparison to Zebra fish, pregnant rats fed one-fiftieth the level of boron as control rats exhibited no impairment in fetal growth or development. Fewer two-cell embryos from the deficient rats, however, reached the blastocyst stage when cultured *in vitro*, suggesting boron deprivation did have an impact at a very early stage of development.

Perhaps the strongest holdup to accepting boron as essential is the failure to define and link a specific organoboron compound with a physiological function. A report of boron associated with a naturally occurring antibiotic is an exception. The data, however, tend to support the notion that boron complexes with biological components are too unstable to be isolated and studied. This clearly has put a damper on the forward thrust of knowing boron's precise metabolic function.

Conclusions

The mineral cofactors described here may be thought of as representing a special subset of the biominerals. Rather than contributing to skeletal mass and fluid homeostasis, however, mineral cofactors are more subtle and are devoted specifically to enzymes. The words 'mineral' and 'cofactor' combine to designate an inorganic component required by an enzyme in order to achieve optimum catalytic efficiency. In seeking a reason for mineral cofactors, one must consider that to meet its functional obligations, an enzyme faces many challenges. The protein surface can easily be modified chemically through interaction with substrates and the enzyme protein can readily lose its biological form through denaturation. Electrons and groups that are transferred to and from substrates have the potential to permanently modify the enzyme. This happens frequently and instead of undergoing repair, old enzymes are replaced by new ones. The mineral cofactors fit into the daily wear and grind by making the enzyme better able to stand up to the harsh environment of their existence. They also have been shown to be effective binders of substrate and to interact with oxidants and reductants in a facile manner. Some trace metals such as Zn can accept electron pairs in forming a covalent attachment that polarizes and facilitates rupture of the chemical bonds in the substrate. Other metals such as copper and iron can accept electrons from the substrate and pass them to oxygen. Catalysis and structure stability are the two primary functions of metals in enzymes. Many organic

factors serve as electron-capturing and group-transferring agents. This suggests that metalloenzymes may backup enzymes with organic cofactors. This view is rather narrow and oversimplified because there are many enzyme-catalyzed reactions where only a metal will suffice, such as in the metalloenzymes that catalyze the destruction of oxygen radicals. In biology seldom does one factor become indispensable. What nutritionists refer to as essential metals are on the same level as vitamins in that they are needed in very small quantities to maintain a status quo system and, like vitamins, available strictly through the diet. One must, therefore, conclude that essential minerals and vitamins have common ground in the enzymes, which they literally permit to function.

See also: Cofactors: Organic

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Organic

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Glossary

Carbanion ion A carbon ion with a negative charge.

Hydride ion A hydrogen atom with two electrons.

Mitochondrion A small sac-like energy producing organelle in a cell.

Introduction

Cofactors are important accessories to biochemical processes. Generally present as small organic compounds or metal ions, cofactors empower enzymes to function at maximal catalytic effectiveness or endurance. A related term, coenzymes, refers to a subgroup of cofactors whose structure in part is derived from water-soluble B vitamins. Historically, cofactors were often inadvertently removed during purification and had to be added back to restore enzyme activity. Today, we regard a cofactor as an obligatory component of the catalytic mechanism. Compounds meeting the criteria are either (1) small organic molecules that bind directly to the enzyme surface forming an active site for the substrate to bind or interact, or assist in these events indirectly, or (2) inorganic ions that bind to specific groups on an enzyme surface and aid in substrate binding, catalysis, stabilizing the transition state, or contributing to the overall stability of the enzyme's structure. Practically speaking, any substance in an assay medium that promotes the catalytic activity or stability of an enzyme is a candidate for its cofactor.

As will be illustrated in this and the following article, cofactors are indispensable adducts of the catalytic machinery of the body and have provided nutritionists with the strongest insights into the essential role of vitamins and trace elements. It is still fashionable to consider coenzymes as vitamin derivatives that bind loosely to enzymes or serve as transient active sites. Cofactors and coenzymes are terms that are used interchangeably. It's important to note, however, that the term 'holo-' is used to refer to an enzyme and its coenzyme or cofactor together as a catalytic unit and 'apo-' when either is missing. Apoenzymes are functionless and are of no benefit to the organism.

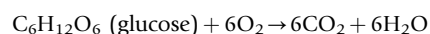
History

Early studies of vitamins found that many, especially the water-soluble B vitamins, formed the nucleus of compounds that partook in enzyme catalysis. The discovery established a bridge between nutrition and the fledgling science of biochemistry. Indeed, many early biochemical investigations were devoted to learning the biological functions of essential nutrients, which included the vitamins. A general principle that emerged at the time was that a vitamin had to be changed to another compound in order to be metabolically functional. With the diet as the only source, it was possible to learn the

specific effects of individual vitamins by omission studies. With deeper insights into biological processes, it was soon realized that canceling an enzyme in a critical biochemical pathway was behind many of the vitamin-deficiency diseases such as beriberi, pellagra, and pernicious anemia. This put dramatic new emphasis on enzyme functions and the search for enzymes that depended on vitamins for function.

Cofactors in Biochemical Pathways

Table 1 lists vitamins and nonvitamins that are known to give rise to many of the organic cofactors in humans. **Figure 1** shows the location of organic cofactors in the biochemical pathway for oxidizing glucose and other biocompounds to CO₂ and H₂O. That overall reaction for glucose is:



One sees that at least seven distinct B vitamin-derived coenzymes are needed to complete the transition. Nicotine adenine dinucleotide (NAD⁺), derived from niacin, is required for the oxidation of glucose to pyruvate and thiamine pyrophosphate (TPP) derived from the vitamin thiamine (sometimes written as thiamin), flavin adenine dinucleotide (FAD) from riboflavin, pantothenic acid from pantothen, and lipoic acid all take part in the oxidation of pyruvate to acetyl-coenzyme A in the middle stage. In addition, flavin mononucleotide (FMN) also from riboflavin and coenzyme Q (CoQ) from ubiquinone take part in completing the oxidation to CO₂ and H₂O in oxidative-phosphorylation pathway in the mitochondria. All told, some 20 organic cofactors engage enzymes in the various biochemical pathways of humans. The section on specific vitamin as cofactors gives a brief description of each cofactor. **Table 2** summarizes the list of key enzymes known to be associated with each coenzyme.

Specific Vitamins as Cofactors

Thiamine (Vitamin B₁)

Best known as the anti-beriberi factor and called at first simply vitamin B by McCollum, thiamine was shown to be involved in the decarboxylation of pyruvate to acetaldehyde in alcohol fermentation and was named 'cocarboxylase' in 1932. Confirmation of its structure as TPP came five years later. Its name is meant to signify a vitamin containing sulfur (*thios* in Greek).

Table 1 Vitamin and nonvitamin cofactors

Name of vitamin ^a		Related coenzymes	Biochemical function
<i>Vitamin cofactors</i>			
Thiamine, thiamin	B ₁	TPP	Carbonyl group transfer
Riboflavin	B ₂	FMN, FAD	Redox reactions
Niacin (nicotinamide)	B ₃	NAD, NADP	Redox reactions
Pantothenic acid	B ₅	Coenzyme A	Acyl group transfer
Pyridoxine	B ₆	Pyridoxal-5'-phosphate	Amine group transfer
Folic acid (Folacin)	B ₉	Tetrahydrofolates	One-carbon transfer
Cobalamin	B ₁₂	5'-Deoxyadenosyl cobalamin	Methylation, rearrangement reactions
L-Ascorbic acid	C	Dihydroascorbate	Collagen, adrenaline synthesis
Calciferol	D	None	Calcium absorption
Tocopherol	E	None	Antioxidant
Biotin	H	Biocytin	CO ₂ fixation
Phylloquinone	K	None	Prothrombin synthesis
Bioflavonoids	P	None	Antioxidant
<i>Nonvitamin cofactors</i>			
p-Aminobenzoate		Tetrahydrofolate	One-carbon transfer
α-Lipoic acid		None	Acetyl group transfer
Betaine		None	Methylating agent
CoQ		Ubiquinone	Electron transfer
PQQ		None	Oxidation reactions
Topa quinone		None	Oxidations reactions
Carnitine		None	Fatty acid transfer
Inositol		None	Membrane lipids
S-adenosyl methionine		None	Methylation reactions
Glutathione		None	Reductions and group transfer
3'-Phosphoadenosine-5'-phosphosulfate		None	Sulfate esterification

^aAlthough codified in vitamin literature at one time, B₄, B₁₀, and B₁₁ have since been abandoned.

Reactions

1. Pyruvate dehydrogenase complex in mitochondria.
2. α-Ketoglutarate dehydrogenase complex in mitochondria.
3. Branch-chain dehydrogenase.
4. Transketolase reactions in pentose pathway and in reductive pentose pathway of photosynthesis.

Reactivity

The structure of thiamine has two rings bridged by a methylene group as seen in **Figure 2a**. The coenzyme (TPP) arises via an ATP-dependent pyrophosphorylation of the primary alcohol group (**Figure 2b**). What may be called the active site of the coenzyme is the carbon in position 2 (C-2) of the smaller five-member thiazolium ring (arrow). A favorable positioning of C-2 between atoms of nitrogen and sulfur cause C-2 hydrogen to exchange protons with water, indicating C-2 can ionize to a carbanion. As a carbanion, C-2 is able to engage positive centers such as carbonyl carbons of α-keto acids and keto sugars. In the reaction with pyruvate, α-ketoglutarate, or branch-chain α-keto acids from valine, leucine, or isoleucine, a carboxyl group is expelled as CO₂ and the electrons remain with the 'active aldehyde' on the C-2 position. Attack on a keto sugar cleaves the first two carbons as a unit, which then attaches to C-2 as an 'active glycoaldehyde' adduct. Yeast disengage active aldehyde as acetaldehyde later to be reduced to ethanol by alcohol dehydrogenase. Bacteria convert 'active

aldehyde' to acetyl-phosphate. In the mitochondria of higher organisms, however, active aldehyde is oxidized by an FAD-containing enzyme (part of the pyruvate dehydrogenase complex) and transferred to lipoic acid (see **Figure 10**), which transfers the highly energetic acetyl group to the thiol group of coenzyme A. As a coenzyme for transketolases in the pentose pathway, TPP takes part in the formation of ribose-5-phosphate, glyceraldehyde-3-phosphate, and erythrose-4-phosphate from sedohepuloose-7-phosphate, xyulose-5-phosphate, and fructose-6-phosphate, respectively. Each sugar phosphate donates an 'active glycoaldehyde' to an aldose acceptor.

TPP is also the coenzyme for branch-chain dehydrogenase, the enzyme that catalyzes the oxidative decarboxylation of α-keto acids derived from leucine, isoleucine, and valine, three essential amino acids. The reaction follows a scheme similar to pyruvate oxidation, only this time the carbon skeleton of the amino acid condenses with CoA.

Riboflavin (Vitamin B₂)

The first hint that McCollum's Vitamin B was in reality a multifactor complex came when yeast void of antineuritis activity still retained growth stimulating activity. Originally called vitamin G, riboflavin was renamed vitamin B₂ when it was recognized to be part of the yeast B complex. The name riboflavin followed the discovery in 1935 of its association with green fluorescent pigment of whey. Today, we regard riboflavin and niacin as the two principal vitamins that give

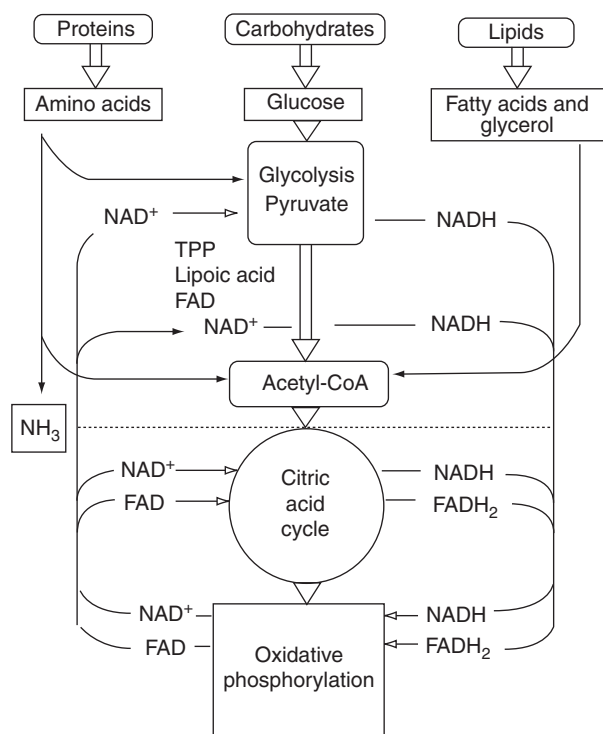
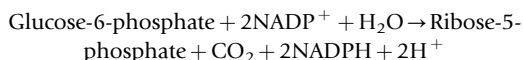


Figure 1 Occurrence of organic cofactors in the glucose oxidation pathway. Only a few key intermediates in the pathway are shown. Components below the dotted line represent reactions taking place in the mitochondria.

rise to coenzymes that function with enzymes known as oxidoreductases. Both coenzymes transport electrons to and from substrates and in so doing form oxidized or reduced products. The two are referred to as 'redox' (an abbreviation for oxidation–reduction) coenzymes for that reason. Riboflavin was identified as the biochemical compound that gave the color to Warburg's 'yellow enzyme', glucose-6-phosphate dehydrogenase (G6PDH). G6PDH was observed to catalyze the transfer of electrons from nicotinamide adenine dinucleotide phosphate (NADPH) to methylene blue, a redox sensitive dye, which lost color on reduction, suggesting that riboflavin probably mediated the electron exchange. G6PDH is a key entrance point for glucose into the pentose pathway and a major contributor of NADPH for the biosynthesis of fatty acids and other fats. The reaction is:



Riboflavin (**Figure 2b**) is associated with two coenzymes, FMN and FAD. FMN is formed by phosphorylating the primary alcohol on the sugar moiety of riboflavin, an ATP-dependent reaction. FAD results from a further condensation of FMN with the 5'AMP moiety of ATP (**Figure 2e**). What may be considered the active site is the isoalloxazine ring, which can exist in both oxidized and reduced states depending on whether electron pairs are absent or present, respectively. Enzymes that contain FAD or FMN are referred to as flavoproteins. FMN is limited to the membrane proteins of the mitochondria electron transport system whereas FAD is found

Table 2 Sample of enzymes associated with each of the coenzymes derived from vitamins

Coenzyme	Enzyme
1. Thiamine pyrophosphate	Pyruvate dehydrogenase complex α -Ketoglutarate dehydrogenase complex Transketolase
2. NAD^+ , NADH	Branch-chain dehydrogenase Glyceraldehyde-3- PO_4 dehydrogenase Pyruvate dehydrogenase complex Alcohol dehydrogenase Lactate dehydrogenase
3. NADP^+ , NADPH	Glucose-6- PO_4 dehydrogenase Glutamate dehydrogenase β -Ketoacyl-ACP synthase
4. FAD, FADH_2	Glucose-6- PO_4 dehydrogenase Succinate dehydrogenase Fatty acyl-CoA dehydrogenase
5. Pyridoxal-5'-phosphate	Amino transferases Glycogen phosphorylase
6. Tetrahydrofolate	Glycine synthase Homocysteine methyltransferase
7. Biocytin	Pyruvate carboxylase Acetyl CoA carboxylase Propionyl CoA carboxylase
8. Coenzyme A (pantothenic acid)	Pyruvate dehydrogenase complex Acetyl-CoA carboxylase Citrate synthase
9. Cobalamin	Homocysteine methyltransferase Methylmalonyl-CoA mutase
10. L-Ascorbate	Prolyl and Lysyl hydroxylase Dopamine- β -monooxygenase

in both membrane-bound and soluble enzymes. The flavin cofactor is bound covalently to the structure preventing disengagement during purification procedures.

Reactions

Flavin enzymes are designed to remove (and add) electrons to and from substrates. In general, flavin coenzymes are stronger oxidizing agents than the pyrimidine coenzymes (NAD^+ , NADP^+) and tend to participate in more complex reactions. Also, flavin coenzymes can accept single electrons from a donor, forming a semiquinone and allowing flavoproteins to take part in reactions that form free radicals. Having a single electron also allow favins to bind molecular oxygen as a hydroperoxyl complex.

Niacin (Nicotinic Acid, Nicotinamide)

Niacin presents an unusual twist in that its parent compound, nicotinic acid, had been known for approximately 70 years (*ca.* 1867) before its activity as a vitamin first became known (*ca.* 1937). If thiamine (B_1) is the anti-beriberi factor, niacin (B_3) is the anti-pellagra factor. Pellagra is a disease characterized by a rash or dermatitis on areas of the skin exposed to sunlight as well as swelling in the legs from the knee on down and a painful flush and rash. Niacin, also called nicotinic acid or nicotinamide, its amide derivative (**Figure 2c**), is the active component of the second major

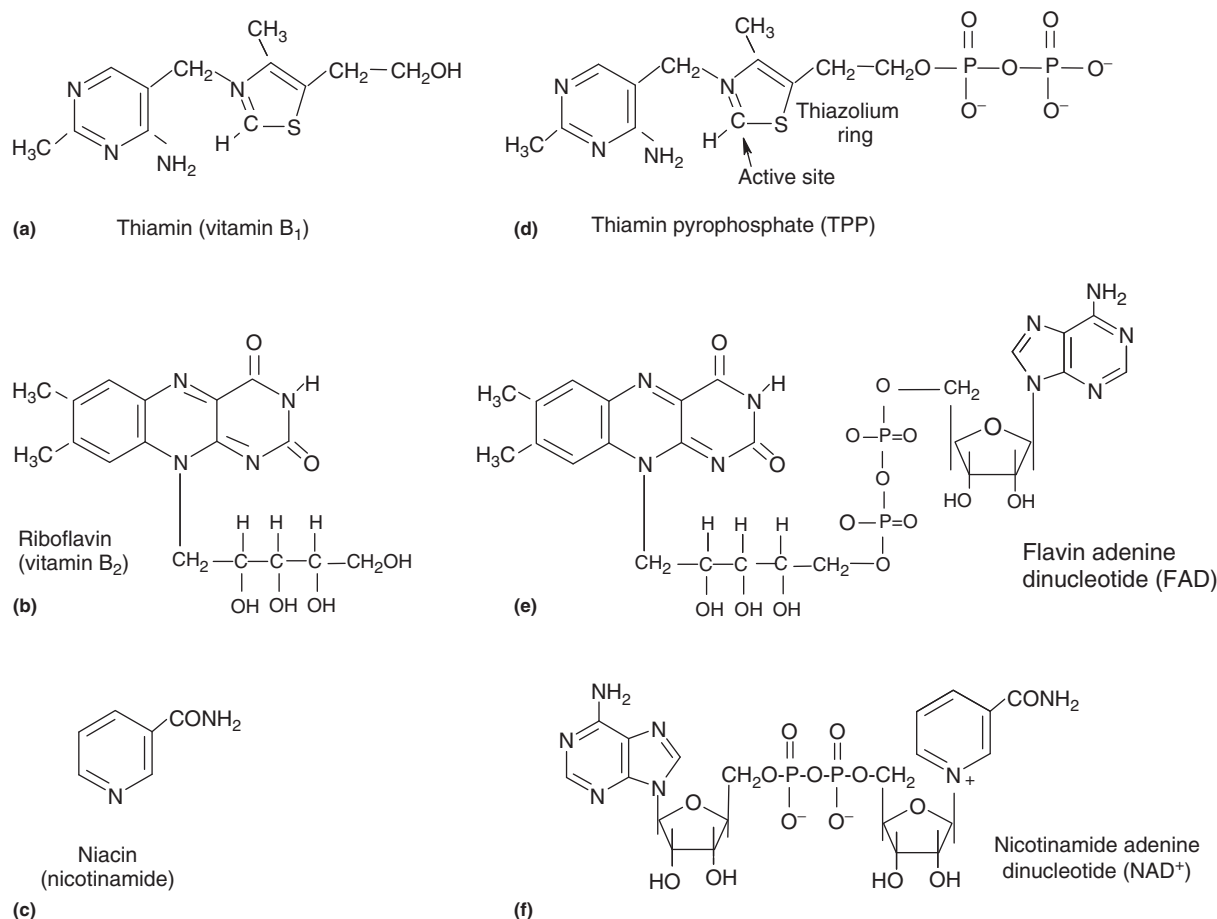


Figure 2 Structural relationship of vitamin-coenzyme for thiamine (B₁), riboflavin (B₂), and niacin. On the left is the structure of the vitamin. Note the prevalence of phosphate groups in (a) thiamine and phosphate and adenosyl groups in the coenzyme forms for (b) riboflavin and in (c) niacin, indicative of a reactions requiring ATP.

redox coenzyme nicotinamide adenine dinucleotide and its phosphate (NAD⁺ and NADP⁺, respectively, **Figure 2f**). As with FMN and FAD, NAD⁺ and NADP⁺ arise by phosphorylation and condensation of the basic vitamin structure with ATP. NAD⁺ differs from NADP⁺ by having a phosphate group on the 3' position of the pentose nearest to the adenine. NAD⁺ and NADH were discovered in dialyzable extracts of yeast, meaning these coenzymes readily disengaged from the enzyme that bound them. The ability to come on and come off an enzyme is fundamental to the electron delivery scheme shown in **Figure 1**.

Reactivity

With few exceptions, the redox related reactions of NAD⁺ and NADP⁺ are with dehydrogenase enzymes i.e., enzymes that catalyze the removal and addition of electrons (as hydride ions) to substrates. A typical reaction in which NAD⁺ is the oxidizing agent is the conversion of L-lactic acid to pyruvate.



The coenzyme, therefore, is a major participant in energy-yielding catabolic reactions. NADP⁺ performs less of a catabolic role, but its reduced form, NADPH, is a major reductant

in anabolic reactions especially the biosynthesis of fatty acids and other lipids.

The active site of both NAD⁺ and NADP⁺ is the nicotinamide ring. The oxidized form nicotinamide has a quaternary nitrogen (four attaching bonds) that is written as a positive charge (**Figure 2f**). The oxidized ring accepts two electrons and one proton from a substrate (literally a hydride ion, H⁻) reducing the ring and abolishing the positive charge on the nitrogen.



More than 200 enzymes are known to catalyze reactions in which NAD⁺ or NADP⁺ accepts a hydride ion from a substrate. Moreover, there is a strong stereospecificity to this reaction. Addition of an H⁻ to the ring can either be in front (A-type) or in the back (B-type), depending on the enzyme. Reducing the ring weakens its bonding to the enzyme and causes the NADH (NADPH) to dissociate and engage other cell components for the purpose of transferring the electrons.

NAD⁺ is also a source of 5'-adenosine monophosphate (5'-AMP) in a limited series of activation or inhibition

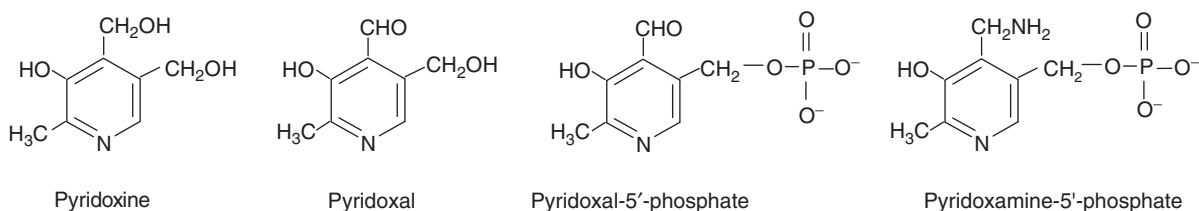


Figure 3 Multiple forms of vitamin B₆.

reactions. The transferred 5'-AMP becomes a leaving group for subsequent bond formation. In DNA ligase in bacteria, for example, the 5'AMP is transferred to a lysine on the enzyme to form an unusual phosphoamide adduct that subsequently is transferred to one of the DNA strands. Attack by the 3' hydroxyl group on the adjacent DNA strand releases the 5'AMP concomitant with forming a phosphodiester bond and sealing the two DNA strands together. A related reaction, referred to as an ADP-ribosylation, results in the nicotinamide group being split from the NAD⁺ and the ribose moiety forming a covalent glycosylamine bond with a protein. ADP-ribosylation of sensitive proteins is one of the deadlier effects of bacterial toxins such as cholera toxin, diphtheria toxin, or pertussis toxin.

Pyridoxine (Vitamin B₆)

Vitamin B₆ was discovered in the 1930s and named pyridoxine because of its structural resemblance to pyridine. Pyridoxine's principal involvement is with a family of enzymes known collectively as amino transferases. These enzymes exchange amine groups from amino acids to α -keto acids. Familiar names include serum glutamate-oxalate transaminase (SGOT). The coenzyme, pyridoxal-5'-phosphate (PLP), is the predominant form and is synthesized in a two-step reaction involving the oxidation of the hydroxymethyl group in the para position on the pyridine ring to an aldehyde, and the phosphorylation of the hydroxymethyl group on the 5 position. PLP is also a coenzyme for glycogen phosphorylase and ornithine decarboxylase. With tetrahydrofolate (see section on Reactivity), PLP takes part in serine to glycine interconversion. On the enzyme surface, the reactive species is not an aldehyde, but rather an aldamine formed by a Schiff base bond between the aldehyde and an ϵ -amino group of lysine in the active site (Figure 3).

Reactivity

The reactivity of PLP is owed to a number of features. First, the carbonyl (aldehyde) on the ring is positioned to engage the amino groups from amino acids and tether the amino acid to the enzyme. Through a series of electron rearrangements promoted by the PLP, the nitrogen on the amino acid substrate is disengaged and the carbon skeleton (an α -keto acid) is set free retaining the amine group on the coenzyme (pyridoxamine-5'-phosphate). The enzyme then binds a second α -keto acid and transfers the amino group generating a new amino acid, and restoring the carbonyl function on the PLP. Other changes can also occur to a tethered structure. An electron rearrangement can result in the loss of a carboxyl group (as CO₂) or a molecule of H₂O. Thus, PLP enzymes also

take part in decarboxylation and dehydration reactions. In the glycogen phosphorylase reaction, the phosphate group of the coenzyme acts as a general catalyst, promoting the attack of phosphate on the glycosidic bond of glycogen.

Folic Acid

Folic acid was first recognized as the yeast or liver factor that cured a severe megaloblastic anemia in chicks, monkeys, and humans. Showing later that the active substance was a growth factor for certain bacteria (*Lactobacillus casei* and *Sterptococcus faecalis*) provided a rapid bioassay for isolating, identifying, and eventually synthesizing the vitamin and its coenzymes. The name folic acid was given in 1941 in recognition of its abundance in leafy green vegetables or 'foliage' and its structure was confirmed as monopterylgutamic acid in 1946. Today, we recognize folic acid as one of our most complex vitamin-coenzymes because of its presence in many biochemical forms. Despite such enormous complexity, however, the biochemical role of folic acid narrows down to a specific set of synthetic reactions whose common denominator is one-carbon units.

The structure of folic acid (N-pteroyl-L-glutamic acid) can be pictured as a composite of three covalently linked molecules: a methylated pteridine ring attached to *p*-aminobenzoic acid (PABA), which in turn is linked via the carboxyl group to the α nitrogen of glutamic acid (Figure 4a). The coenzyme form is tetrahydrofolate (FH₄) formed in mammals by adding four electrons and four hydrogens to the pteridine ring (Figure 4b). The reduction is catalyzed by dihydrofolate reductase with NADPH as the electron donor. The addition of one or more glutamic acid residues completes the structure. In the reductive step, a new asymmetric center is generated at C-6 and appears to be critical to the biological role because only one stereoisomer of this center is active. FH₄ may have up to seven glutamic acid residues and exist in many different chemical forms, most of which are inconvertible.

The basic reactions take part at the N⁵ and N¹⁰ positions on the molecule, which serve as attachment points for one-carbon units in transit (Figure 4c). N¹⁰-formyl- and N⁵,N¹⁰-methenyl-FH₄ are two synthetic forms that are biologically active. Complexes of N⁵-formyl-FH₄ (folinic acid) transfer formyl groups to specific substrates. Active folic acid derivatives have carbon in the oxidation state of formate as well as formaldehyde (methylene) and a methyl derivative, N⁵-methyl-FH₄, is known to take part in the enzymatic conversion of homocysteine to methionine. These observations reveal that the family of folic acid coenzymes is quite complex but all seem to involve the attachment of a single carbon atom to the substrate.

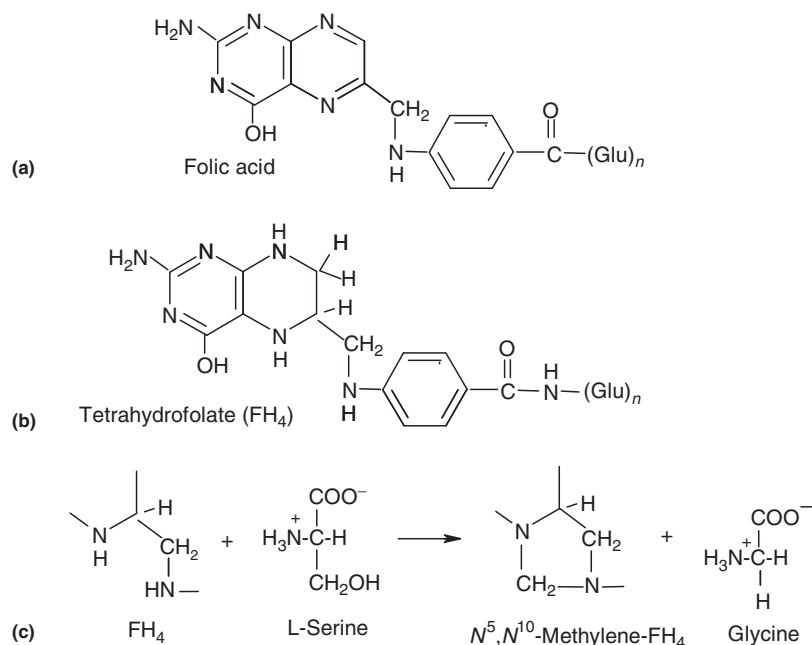
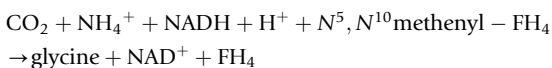


Figure 4 Folic acid and its coenzyme form. Bottom shows the specific function of one of the tetrahydrofolate derivatives, *N*⁵,*N*¹⁰-methylene, in the interconversion of serine and glycine.

Reactivity

Enzymes that require folic acid participate in what is referred to as 'one-carbon metabolism'. These take the form of group transfers involving methyl groups, formyl groups, formimino groups, and methylene groups. Folic acid does not take part in acetylations or carboxylations. A typical reaction in higher vertebrates is the synthesis of glycine by the enzyme glycine synthase:



In perhaps its only major requirement as a methyl group donor, *N*⁵-methyl-FH₄ is needed by the enzyme homocysteine methyltransferase to synthesize (regenerate) methionine from homocysteine. This reaction also uses a methylated derivative of vitamin B₁₂ to mediate the group transfer. Another important reaction is the reversible conversion of serine to glycine. As shown in **Figure 4c**, the reaction requires the *N*⁵,*N*¹⁰-methylene-FH₄ derivative. Today, the list of folate-catalyzed reactions is quite large and includes one-carbon units in the synthesis of a purine ring of nucleic acids, methylation of DNA, RNA, thymidine biosynthesis, choline and S-adenosylmethionine (SAM) biosynthesis, and histidine and tyrosine catabolism.

Biotin

Early interest in biotin involved the so-called egg white injury factor. When it was confirmed that egg white injury was caused by a deficiency and not a toxicity, pursuit of the missing substance led eventually to the discovery of biotin. Research on the vitamin brought a new concept to nutrition, that of the 'antivitamin' or substances capable of negating the action

of vitamins before their use as cofactors. In the case of biotin, the 'antivitamin' turned out to be the protein avidin, which bound biotin tenaciously and limited its intestinal absorption.

Reactivity

Biotin can be thought of as another one-carbon cofactor, but for biotin the one carbon is CO₂. Thus, biotin-requiring enzymes catalyze carboxylation, decarboxylation, or transcarboxylation reactions. The active form of biotin is 'biocytin' (*ε*-*N*-biotinyl-L-lysine), which is formed by the covalent attachment of the biotin side chain to the *ε*-amino group of a lysine residue on the apoenzyme as catalyzed by a specific synthetase (**Figure 5b**). The condensation requires ATP and precedes via a biotiny-AMP intermediate with the apoenzyme catalyzing formation of the amide bond. The resulting unique structure combines the aliphatic chains of biotin and lysine permitting the ring structure of biotin to extend approximately 14 Å from the enzyme's surface (**Figure 5b**).

The active site on a biotinyl group is one of the N in the 5-member ring (**Figure 5c**). An *N*-carboxyl derivative serves as a donor of CO₂ to an appropriate substrate acceptor. The reaction occurs in two steps and requires an ATP-dependent formation of a carboxy biotinyl enzyme. If the enzyme is a carboxylase, there are two main substrate types: (1) acyl-CoA derivatives, which include acetyl CoA, propionyl CoA; and (2) simple *α*-keto acids such as pyruvate. Each substrate must contain a carbonyl group adjacent to or conjugated with the carbon receiving the carboxyl group from carboxy biocytin. Perhaps the most familiar biotin carboxylase enzymes in mammalian systems are acetyl CoA carboxylase in fatty acid biosynthesis, propionyl CoA carboxylase in odd-chain fatty acids catabolism, pyruvate carboxylase in gluconeogenesis, and *β*-methylcrotonyl CoA carboxylase in leucine catabolism.

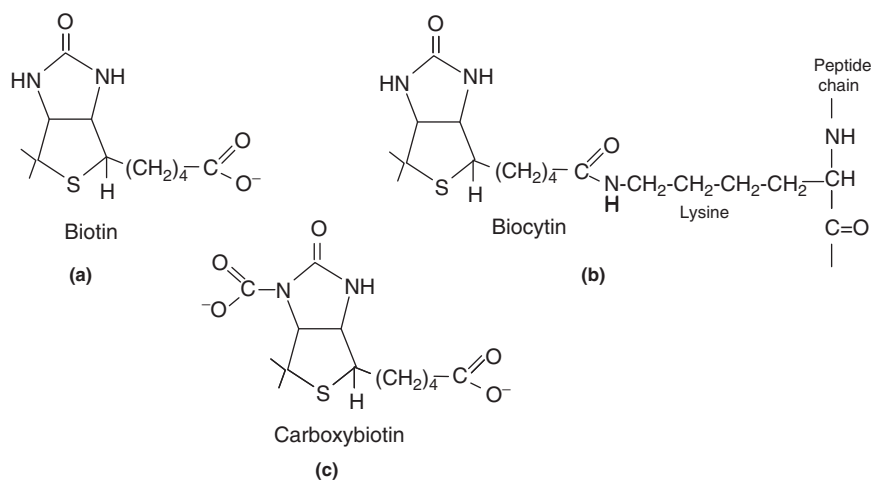


Figure 5 Biotin and its coenzyme. (a) Biotin as a vitamin, (b) biotin attached to the ϵ -amino group of lysine (biocytin), and (c) the carboxy derivative of biocytin prepared to donate CO₂ to a substrate.

Pantothenic Acid

Pantothenic acid was named by Roger Williams who recognized its ubiquitous (pantos, L. everywhere) occurrence in tissues of all organisms and all food sources. The history of its discovery gives two twists. One is that the coenzyme form, Coenzyme A (CoA), was discovered long before the vitamin and, second that investigations of the substructure of CoA led to an understanding of how the coenzyme was synthesized. CoA was known to be a dialyzable cofactor essential for the acetylation of sulfonamide and choline. Indeed, the 'A' designation in CoA recognized an importance in acetylation reactions. The first clue to the vitamin's structure came when digests of CoA with intestinal phosphatase and liver extracts were found to contain β -alanine and pantothenic acid as hydrolysis products. Treating CoA with a specific 3' nucleotidase inactivated the coenzyme and a specific pyrophosphatase cleaved the coenzyme to a pantothenic acid-containing product that could be restored to CoA by adenylation with ATP. These studies showed that pantothenic acid was an essential component of CoA and had been locked into the structure of a rather complex coenzyme (**Figure 6**).

Reactivity

As a major component of CoA and its derivatives, pantothenic acid is involved in acetylation reactions, which include the synthesis of acetoacetyl-CoA, a precursor of cholesterol, and the biosynthesis of citrate from acetate. CoA can engage in acyl thiotransfer reactions such as accepting the acetyl groups from lipoic acid and forming acetyl-CoA as well as fatty acyl CoAs. Pantothenic acid is also found as a prosthetic group (4'-pantothenic) attached to a serine residue of acyl carrier protein (ACP), which plays a prominent role in the biosynthesis of fatty acids.

Cobalamin (Vitamin B₁₂)

Few vitamins have been more challenging to structure-function studies than vitamin B₁₂. Among its many unique features, B₁₂ is the only vitamin-coenzyme known to have a

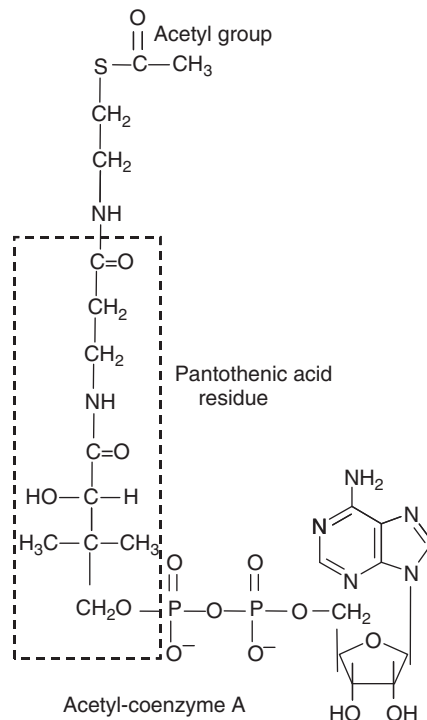


Figure 6 Pantothenic acid and the structure of coenzyme A.

transition metal ion (cobalt) coordinated to its structure. The metal allows some unusual chemistry. The vitamin is present in a variety of foods but is almost totally lacking in plants. Although cobalamin can be synthesized *de novo* by intestinal flora, the absorption site anatomically is before the synthesis site in the gut, which means little benefit is derived from endogenous synthesis. Isolating the active form of the vitamin meant developing an *in vitro* assay for 'pernicious anemia', one of the deficiency symptoms. In 1950, Shive introduced an assay in which homocysteine was converted to methionine in a B₁₂-dependent reaction. A second assay showed that the derivative 5-deoxyadenosyl cobalamin was essential for the

conversion of L-glutamate and β -methyl aspartate. The latter discovery led to the isolation of 5'-adenosylcobalamin, the principal active form of the vitamin.

Reactivity

The core of the vitamin consists of a corrin ring with a central cobalt atom. Corrin contains four pyrrole rings linked together and vaguely resembling structurally the porphyrin ring in heme (Figure 7). An inactive form of the vitamin contains a displaceable CN group bound to the cobalt; hence the early name cyanocobalamin for one of its more familiar forms (Figure 7). The cobalt atom in the ring can have a +1, +2, or +3 oxidation state. The fifth valence (below the ring plane) has a dimethylbenzimidazole attached to the cobalt and the six can be either a methyl group, an -OH group, or a

5'-deoxyadenosyl group depending on the reaction or enzyme. As noted, 5'-deoxyadenosylcobalamin is the most common form of the coenzyme. The 5'-deoxyadenosylcobalamin arises by an attack on the 5'-carbon of ATP by the Co^+ , which displaces the entire triphosphate group of ATP, a rare action in biochemistry. Known enzymes that require B_{12} fit one of two functional categories: those that transfer methyl groups from the coenzyme to the substrate, and those that take part in positional rearrangement of neighboring groups on the substrate, or group transfer reactions.

As noted above, methylation reactions in mammalian systems that involve B_{12} are limited to the transfer of a methyl group to homocysteine to form methionine. Remember, N^5 -methyl-THF is the methyl group donor in the reaction and B_{12} mediates the transfer. Restoring the methyl group on methionine primes the system to further methylation because methionine itself, acting through its active form, SAM, is a primary donor of methyl groups to other substrates.

Of late, there has been considerable interest in vitamin B_{12} reactions that have free radicals as intermediates. This may be one of the principal advantages of the coenzyme, i.e., the ability to form and retain a stable free radical in its structure. The stability of the free radical owes to the unusual chemistry of the cobalt ion.

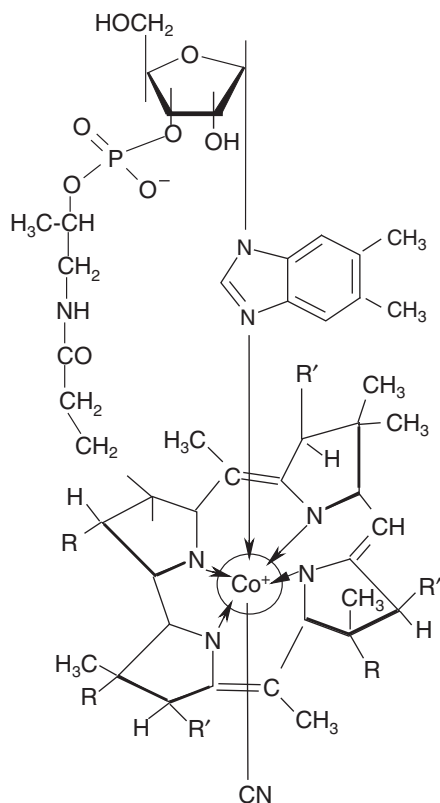


Figure 7 Cyanocobalamin, one of the forms of vitamin B_{12} . Shown are the corrin ring and the attachment of the dimethylbenzimidazole to the cobalt. The cyano group is shown attached to the cobalt below the plane of the ring.

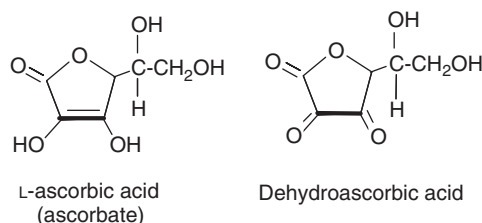


Figure 8 Reduced (right) and oxidized (left) forms of L-ascorbic acid.

Ascorbic Acid

The vitamin (L-ascorbic acid) linked with scurvy is known to be a cofactor for two enzymes that take part in the biosynthesis of collagen, the major connective tissue protein; the formation of hydroxyproline and hydroxylysine residues as catalyzed by prolyl hydroxylase and lysyl hydroxylase enzymes, respectively. Collagen is an essential component of the extracellular matrix. As a cofactor for dopamine- β -monooxygenase, L-ascorbic acid is also required for the synthesis of adrenaline (epinephrine) and noradrenalin (epinephrine) in the adrenal medulla. Other important noncofactor roles of L-ascorbate are as an antioxidant in cells and blood (Figure 8).

Vitamin K

The antihemorrhagic role of the vitamin K had been established long before it was realized that the vitamin with no structural modification was essential for the biosynthesis of functional prothrombin. The two forms of the vitamin, phyloquinone (vitamin K_1), and menadione (vitamin K_2) differ only in the structures of their side chains (Figure 9).

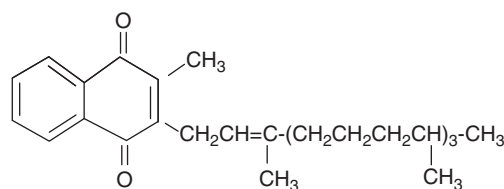


Figure 9 Phyloquinone, one of the active forms of vitamin K (vitamin K_1).

Reactivity

Vitamin K takes part in an extensive series of carboxylation reactions involving all the glutamic acid residues in the first half of prothrombin, a blood clotting factor. The carboxy-glutamic acid residues or 'gla' that now occupy this region of the prothrombin molecule are able to bind the calcium ions needed to catalyze formation of thrombin. Carboxylation is dependent on oxygen and uses the hydroquinone form of the vitamin as the driving force. Warfarin, a clotting inhibitor, interferes with the carboxylation reaction, which explains the basic mechanism of this inhibitor. A synthetic substrate, Pro-Leu-Glu-Glu-Val, has been found to substitute for the prothrombin in the reaction, opening the way to learning the finer details of the reaction mechanism and the specific role of vitamin K.

Nonvitamin Cofactors

In addition to the above vitamin-coenzymes, there is a list of cofactors that do not fit the general category of vitamin-derived. Nonetheless, these organic cofactors are essential components of the catalytic mechanisms of important enzymes or membrane-bound enzyme systems.

Lipoic Acid

Lipoic acid's role as a growth factor for microorganisms and as a cofactor for biochemical reactions in all organisms is well established. The cofactor was discovered originally in the conversion of pyruvate to acetate and as a factor essential for the oxidation of pyruvate. Lipoic acid is known to occur in α -keto acid dehydrogenases from a variety of organisms. It is normally bound to the ϵ -amino group of a lysine residue (analogous to biotin) allowing the cofactor to extend out and away from the enzyme surface as a 'swinging arm'.

Reactivity

The reactions taking place in the pyruvate dehydrogenase complex best reveal the cofactor function of lipoic acid. As a

cofactor, lipoic acid (6,8 dithiooctanoic acid) exists in both an oxidized (disulfide) and reduced form (Figure 10). The disulfide form oxidizes active acetaldehyde bound to TPP simultaneously with the transfer of the acetate product to one of the $-SH$ groups of the now reduced lipoic acid. As a thioester, the acetate group is subsequently transferred to coenzyme A forming acetyl CoA and regenerating a free $-SH$ group on lipoic acid. The reduced lipoic acid, which still contains the electrons is then oxidized by a flavoprotein (FAD) restoring the disulfide group of lipoic acid for another round of catalysis. Eventually $FADH_2$ passes the electrons to NAD^+ , which links to the electron transport chain of the mitochondria. Lipoic acid is thus an oxidizing agent and a carrier of acetate in the reaction. One can picture the long arm of the cofactor swinging between sites on the subunits of the pyruvate dehydrogenase complex in order to perform the multiple reactions in synchrony with the catalytic events taking place.

Carnitine

Carnitine is readily synthesized from lysine. As a cofactor, carnitine takes part in the membrane-bound enzyme system that transports fatty acids into the mitochondria for energy oxidation. Two enzymes, carnitine acyl transferase I and carnitine acyl transferase II, comprise a cycle that delivers the fatty acid as an acyl carnitine derivative to the interior of the mitochondria and returns the carnitine to the cytosolic side for further transport (Figure 11). The structure of carnitine with its hydroxy group on C-3 is ideally suited for forming an acyl bond with a fatty acid.

CoQ (Ubiquinone)

First detected in lipid extracts of mitochondria and identified as a quinone, CoQ was so named to signify its cofactor role in oxidation reactions. A second group of investigators discovered a cofactor that had ubiquitous occurrence in oxidative processes, which they named ubiquinone. In time, CoQ and ubiquinone were found to be the same compound. Early studies on the mitochondria electron transport chain showed at

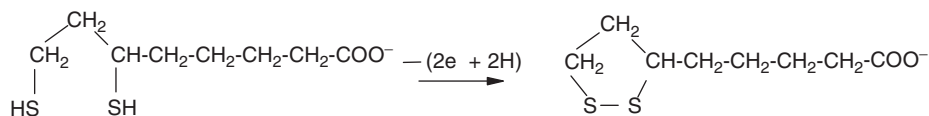


Figure 10 Reduced (right) and oxidized (left) forms of lipoic acid.

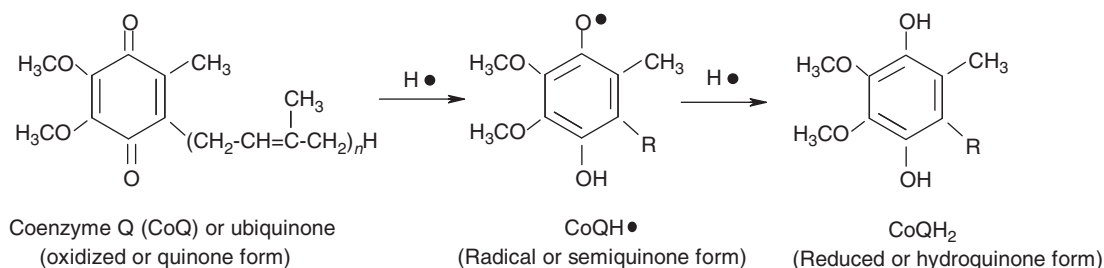


Figure 11 Multiple biochemical forms of coenzyme Q. The oxidized form is shown on the left, the reduced on the right. The semiquinone free radical mediates the movement of electrons between the two.

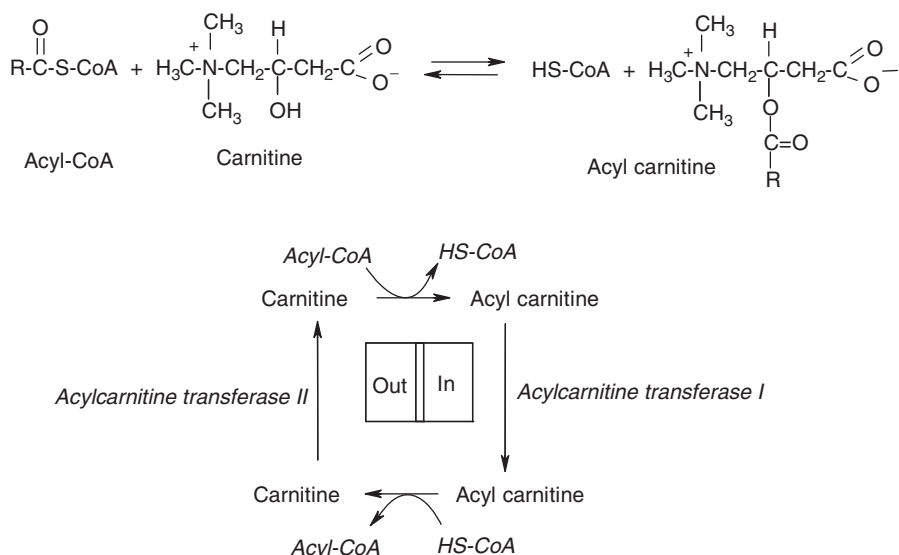


Figure 12 Carnitine-dependent transfer of fatty acyl groups. The diagram below shows the two transferase enzymes, acylcarnitine transferase I and II, that are the carriers. Note that the acyl group is transferred to the carnitine from CoA and returned to CoA inside the mitochondria.

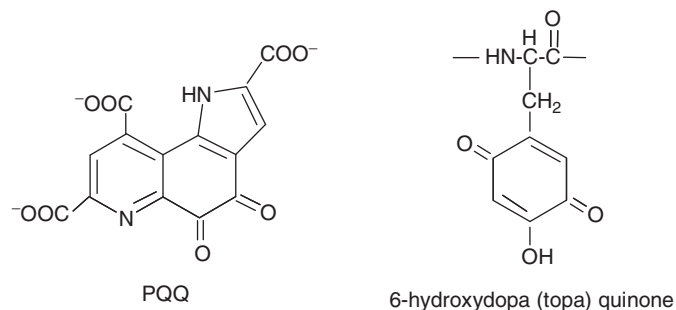


Figure 13 Comparison of structures of pyrroloquinoline quinone (PQQ) with topa quinone. Cofactor on the right has been identified with mammalian systems.

least three complexes required CoQ and recognized its essential role in the electron transfer process overall. CoQ can be synthesized in man from tyrosine in a rather complex synthesis.

Reactivity

CoQ and its reduced form CoQH_2 are designed to handle electron pairs in transit in oxidation–reduction reactions. A third form, semiquinone (CoQH), exists as a stable radical and is capable of a one-electron transfer (Figure 12). Because of its ability to deal with electrons on a single or paired bases, CoQ takes part in electron transport chains. Its lipid nature allows the cofactor to bind firmly to the cell membrane. Besides being a prominent carrier of electrons in the electron transport chain of mitochondria, CoQ is known to be a source and mediator of protons that are pumped across the inner mitochondrial membrane to form the high energy proton gradient associated with oxidative phosphorylation.

Pyrroloquinoline Quinone (PQQ) and 6-Hydroxydopa (topa) Quinone

A cofactor known to be present in methylogenic bacteria and other microorganisms, PQQ was considered at first to be a

cofactor for mammalian copper amine oxidases and other copper enzymes. Its essential nature led some workers to consider PQQ another vitamin. This however, turned out not to be the case and PQQ as a cofactor has now been relegated to the world of microorganisms.

What at first was thought to be PQQ in copper oxidases turned out to be a cofactor with quinone properties that was derived by modifying a select tyrosine residue in the enzyme (Figure 13). The synthesis of 6-hydroxy (topa) quinone, a derivative of tyrosine, requires copper in an apparently autocatalytic reaction. Though rare and limited, this most unusual biochemical reaction opens a new chapter on cofactors by showing that some enzymes have a limited but specific capacity to synthesize cofactors on their surface through modification of existing amino acid side chains.

Other

There are a number of organic compounds that occur naturally that do not quite fit the description of cofactors, yet have been identified with stabilizing enzymes or in taking part in a select series of reactions. We include them here with

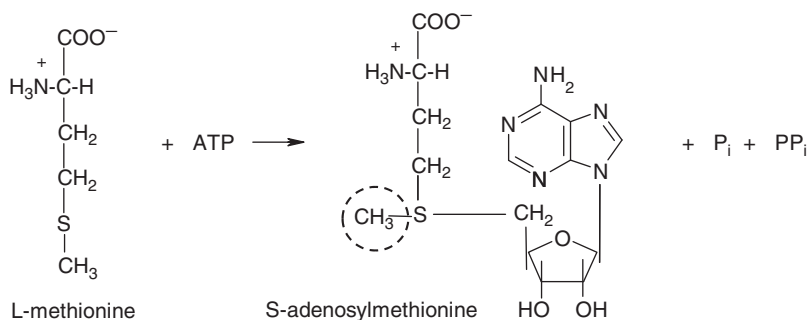


Figure 14 Synthesis of SAM from methionine. Note the favorable positioning of the methyl group of methionine as a result of the condensation with ATP.

the caveat that some may behave more as substrates than cofactors.

Glutathione

Glutathione is a naturally occurring tripeptide that is known to exist in millimolar quantities in cells. The reduced form (GSH) has an exposed –SH group associated with an internal cysteine residue that has been shown to figure prominently in the stability of enzymes that have –SH groups at the active site. Glutathione partakes in many biological reactions such as amino acid transport, heavy metal transport, and antioxidant activity. Its cofactor role, however, should not be overlooked because GSH has been shown to activate many enzymes or retain their catalytic effectiveness during assays. One suspects, with justification, that this could be one of the functions of GSH *in vivo*.

Betaine

Betaine (*N,N,N*-trimethylglycine) arises from choline by an oxidation reaction. The structure is a trimethyl derivative of glycine. Betaine occurs in very small quantities in cells and has been shown to be a methyl donor for a limited number of reactions, notably synthesis of methionine from homocysteine.

SAM

To consider SAM as a cofactor is to recognize its role in a multitude of reactions that transfer methyl groups to substrates. Thus, SAM is involved in an extensive series of methylation reactions that surpass methylations of either *N*⁵-methyl-THF or methyl cobalamin combined. The methyl group transferred is the terminal carbon of methionine. To activate the methyl group, methionine reacts with ATP, adding an adenosyl group to the sulfur atom and causing a high energy methyl donating species to form (Figure 14). Although SAM is perhaps more of a substrate than a cofactor, its inclusion here is to denote the importance of methionine and its reactive form, SAM, in a series of extremely important biosynthetic reactions.

3'-Phosphoadenosine-5'-Phosphosulfate (PAPS)

PAPS is a cofactor for sulfation reactions, a process confined largely to plants and bacteria, but an important metabolic reaction in humans. PAPS serves as an active agent for sulfate esterification, as in the synthesis of sulfated polysaccharides such as chondroitin sulfate, keratin sulfate, and heparin.

Conclusions

Of the 20 or so organic cofactors that have been discovered over the years, the structures of more than half are derived from the nucleus of vitamins, primarily the water-soluble B vitamins. As companions to enzymes, organic cofactors relate to all forms of life. Although we may think of vitamins as needed only for higher organisms, many organic factors were designed to serve exclusively with enzymes and imperfections in enzyme systems that gave vitamins an essential character were revealed in bacteria and yeast systems long before they became known in humans. The carry over between cofactor-dependent reactions in microorganisms and humans has been remarkable, an illustration of the structure–function principle of biochemistry. Still, we must not overlook the fact that a study of cofactors has brought a sharper focus to the role of enzymes in human health and nutrition. Whereas mutated bacteria may fail to grow for want of a vitamin, a susceptible human will develop a deficiency symptom. Both need the vitamin factor in a failing enzyme system in order for that system to perform at a healthy capacity. Nutritionists are challenged to learn the function of all cofactors because that information provides fundamental insights into a chemical blue print that applies to a wide spectrum of different organisms at the molecular level.

See also: Amino Acids: Chemistry and Classification; Metabolism; Specific Functions. Ascorbic Acid (Vitamin C): Deficiency States; Physiology, Dietary Sources, and Requirements. Biotin: Physiology, Dietary Sources, and Requirements. Choline and Phosphatidylcholine. Cofactors: Inorganic. Folic Acid. Niacin and Pellagra. Pantothenic Acid. Riboflavin. Thiamin: Beriberi; Physiology. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources and Requirements. Vitamin B₆: Physiology. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements. Vitamin D:

Physiology, Dietary Sources, and Requirements. **Vitamin E:** Metabolism and Requirements; Physiology and Health Effects. **Vitamin K**

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Structure, Function, and Disorders

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Glossary

Diarrhea Defined as either a decrease in stool consistency or an increase in stool frequency and volume.

Enteric nervous system The ENS operates both in conjunction with and independent of the peripheral nervous system.

Inflammatory bowel disease A chronic inflammatory condition of unclear etiology, categorized by location, extent, severity of disease, in addition to being a strictly mucosal process versus a transmural process.

Polyps Intestinal polyps are intraluminal protuberant tumors characterized by their gross morphological appearance, location(s), numbers, size, and presence (pedunculated) or absence (sessile) of the stalk.

Prebiotics Naturally occurring nutrients that support the intestinal microbiome.

Probiotics Bacteria present in nature and in naturally fermented foods that have benefits for the host following ingestion.

Protein losing enteropathy Protein losses occurring from the colon, usually of an exudative process, and not only related to inflammatory conditions, but also to perturbations in oncotic and hydrostatic pressure equilibrium.

The intestinal microflora and the microbiome The complement of bacteria that normally resides in the colon.

Structure and Function

Gross Morphology

The colon is a continuous structure originating at the ileocecal valve and extending to the anus. The cecum is the first part of the colon, which lies in a posterior position at the right iliac fossa, and has an ovoid-like shape. This cavity is more generous in proportion than other compartments of the colon. The appendix (a blind-ending outpouching) originates in the cecum and its opening is usually visible during colonoscopy.

The ascending colon runs cephalad and anteriorly from the cecum to just inferior to the liver, to the hepatic flexure, emerging into the peritoneum. The transverse colon continues from the hepatic flexure to the splenic flexure, from where it travels distally, and once again posteriorly to the sigmoid colon, an S-shaped, tortuous, narrow peritoneal structure. At the peritoneal reflection the rectum arises and, closely following the sacral curve leads to the anal canal. The rectum is a vault-like structure, which can distend in order to accommodate fecal load. The anal canal bears two sphincters, an internal and an external anal sphincter. The internal sphincter is comprised of inner circular smooth muscle fibers, and a distal external fiber on the other side of a muscular pelvic diaphragm. The fibers of the external sphincter are intertwined with those of the levator ani, tethered anteriorly and posteriorly to the perineal body and the coccyx, respectively (Figure 1).

With respect to colonic mobility within the abdominal, peritoneal, and pelvic cavities, the cecum and flexures are less mobile, with the sigmoid colon being the most mobile.

The transverse colon supports the greater omentum and has a variable degree of mobility.

Cross-sectionally, the colon has an external longitudinal muscle and an inner layer of circular musculature, the former has a coalescence of fibers forming band-like structures known as teniae. These teniae are particular to the large intestine, and are located at one-third of the circumference from each other, and run continuously from one end of the colon to the other. Haustra are hemilunar-like outpouchings, which are present between teniae. The more proximal rectal tenial fibers surround the rectum; the inner fibers form the internal anal sphincter. The external fibers are intertwined with those of the levator ani, and sandwiched between fibers running anterior to posterior, from the peroneal body to the coccyx, forming the external sphincter.

Vasculature

The ascending colon and portions of the transverse colon are perfused by branches of the superior mesenteric artery, with the remainder of the colon receiving arterial blood from tributaries of the inferior mesenteric artery. Distal iliac arterial branches perfuse the anal canal. Venous drainage is achieved via the superior and inferior mesenteric veins lying in close approximation to their arterial counterparts, and subsequently dumping into the portal vein.

Additional gross morphologic structures include 'lymphatic vessels,' in close approximation to the vasculature, leading to lymph nodes in the celiac, superior, and inferior preaortic regions. Perianal drainage is via the inguinal lymph nodes.

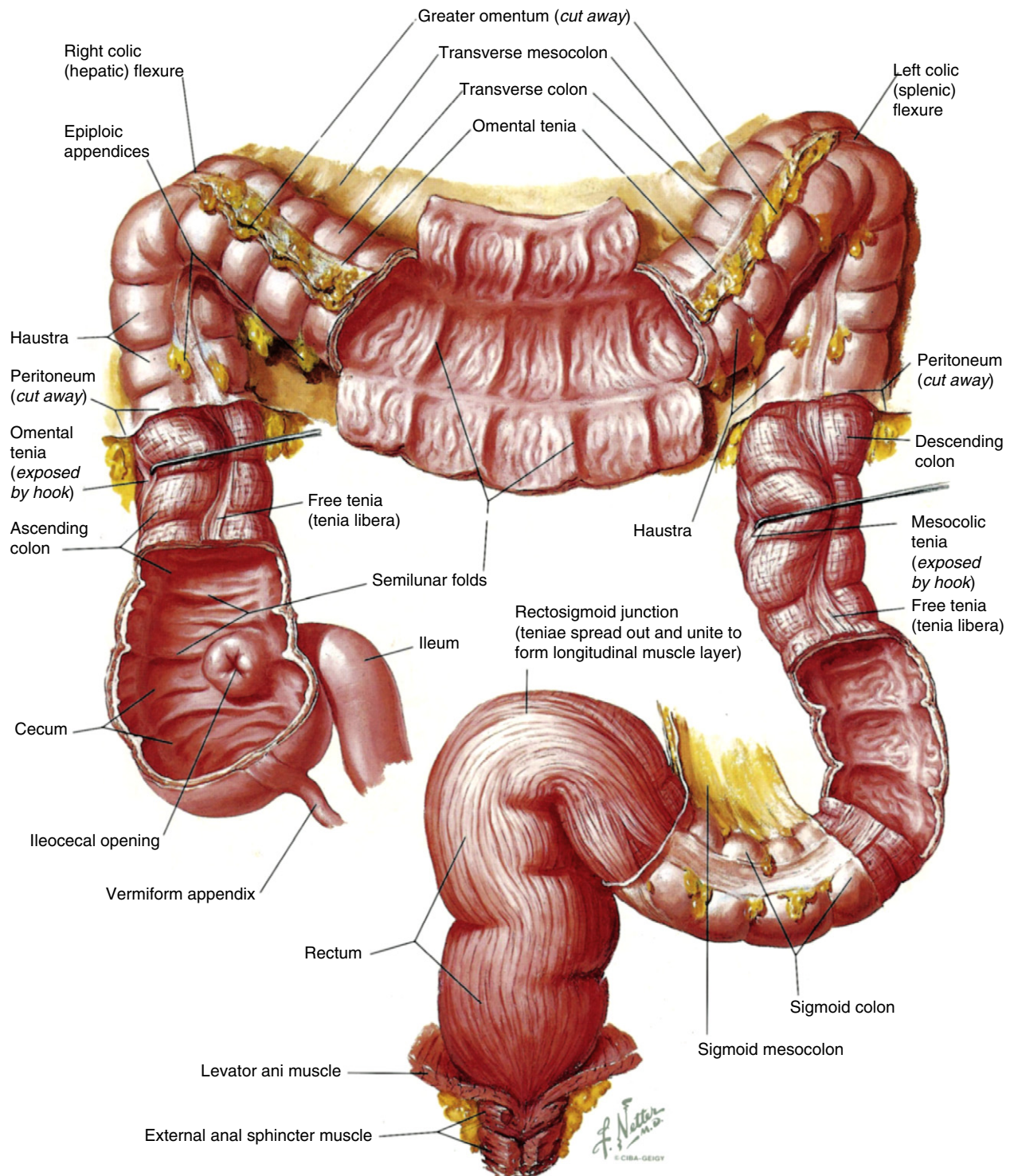


Figure 1 Mucosa and musculature of the large intestine. Reproduced from Netter F (1995) *Atlas of Human Anatomy*. Geneva: Ciba-Geigy, with permission from Anatomy Atlas.

Innervation

Parasympathetic innervation to the proximal colon is provided via the vagus nerve; the distal colon and rectum are innervated via pelvic parasympathetic fibers. The sympathetic

nervous system innervates the proximal colon via lower thoracic fibers, and the distal colon and rectum via lumbar fibers. Prevertebral sympathetic vertebrae receive fibers from neurons projecting out of the gut.

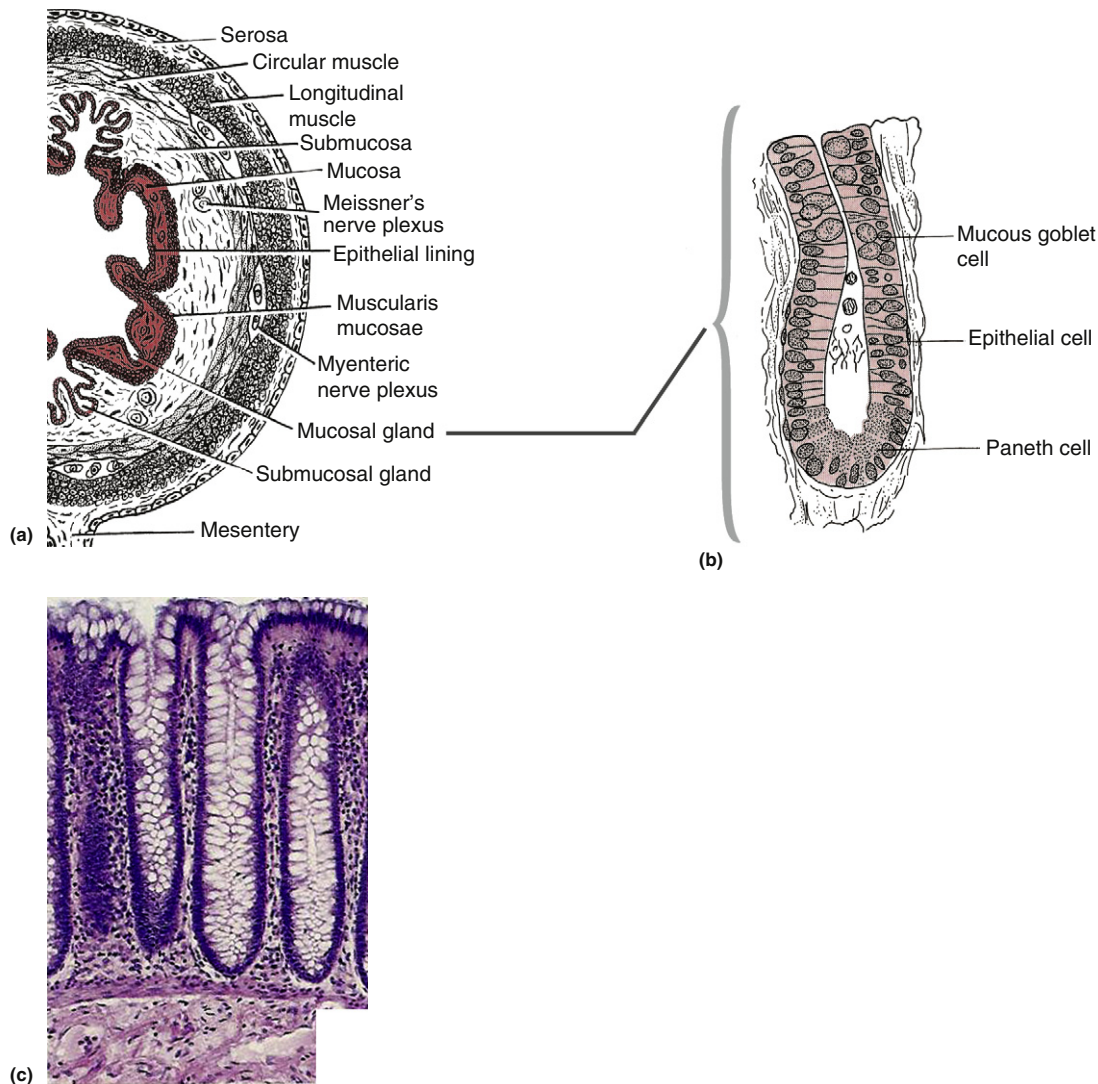


Figure 2 (a) and (b): Cross sections of the gut and a colonic crypt. Reproduced from Guyton AC (1991) *Textbook of Medical Physiology*, 8th edn. Philadelphia: WB Saunders Company. (c) H and E stain of a typical colonic crypt. Reproduced from Burkitt HG, Young B, and Heath JW (1993) *Wheater's Functional Histology*, 3rd edn. London: Churchill Livingstone.

Histology

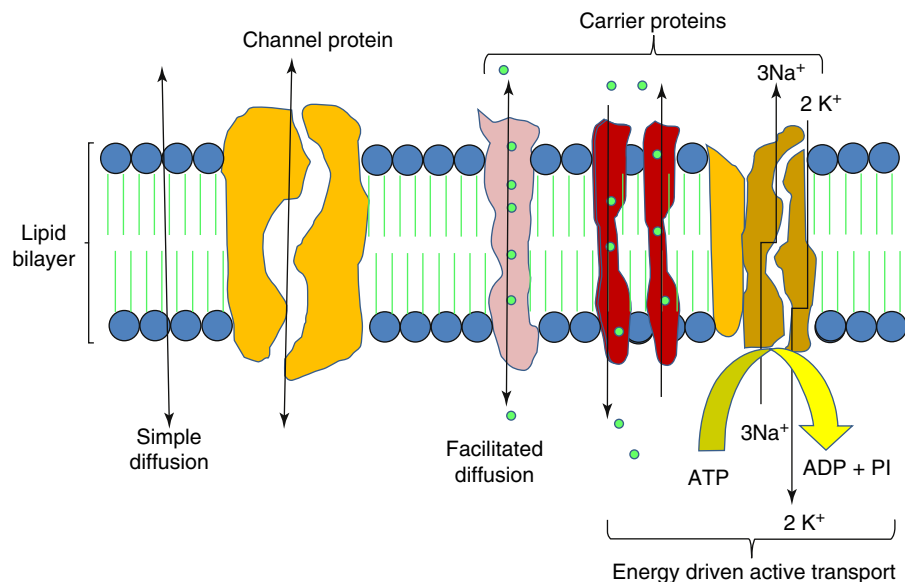
Cross-sectionally, the intestinal wall is divided into four layers, with the serosa, a monolayer of mesothelial cells comprising the outermost, followed by the muscularis externa. These muscle layers comprise an external longitudinal layer and an internal circular layer. Sandwiched in-between these two layers lies Auerbach's (myenteric) plexus. The submucosa is the next more medial layer; a rich admixture of cells, including structural elements such as fibroblasts and dense connective tissue, immunologically important cells (plasma cells, lymphocytes, macrophages, eosinophils, mast cells) in addition to vascular tissue and innervation to Meissner's plexus (ganglion cells), and lymphatics comprise this layer. The muscularis mucosa, a thin sheet of smooth muscle, separates the deeper submucosa from the mucosa. The lamina propria runs interior to this layer, and is composed of connective tissue, and is lined by the luminal epithelium (Figure 2(a)).

The intestinal epithelium is a tight monolayer of cells that functions to absorb nutrients, electrolytes, and liquids, as well as to secrete mucus and fluids. The epithelial surface is punctuated by numerous tightly packed crypts, which contain epithelial precursor cells, enteroendocrine cells, other undifferentiated cells, and Paneth cells. Goblet cells, which secrete mucin, are also located in the crypt (Figures 2(b) and 2(c); Table 1). As undifferentiated and precursor cells mature, they migrate superiorly to the surface and to the monolayer of absorptive cells present in crypts. The average lifespan of a colonocyte is 3–6 days.

The absorptive colonocyte develops short microvilli while in the colonic crypt, which elongate during its migration to the surface. The hydrophobic lipid bilayer of the colonocyte epithelium prevents passive transport of charged particles. The epithelial membrane contains specific protein transporters, carrier proteins, and channels allowing electrolyte transport.

Table 1 Colonic cell types

Cell type	Location	Function(s)
Stem cells	Crypt (base) <ul style="list-style-type: none"> • Nonmigratory until differentiated 	Pluripotent
Undifferentiated crypt cell	Crypt	Secrete water and chloride into intestinal lumen
Paneth cells	Crypt base <ul style="list-style-type: none"> • Nonmigratory • Basophilic cytoplasm • Proximal one-third of colon only 	<ul style="list-style-type: none"> • Growth factor secretion and digestive enzyme synthesis • Antimicrobial peptide synthesis and release
Goblet cells	Colonic crypt <ul style="list-style-type: none"> • Most common cell type in the colon 	Mucin release
Enteroendocrine cells	Mostly in small intestine <ul style="list-style-type: none"> • Basolateral membrane 	Receptor-mediated epithelial cell function modulators <ul style="list-style-type: none"> • Digestive enzyme synthesis (small intestine)
Enterocytes	Predominantly small intestinal; present in the colon	<ul style="list-style-type: none"> • Ion transporters and channels involved in fluid and electrolyte transport
M cells	Small and large intestines <ul style="list-style-type: none"> • Overlying lymphoid follicles 	Bind, process, and present antigens to components of the mucosal lymphoid immune system
Intraepithelial lymphocytes	Small and large intestines <ul style="list-style-type: none"> • Basolateral membranes 	<ul style="list-style-type: none"> • Memory T cells • Mucosal immune defense

**Figure 3** Electrolyte transporters and the cell membrane.

The electrochemical gradient formed by active transport facilitates passive flow across cell membranes.

Function

Electrolyte Transport: Ion Channels

Fluids and electrolytes are absorbed via one of two pathways, transcellular versus paracellular. Active and passive transport systems exist via both these pathways.

There is a clear polarity to the distribution of protein transporters, channels, and pumps distinguishing the apical from the basolateral membrane. Active transport utilizes transcellular, energy-driven protein pumps or channels to facilitate passage of electrolytes from an area of low concentration to one of high concentration/electrochemical gradient. A prime example of this is the Na-K-ATPase pump, the principal pump present along the basolateral membrane. The net effect of the three Na ions expelled for every two K ions accepted into the cell is a lowered intracellular Na content, and resultant net negative charge. The negative charge formed by

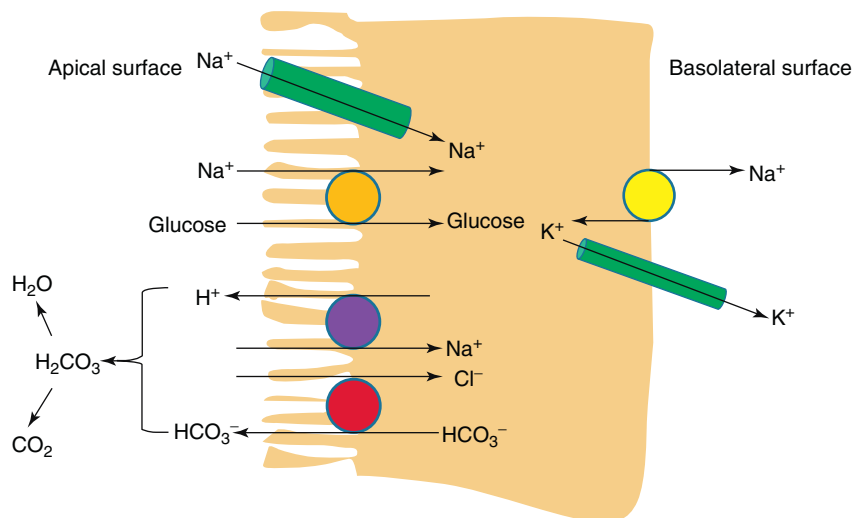


Figure 4 Electrolyte transport at the colonocyte level.

this active transport creates an electrochemical gradient facilitative to the passive flow for other ions across the cell membrane, a process known as secondary active transport (Figures 3 and 4).

Ion transporters may be additionally subclassified into symporters, in which ions move in the same direction, or antiporter, in which case ions move in opposite directions across the cell membrane. Cotransport of ions with other molecules, such as that of Na and glucose. The intracellular concentration of glucose is regulated both by uptake at the apical surface as well as by exit through the basolateral membrane, allowing for conditions favorable to uptake from the lumen. The Na-glucose transporter system allows for therapeutic interventions, such as the use of oral rehydration solution (ORS) in cases of severe diarrhea related to cholera or other processes. Similar cotransporters exist linked to the transport of bile salts and amino acids (Table 2).

Although sodium is the primary cation involved in ion transport, short-chain fatty acids (SCFAs) constitute the primary anion in the colon and primary metabolic fuel for colonocytes. Their transport is postulated to be linked to Na-H transporters and pH, specific bicarbonate linked transporters, and the concentration gradient across cell membranes. Chloride transport occurs via both active and passive processes, and is the major intestinal anion involved in the intestinal secretion of fluids.

Colonic smooth channels also possess ion channels, and are involved in active and secondary ion transport processes involving calcium. The electrochemical gradient formed by the activity of these ion channels facilitates the function of smooth muscle action potential generation on depolarization. With the generation of smooth muscle action potentials attaining threshold voltage, contractility of the smooth muscle is possible. The efflux of calcium into these active transport channels activates the process of contraction. Interaction with the enteric nervous system (ENS) stimulates the release of calcium ions stored in intracellular stores. The function of ion channels can be modified by calcium channel-blocking drugs. This contractile activity, when occurring in a coordinated

fashion and modulated by neurotransmission, effects peristalsis and colonic motility, which are discussed in this article.

Fluid Transport

There is an evident heterogeneity to the mucosal epithelium dependent on location in the alimentary canal, in several aspects. The type, variety, and number of ion transporters, channels, and carrier proteins vary from region to region, i.e., from jejunum to colon. Additionally, the nature of inter-epithelial cell junctions varies from the proximal to distal intestinal tract, influencing the 'leakiness' of the respective regions. Finally, a clear gradient in cell composition and function between colonic crypt cells and those on the surface exists. Physiologic heterogeneity follows the aforementioned patterns, defining tissue function in these respective areas. For example, the colonic crypts have more of a secretory function, whereas the villus structures seen most notably in the jejunum exhibit a greater absorptive function. This heterogeneity is key in understanding changes in intraluminal osmolality and fluid shifts that occur in the intestine.

Approximately 98% of the daily fluid load handled by the intestine is reabsorbed – approximately 9 l day⁻¹. Of this, the jejunum absorbs 85% and the colon absorbs approximately 13% or 1.5 l.

Passive reabsorption of water occurs in the intestines, regulated primarily by electrolyte transport, i.e., following an osmotic gradient. Na-driven/related transport mechanisms are the primary driving force allowing water absorption. This osmotic gradient facilitates water absorption via both transcellular and paracellular pathways.

Transcellular water transport mechanisms such as aquaporins, or water channels, have been described. The paracellular pathway of water transport has been studied extensively, a process often described as 'solvent drag' (Figure 5).

The leakiness of paracellular pathways that varies by location in the lower alimentary tract (more prominent in the jejunum, with subsequent decrease distally), and the

Table 2 Electrolyte transport: examples

<i>Ion</i>	<i>Transporter</i>	<i>Location</i>	<i>Type</i>	<i>Function(s)</i>
Na	Na, K-ATPase	Basolateral membrane	Active; antiport	Principal ion involved in water absorption
	Na-H exchangers	Apical and basolateral membrane	Secondary; antiport	
Na and Cl	NaCl	Apical	Antiport; passive; electrochemically neutral	
Cl	Protein channel	Apical	Diffusion; passive (secretion) and some active transport proteins at the apical surface (absorption and secretion), including CFTR	Principal ion involved in water secretion
				Basal rate of secretion influenced by several mediators (endocrine, paracrine, neural, luminal, etc.)
Cl	Protein channel			
K	Protein channel		<ul style="list-style-type: none"> • Antiport active transport (basolateral membrane) • Active secretion at the apical membrane; linked to Cl transport function • Absorptive active apical K-ATPase pumps in distal colon 	
HCO ₃		Apical and basolateral channels;	<ul style="list-style-type: none"> • Alkaline phosphatase linked • Passive transport mechanisms • Na-HCO₃ cotransporter postulated • CFTR-synchronized apical channel and Cl-HCO₃ exchanger postulated 	
Short-chain fatty acids	Apical		<ul style="list-style-type: none"> • Postulated link to Na-H ion transport 	Principal anion of the colon

CFTR, cystic fibrosis transmembrane conductance regulator.

magnitude of the osmotic gradient (also effected by dietary Na content) are important factors affecting solvent drag. The nature of the intercellular junctions in a particular region of the colon determines the permeability, or 'leakiness' of that particular epithelial area. Several intercellular structures have been described, including the *zona occludens* (tight junction), desmosomes (connections between cells), and the *zona adherens*, the latter function in cell adhesion and therefore contribute to maintaining cellular polarity across the membrane. *Zona occludens* are more apical in location and form junctional complexes between cells. It has been postulated that these junctional complexes may be more dynamic than previously believed, responding to signaling mechanisms and subject to regulation, thereby influencing their function and resultant permeability characteristics (Figure 5).

The ENS and Gastrointestinal Motility

The ENS operates both in conjunction with and independent of the peripheral nervous system. Nerve plexi exist within the bowel wall, with Auerbach's plexus sandwiched between

longitudinal and circular muscle layers, and Meissner's plexus located more medially in the submucosa.

The ENS is the largest component of the autonomic nervous system, based on nerve cell number.

Interstitial cells of Cajal, a cell type unique to the alimentary tract, are present medial to the inner smooth muscle layer. These specialized cells interact with myenteric neurons, and are thought to exhibit independent electrical activity, generating and transmitting slow waves to smooth muscle, functioning as pacemakers for colonic motility. The ENS is capable and does functions independent of the central nervous system (CNS), with reflex activity, in response to luminal stimuli, including muscle contraction and coordination – i.e., motility, blood flow, and glandular secretion. Modulation of the ENS is via the sympathetic and parasympathetic nervous system.

Colonic Motility

The colon functions to delay passage of luminal contents to allow for water absorption, and to allow for mixing of luminal contents with the mucosa, to store fecal matter before defecation, and to propel contents forward during defecation.

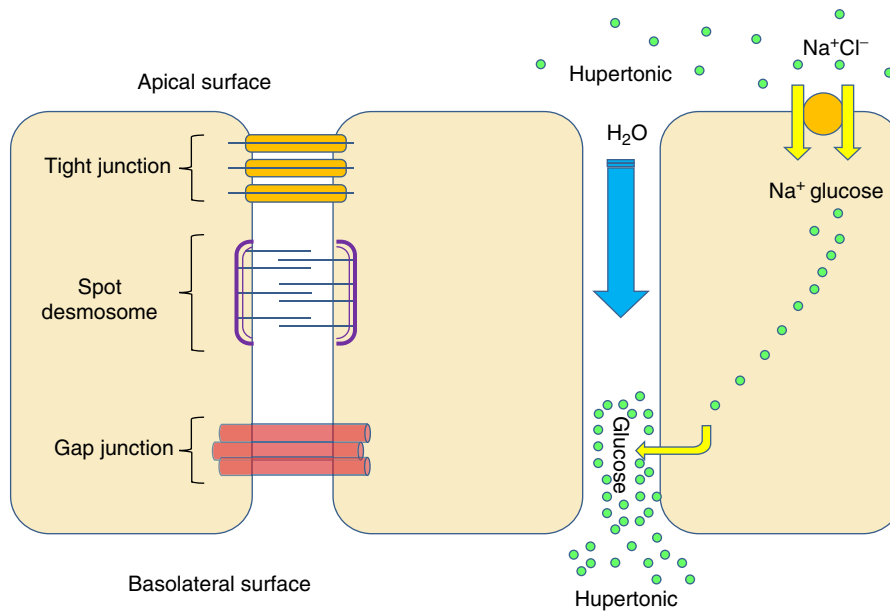


Figure 5 Intercellular junctions and fluid transport across the cell membrane.

The frequency and duration of propagative, high-pressure waves in the colon in part is determined by pressure exerted by the intraluminal contents (mechanical) and degree of stretch stimulation, (chemical) composition of the contents, and by other stimuli interacting with the colon.

The gastrocolic reflex, an anterograde postperistaltic process, occurs following a meal, originating proximally and propagating anterograde. Both the caloric content and the fat composition of the meal bear influence on colonic peristalsis. Likewise, gastric distention by food contents, water, or gas also has a stimulatory effect. Gastrointestinal hormones secreted in response to a meal, such as cholecystokinin, are thought to mediate peristaltic responses to a (fatty) meal. Irritant laxatives also stimulate peristalsis, even when administered rectally. Opiates are known to inhibit the ENS, and, as a consequence, retard peristalsis. Colonic motility diminishes significantly during sleep, resuming on awakening.

Motor activity varies by colonic region, in degree, frequency, amplitude, velocity, being propagative versus non-propagative (the latter are more common in the distal colon than the former), relative distance of propagation, and direction of propagation (anterograde vs retrograde, the latter most commonly seen in the proximal colon). Approximately one-third of these colonic peristaltic waves are propulsive, and the ones associated with propulsion of stool tend to be slower, yet greater in amplitude.

Defecation

Defecation involves the integration of peristaltic activity in most colonic regions, and not solely in the anorectal region. In the predefecatory phase, approximately an hour before actual defecation, the majority of the colon exhibits an increase in propulsive peristaltic waves, first in the proximal colon, then advancing distally.

The sensation of defecatory urge is not evident until approximately 15 min before defecation. At that time, there is a

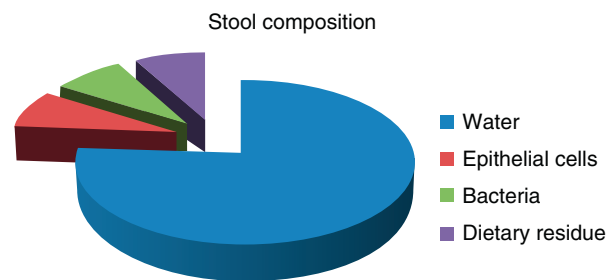


Figure 6 Fecal composition.

marked increase in propagative peristaltic activity, originating more distally in the colon. Each of these late propagative waves successively originates 'proximate' to its preceding one, with 'greater' amplitude, and present over a 'greater' distance of colonic length.

Stool contact with the receptors in the upper anal canal can effect relaxation of the inner anal sphincter. In addition, stretch receptor stimulation of the rectal-vault walls results in the urge to defecate. Failure of relaxation of the external anal sphincter (which is under voluntary control) results in retrograde passage of stool into the rectum, with subsequent diminishing of more proximal peristaltic propagative waves, thereby maintaining continence when immediate defecation is not desirable or convenient.

Evacuation of the rectum and defecation require correcting the angle of the anal canal in the anterior-posterior plane; this is accomplished by assuming a squatting position. Contraction of the abdominal musculature and of the diaphragm, with a relaxed pelvic floor facilitates defecation, even in the absence of colonic peristalsis.

Stool size and consistency vary based on diet and water intake, and transit time determines size and consistency of the stool, as well as bacterial content (a major component of stool).

Higher water content tends to result in larger, softer stools. The more fusiform-shaped the stool is, the less likely that its passage is associated with straining. The transit time through the colon is inversely related to the stool's water content and, hence, its consistency (Figure 6).

Colonic Immune Function and Colonic Microflora

The immune system of the gastrointestinal tract functions in defending against infection (bacterial, viral, and parasitic) and luminal antigens ingested/formed by bacteria. Nonspecific and specific mechanisms exist.

The mucin secreted by colonic goblet cells functions as a barrier for the mucosal surface. Mucosal integrity serves an important barrier to luminal pathogens. Interepithelial cell junctions function both to control permeability as pertains to fluid and electrolyte absorption, as well as to preventing pathogen access beyond this layer.

The enteric immune system is vast and complex; it functions to interact with the rest of the immune system, as well as with luminal contents. Gut-associated lymphoid tissue consists of both discretely organized tissue, such as Peyer's patches (lymphoid follicles with proliferative potential in response to antigen presentation) containing M cells, and the more diffuse lymphocytes and macrophages distribution among the submucosa, mucosa, and lamina propria. M cells function in antigen sampling of intraluminal contents by binding antigens, endocytosis, antigen processing, and subsequent interaction with lymphocytes and macrophages within Peyer's patches, eliciting host responses. The lymphocyte complement of Peyer's patches originates in either the bone marrow or the thymus, enters the systemic circulation to migrate to Peyer's patches, interacts, returns to the intestinal mucosa or, via mesenteric lymph nodes, reenters the systemic circulation to reach other organs.

The gastrointestinal tract houses up to 80% of the body's immunoglobulin-producing cells. Intraepithelial T lymphocytes, plasma cells, macrophages, dendritic cells, eosinophils, and mast cells also function in a specialized manner.

Secretory immunoglobulin A (IgA) is an important host immune-defensive mechanism. Unlike the monomeric, systemic form of IgA, intestinal secretory IgA is polymeric (specifically, dimeric) in nature. This dimeric immunoglobulin is secreted by B lymphocytes situated in the lamina propria, and contains a unique 'J' chain instrumental in polymer formation. This IgA binds to the Ig receptor of the epithelial cell on the basolateral membrane, and, following endocytosis and transport across the cell, is secreted from the apical side.

Secretory IgA binds to intraluminal antigens, including dietary ones, and functions in preventing their absorption. Additionally, secretory IgA has the ability to bind to microorganisms, hence preventing adherence, colonization, and invasion. Secretory IgA is secreted in breast milk, and in the breastfed neonate and infant confers a degree of passive immunity to infection by limiting luminal contents from interacting with, or directly binding to/involving the mucosa.

Interaction of intraluminal bacteria with the immune system may affect intestinal permeability, and may modulate the intestinal immune system. Certain bacterial species are believed to interact with other enteric flora as well as with the

host immune system to effect a healthier gastrointestinal tract and enhanced nutrient digestion; organisms studied include *Lactobacillus*, *Vibrio* species, and *Saccharomyces* (commonly referred to as probiotics).

Regulation of quantity of bacteria, in addition to the specific profile of bacterial species present is dependent on a host of factors, including gastric acid output, gastrointestinal motility, luminal contents, and the milieu created therein. Additionally, the intraluminal environmental milieu is affected by the specific properties of different species of bacteria, their interactions with other luminal species, and with the host itself.

The Colonic Microbiome

The colon accommodates the largest number of enteric flora, on the order of 10^{10} – 10^{12} greater than 100 000 the number of flora and more than 100-fold greater diversity of species than any other location in the alimentary canal. Efflux of bacteria into the ileum is hindered by the ileocecal valve, which functions to restrict several of these bacterial species from entering large intestine. The majority of these colonic bacteria are anaerobic in nature. (Table 3).

The enteric flora plays several important roles, including interaction with the enteric immune system, effecting cellular immune activity, associated with the size and number of Peyer's patches present, influencing intestinal motility, and nutritively important functions, including bile salt deconjugation (facilitates

Table 3 Colonic enteric flora

Bacterial genus	Prevalence (%)	Total count (CFU g ⁻¹ or ml)
Anaerobes		10^{10} – 10^{12}
• <i>Bacteroides</i>		10^9 – 10^{12}
• <i>Porphyromonas</i>		
• <i>Bifidobacterium</i>		
• <i>Lactobacillus</i>		
• <i>Clostridium</i>		
• <i>Peptostreptococcus</i>		
• <i>Peptococcus</i>		
• <i>Methanogens</i>		
Facultative aerobes		10^2 – 10^9
• <i>Enterococcus</i>		
• <i>Escherichia coli</i>		
• <i>Staphylococcus</i>		
• Other Enterobacteriaceae		

Table 4 Examples of biochemical reactions by intestinal flora

Reaction type	Reaction	Example substrate
Hydrolysis	Amides	Methotrexate
	Glucuronides	Estradiol-3-glucuronide
Dehydroxylation	Decarboxylation	Amino acids
	Deamination	Amino acids
	Dehydrogenase	Bile acids, cholesterol
Reduction	Double bonds	Unsaturated fatty acids
	Acetylation	Histamine

enterohepatic circulation of bile salts), bilirubin metabolism (deconjugation and urobilin formation, allowing excretion), mucin degradation, and lipid metabolism (generation of SCFAs). Androgens and estrogens are hydrolyzed facilitating reabsorption and conservation of these sterols, whereas cholesterol is processed into coprostanol, a nonabsorbed sterol. Ammonia- genesis via protein and urea degradation may play a role in hepatic encephalopathy (Table 4).

Consumption of lipids, carbohydrates, and protein also occurs by colonic bacteria, in addition to that of vitamins (vitamin B₁₂ and folic acid are consumed; vitamin K and biotin are produced by these bacteria).

The development of the colonic microbiome is influenced by many factors, including environmental factors such as site of birth (home vs hospital, rural vs urban hospital setting), of antibiotic exposure perinatally, of oxygen deprivation, and by mode of birth (vaginal vs cesarian). Although it is well established that the microbiome bacterial profile is largely determined after birth and with the development of immunological tolerance within the first 2–3 day of life, with a preponderance of facultative anaerobes, it is also recognized that the dietary composition plays an important ongoing role in the maintenance and character of the microbiome. Differences in the microbiome may exist in children who are breast versus formula fed, and the composition of the breast milk in turn is modulated by maternal dietary intake. Dietary fiber in particular is very important throughout the life cycle, as bacterial fermentation produces SCFAs, which are the preferred colonocyte energy substrate. Butyrate is one such SCFA of particular importance.

The composition of the intestinal microbiome has been associated to specific disease states, including obesity, inflammatory bowel disease (IBD), and cystic fibrosis (CF). In all these conditions, in addition to the microbiome being varied from their respective healthy counterparts, it is thought to be reduced in terms of the diversity.

In the case of obesity, a varied proportion of the intestinal microflora phyla (more Firmicutes and less Bacteroidetes) have been observed in the obese versus lean individuals. From a microbial metagenomic perspective, these bacteria are enriched in genes associated with energy harvest. What is not clear at present is if this association is causative or reflective of being obese or not. What these observations do is to raise interesting questions regarding whether if the intestinal microbiome contributes significantly to energy balance in their respective human hosts. The intestinal microflora had previously not been considered to contribute to host energy balance in a clinically significant manner.

In IBD and CF, the microbiome differs from that of healthy individuals. In the case of IBD, there is a decrease in Bacteroidetes and Firmicutes and increase in Proteobacteria – i.e., there is a decrease in the protective flora and a corresponding increase in detrimental flora. This microbiome is thought to play a causative role in disease pathogenesis in susceptible individuals. Interestingly, in comparison to healthy individuals, the colonic butyrate production in individuals with ulcerative colitis (UC) is reduced. This may be indicative of both dietary differences between these two groups (a known risk factor for the development of IBD in genetically susceptible individuals) and possibly in the pathogenesis of the disease process.

Mucus secreted by colonic goblet cells forms a physical barrier to bacterial pathogens known as the unstirred layer, and is routinely cleared by the colon. In CF, as with pulmonary secretions, colonic secretions are underhydrated, and this renders the unstirred mucus layer thicker and less able to clear trapped bacteria, which is thought to contribute to colonic inflammation frequently seen in this condition.

Modulation of the intestinal microbiome is possible by dietary changes, the use of antibiotics, and of probiotics. In the case of IBD, the colonic microflora has often been a target for therapy, with the use of antibiotics useful as therapy for the disease, while colonic diversion is another modality sometimes used. Another strategy studied in digestive and inflammatory disorders of the colon involves use of probiotics and of prebiotics (mostly dietary oligofructases) to modulate and change the profile of the microbiome. Specifically, supplementation of single and multiple species of these probiotics has been studied in the prevention and treatment of antibiotic-associated diarrhea, bacterial overgrowth in short bowel syndrome, rotaviral infections, refractory post IBD resection-related pouchitis, irritable bowel syndrome and necrotizing enterocolitis, and for the treatment/prevention of recurrent *Clostridium difficile* colitis. In CF animal models, the use of probiotics and bowel hydration retention agents such as polyethylene glycol 3350 has been associated with a decrease in colonic markers of inflammation, suggesting that altering the intraluminal microbiome and intestinal milieu may have significant impact on health and disease states. The use of probiotics in other conditions that extend beyond the gastrointestinal system (such as allergic conditions and pulmonary disease) remains an active area of research. It is highly likely that the relationship between host and microbiome is dynamic and bidirectional, and that the effects extend beyond the colon, from a nutritional, metabolic, and immunological basis. However, many studies of probiotics have not yielded clinically significant results, and caution in interpreting these findings and in consideration to the roles and types of probiotics in use should be exercised. The human host develops immunotolerance within the first 2–3 days of life, and so if probiotic therapy is started, it may need to be continued to maintain/sustain microbiome changes; cessation of supplementation may allow the microflora to return to its preprobiotic supplementation profile. Some specificity of the type of probiotic for different conditions may need to be considered. Probiotic use may be contraindicated in immunocompromised individuals where the risks of bacterial translocation and sepsis are increased, and are currently contraindicated for use in pancreatitis, where their use has been associated with adverse outcomes.

The Colon and Energy Metabolism

Although the role of the colon in fluid and electrolytes transport is well known, until recently, less was known about the colon and energy metabolism. The intestinal microflora-mediated fermentation of dietary fiber and utilization of SCFAs (acetate, propionate, and butyrate) are important in the maintenance of the colonic microbiome. SCFAs are also

absorbed and contribute approximately 200 kcal d^{-1} to the human host.

The role of the colon in energy retention is of increased importance in cases of short bowel syndrome related to resection, and in relation to the small bowel length. In cases of small bowel resection, hypertrophy of colonic tissue occurs which enhances fluid and electrolyte retention capacity; however, malabsorption of long-chain fatty acids and carbohydrates may still occur. Although it is understood that the sites of small bowel resection and amount of resection are important (in addition to the status of the ileocecal valve, an important antibacterial barrier for the small bowel), the role of colonic retention may also be important. In this setting, colonic energy retention of fermentable carbohydrates – dietary fiber – into SCFAs and their absorption increases. In individuals with small bowel resections, dietary modification to increase fermentable carbohydrates may be an important strategy in decreasing malabsorption and maldigestion.

The Colon and Nitrogen Metabolism

Colonic nitrogen metabolism involves both protein and nonprotein sources. Protein catabolism in the gastrointestinal tract can account for 10% of total body protein metabolism. The intestinal microbiome contributes much of the fecal matter and intraluminal nitrogen content and, as such, the majority of fecal nitrogen losses. Colonic ammonia specifically is the main nonprotein source of nitrogen in the colon. This ammonia is obtained by the hydrolysis of urea by colonic bacteria, and is utilized for bacterial protein synthesis. Intraluminal ammonia is also enterohepatically recirculated, and is reconverted to urea by the liver. Interestingly, increasing dietary protein intake does not increase fecal nitrogen excretion; however, increase in dietary fiber is associated with increased luminal bacterial utilization of the nitrogen (ammonia) for protein synthesis, thereby increasing fecal nitrogen excretion.

Altering the intestinal nutrient exposure, bacterial load, and pH can disrupt this enterohepatic recycling of urea and trapping it in the intestinal lumen. This is a desirable strategy employed in liver failure to decrease blood and hepatic ammonia levels. Specifically, lactulose, a nonabsorbable sugar is enterally administered to lower intestinal pH and to drive bacterial fermentation and protein synthesis, thereby trapping the nitrogen in the fecal material.

Disorders of the Colon

Diarrhea

Diarrhea is defined as either a decrease in stool consistency or an increase in stool frequency and volume. It results from a complex interplay between colonic epithelial cell function, luminal factors, intestinal motility, and other factors.

Intestinal motility also influences stool volume and consistency. The ENS, with some modulation by the autonomic nervous system, is the primary regulator of gastrointestinal motility. Neuropeptides, gastrointestinal hormones, and luminal stimuli, such as dietary factors and interactions with

bacteria, influence colonic motility. Disruptions in these systems can and do influence stool consistency and frequency.

Mechanisms of diarrhea can also be viewed from the perspective of absorptive capacity of the small intestine and colon; diarrhea results when this threshold is exceeded.

From a pathophysiological perspective, four mechanisms of diarrhea are traditionally described as of one of the following four types:

- Osmotic
- Secretory
- Motility
- Inflammatory

A degree of overlap occurs between these different types of diarrhea.

Osmotic diarrhea occurs when there is a failure to absorb a solute (usually a carbohydrate) in the proximal small intestine, thus not rendering the fluid isotonic, as would regularly occur, but, rather, hypertonic. Although electrolytes may be reabsorbed, the carbohydrate is not; rather, a portion of it is metabolized by enteric flora to SCFAs, carbon dioxide, hydrogen, and methane. With sodium and other electrolytes absorbed readily by the colon, and resultant low sodium concentration in the lumen, compounded by the presence of nonabsorbed carbohydrate, the high osmotic gradient draws fluid into the lumen and results in diarrhea. This type of diarrhea is characterized by a significant osmotic gap that can be calculated; an additional clinically significant feature of this type of diarrhea is that it diminishes on cessation of enteral intake. Malabsorbed carbohydrate and its metabolites also effect a lowering of the pH of the stool. Lactose deficiency is a good example of osmotic diarrhea in both children and adults. Ingestion of nonabsorbable sugars, such as sorbitol, can also lead to osmotic diarrhea. Excessive intake of carbohydrate-rich and, in particular, simple-sugar-rich beverages can contribute to osmotic diarrhea in children, and can exacerbate pre-existing diarrheal disease. Osmotic diarrhea resulting from excessive carbohydrate and/or simple sugar intake usually improves to resolves with reduction to cessation of dietary intake of these particular nutrients.

Secretory diarrhea occurs when the net secretion of fluids and electrolytes from the colon exceeds their absorption. This type of diarrhea exists independent of eating, and is not influenced by fasting or bowel rest. The prototypical example of pure secretory diarrhea (i.e., in the absence of inflammation or blood present in the stool) is of congenital chloride transport defects, and of gastrointestinal hormonal disorders, such as in Zollinger–Ellison syndrome and in disorders of vasoactive intestinal peptide or in other neuroendocrine tumors (Figure 7).

Cholera occurs when the toxin interacts with the colonocyte stimulating chloride, potassium, and bicarbonate secretion, via toxin A stimulation of cyclic adenosine monophosphate (cAMP); some degree of inflammation may accompany this. ORS, which contributes fluid, sodium, and glucose relies on cellular mechanisms to effect rehydration, and is the mainstay of therapy.

Motility disorders influence intestinal function as pertain to absorption; whereas decreased transit enhances absorption

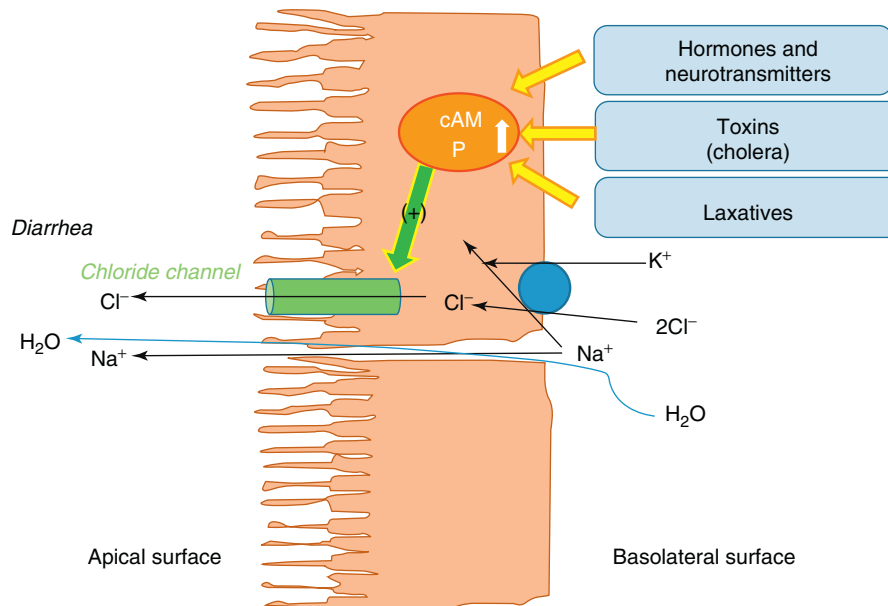


Figure 7 Diarrhea and chloride transport across the small intestine epithelium.

of nutrients, significant decreases in motility can result in stasis. Deconjugation of bile acids by enteric flora can result in malabsorption and inflammation. Increases in motility can occur in the clinical picture of an inflamed colon, which can occur in infants and adults alike. Acute hormonal influences are more common in the adult population, such as those seen with thyrotoxicosis and carcinoid syndrome. Pharmacological agents or substance abuse can also influence motility.

Inflammatory diarrhea results in secretion of mucus, and, typically, with the presence of blood in the lumen, which in itself is a cathartic agent. The integrity of the epithelial barrier is often compromised, with resultant exudation of water and proteins. Bacterial invasion of the mucosa may occur, and is one example of inflammatory diarrhea. Additional disorders that can cause inflammatory diarrhea include allergic colitis and IBD.

Lastly, diarrhea can be categorized clinically into acute and chronic forms, with the latter being defined in persistence of symptoms for more than 3 weeks. Each type of diarrhea can be further clinically divided based on age with respect to likelihood of cause.

Protein Losing Enteropathy (PLE)

Albumin is the main protein maintaining oncotic pressure in the vascular compartment. Intracellular fluid status is maintained by an equilibrium of the oncotic pressure and hydrostatic pressure. Under normal circumstances, intact mucosal barrier and intercellular junctions function to prevent protein losses from the extracellular spaces. Protein losses can occur via the intestines, and can be categorized as conditions associated with mucosal disruption (functional or structural), or those with lymphatic obstruction plus increased hydrostatic pressure in the lymphatic vessels driving the process. The conditions associated with mucosal disruption can be further subdivided into inflammatory, infectious, and noninfectious processes. Another way to characterize these conditions are

Table 5 Classification of PLE

- Inflammatory
 - Non-infectious
 - Eosinophilic gastroenteropathy
 - Cow's milk protein allergy/intolerance
 - Celiac disease
 - Graft versus host disease
 - Crohn's disease
 - Intestinal polyposis syndromes
 - Necrotizing enterocolitis
 - Malnutrition
 - Anastomotic ulceration
 - Infectious
 - CMV gastritis (Menetriere's disease)
 - Rotavirus
 - helicobacter pylori
 - giardia lamblia
 - clostridium difficile
 - salmonella
 - strongyloides stercoralis
- Lymphatic obstructive
 - Lymphangiectasia
 - Cardiac disease /post surgical (Fontan)

whether or not they are associated with normal versus low serum albumin levels; specifically, low albumin states occur in conditions where protein synthesis cannot maintain equilibrium with turnover and needs. Stool alpha-1 antitrypsin status determination is the preferred method of diagnosis of PLE, as it is a protease inhibitor, which is not absorbed by the intestine, but is secreted into the gastrointestinal lumen, where it is not degraded by the intestinal microflora, and is readily

Table 6 Bacterial pathogens grouped by pathogenic mechanism

Adherent	Invasive	Toxigenic	Cytotoxic
Enteropathogenic <i>E. coli</i>	<i>Shigella</i>	<i>Shigella</i>	<i>Shigella</i>
Enterohemorrhagic <i>E. coli</i>	<i>Salmonella</i>	Enterotoxigenic <i>E. coli</i>	Enteropathogenic <i>E. coli</i>
Enteraggregative <i>E. coli</i>	<i>Yersinia enterocolitica</i>	<i>Yersinia enterocolitica</i>	Enterohemorrhagic <i>E. coli</i>
Diffuse-adherent <i>E. coli</i>	<i>Campylobacter jejuni</i>	<i>Aeromonas</i>	<i>Clostridium difficile</i>
	<i>Vibrio parahaemolyticus</i>	<i>Vibrio cholerae</i>	

measurable and quantifiable. Examples of conditions associated with PLE are presented in [Table 5](#).

Infections and Enteric Parasites

In order for viral and bacterial agents to cause inflammatory disease involving the gastrointestinal tract, nonspecific host defense factors of gastric acidity, gastrointestinal motility, enteric flora, barrier functions of mucus secretion and mucosal integrity (in some cases), and of specific enteric mucosal immunity and systemic immune mechanisms have to be overcome.

These infections can result in symptoms of vomiting, diarrhea, and abdominal pain, in addition to systemic effects such as fever. Clinical symptoms vary according to the pathogen.

Bacterial virulence is facilitated by enterotoxin secretion (which may be site specific in its action, secreted before introduction, or while within the lumen), adherence and invasion of the mucosa, and cytotoxin production, which function to disrupt mucosal and cellular function.

Bacteria can be classified based on their pathological mechanism ([Table 6](#)) as well as by their site of activity, and the nature of clinical signs and symptoms manifested. Signs and symptoms vary significantly by pathogen and age at presentation, with some forms presenting as crampy abdominal pain with watery diarrhea of relatively short duration, to frankly bloody diarrhea, and systemic signs and symptoms of inflammation with frank sepsis and shock possible. Common bacterial, viral, and parasitic infections involving the colon are outlined in [Tables 7](#) and [8](#).

Polyps

Intestinal polyps are intraluminal protuberant tumors characterized by their gross morphological appearance, location(s), numbers, size, and presence (pedunculated) or absence (sessile) of the stalk ([Figure 8](#)). Additional salient features include specific histological features used to discriminate between types and aid in predicting malignant potential. Extraintestinal manifestations are also associated with specific polyposis syndromes. Age at occurrence is important with respect to clinical significance and malignant potential; family history of polyps or of polyposis syndromes can also be predictive of disease evolution and aid in screening and surveillance of family members.

In children, juvenile polyps are the most frequently occurring kind, accounting for approximately 90% of colonic polyps. There are also many other types of polyps and polyposis syndromes, which are reviewed in [Tables 9](#) and [10](#). The age of the subject, family history and inheritance patterns,

number and location of polyps, and histology guide to the frequency of surveillance colonoscopy. Symptoms of rectal bleeding usually bring these patients to the attention of a physician. Polyps can cause clinically significant – yet often painless – bleeding so as to cause anemia, and can be linked to abdominal pain, rectal prolapse, or lead points associated with intussusceptions.

IBD

The phrase IBD encompasses UC and Crohn's disease. Indeterminate colitis is a diagnosis attributed to a condition in which a clear distinction cannot be made between the two aforementioned forms of IBD, as opposed to a heterogeneous group of diseases that present a wide clinical and histological spectrum.

Epidemiology

IBD presents in a bimodal fashion as pertains to age, first in late adolescence or early adulthood, and a smaller peak in the fifth decade of life. Overall, the sexes are equally affected for UC; in adults, the incidence of Crohn's disease is 20–30% higher in women.

The second half of the twentieth century has seen the incidence of UC remain stable over time; Crohn's disease has demonstrated a marked increase across all age groups since 1950. Although IBD can affect all races, Caucasians are affected markedly more than Africans, or people of African origin. Ashkenazi Jews have a markedly increased risk of IBD compared with other Jewish groups. The incidence in the Ashkenazi Jewish population roughly parallels that of the respective geographical community in which they reside, albeit at a level which can be three- to four-times that of that general population, suggesting a genetic predisposition. The majority of individuals affected by both disorders are in North America and Northern Europe. The remainder of Europe, Latin America, and Australia has lower incidence rates, with rare cases occurring in Africa and Asia.

Etiology

The exact etiology of IBD is unclear and is an area of active research. A multifactorial interaction between genetic predisposition, environmental stimuli, endogenous triggers, immunological dysregulation, and modifying factors is postulated, and is discussed below.

Genetics

A positive family history in a first-degree relative confers significant risk, between 10% and 20% risk of disease occurrence of

Table 7 Bacterial enteric infections

Name	Epidemiology and pathogenesis	Clinical features	Diagnosis and treatment
<i>Shigella</i> <i>S. dysenteriae</i> <i>S. sonnei</i> <i>S. flexneri</i> <i>S. boydii</i>	<ul style="list-style-type: none"> Acute infection Highly contagious; low infective dose (10–100 organisms) 	<ul style="list-style-type: none"> Bacterial dysentery <ul style="list-style-type: none"> Crampy abdominal pain and watery stools; Progressive to bloody, mucoid, pus-laden stools Tenesmus Fever Meningismus Febrile seizures in younger patients Hemolytic uremic syndrome (HUS possible) 	<p>Diagnosis</p> <ul style="list-style-type: none"> Crypt abscesses Lymphatic hypertrophy Necrosis Elevated WBC count Stool culture <p>Treatment</p> <ul style="list-style-type: none"> Fluid and electrolyte replacement Hand washing to prevent transmission Limited role of antibiotics
<i>Salmonella</i> <i>S. typhi</i> <i>S. paratyphi</i> <i>S. enteritidis</i>	<ul style="list-style-type: none"> Infective load: 10^3–10^5 organisms Reservoirs: poultry and eggs, lizards, amphibians Raw/undercooked foods Mucosal invasion (jejunum and colon) Inflammatory response with active secretion 	<ul style="list-style-type: none"> Five clinical presentations <ul style="list-style-type: none"> Acute gastroenteritis (12–72 h incubation) Focal, nonintestinal infection Bacteremia Asymptomatic carrier state Enteric fever, abdominal cramping, nausea, vomiting, bloody, mucoid stools, rose spots on the trunk, leucopenia, prolonged excretion possible, variable by age, carrier state not uncommon 	<p>Diagnosis</p> <ul style="list-style-type: none"> Stool culture <p>Treatment</p> <ul style="list-style-type: none"> Supportive Very limited role for antibiotics
<i>Campylobacter</i>	<ul style="list-style-type: none"> Transmission by contaminated foods (poultry, eggs, milk; water; and domestic animals) Initial site(s): jejunum colon 	<ul style="list-style-type: none"> Incubation period of 2–11 days Fever prodrome Severe diarrhea, tenesmus, and abdominal pain 	<p>Diagnosis</p> <ul style="list-style-type: none"> Incubation for culture <p>Treatment</p> <ul style="list-style-type: none"> Supportive Role for antibiotics for limiting excretion period and duration of illness
<i>Clostridium</i> <i>C. difficile</i>	<ul style="list-style-type: none"> Enteric flora Toxin A (enterotoxin: alters permeability; inflammation mediator) Toxin B (cytotoxin) Pseudomembranes 	<ul style="list-style-type: none"> Antibiotic-associated diarrhea May be restricted to the right colon 	<p>Diagnosis</p> <ul style="list-style-type: none"> Stool <i>C. difficile</i> toxins A and B ELISA Endoscopy and histology: pseudomembranes (mucin, fibrin, polymorphonuclear lymphocytes, necrotic debris) Erythema Edema Friability Apthous ulcers <p>Treatment</p> <ul style="list-style-type: none"> Supportive therapy Cessation of offending antibiotic Metronidazole as first-line agent Vancomycin secondary agent Probiotics useful in relapse prevention
<i>Yersinia</i> <i>Y. enterocolitica</i>	<ul style="list-style-type: none"> Transmission <ul style="list-style-type: none"> Contaminated pork, Enterotoxin elaboration 		<p>Diagnosis</p> <ul style="list-style-type: none"> Cultures are not very accurate Endoscopy: mucosal ulcerations, friability throughout colon and terminal ileum possible Histology: lamina propria infiltration B inflammatory cells; ulcerative and necrotic areas Dilated crypts <p>Treatment</p> <ul style="list-style-type: none"> Antibiotics

(Continued)

Table 7 Continued

Name	Epidemiology and pathogenesis	Clinical features	Diagnosis and treatment
<i>Aeromonas</i> <i>Hydrophila</i>	Water contaminants	Three symptoms <ul style="list-style-type: none"> • Mild watery diarrhea • Bloody diarrhea • Persistent diarrhea 	Diagnosis <ul style="list-style-type: none"> • Stool culture Treatment <ul style="list-style-type: none"> • Antibiotics
<i>E. coli</i> Enteropathogenic <i>E. coli</i> (EPEC)	<ul style="list-style-type: none"> • Localized adherence to enterocytes • Signal transduction • Intimate adherence and effacement 	<ul style="list-style-type: none"> • Diarrhea • Vomiting • Malaise • Fever • Mucoïd, nonbloody stools • Two-week duration 	Diagnosis <ul style="list-style-type: none"> • Presence of adherent organisms on small intestinal/rectal biopsy Treatment <ul style="list-style-type: none"> • Antibiotics
Enterotoxigenic <i>E. coli</i> (ETEC)	Enterotoxin elaboration <ul style="list-style-type: none"> • Heat-labile (LT) toxin • Heat-stable (ST) toxin • Fimbriae-based attachment • Stimulate adenylate cyclase (LT) and guanylate cyclase (ST) to secrete fluid 	<ul style="list-style-type: none"> • Nausea • Abdominal pain • Watery diarrhea • Traveler's diarrhea 	Diagnosis <ul style="list-style-type: none"> • Bioassays • Immunoassays • Gene probes for ST or LT Treatment <ul style="list-style-type: none"> • Supportive • Antibiotics decrease duration of excretion; not recommended for children
Enteroinvasive <i>E. coli</i> (EIEC)	<ul style="list-style-type: none"> • Colonize colon • Invade tissue • Replicate within cells • Secretory enterotoxins 	<ul style="list-style-type: none"> • <i>Shigella</i>-like <ul style="list-style-type: none"> – Watery diarrhea – Bloody mucoïd, pus-laden diarrhea – Tenesmus and fever 	Diagnosis <ul style="list-style-type: none"> • Bioassays • Serotyping • ELISA Treatment <ul style="list-style-type: none"> • Supportive • Limited antibiotic role
Enterohemorrhagic <i>E. coli</i> (EHEC)	<ul style="list-style-type: none"> • Part of normal enteric flora in healthy animals • Cytotoxin similar to shiga toxin • Adherence • Transmission <ul style="list-style-type: none"> – Contaminated, undercooked meat – Unpasteurized apple cider – Children and the elderly more prone to HUS 	<ul style="list-style-type: none"> • Hemorrhagic colitis <ul style="list-style-type: none"> – Crampy abdominal pain – Watery diarrhea progressing to bloody stools – Absence of fever • Hemolytic uremic syndrome (HUS) 	Diagnosis <ul style="list-style-type: none"> • Serotyping • Serum antibody tests • Cytotoxin bioassays • DNA hybridization • PCR-based tests • ELISA Treatment: <ul style="list-style-type: none"> • No effective therapy • Supportive care <ul style="list-style-type: none"> – Dehydration correction – Management of electrolyte abnormalities – Blood transfusions as necessary
Enteroaggregative <i>E. coli</i> (EAEC)	<ul style="list-style-type: none"> • Localized adherence likely (HEp-2 or HeLa cells) • Enterotoxin • Increased intestinal mucus secretion 	<ul style="list-style-type: none"> • Diarrhea <ul style="list-style-type: none"> – Watery – Mucoïd – Persistent 	Diagnosis <ul style="list-style-type: none"> • DNA probes
Diffuse adherent <i>E. coli</i>	<ul style="list-style-type: none"> • Diffuse adherence (HEp-2 or HeLa cells) likely 	<ul style="list-style-type: none"> • Diarrhea 	Diagnosis <ul style="list-style-type: none"> • DNA probes

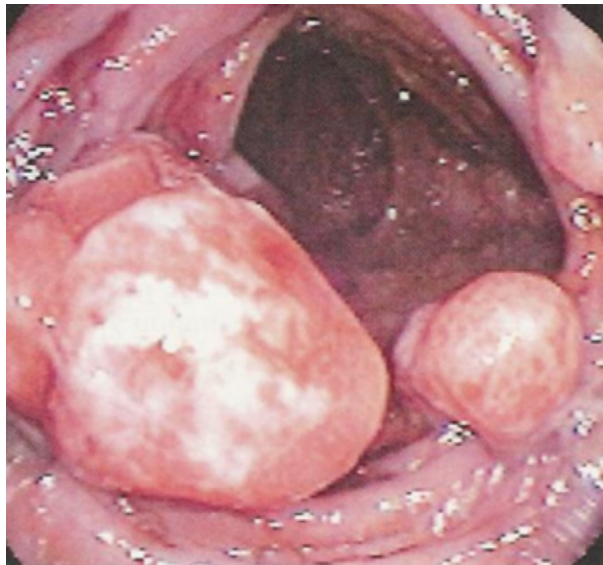
either disease type in a first-degree relative. Northern European, North American populations, and in particular globally the Ashkenazi Jewish population have the highest risk of the disease.

A high rate of concordance among Swedish monozygotic twins versus dizygotic twins has been reported in Crohn's disease (44% vs 3.8%). In the same study, the incidence rates observed in monozygotic twins for UC were (6.3%). These

data, although supportive of a genetic role, show less than 100% penetrance, suggesting that whereas genetics are more important in Crohn's disease than in UC, environmental influences play a significant role. Simple Mendelian models of inheritance are inadequate to address the complex inheritance patterns of IBD. Candidate gene studies have suggested modest HLA associations, which differ in different

Table 8 Additional colonic pathogens

Name	Pathogenesis	Clinical symptoms	Diagnosis and treatment
Ameba <i>Entamoeba histolytica</i>	<ul style="list-style-type: none"> • Travel to endemic areas a risk factor. Large intestinal commensal organism • Transmission: <ul style="list-style-type: none"> – Person to person contact – Contaminated food/water (cysts) – Cysts transform into trophozoites at the terminal ileum – Invade mucosa and submucosa 	<ul style="list-style-type: none"> • Acute onset • Fulminant colitis <ul style="list-style-type: none"> – Bloody, mucoid diarrhea – Abdominal distention – Abdominal pain • Perforation possible • Hepatic abscesses possible 	Diagnosis <ul style="list-style-type: none"> • Histopathology <ul style="list-style-type: none"> – Hyperemia and edema – Acute inflammation – Microulceration – Flask ulcer formation • (Fresh) stool examination for cysts or trophozoites Treatment <ul style="list-style-type: none"> • Iodoquinol • Metronidazole
Helminths <i>Trichuris trichura</i> (whipworm)	<ul style="list-style-type: none"> • Primarily colonic 	<ul style="list-style-type: none"> • Heavy infestations associated with (bloody) diarrhea • Rectal prolapsed 	Diagnosis <ul style="list-style-type: none"> • Stool assays Treatment <ul style="list-style-type: none"> • Thiabendazole • Mebendazole
Schistosomiasis <i>S. mansoni</i>	<ul style="list-style-type: none"> • Snail as pathogen • Fresh water contamination 	<ul style="list-style-type: none"> • Dysenteric-like illness • Bloody diarrhea • Perianal fistulae 	Diagnosis <ul style="list-style-type: none"> • Endoscopic <ul style="list-style-type: none"> – Focal and diffuse fibrosis – Intraluminal – Granulomatous masses (bilharziomas) • Stool exam for viable eggs Treatment <ul style="list-style-type: none"> • praziquantal

**Figure 8** Endoscopic view of a colonic polyp.

populations. Systemic genome searches more recently employed in families with several members have IBD employing linkage analyses. Evidence of the *NOD2* gene on chromosome 16 being involved with cases of Crohn's disease has led to it

being labeled as the IBD1 gene locus. This gene is involved with the encoding of a protein associated with monocytic nuclear factor- κ B; this protein and pathway are involved with the interaction of monocytes with bacterial peptidoglycans. Note that only approximately 30% of individuals with Crohn's disease test positive for this particular gene mutation.

Environmental Influences

The rapid increase of Crohn's disease over the past 50 years, increasing trends in immigrant populations, as well as incomplete genotype-phenotype associations have promoted attention to environmental factors. In particular, the search to identify an antigenic trigger to the enteric immune system has been pursued by several investigators. Postulated microbial intraluminal triggers have included mycobacterium and viruses. Dietary antigens or toxins have not been identified; diet westernization has been explored and remains an active area of research. Environmental exposures early in the life cycle such as birth environment, nutritive factors (breast vs formula fed, with the former thought to have a protective effect) may alter the risk for developing disease in susceptible individuals/groups. Additional modulating factors include smoking and the use of oral contraceptives.

Table 9 Hamartomatous intestinal polyps

<i>Syndrome</i>	<i>Location of polyps</i>	<i>Pathology</i>	<i>Extraintestinal abnormalities</i>	<i>Cancer risk</i>
Juvenile polyposis	Colon; some small intestinal involvement	<ul style="list-style-type: none"> Up to 3 cm in size Mucus retention and inflammatory cells in the lamina propria cysts Mostly pedunculated 		Colonic: low risk
Peutz–Jeghers	Mostly small intestinal; some gastric and colonic involvement	<ul style="list-style-type: none"> 1–3 cm in size Either sessile or pedunculated Glandular epithelium and smooth muscle branching 	Macular pigmentation on hands, lips, and mouth	Up to 18x vs the general population; lower than other polyposis syndromes
Cowden's syndrome	Colon and stomach	<ul style="list-style-type: none"> Multiple polyps Hamartomatous 	<ul style="list-style-type: none"> Lipomas Papillomas Orocutaneous hamartomas 	<ul style="list-style-type: none"> Fibrocystic or fibroadenomatous, ductal breast cancer Nodular thyroid hyperplasia or follicular adenoma

Table 10 Polyposis syndromes

<i>Type/syndrome</i>	<i>Location(s)</i>	<i>Histology</i>	<i>Clinical features</i>	<i>Cancer risk</i>
Familial polyposis coli	Colonic: fundic gland hyperplasia (stomach)	<ul style="list-style-type: none"> Thousands of adenomas Elevated ornithine decarboxylase levels APC gene 	<ul style="list-style-type: none"> Apparent after puberty Diarrhea as most common symptom Abdominal pain Hypertrophic retinal lesions 	<ul style="list-style-type: none"> Thyroid cancer Pancreatic cancer <p>Risk of colon approximately reaches 100% by 55 years of age</p>
Gardner's Syndrome	Colon, stomach, duodenum, small intestine	<ul style="list-style-type: none"> 2–5 mm sessile Adenomas in the antrum and periampullar regions More than 1000 at a time 	<ul style="list-style-type: none"> Triad of: <ul style="list-style-type: none"> Polyps Osteomas Soft tissue tumors Dental abnormalities 	<ul style="list-style-type: none"> Duodenal tumors at highest risk Associated risk of: <ul style="list-style-type: none"> Pancreatic carcinoma Ampullary cancer Hepatoblastoma
Turcot's syndrome	Colonic	Adenomatous polyps	<ul style="list-style-type: none"> Present in adolescents with cancer; family history Autosomal recessive 	<ul style="list-style-type: none"> Associated neural tumors <ul style="list-style-type: none"> Medulloblastomas Gliomas
Cronkhite–Canada Syndrome	<ul style="list-style-type: none"> Throughout gastrointestinal tract 	<ul style="list-style-type: none"> Adenomatous lesions within adenomatous polyps 	<ul style="list-style-type: none"> Alopecia Nail dystrophy Brown macular skin lesions Edema related to protein losing enteropathy 	<ul style="list-style-type: none"> Five percent of cases evolve into gastrointestinal carcinomas
Inflammatory polyposis	Colonic: pseudopolyps	<ul style="list-style-type: none"> Pleomorphic regenerative tissue 	<ul style="list-style-type: none"> Systemic signs and symptoms of inflammation (Section IBD) 	<ul style="list-style-type: none"> Colonic; risk of cancer from inflammatory bowel disease (Crohn's disease and ulcerative colitis)

The incidence and prevalence of IBD are more in the developed world, and the global distribution is inverse to that of where geohelminthic worm infections are endemic.

Furthermore, there are nutritional factors also implicated in the increasing incidence and prevalence of IBD in the developing world. Transition from traditional to the modern western diet has been identified as a risk factor for many noncommunicable diseases, including IBD. In particular, decreasing fruit, vegetable, and fiber intake, increasing terrestrial animal protein intake, and

decreased marine and plant-based protein intake, and changing profiles of types and amount of dietary fat intake (i.e., less ω -3 polyunsaturated fatty acids, higher intakes of ω -6 polyunsaturated fatty acids) are among implicated risk factors for the development of IBD in susceptible individuals.

Pathogenesis

The interactions between the enteric immune system and the intestinal lumen are dynamic; some degree of inflammation in



Figure 9 Distribution of ulcerative colitis.

response is ever present in the normal mucosal lamina propria of the colon and small intestine, which see a very large antigenic load daily. An intact mucosal barrier, in addition to normally functioning immunoregulatory mechanisms prevent this interaction progressing to the level at which tissue injury occur.

Current chronic, inflammatory relapsing disease processes may represent either an inappropriate persistent immune response to a luminal antigen/stimulus, versus an appropriate immune response to a persistent, abnormal stimulus, versus perhaps a prolonged immune response to a ubiquitous stimulus.

Enteric flora may play a role in this process, although no evidence to date points strongly to a single pathogen. Defective mucosal barrier function and increased intestinal permeability – the latter being documented in patients with IBD and in up to 10% nonaffected first-degree relatives – may also be involved.

The immune response is primarily T-cell mediated, of a Th-1 nature. Abnormalities of interleukin-12 (IL-12), interferon gamma (INF- γ), and tumor necrosis factor alpha (TNF- α) have also been implicated in the immunological pathogenesis of this disease. White blood cells respond to these inflammatory mediators and proliferate the immune response. These recruited cells synthesize agents such as arachidonic acid metabolites, platelet activating factor, proteases, free radicals such as reactive oxygen species – all of which can and do cause direct injury to cells and the mucosa.

Pathology

Pathology differs between these two disorders, both in terms of anatomical distribution and tissue involvement.

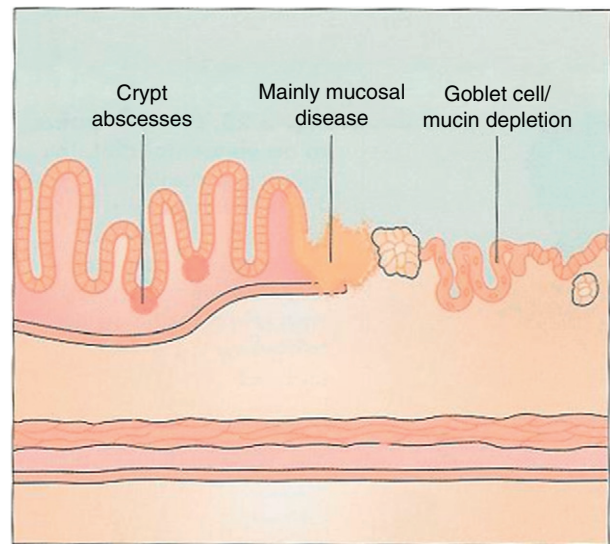


Figure 10 The mucosa in ulcerative colitis. Reproduced from Kelly DA and Booth IW (1996) *Pediatric Gastroenterology and Hepatology*. London: Mosby-Wolfe.

Ulcerative Colitis

UC is limited to the colon and rectum, usually beginning distally in the rectum and extending to varying lengths proximally, by definition, in a continuous fashion (**Figure 9**). Usually, a clear distinction can be made where disease ends and normal mucosa can be appreciated grossly, or endoscopically. The gross appearance of the mucosa is dependent on the severity of the disease process. Mild disease presents with a diffuse erythema and loss of the characteristic appearance of the vasculature. Numerous small, superficial ulcerations, exudates, and bleeding are seen in moderate disease; larger, deeper ulcerations increased exudates, and the development of pseudopolyps, loss of normal gross architectural landmarks such as the folds diminish. Microscopically, UC is limited to the mucosa; with more severe disease, deeper layers may show a degree of involvement, with inflammatory cell infiltrates, shortening, branching, and decreases in the number of crypts as well as crypt abscesses can also be seen (**Figure 10**).

Crohn's Disease

Crohn's disease can involve any part of the alimentary tract from the mouth to the anus, and frequently does so in a discontinuous fashion, leaving 'skip areas' – regions which are grossly and histologically normal (**Figure 11**); in the colon, this lends a cobblestoned appearance. Macroscopically, wall thickening is evident in long-standing disease. This disease, by definition, is a transmural process (**Figure 12**). With chronic disease, fibrostenosis occurs, narrowing the intestinal lumen. Strictureing disease may follow fibrosis of superficial and deeper layers of the intestinal wall, which are evident on radiographic studies (**Figure 13**).

The mesentery may also demonstrate inflammation, with adhesion and fixation of the colon a consequence of the transmural inflammatory process. Adjacent loops of bowel may become matted together. As luminal diameter narrows, intraluminal

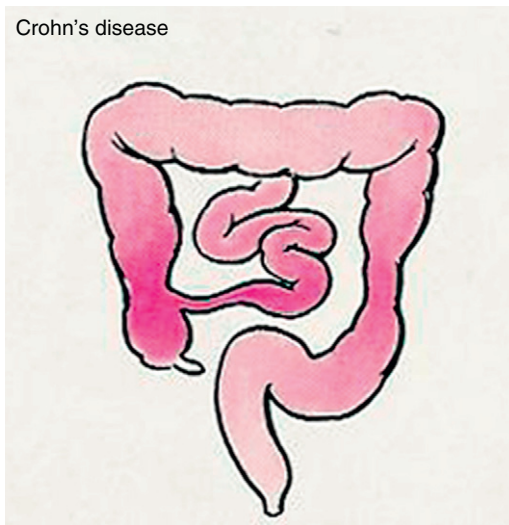


Figure 11 Distribution of Crohn's disease.

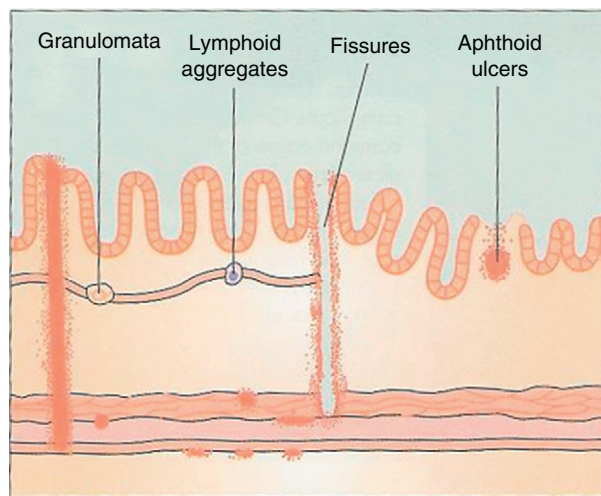


Figure 12 The mucosa in Crohn's disease. Reproduced from Kelly DA and Booth IW (1996) *Pediatric Gastroenterology and Hepatology*. London: Mosby-Wolfe.

pressure may increase; in the face of nonabating inflammation, this transmural process may lead to fistula formation. Enterointeric fistulas are limited to the bowel; enterovaginal, enterovesicular, and enterocutaneous fistulization may occur. Inflammatory intra-abdominal masses called phlegmons may also form by this fistulization process.

The endoscopic appearance of Crohn's disease varies both by location and by time relative to the disease evolution. Intestinal Crohn's disease may initially present with aphthous ulceration overlying Peyer's patches in the colon. Ulcerations eventually grow in diameter and progress in depth, with frankly friable, exudative lesions.

Histological findings of affected areas include intense inflammatory cell infiltrates extending into the crypts, with

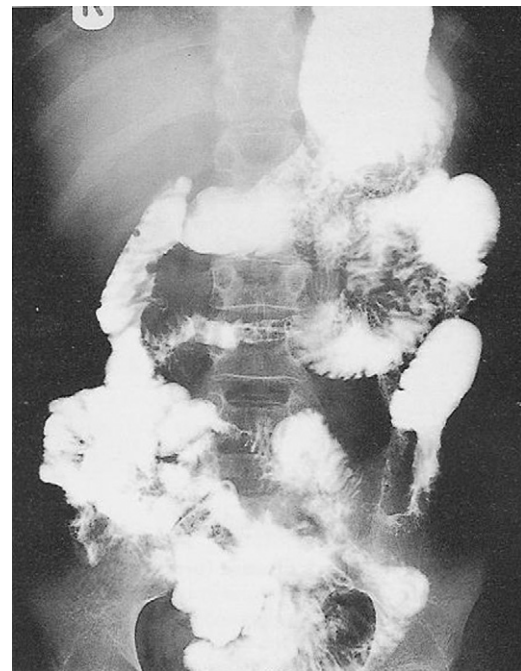


Figure 13 Small bowel stricturing noted on small bowel follow through series in a child with Crohn's disease. Reproduced from Kelly DA and Booth IW (1996) *Pediatric Gastroenterology and Hepatology*. London: Mosby-Wolfe.

shortening and forking of these structures, and associated abscesses. The inflammation is transmural; fibrosis and histiocyte proliferation are also seen. Noncaseating granulomatous submucosal and mucosal lesions, which are a hallmark of this disease, are not found in a majority of biopsy specimens. Granulomas can also be seen in intestinal infections such as in intestinal tuberculosis and sarcoidosis.

Even macroscopically normal-appearing tissue may yield histological findings of inflammation compatible with Crohn's disease, thus indicating that examination of the entire alimentary canal with surveillance biopsies is required before arriving at a diagnosis.

Inflammatory Bowel Disease and the Terminal Ileum

The terminal ileum is one of the most commonly involved sites in the intestine for Crohn's disease, originating often at the lymphoid follicle; strictures may form. A phenomenon of ileal involvement has been postulated in cases of apparent UC that involve the ileum, in which cecal inflammation is postulated to 'backwash' into the ileum; this finding being consistent with UC is controversial.

Extraintestinal Manifestations

Extraintestinal manifestations are common in both Crohn's disease and UC, including ophthalmologic (uveitis), joint involvement (arthralgias and arthritis of the large joints), the skin, hepatobiliary system, pancreas, renal system, and vascular system, most commonly. Anemia and weight loss are common at the time of presentation. Growth and pubertal delay are very common at the time of presentation in children; short stature occurs in up to 50% of children. Some of these

findings relate to the inflammatory process itself; others are linked to malnutrition associated with IBD.

Perianal disease with fistulization and skin tags are perhaps the most common extraintestinal abnormality associated with Crohn's disease.

Nutritional Consequences of Inflammatory Bowel Disease

Malnutrition includes acute weight loss, partly attributable to anorexia associated with inflammation, and partly to the disease process itself, i.e., inadequate intake as well as of excessive (malabsorptive) losses. An example delineating all of these mechanisms is of anemia, which can result from frank blood loss from associated gastrointestinal bleeding, anemia of chronic disease mediated by the inflammatory mediators, anorexia with decreased dietary iron intake, and, as in the case of duodenal and jejunal disease activity (as can occur in Crohn's disease), with decreased absorption.

Intestinal disease can result in both decreased nutrient absorption as well as disruption of the mucosal barrier resulting in exudation of proteins, a process known as PLE. The latter can result in hypoalbuminemia; third spacing of fluids as a result of decreased intravascular oncotic pressure can occur. Increased energy expenditure as a consequence of inflammation is noted, particularly in the febrile state, or with sepsis. Inflammation and discomfort also contribute to decreased enteral intake – factors contributory to a catabolic state.

In addition to iron, other mineral and trace element deficiencies are noted in IBD. Iron deficiency has been discussed above. Zinc is intimately associated with gut mucosa, and is susceptible to deficiency; low albumin levels resulting from PLE and increased intestinal epithelial cell turnover probably represent a significant source of zinc depletion. Vitamin B₁₂ and folic acid deficiencies have also been documented among the water-soluble vitamins, particularly when the terminal ileal disease is noted. Vitamin D deficiency is the most common among the fat-soluble vitamins.

Treatment of IBD

Medical approaches: Several anti-inflammatory treatment modalities have been employed in the treatment of IBD. Their use is dictated by disease type, location, extent, and severity. Steroids provide the cornerstone of initial therapy for acute inflammation. Five aminosalicylate derivatives, use of anti-metabolites such as azathioprine and 6-mercaptopurine methotrexate, and newer biological agents including anti-TNF- α are currently employed.

Nutritional therapies including semielemental enteral feedings or parenteral therapy have a role in the management of IBD. Although enteral therapy is not considered a first-line therapy for UC at the present in the USA, its use as such is popular in Europe and Canada, and allows for steroid sparing/

avoidance. The time to onset of remission using enteral therapy in Crohn's disease is much less with steroids than with enteral therapy, however, with the former occurring typically within 2 weeks, the latter taking usually 6–8 weeks to achieve similar clinical remission. Smaller studies have been conducted employing low-fat diets, lower processed sugar foods, and employing polyunsaturated fatty acids, such as in fish oils, which may play a role in the treatment of disease. The data regarding use of ω -3 fish oil supplements for the treatment of IBD currently do not support routine use as a main-line therapy, but may have an adjuvant role in maintaining disease remission.

Surgical treatment is indicated in UC when acute, fulminant disease does not respond to medical therapy, or persistent chronic disease which is refractory to medical (steroid) therapy and when the diagnosis has been confirmed, i.e., that Crohn's disease has been ruled out. Colectomy is curative in such instances.

Crohn's disease is more complex, and surgical intervention, limited to involved segments only, is not curative. Failure of medical therapy to reduce inflammation, critical stenosis of the involved segments with fibrosis leading to obstruction, perforation, fistulization, and abscess formation not amenable to medical therapy and frank gastrointestinal hemorrhage are indications for surgical intervention. Re-activation of disease can occur postoperatively, at the site of anastomoses or elsewhere.

The natural history of IBD is such that long-standing disease increases the risk of colonic dysplasia, particularly in the case of UC, besides being a curative intervention, making the case for colectomy more attractive in older patients. Ileoanal continuity can be achieved by means of surgical anastomoses. Pouchitis secondary to bacterial overgrowth, smoldering pockets of disease activity that may not have been resected or have become evident after resection, and loss of continence are common complications of these procedures.

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COPPER

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Introduction

Copper (atomic number 29 and atomic weight 63.55) is an essential trace element. It was one of the first metals to have been isolated and used by man, possibly approximately 10 000 years ago in the area between present day Greece, and India. Certainly, the metal had been isolated before 3500 BC, which is when it was realized that adding tin (one part to nine parts copper) produced a harder more versatile material, namely bronze. After iron and aluminum, copper is the most widely used metal. Its abundance in the Earth's crust is 68 ppm, and although small amounts of metallic copper can be found naturally, 50% of copper occurs with iron as sulphide ores, chalcopyrite, and bornite; other ores include chalcocite and covellite, both sulphides; the carbonates malachite and azurite, and cuprite which is an oxide. In its biological role and activity, copper is bound in proteins by N and S ligands provided by the amino acids histidine and cysteine, and it has a major role in the storage and utilization of oxygen. Copper has two oxidation states that are of physiological importance these are the cuprous (Cu^+) and cupric (Cu^{2+}) states. Cuprous copper is easily oxidized to the cupric state but this transition is controlled when the copper is bound in a complex as a cuproprotein. The two states have different coordination chemistries; cupric copper favors a planar configuration with 4N ligands, whereas cuprous ions form tetrahedral complexes involving 4S ligands. More complex structures involving two or more copper atoms and others with Cu^{3+} and Cu^{4+} states are known but as yet their functions have not been fully characterized. One exception to this generalization is hemocyanin which is found in molluscs and arthropods including crustaceans. Hemocyanin contains two Cu^+ atoms that interact to bind molecular oxygen reversibly at the low oxygen tensions and temperatures of the environment at which these creatures live; it is not found in mammals in whom hemoglobin has the same role. However, many cuproproteins found in humans and other mammals are ubiquitous and highly conserved thereby encouraging speculation that, like iron, copper may have been functionally important before an aerobic environment evolved.

The oxidation of cuprous to cupric copper generates free radicals which, in turn, can cause extensive functional and architectural tissue damage by oxidizing organic molecules such as lipids, proteins, and nucleic acids. Cu^{3+} is a more powerful oxidant than the two usual states. Thus to acquire, distribute, use, and excrete copper, organisms have had to

evolve a system of specific protein carriers which forestalls producing free copper ions and thereby minimizing the risk of oxidative toxicity. Similar systems of chaperoned traffic exist for other essential transition elements such as iron, zinc, and manganese. Since these elements have similar physicochemical properties, interactions may occur between them at separate stages of their respective trafficking systems, however, at normal levels of exposure each system has an accumulative specificity and selectivity which discriminates effectively for their delivery to their respective depots and functional sites.

Copper Function

Copper-dependent enzyme activities are crucial to energy metabolism and muscle efficiency, connective tissue and bone formation, neurotransmission and catecholamine synthesis, the turnover of peptide hormones, free radical elimination, and iron trafficking and hemoglobin formation. The features of copper deficiency are described later. Genomic analyses indicate the existence of many potential copper binding motifs for which the proteins and functions have yet to be identified (Table 1).

Dietary Sources and Reference Values

The copper content of some common foods is shown in Table 2. Good dietary sources of copper are seeds, grains, nuts (cashews and pecans), beans, wheat bran, crustacea, shellfish, cocoa products, and liver. Although copper is naturally abundant in the liver, the amount in the livers of farmstock may have been enhanced either from the animals having fed in areas which had been surface treated with slurry which may also have contained high levels of cadmium, which like copper, accumulates in the animals' livers and thus presenting a risk of high exposure to cadmium; or in the case of pigs from copper given to them as a microbicide to alter the gut flora or prevent enteric infections, and improve their growth efficiency (i.e., thriftiness). These practices and their regulation should be considered in the appropriate perspective when preparing dietary advice for individuals and populations. The entry of copper into the food chain may be limited by the redox potential, alkalinity, and sulfur and molybdenum content of the soil. Thus in some areas, copper deficiency may be endemic in grazing livestock despite copper being abundant in the soil.

Table 1 Copper-dependent enzyme activities

Cytochrome <i>c</i> oxidase	Mitochondrial oxidative phosphorylation transfer of electron to O ₂
Dopamine- β -hydroxylase	Norepinephrine and epinephrine synthesis
Tyrosinase	Tyrosine \rightarrow dopa \rightarrow dopaquinone \rightarrow melanin production
Peptidylglycine α -amidating mono-oxygenase	Neuropeptide synthesis including melanocyte stimulating hormone
Tryptophan 2,3-dioxygenase	Tryptophan to <i>N</i> -formyl-L-kynurenine
Indole 2,3-dioxygenase	Indole to 2-formylaminobenzaldehyde
Monoamine	Degradation of amines, e.g., serotonin, catecholamines, dopamine, and tyramine
Diamine oxidase	Degrades histamine and polyamines
(Cu-Zn) superoxide dismutase	Cytosolic antioxidant; $2O_2^- + 2H^+ \rightleftharpoons H_2O_2 + O_2$ Superoxide conversion
Lysyl (and hydroxylysine) oxidase	Collagen and elastin cross linking; lung, bone matrix, cardiovascular integrity
Uricase	Hepatic and renal metabolism of uric acid
Hephaestin	Ferroxidase for cellular export of iron
Ceruloplasmin	Plasma metallo/ferroxidase
Thiol oxidase	Formation of disulphide linkages

Table 2 Illustrative copper contents of foods

<i>Food</i>	<i>Copper content ($\mu\text{g}/100\text{ g wet weight}$)</i>	<i>Size of typical serving (g)</i>	<i>Copper in a typical serving (wet weight) (mg)</i>
Fish	61	120	0.07
Turkey	71	120	0.09
Chicken	34	120	0.04
Hamburger	95	120	0.11
Roast beef	82	120	0.10
Steak	120	120	0.14
Sheep liver	15 700	120	18.9
Pork liver	14 100	120	17.0
Egg	80	40	0.03
Hard cheese	43	120	0.05
Whole wheat	107	30	0.03
Scallops	608	120	0.03
Clams	739	120	0.73
Crab	175	120	0.89
Shrimp	175	120	0.21
Oysters	289	120	0.35
Mussels	475	120	0.57
Lobster	3 600	120	4.40
Chocolate	118	15	0.02
Milk	33	120	0.04
Peas	238	120	0.29
Soy beans	109	120	0.13
Avocado	168	120	0.20
Raisins	168	30	0.05
Peanut butter	853	30	0.26

The copper content of water delivered by copper piping can contribute significantly to intakes. Soft acidic water can leach copper from the piping. If the copper content of water is $<0.1\text{ mg l}^{-1}$ water contributes less than 10% of daily intake of copper, whereas if it is $>1\text{--}2\text{ mg l}^{-1}$ up to 50% (i.e., 0.5 mg) of daily intake may derive from water. Whether or not this constitutes a potential benefit or hazard needs to be determined by a case-by-case risk assessment; this applies also to assessing the potential impact of using utensils made of copper alloys (e.g., brass) or of copper for cooking or for storing water, milks, and drinks.

The dietary reference intakes for copper are given in **Table 3**. These values were developed for North America but

most advisory bodies give similar values either as a single value, or as a range (e.g., $1.0\text{--}1.2\text{ mg day}^{-1}$ in Australia and New Zealand). Some bodies do not think that additional intakes are necessary during pregnancy or lactation, considering that maternal adaptation such as increased absorption or mobilization of endogenous depots would meet the needs of pregnancy and lactation. The uncertainty of the information on copper nutrition in humans has meant that rather than estimating a range of lower, average, and population intake reference values, most agencies provide figures that are seen as adequate intakes. Failure to meet these intakes is not diagnostic of deficiency, though it would be an indicator of a risk of deficiency. These uncertainties are the source of variability

Table 3 Dietary reference intakes for copper in North America

Age	RDA (mg day ⁻¹)
<6 months	0.2
6–12 months	0.3
1–3 years	0.34
4–8 years	0.44
9–13 years	0.7
14–18 years	0.89
19–>70 years	0.9
Pregnancy	1.0
Lactation	1.3

Source: Reproduced from IOM recommendations.

amongst the values produced by different advisory bodies and because such variations, no matter how small, could be barriers to trade there are international initiatives to harmonize approaches for the setting of reference values. The dietary intake of copper is approximately 1–2 mg day⁻¹, but individual intakes of copper vary widely over 2–3 weeks, according to the pattern of intakes of particular high sources of copper. Few dietary intake surveys are long enough to capture this variability. A tolerable upper intake limit, based on the risk of liver damage, of 10 mg for daily intakes of copper over a lifetime has been advised in North America. In the European Union, an upper level of 5 mg has been recommended. Some would regard this value as cautious.

Copper Trafficking and Kinetics

Knowledge of the systemic use of copper has been deduced from studies in cell line systems *in vitro*, bacteria, yeasts, drosophila, xenopus, zebra fish, mice, sheep, and humans. Knockout models and inborn errors of metabolism in mammals have contributed appreciably, as have molecular biological techniques which have enabled the integration of information from these sources, to provide a mechanistically plausible model for the absorption and systemic fate of copper in humans. Studies of these processes are hampered because the abundance of two natural stable isotopes of copper ⁶³Cu and ⁶⁵Cu of 69% and 31% make them difficult to use in tracer studies, and the short half-lives of its radio isotopes (⁶⁷Cu – 61.9 h and ⁶⁴Cu – 12.9 h) limit their value as tracers also.

The principal proteins in the trafficking pathways for copper are a high-affinity copper transporter, CTR1, and a low-affinity transporter, CRT2 (there may be a third CTR) which are responsible for the energy independent influx of copper into cells; two carriers involved with the energy-dependent efflux of cupric copper from cells, ATP7A ATPase and ATP7B ATPase; a depot protein, metallothionein, or more specifically isometallothioneins (MTN); and a number of target-specific chaperone carrier proteins that take copper to its functional sites. Additionally, there is a copper transport protein, transcuprein, in the circulation. These proteins are homologous to a varying extent to proteins with similar functions in bacteria and other species, in some of which proteins such as CTR1 homologs are essential for embryonic morphogenesis and cell differentiation, thereby raising interest in the copper

dependence of this role, and speculation that CTR may have similar functions in human embryogenesis.

ATP7A and ATP7B transfer copper to apoenzymes, or into vesicles for export from the cell. The loss of either role is responsible for a specific disturbance of copper turnover; the absence of a normally functioning ATP7A is associated with Menkes' disease and that of ATP7B with Wilson's disease. These are discussed later. The difference between these two diseases and their variants can be appreciated from the different distributions of the two transporters. Although both are ubiquitous, ATP7A predominates in the kidneys, lungs, blood–brain barrier, gastrointestinal tract, and muscle, whereas ATP7B predominates in the hepatocytes where it is responsible for the synthesis of caeruloplasmin, and for the excretion of copper into the bile. There is little ATP7A in the liver. Within cells both ATP7A and ATP7B are usually distributed around the nucleus where they donate copper to apoenzymes in the *trans* Golgi network (TGN). Cisplatin and related anticancer agents are trafficked via CTR1 which mediates their uptake; additionally cisplatins are excreted by ATP7B into the bile and resistance to these compounds is associated with an upregulation of ATP7B.

MTN are ubiquitous intracellular monomeric polypeptides with a relative molecular mass of 6500 that contain 60 amino acids 30% of which are cysteine. MTN binds 6–10 atoms of copper, and it is induced among other things by endotoxemia, infections, calorie restriction, glucocorticoids, exercise, oestrogens, and hypothermia, as well as by zinc and probably, high exposure to copper. MTN may have a role as a sequester of excess copper or a mobile depot of the metal.

Caeruloplasmin contains 60–70% of copper in the circulation. It binds six atoms of copper and was thought to be a copper transport protein. However, evidence that caeruloplasmin has cuprous oxidase activity and ferroxidase activity, and a role in facilitating the binding of manganese to transferrin has prompted the revised concept that caeruloplasmin, and the related hephaestin are metallo-oxidases rather than just ferroxidases.

The copper content of some tissues in adults and infants, and in the functional absence of ATP7A (Menkes' disease) is shown in Table 4. The adult distribution of copper is acquired during infancy and early childhood. Overall, an adult human contains 50–120 mg of copper: 40% of this is in muscle; 15% in hepatocytes; 10% in brain, and 6% in blood. Approximately 60% of the copper in red blood cells is in superoxide dismutase. In plasma, 60–70% of copper is found in caeruloplasmin, 10–30% is associated with a transport protein transcuprein, and 15–20% is bound to albumin and amino acids.

Copper Absorption

The intestine absorbs 5–90% or more of ingested copper depending on the character and form of the copper intake. The higher figure relates to the intakes of copper in solutions among which copper oxides are less well absorbed than acetate, sulphate, gluconate, and citrate salts. Otherwise, absorption varies considerably, approximately 10–60%, depending on the amount in the diet, the character of the diet and the host's need for copper. Copper is released from the

Table 4 Tissue copper content in adults, infants, and infants with ATP7A dysfunction (Menkes' Syndrome)

<i>Copper content ($\mu\text{g g}^{-1}$) (wet weight)</i>			
<i>Organ</i>	<i>Adults</i>	<i>Infants</i>	<i>Menkes' Syndrome</i>
Placenta	—	4.1–7.5	8.3–14.5
Liver	4.2–16.9	30–80	3–12
Brain	3.6–7.5	0.3–1.2	0.2–1.04
Intestine	1.2–3.4	4.1–7.5	6.4–12.4
Muscle	0.6–1.4	0.25–1.02	1.7–2.6
Spleen	0.9–1.7	0.6–1.9	6.4–15.4
Kidney	2.1–3.7	0.5–1.9	5.9–36.8
Lung	1.02–2.0	0.4–1.0	1.8–4.6

Source: Reproduced with permission from Aggett PJ (1998) Neonatal trace element metabolism. In: Cowett RM (ed.) *Principles of Perinatal-Neonatal Metabolism*, 2nd edn., ch. 41, pp. 909–941. New York: Springer-Verlag.

dietary matrix by gastric acidity and proteolytic activity. Gastric mucosal uptake and transfer of copper have been modeled in animal models and plasma appearance curves after ingesting copper salts suggest that gastric absorption occurs in humans but this is probably not a major route in normal physiology. The predominant site for uptake of copper in adults is the duodenum and proximal jejunum; however, in young animals the uptake of copper extends throughout the small intestine.

The intraluminal solubility of copper and thus its availability for intestinal uptake is enhanced by an acid milieu, anions such as sulfate and nitrate, and low molecular weight ligands including sulfur amino acids, histidine, lactose, glucose, and starch, presumably after its hydrolysis to glucose. The uptake of copper from foodstuffs is impaired by Maillard reaction products which are produced during food preparation, and by interactions involving phosphate compounds (such as phytate), amino acids, magnesium, and calcium which precipitate copper in the gut lumen. Similarly vitamin C, by oxidizing cuprous copper to cupric, impairs copper uptake in animal models, but this does not necessarily happen in humans. A phenomenon that has not been fully explained is that high fructose and sucrose intakes apparently increase the risk of marginal intakes of copper, whereas isocaloric intakes of complex carbohydrates do not. This was first observed experimentally and may not be relevant to humans. Nonetheless, given concerns about the adequacy of copper intakes and the character of Western diets, some investigators regard this to be of public health interest, particularly in the light of the possible impact of low copper intakes on cholesterol and triacylglycerol metabolism. This association is not predictable, and neither is the effect of coincidental intakes of zinc and iron on copper utilization. When given in solution with copper both iron and zinc reduce the systemic absorption and use of copper. Reduced red cell superoxide dismutase activity has been seen in infants given iron supplements, and iron supplements impair the utilization of copper when given during the management of pan-malnutrition. However, when given in the complex milieu of diets and infant formulas the interactions are not always observed. In another context mentioned later persistent intakes of zinc can block intestinal transfer of copper by inducing enterocytic MTN.

The first stage of copper uptake is its aggregation by the mucus and glycocalyx on the enterocytic microvilli, whence it reaches membrane-associated reductases (STEAP2 and Dcytb) which convert any residual cupric to cuprous copper for translocation into the enterocyte. There are at least two mechanisms by which enterocytes take up copper; one which is dependent on CTR1, possibly with CTR2, accounts for 70% of uptake, and one which probably uses the divalent metal transporter (DMT1). The former mechanism involves copper uptake into subapical vesicles in the enterocyte, and because this process occurs in the absence of CTR1, and has not been associated with DMT1-mediated uptake of other metals, it is thought that copper may be first taken up by endocytosis, it is not clear whether the metal is reduced before endocytosis or within the endosome. Then CTR1 transfers the cuprous metal out of the vesicle. This endocytic route would be consistent with early literature reports of copper uptake. However, this mechanism has not been confirmed and the location of CTR1 at the apical membranes or sub apical vesicles of enterocytes, or even at the basolateral membranes is uncertain. Similarly there is uncertainty about copper trafficking in liver cells and other tissues. In the enterocyte, copper is either bound to metallothionein or to a chaperone, Atox1, which translocates the copper to ATP7A for vesicular transfer of the metal across the basolateral membrane into the portal circulation.

In the portal circulation, copper has a number of carriers. These include transcuprein, albumin, and complexes with amino acids usually histidine, threonine, and glutamine. Copper is transported in the portal circulation to the liver and then to the systemic circulation. Copper complexed with phosphatidic acid and fatty acids has been found in mesenteric lymph; this implies that some copper may bypass the liver and reach the systemic circulation via the thoracic duct. Usually, within 2 h of ingestion nearly all absorbed copper is taken up by the liver. This is mediated by cell membrane bound reductases and CTR1. The liver is the primary depot for copper, and the principal mediator of its systemic homeostasis.

In the hepatocyte, the copper joins a high turnover labile pool, probably centered on copper–glutathione complexes from which the metal is distributed to at least four targets: (1) a metallothionein pool; (2) the copper chaperone for Cu, Zn – SOD(CCS) to SOD1; (3) a series of chaperones involving COX 17 (with COX 19 and COX23) to COX 11 and SCO1 the chaperone; taking copper to mitochondrial inner membrane cytochrome *c*-oxidase or (4) to Atox1, for transport to ATP7B in the TGN which incorporates copper into apoproteins to form caeruloplasmin, and other cupric enzymes. It is noteworthy that knockout models of ATOX1 accumulate intracellular copper and develop a copper-deficient phenotype. Caeruloplasmin is then secreted into the circulation or excreted directly in the bile.

Copper Distribution

Copper is probably distributed to peripheral tissues by the same complexes that are formed in the portal circulation. Caeruloplasmin had long been thought to be a systemic transport protein for copper but this is probably not so; inherited deficiency of caeruloplasmin has little effect on copper

trafficking and function, but has appreciable effects on iron utilization. Peripheral tissues are thought to take up and utilize copper in the same way as by the liver. ATP7A is essential for the adequate distribution of copper across the blood–brain barrier. In tissues, CRT2 has been localized with lysosomal vesicles and it is surmised that CRT2 is involved with the recycling of copper from hydrolyzed protein.

Copper Excretion and Homeostasis

The major route for systemic homeostasis of copper at customary intakes is hepato-biliary excretion; this accounts for 98% of excretion, and the rest is lost via the urine. Hepato-biliary excretion involves ATP7B, and in a minor capacity ATP7A, secreting copper as a variety of complexes into vesicles which merge with the hepatocytic apical plasma membrane and transfer the copper and complexes into the bile for elimination. The excreted copper is not reabsorbed and is lost in the feces.

The excreted copper pool may be supplemented by copper acquired from senescent cells and partly degraded caeruloplasmin.

At customary intakes, the amount of copper in the body is regulated by the changes in the amount of copper excreted by the liver. Usually, this entails the body retaining approximately 15% of absorbed copper and excreting the rest. The amount retained compensates loss of copper from shed epithelia (skin, intestinal, and other mucosa) and hair, menstruation, and adventitious blood loss. At times of increased copper need from potential deficiency, arising from inadequate intakes and depleted depots or from new tissue synthesis during growth or nutritional rehabilitation, hepatic excretion of copper is reduced, and intestinal uptake is increased; whereas with excessive copper exposure, hepatic excretion is increased, intestinal uptake and transfer are reduced, and at really high exposures increased metallothionein levels in the gut mucosa and liver, respectively, reduce intestinal transfer of the element, and increase its deposition in the liver. All these phenomena occur over a wide range of intakes and are deduced from a variety of studies in different models, but the adaptive phenomena at copper intakes between 0.8 and 7.5 mg at which absorption was 56% and 12%, respectively, have been well documented in humans. Note though that although the % absorption declined, the absolute amount of copper increased at the higher intake.

Studies at the cellular level show that when there is an increased need for copper both ATP7A and ATP7B traffic to and become tightly associated with the TGN; CRT1 increases in several tissues, and aggregates near the plasma membrane. In the enterocytes, CRT1 aggregates at the apical membrane.

High copper states in hepatocytes induce a migration of ATP7B from the TGN to cytosolic vesicular compartments that support biliary elimination of copper. In enterocytes, and other cells, increased copper leads to the endocytosis and degradation of CRT1, as well as the relocation of ATP7A and ATP7B to cytosolic vesicles at the basolateral plasma membrane for transfer of copper into the blood. It is not known how these processes are regulated. A mechanism for the posttranslational regulation of ATP7B has been

demonstrated. This involves a protein, COMMD1, which stabilizes ATP7B. However, the stability of COMMD1 itself depends on the protection from proteomic degradation which is mediated by X-linked Inhibitor of Apoptosis (XIAP). XIAP appears to be a copper sensor in that when it binds to copper it loses its inhibitory action on the proteomic degradation of COMMD1 and in turn ATP7B is degraded.

Inborn Errors of Metabolism

Collectively inborn errors of copper trafficking have contributed considerably to the understanding of the systemic use of copper. A source of detailed information on these is the Website *Online Mendelian Inheritance in Man*. Two conditions, Menkes' and Wilson's diseases with respective incidences of 1:300 000 and 1:30 000 have captured interest.

The role of copper in the neurodegenerative condition, Menkes' disease was suspected when its phenotypic similarity to copper deficiency in sheep was realized. The defect is in the X chromosome gene for ATP7A and because this is a recessive defect Menkes' Disease usually affects boys. The synthesis of a dysfunctional ATP7A results in the affected boy not being able to transfer copper out of the intestine effectively, and the copper which is transferred is neither distributed effectively around the body or within organs and cells. The latter may accumulate copper but are unable to transfer it to apoenzymes: an illustration of this is that patients with Menkes' have low circulating levels of copper and functioning caeruloplasmin, but the circulating level of immunoreactive apo-caeruloplasmin may be normal. Since ATP7A is responsible for the transfer of copper to the brain, this organ is affected by a severe copper deficiency. There is an accumulation of copper in the placenta, but the body has little or no features of copper deficiency when born. The boy presents after 3 months of age with developmental regression, apneic episodes, failure to thrive and a propensity to infection. This is a heterogeneous condition with a number of defects affecting the allele, and the functional implications of these are being explored in a number of mouse models of ATP7A dysfunction.

Defects in ATP7B synthesis result in Wilson's disease, in which there is an inability to transfer copper to apoproteins, including caeruloplasmin, in the TGN and to excrete copper into the bile. There are more than 60 genetic mutations and the genotype can to a certain extent predict both the age of onset of the disease, which has been as early as 4 years, and its character. Usually, copper slowly accumulates in the liver and leaks into the circulation where it is deposited in a variety of tissues predominant among which, possibly because ATP7A is functioning normally, is the central nervous system (CNS). The onset of the disease is insidious; early biochemical evidence of liver damage is not detected because the patients have no symptoms or signs to prompt any investigations. However, liver damage has been found at 1 year of age in the sibling of a known case. Other than liver disease resembling hepatitis, or in later presentations, hepatic cirrhosis, the first evidence of disease is often deterioration in school work, and neuromotor and psychiatric defects. Overall, in cases that have not been diagnosed until adulthood most tissues in the body are

affected by copper deposition and associated oxidative damage. On a positive note if the condition is detected early patients can be decoppered with clinical improvement by using a chelating agent such as D-penicillamine or trientine, with the use of large doses of oral zinc which induces enterocytic metallothionein thereby blocking the transfer of copper taken up from the gut, and of tetrathiomolybdate which was initially thought to mimic the cause of copper deficiency in livestock by complexing dietary copper and preventing its uptake by the gut, but which is now thought to block the function of ATOX1 and other metallochaperones.

Hypoceruloplasminemia is manifest by low circulating levels of caeruloplasmin and consequently low plasma copper levels, but with no other abnormalities. However, aceruloplasminemia in which there is a complete loss of ferroxidase (metallo-oxidase) activity has marked effects on iron metabolism with tissue deposition of iron in tissues, including the CNS and basal ganglia, causing oxidative damage to tissues and resulting in endocrine and severe neurological disturbances. There have been case descriptions of humans with defects of the cytochrome *c* oxidase copper chaperones; a defect in SCO1 caused neonatal liver failure and encephalopathy, and defective SCO2 was linked to heart, brain, and muscle damage in an infant. Not all of these effects can be accounted for by loss of cytochrome *c* oxidase activity, so perhaps the SCOs have other roles.

Measuring Copper Status

It is difficult to determine by a single measurement if an individual's copper status is deficient, adequate, or excessive. Easily accessible markers such as serum copper or caeruloplasmin concentrations represent a small proportion of the body pools of copper and are susceptible to many confounders. Since caeruloplasmin can contain up to 95% of the serum copper, measuring serum and plasma copper or caeruloplasmin is essentially measuring the same thing. However, caeruloplasmin levels, and copper, are increased as a component of the acute phase 1 reaction by infection, pregnancy, inflammation, and by estrogen, and hypoxia among other factors. Levels are low in copper deficiency but in human conditions the deficiency is invariably accompanied by conditions that increase caeruloplasmin levels, thus obscuring the deficiency marker. Only with severe copper deficiency are serum caeruloplasmin and copper levels reduced. Other approaches have been to measure copper-dependent enzyme activities in tissues such as erythrocyte superoxide dismutase, or using a tissue with a rapid turnover, such as platelet cytochrome *c* oxidase. Strategies based on measuring the non-caeruloplasmin component of circulating copper, multiple indices, or genomic and postgenomic markers of homeostatic adaptation are being explored. Currently, suspicion and alertness to the possibility of copper deficiency coupled with monitoring any response to copper supplements are the best clinical approaches to diagnosing copper deficiency, but this approach is not useful for large populations or for the subtle deficiency associated with multiple micronutrient deficiencies.

Copper Deficiency

Copper deficiency is seldom an isolated phenomenon, although it might occur as such in infants, children, and adults on synthetic diets, and parenteral or enteral nutrition. The appearance of copper deficiency in both term and ex-preterm infants develops when their hepatic deposits of copper are exhausted. Copper-deficient infants have presented at 4 weeks and 8 months of age. At 26 weeks of gestation the fetal liver has accrued 3 mg of copper, and at term this depot approximates 10–12 mg so early presentation of copper deficiency may involve defective accumulation of copper *in utero*. In recent clinical practice, copper deficiency is emerging as a complication of gastric surgery in the management of obesity.

The most common cause of copper deficiency is a complex of malabsorption and increased losses from the body as a result of hemolytic anemias, and gut infections and parasitism, which cause protein losing enteropathies. These circumstances are widespread affecting perhaps 25% of the global population, in whom the effects on copper nutriture would be compounded by an absolute or relatively inadequate intake of copper.

The features of copper deficiency observed in infants and children with copper deficiency are listed in **Table 5**. They are the features of marked copper deficiency; the features in classic Menkes' disease are more marked with varicose vasculature, hernias, as well as neurodegeneration secondary to defective synaptogenesis and axon formation.

The propensity to infections is attributable to neutropenia and reduced microbicidal activity of the cells secondary to loss of the respiratory burst and reduced superoxide dismutase activity. In adults, experimental diets providing 0.7–1.0 mg of copper daily have induced cardiac dysrhythmias, conduction defects, bradycardia, and elevated low-density lipoprotein (LDL) cholesterol levels and reduced high-density lipoprotein (HDL) cholesterol levels in the circulation. These changes

Table 5 Features of copper deficiency in infants and children

(Hypocupraemia, and hypoceruloplasminaemia) ^a
Hypochromic microcytic iron resistant anemia,
Arrested maturation of erythroid and myeloid bone marrow
Neutropenia and propensity to infections
Hypotonia and muscle weakness, poor feeding
Failure to thrive
Pallor
Hypothermia, apneic episodes,
Skeletal change
Scurvy like bone changes: metaphyseal irregularities, epiphyseal flaring and cupping, bony spurs, and chip fractures
Epiphyseal porosis and separation
Periosteal reaction and subperiosteal new bone formation
Wormian bones, delayed bone maturation
Osteoporosis, fractures
Abnormal elastic and connective tissues, hernias tortuous vasculature, varices and aneurysms
Fishy odor: Trimethylaminemia ^a
Hypoproteinemia with edema
Neurodegeneration, developmental regression

^aNot always evident.

might have arisen from the character of the diet, and the phenomena have not been reproduced or observed using more usual diets. Nonetheless, there are concerns that copper deficiency is widespread in the population and partly responsible for common defects in lipid metabolism with adverse sequelae on cardiac health.

Copper Excess

Exposure to excess copper can occur acutely, usually by ingestion of copper salts as free solutions, or by chronic exposure over an extended period of time. The upper levels mentioned earlier are set to help manage the latter risk. However, they do not prevent large systemic burdens of copper arising from failure to excrete copper as may occur with hepato-biliary obstruction, and Wilson's disease. Additionally, patients receiving renal dialysis via a copper-based dialysis membrane may accumulate copper.

Ingested copper may come from water supplied via copper pipes. The metallic and salty bitter taste of copper in the water can be detected at concentrations of 2.5–3.5 mg copper per l. This is just below the threshold (4–5 mg l⁻¹) at which nausea, retching, vomiting, and abdominal pain develop. The current regulatory limit advised by the World Health Organization for the copper content of water is 2 mg l⁻¹, but a lower limit may be introduced. A high copper content in water might cause hair to turn green. Selfpoisoning with copper salts, usually in suicide attempts, involves doses of 20–70 g of copper. In these circumstances, the above clinical features rapidly progress to hematemesis, diarrhea, and hypovolemic shock all of which reflect intestinal oxidative damage; following this systemic damage resulting in hemolysis, renal and liver failure develop. The stools and vomit may be green.

Chronic copper exposure from copper contamination in food storage and preparation was thought to contribute to the etiology and pathophysiology of the accumulation of copper seen in Indian Childhood Cirrhosis (ICC) (Idiopathic Copper Toxicosis), but it is now uncertain if the accumulated copper is a primary feature, or a secondary and variable sequel of exposure to another hepatotoxin. Thus the precise role of the increased copper deposition as a toxicant is uncertain. ICC may be an ecogenetic condition in which patients have genetic predisposition to be abnormally sensitive to an environmental exposure. This is probably the case in Bavarian or Tyrolean liver disease that seems to be an autosomal recessive trait which only emerges with exposure to elevated copper intakes from the use of copper cooking utensils.

In contrast to the earlier comments on a possible endemic copper deficiency in Western populations, there are also concerns that the public is exposed to too much dietary copper, for example, via water supplies and that this predisposes the population to copper overload and an increased exposure to

oxidative damage, which in the CNS might contribute to the development of Alzheimer's disease.

Conclusion

Copper is an essential micronutrient with extensive roles in enzyme activities that are responsible for the integrity of connective tissue and bone matrix, the development of the nervous system, and the functioning of neurotransmitters, and, not least for effective energy metabolism. Two inborn errors of metabolism affecting copper have provided extensive information on severe copper deficiency and copper overload. These are Menkes' disease and Wilson's disease, respectively, and their respective fundamental defects are the dysfunction of ATP7A and ATP7B which are energy-dependent copper transport proteins. The features of these conditions have facilitated a better understanding of how the body distributes and uses copper, and also of the subtler features of copper deficiency in particular. This has increased awareness that copper deficiency is probably a treatable component of most if not all malnutrition states. In public health nutrition there is probably a need for a thorough risk assessment of the risk of copper deficiencies and excesses in all populations but this will be difficult to achieve until sound markers of adequacy, deficiency, and excess are available. It would be hoped that such markers could be forthcoming from increased information on the homeostatic control of copper turnover at cellular, organ and systemic levels.

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CORONARY HEART DISEASE

Contents

Lipid Theory

Prevention

Lipid Theory

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Introduction

Arteriosclerosis is a group of conditions characterized by thickening and stiffening of the arterial wall. Atherosclerosis is characterized by the formation of atheromas (lipid-laden plaques) in medium to large arteries. These are associated with calcifications of the arterial wall along with other changes. Atherosclerosis can cause clinically important vascular pathology by slow encroachment on the lumen as in occlusive coronary artery disease. Alternatively, because the endothelium overlying atheromata is abnormal, platelet aggregation occurs, and this promotes occlusive clot formation. Lastly, rupture of the plaque can also lead to rapid vascular occlusion with clot. Over the years there have been a variety of hypotheses seeking to explain the development of arterial lesions; these hypotheses have become increasingly complex as our biochemical and molecular biological skills and knowledge increase.

Arterial fatty streaks are ubiquitous in humans, and appear early in life. The fatty streak is comprised of lipid-rich macrophages and smooth muscle cells. Macrophages that accumulate lipid and are transformed into foam cells may be involved in the transformation of the fatty streak to an atherosclerotic lesion. In susceptible persons, the fatty streaks may progress to fibrous plaques. Fibrous plaques, at their core, consist of a mixture of cholesterol-rich smooth muscle and foam cells. This core may contain cellular debris, cholesteryl esters, cholesterol crystals, and calcium. The fibrous cap consists of smooth muscle and foam cells, collagen, and lipid. The final stage in this process is the complicated plaque, which can obstruct the arterial lumen. Rupture of the cap may lead to clot formation and occlusion of the artery.

There are several theories of atherogenesis and these may eventually be shown to be interactive. The lipid hypothesis suggests that persistent hyperlipidemia leads to cholesterol

accumulation in the arterial endothelium. Hypercholesterolemia may activate protein growth factors, which stimulate smooth muscle cell proliferation.

The lipid infiltration hypothesis proposes that elevated low-density lipoprotein (LDL) levels increase LDL infiltration which, in turn, increases the uptake of epithelial cells, smooth muscle cells, and macrophages. This cascade leads to cholesterol accumulation and, eventually, atheroma formation. The endothelial injury may arise from the action of oxidized lipid.

The endothelial injury hypothesis may help to explain the focal distribution of atheromas, which is not adequately accounted for by the lipid hypothesis. The endothelial injury hypothesis asserts that plaque formation begins when the endothelial cells that cover fatty streaks separate thus exposing the underlying lesion to the circulation. This may lead to smooth muscle proliferation, stimulated by circulating mitogens, or may cause platelet aggregation leading to mural thrombosis.

Another hypothesis relating to atherogenesis is the response-to-injury hypothesis. In this hypothesis the injury may be due to mechanical factors, chronic hypercholesterolemia, toxins, viruses, or immune reactions: these increase endothelial permeability, and lead to monocyte adherence to the epithelium or infiltration and platelet aggregation or adherence at the site of the injury. Injury releases growth factors that stimulate proliferation of fibrous elements in the intima. These growth factors may arise from the endothelial cell, monocyte, macrophages, platelet, smooth muscle cell, and T cell. They include the epidermal growth factor, insulin-like growth factors, interleukins 1 and 2, platelet-derived growth factors, transforming growth factors α and β , and tumor necrosis factors α and β , among others. Monocytes and smooth muscle cells carry the 'scavenger' receptor, which binds oxidized but not native LDL in a nonsaturable fashion. Uptake of oxidized LDL converts macrophages and smooth muscle cells into foam cells. Another theory of atherogenesis suggests that it begins as an immunological disease, which starts by an autoimmune reaction against the heat stress protein, HSP60.

[†]Deceased

There have been suggestions that oxidized LDL may be an underlying cause of arterial injury.

The term 'atherosclerosis' is derived from the Greek words *athere*, meaning gruel, and *skleros*, meaning hardening. The term was coined by Marchand in 1904 to describe the ongoing process beginning with the early lipid deposits in the arteries to the eventual hardening. The World Health Organization (WHO) defines atherosclerosis as a 'variable combination of changes in the intima of the arteries involving focal accumulation of lipids and complex carbohydrates with blood and its constituents accompanied by fibrous tissue formation, calcification, and associated changes in the media' – a decidedly more complex concept than attributing it all to the dietary cholesterol.

Discussions of the etiology of heart disease always describe it as a life-style disease and list a number of risk factors, which include family history, hypercholesterolemia, hypertension, obesity, and cigarette smoking. Having listed these factors, discussion generally reverts to serum cholesterol and its control.

The fasting blood plasma of a healthy individual is a clear, straw-colored liquid, which may contain 400–800 mg of lipids per 100 ml. This clear solution, which is high in lipids, is made possible by the water-soluble complex of lipids with protein, the lipoproteins. A generalized view of lipoprotein metabolism is provided in Figure 1. The existence of soluble lipid-protein complexes in serum was suggested about a century ago. Precipitation of a lipoprotein from horse serum was achieved in 1929 and classes of lipoproteins were ad-duced from studies using moving boundary electrophoresis. The critical experiments were carried out by Gofman and his group in the 1950s. They demonstrated that classes of lipoprotein complexes could be identified by their flotation characteristics in the analytical ultracentrifuge. These complexes were separable because they possessed different hydrated densities and they were defined initially by Svedberg units of flotation (S_f). The lipoproteins vary in chemical composition and although it is common to provide tables describing lipoprotein composition, the values are generally average values. This is so because the lipoproteins exist in a dynamic state exchanging their lipid components with those of tissues or other lipoproteins. Since identification is made according to a physical property, that is, hydrated density, it is evident that different agglomerates of lipid and protein may have similar hydrated densities. In general, the lipoproteins are a series of macromolecules that, as they progress from low to high density, display decreasing triacylglycerol content and increasing cholesteryl ester, phospholipid, and protein.

Table 1 describes the major lipoproteins. Their chemical composition is described in Table 2.

As research continues and as analytical methodology becomes more precise we find a higher resolution of some lipoprotein classes and better definition of their roles. One example is lipoprotein (a) (lp(a)), first described in 1963. Lipoprotein (a) is an LDL whose normal apoprotein (apo B) is linked to an additional protein, apoprotein a, via a disulfide bridge. Lipoprotein (a) interferes with normal fibrinolysis leading to an increased prevalence of blood clots, and is thought to present an especially high risk for myocardial infarction. Characteristics and functions of lipoproteins are described in Table 3.

The molecular size influences the ease with which LDL particles can enter the arterial wall. Diabetic rabbits have greatly elevated plasma lipid levels but display surprisingly little atherosclerosis. The reason for this apparent discrepancy

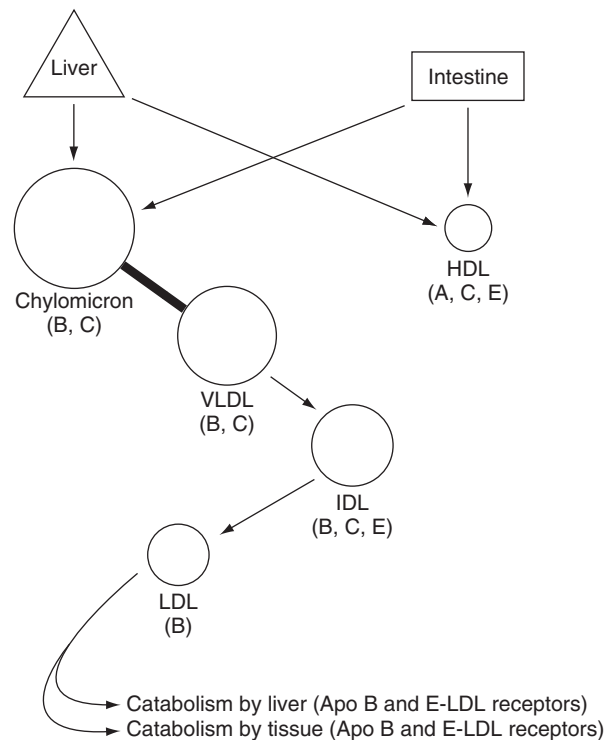


Figure 1 Outline of lipid metabolism. Letters in parentheses refer to apolipoproteins (apo). HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein.

Table 1 Major plasma lipoproteins

Lipoprotein class	Size (nm)	Mol. wt	Density ($g\ ml^{-1}$)	Electrophoretic mobility	Origin	Major apoproteins
Chylomicron	100–400	10^6 – 10^7	< 0.95	Origin	Intestine	A-I, B-48, C-II, C-III, E
VLDL	40–70	5×10^3	0.95–1.006	Prebeta	Liver	B-100, C-II, C-III, E
IDL	30–40	4.5×10^3	1.006–1.019	Between prebeta and β	Catabolism of VLDL	B-100, C-II, C-III, E
LDL	22.5–27.5	2×10^3	1.019–1.063	β	Catabolism of VLDL and IDL	B-100
HDL	7.5–10	0.4×10^3	1.063–1.210	α	Liver, intestine	A-I, A-II, C-II, C-III, E

VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

is that the lipoproteins of diabetic rabbits are rather large in size and do not penetrate the artery. Since 1982, we have known of an array of LDL particles ranging from small and dense to large and comparatively light. An LDL pattern characterized by an excess of small, dense particles is associated with a threefold increased risk of myocardial infarction, independent of age, sex, or body weight. Commonly, LDL is known as the 'bad' cholesterol and high-density lipoprotein (HDL) as the 'good' cholesterol. These recent findings indicate the presence of 'good, bad' cholesterol and 'bad, good' cholesterol.

Among the apolipoproteins, polymorphism of apoprotein E apparently dictates a subject's chances for successful

treatment of lipidemia. The apoE alleles are designated as E2, E3, and E4. The most common pattern (55%) is homozygosity for E3, which gives rise to the E3/E3 phenotype. The next most common phenotype is E3/E4 (26%). The least frequently observed phenotype is E2/E (1%), which is often associated with type III hyperlipoproteinemia. There is some evidence suggesting that subjects bearing the E4 allele have higher levels of LDL than those with the E3/E3 pattern; they may also be more prone to Alzheimer's disease. **Tables 4 and 5** list primary and secondary dyslipoproteinemias.

Determinants of Serum Cholesterol – Cholesterol and Cholesterolemia

In 1913, Anitschkow showed that it was possible to establish atherosclerosis in rabbits by feeding cholesterol. Since then virtually all research on atherosclerosis has centered on cholesterol – circulating cholesterol and dietary cholesterol. The epidemiological data suggest a role for dietary fat, and hypercholesterolemia has been established as a principal risk factor for atherosclerosis. The lipid hypothesis was developed from the data obtained in the Framingham study, which suggested a curvilinear relationship between risk of atherosclerosis and plasma or serum cholesterol levels. However, studies of actual cholesterol intake as it affects cholesterol levels have yielded equivocal results.

Table 2 Plasma lipoprotein composition

Lipoprotein	Composition (wt%)				
	FC	CE	TAG	PL	PROT
Chylomicron	1	3	90	4	2
VLDL	7	14	55	16	8
IDL	6	22	30	24	18
LDL	7	48	5	20	20
HDL	4	15	4	27	50

FC, free cholesterol; CE, cholesteryl ester; TAG, triacylglycerol; PL, phospholipid; PROT, protein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

Table 3 Characteristics and functions of major apolipoproteins

Apolipoprotein	Lipoprotein	(Approximate molecular weight (kD))	Source	(Average plasma concentration (mg dL ⁻¹))	(Physiologic) function
A-I	HDL, chylomicrons	28	Liver, intestine	100–120	Structural apoprotein of HDL, cofactor for LCAT
A-II	HDL, chylomicrons	17	Intestine, liver	35–45	Structural apoprotein of HDL, cofactor for hepatic lipase
A-IV	HDL, chylomicrons	46	Liver, intestine	10–20	Unknown
Apo (a)	Lp(a)	600	Liver	1–10	Unknown
B-48	Chylomicrons	264	Intestine	Trace	Major structural apoprotein, secretion and clearance of chylomicrons
B-100	VLDL, LDL	550	Liver	100–125	Ligand for LDL receptor, structural apoprotein of VLDL and LDL
C-I	Chylomicrons, VLDL, HDL	5.80	Liver	6–8	Cofactor for LCAT
C-II	Chylomicrons, VLDL, HDL	9.10	Liver	3–5	Cofactor for LCAT
C-III	Chylomicrons, VLDL, HDL	8.75	Liver	12–15	Inhibitor of LPL, involved in lipoprotein remnant uptake
E-2	Chylomicrons, VLDL, HDL	35	Liver, peripheral tissues	4–5	Ligand for cell receptor
E-3	Chylomicrons, VLDL, HDL	35	Liver, peripheral tissues	4–5	Ligand for cell receptor
E-4	Chylomicrons, VLDL, HDL	35	Liver, peripheral tissues	4–5	Ligand for cell receptor

HDL, LDL, VLDL, high-, low-, and very-low-density lipoprotein; LCAT, lecithin-cholesterol acyltransferase; LPL, lipoprotein lipase.

Table 4 The primary dyslipoproteinemias

Type	Changes in plasma		Apparent genetic disorder	Biochemical defect
	Lipids	Lipoproteins		
I	TAG ↑	CM ↑	Familial LPL deficiency	Loss of LPL activity
II-a	C ↑	LDL ↑	Familial hypercholesterolemia	Deficiency of LDL receptor and activity
II-b	C ↑, TAG ↑	LDL, VLDL ↑	Familial combined hyperlipidemia	Unknown
III	C ↑, TAG ↑	β-VLDL ↑	Familial type III hyperlipidemia	Defect in TAG-rich remnant clearance
IV	TAG ↑	VLDL ↑	Familial hypertriacylglycerolemia	VLDL synthesis ↑, catabolism ↓
V	TAG ↑, C ↑	VLDL ↑, CM ↑	Familial type V hyperlipoproteinemia	Lipolysis of TGA-rich LP ↓, Production of VLDL TAG ↑
Hyper Lp(a)	C ↑	Lp(a) ↑	Familial hyper apo(a) lipoproteinemia	Inhibits fibrinolysis
Hyperapoβ-lipoproteinemia	TAG ↑	VLDL, LDL ↑	Familial type V hyperlipoproteinemia	CETP deficiency
Familial hypoβ-lipoproteinemia	C ↓, TAG ↓	CM ↓, VLDL ↑, LDL ↓	?	Inability to synthesize apo B-48 and apo B-100
A-β-lipoproteinemia	C ↓, TAG ↓	CM ↓, VLDL ↓, LDL ↓	?	Apo B-48 and apo B-100 not secreted into plasma
Hypo-αlipoproteinemia	C ↓, TAG ↓	HDL ↓	?	LCAT deficiency
Tangier disease				Apo A-I ↓, apo C-III ↓
Fish eye disease				Abnormal apo A-I, and apo A-II metabolism

C, cholesterol; CM, chylomicrons; CETP, cholesteryl ester transfer protein; HDL, LDL, VLDL, high-, low-, and very-low-density lipoprotein; LCAT, lecithin-cholesterol acyl-transferase; LPL, lipoprotein lipase; TAG, triacylglycerol.

Table 5 Secondary dyslipoproteinemias

Type	Associated disease	Lipoproteins elevated	Apparent underlying defect
I	Lupus erythematosus	Chylomicrons	Circulating LPL inhibitor
II	Nephrotic syndrome, Cushing's syndrome	VLDL and LDL	Overproduction of VLDL particles, defective lipolysis of VLDL triglycerides
III	Hypothyroidism, dysglobulinemia	VLDL and LDL	Suppression of LDL receptor activity, overproduction of VLDL triglycerides
IV	Renal failure, diabetes mellitus, acute hepatitis	VLDL	Defective lipolysis of triglyceride-rich VLDL due to inhibition of LPL and HL
V	Noninsulin dependent diabetes	VLDL	Overproduction and defective lipolysis of VLDL triglycerides

HDL, LDL, VLDL, high-, low-, and very-low-density lipoprotein; HL, hepatic lipase; LPL, lipoprotein lipase.

Several studies have shown that the addition of one or two eggs to their daily diet did not influence serum cholesterol levels of free-living subjects. Data from the Framingham study show no correlation between cholesterol intake and cholesterol level. So we are left with the anomalous situation that blood cholesterol is an indicator of susceptibility to coronary disease but it is relatively unaffected by dietary cholesterol. It is of interest to point out that we are also seeing a correlation between low plasma or serum cholesterol levels and noncoronary death.

Early epidemiological evidence pointed to dietary saturated fat intake as a more important factor than dietary cholesterol in determining serum cholesterol levels. Major national and international prevention programs were mounted on the basis of reducing saturated fat intake. More recently, detailed analyses of previous studies appear to indicate that reducing

saturated fat intake *per se* may not be sufficient advice to reduce atherosclerosis risk. Instead, meta-analyses show that only when saturated fat is replaced by polyunsaturated fat or monounsaturated fat is there a meaningful reduction in serum cholesterol levels and on coronary risk. Conversely, replacing saturated fat with carbohydrates may actually increase the risk, particularly in the case of refined carbohydrates.

Further studies on specific saturated fats yielded more insight on the role of fat on hypercholesterolemia and atherosclerosis. Metabolic studies have shown that lauric ($C_{12:0}$), myristic ($C_{14:0}$), and palmitic ($C_{16:0}$) acids raise both LDL and HDL cholesterol levels, and that oleic ($C_{18:1}$) and linoleic ($C_{18:2}$) acids raise HDL and lower LDL levels slightly. Thus, the type of fat is the determining factor in considering dietary fat effects on serum cholesterol. Experiments in which subjects were fed low or high levels of cholesterol in diets containing

high or low ratios of saturated to polyunsaturated fat have been reported. When the fat was homologous, changing from low to high dietary cholesterol raised serum cholesterol concentration by 2%.

In nature most, but not all, unsaturated fatty acids are in the *cis* configuration. The major source of fats containing *trans* unsaturated fatty acids (*trans* fats) in the diet of developed nations is hydrogenated fat, such as is present in commercial margarines and cooking fats. In general, *trans* fats behave like saturated fats and raise serum cholesterol levels, but have not been found to be more atherogenic than saturated fats in studies carried out in rabbits, monkeys, and swine. The concerns relative to *trans* fat effects have led to recommendations that the levels of *trans* fats present in the diet be reduced as much as possible, which means less than 1% of caloric content.

Protein

The type of protein in the diet also influences cholesterolemia and atherosclerosis. In animal studies in which the sole source of protein is of animal or plant origin, the former is more cholesterolemic than atherogenic. However, a 1:1 mix of animal and plant protein provides the higher grade protein of animal protein and the normocholesterolemic effects of plant protein. The results underline the need for a balanced diet.

Fiber

Dietary fiber may influence lipidemia and atherosclerosis. Substances designated as insoluble fibers (wheat bran, for instance) possess laxative properties but have little effect on serum lipid levels. Soluble fibers (gel-forming fibers such as pectin or guar gum) influence lipidemia and glycemia. Oat bran, which contains β -glucans, which are soluble fibers, will lower cholesterol levels despite its designation.

Variations in Cholesterol Levels

Ignoring the differences of technique involved in cholesterol measurement in the laboratory – variations that are amenable to resolution – there are physiological considerations that should be recognized. Age, gender, genetics, adiposity, and personality traits can affect cholesterol levels, as can diseases unrelated to coronary disease. Stress (job stress, deadlines, and examinations) can lead to increased cholesterol levels.

A definite seasonal variation in cholesterol levels (usually higher in winter months) has been seen in a number of studies. Scientists from the National Institutes of Health in the United States carried out one of the finest studies in this area. They carefully examined the data from the 10 American Lipid Research Clinics. They observed that the etiology of their findings was unknown but they found the total and LDL cholesterol levels varied inversely with length of day. The level of HDL cholesterol varied much less, but its variation was correlated directly with ambient temperature. The foregoing does not reduce the importance of measuring cholesterol

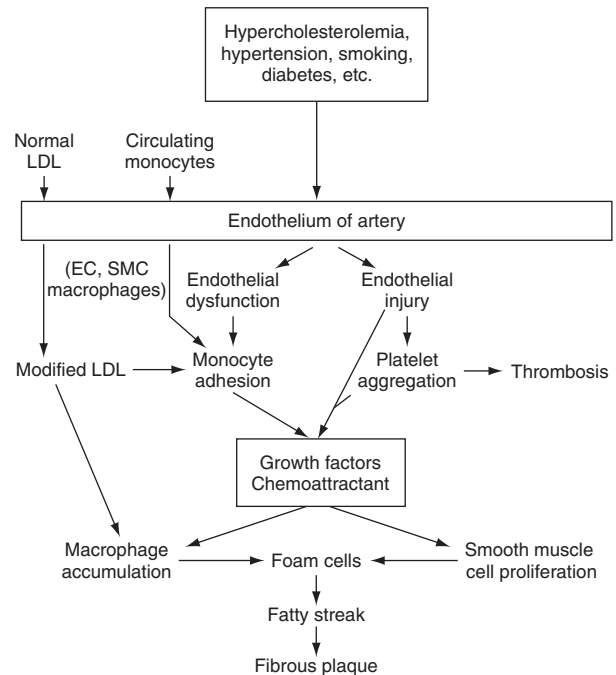


Figure 2 Factors involved in formation of the atherosclerotic plaque.

levels but makes it important to take into consideration the subjects' physical and mental state as well as time of year.

Figure 2 attempts to summarize the many factors now considered to play a role in the formation of the atherosclerotic plaque.

See also: Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Coronary Heart Disease: Prevention. Diabetes Mellitus: Etiology and Epidemiology. Fats and Oils. Hyperlipidemia: Prevention and Management; Overview. Lipoproteins

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Prevention

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Abbreviations

MUFA monounsaturated fatty acids
PUFA polyunsaturated fatty acids

SFA saturated fatty acids
t-FA trans-fatty acids

Introduction

Coronary heart disease (CHD) is the leading cause of death in the world. Although it is well established as the foremost contributor to mortality in most developed countries, it is also a major and rapidly rising cause of death in many developing countries. Global health transitions, which have seen substantial changes in age-specific coronary mortality rates across the world, in the past half a century, have also been associated with nutrition transitions, which explain a large part of the rise or fall of CHD-related death rates.

Diet and nutrition have been extensively investigated as risk factors for CHD. Many dietary factors have been linked directly to an increased or decreased risk of CHD or to major established risk factors of CHD like high blood pressure (HBP), disordered blood fats (dyslipidemia), diabetes and metabolic syndrome, overweight and obesity, and also to emerging risk factors like inflammatory markers and homocysteine. Nutrition influences atherogenesis, thrombosis, and inflammation—all of which are interconnected pathways that lead to CHD.

Observational epidemiological studies and clinical trials have contributed to a wide body of knowledge on the role that some nutrients (like saturated and trans-fats, salt, and refined carbohydrates) play in increasing the risk of CHD and of the protective effect of other nutrients (such as fruit and vegetables, polyunsaturated fats, nuts, and fish) against CHD. This knowledge has been successfully applied both in public health and in clinical practice, to reduce the risk of CHD, in populations as well as in individuals. This article summarizes the present state of that knowledge, as relevant to prevention of CHD.

Global Trends in CHD as a Reflection of Nutrition Transition

CHD accounted for 7.2 million deaths in 2004, which forms a large fraction of not only the total number of deaths worldwide due to cardiovascular diseases (17.1 million) but also of the global total of deaths from any cause (57 million). Although age-specific coronary mortality rates have declined in the industrial countries, over the past few decades, the absolute burdens of CHD continue to be high. Notably, CHD death rates are rising in the developing countries, where about half of these deaths occur below the age of 70 years, entailing high loss in potential productive years of life as well as in national incomes. In the INTERHEART study, which examined risk factors for myocardial infarction (MI) in 52 nations

worldwide, the mean age for first MI in South Asia was 53 years compared to 58 years in other parts of the world. In Eastern and Central Europe CHD mortality rates rose sharply in the 1980s and 1990s but are recently showing signs of stabilization, albeit at high levels.

These changes in CHD mortality rates have accompanied well documented or clearly discernible shifts in the nutritional state of the populations. The decline of CHD mortality in Western and Northern Europe was linked to a reduction in the consumption of unhealthy fats (saturated fats and trans-fats) and salt as well as an increased consumption of fruits and vegetables. This is best documented in Netherlands and Finland. Similarly, the decline of CHD mortality in Poland was explained by the increase in fruit and vegetable consumption and growing substitution of vegetable fats for animal fats. Similar evidence of a favorable nutrition transition preceding the decline in CHD mortality rates is available from other developed countries like USA, Canada, Australia, and New Zealand.

The developing countries have, however, witnessed a recent transition in the opposite direction. China, for example, has experienced a large increase in fat consumption over the past two decades, accompanied by a progressive rise in the mean plasma cholesterol levels of the population as well as in the CHD mortality rates. Other developing countries too are increasingly adopting unhealthy dietary patterns, which augment the risk of CHD. In India, there has been a progressive increase in the consumption of dairy products, sugars, and edible oils, most of which are very high in saturated and trans-fats.

Understanding the Links between Nutrition and CHD

The pathogenesis of CHD is mediated through the interconnected pathways of atherogenesis (fat deposition in the walls of the coronary arteries to form plaques), thrombosis (blood clotting over disrupted plaques), and inflammation (which initially damages the blood vessel walls and continues to destabilize the plaques). Nutrition has a major role in influencing each of these pathways and often provides the connecting link between them.

Major coronary risk factors include an abnormal blood lipid profile (especially plasma cholesterol and its subfractions), HBP, and diabetes. Overweight and obesity (both the general and central patterns) are also associated with an increased risk of CHD. Nutrition has a powerful influence on all of these risk factors, with an unhealthy diet pattern tending to elevate them and a healthy diet pattern reducing the levels of

risk. Diet becomes especially important in the context of the metabolic syndrome (a complex of central obesity, HBP, dyslipidemia, and glucose intolerance), an entity, which has been identified as a major risk factor for CHD. Nutrition is also linked to the propensity to develop cardiac arrhythmias, and is an important predictor of sudden cardiac death. These links between dietary patterns and several specific nutrients not only manifest as fat deposition in the arteries, plaque growth, plaque instability, and thrombosis but are also evident much earlier in the natural history of CHD, as endothelial dysfunction (inability of the arteries to dilate normally), elevated levels of inflammatory markers (such as C Reactive protein), and increased intimal medial thickness of arterial walls. These precede and predict the clinical manifestation of CHD.

Nutrients and CHD

Dietary Fats: Cholesterol

The relationship between dietary fats and cardiovascular disease (CVD), especially CHD has been extensively investigated, with strong and consistent associations emerging from a wide body of evidence accrued from animal experiments, as well as observational studies, clinical trials, and metabolic studies conducted in diverse human populations. This relationship was initially considered to be mediated mainly through the atherogenic effects of plasma lipids (total cholesterol, lipoprotein fractions, and triglycerides). The effects of dietary fats on thrombosis and endothelial function as well as the relationship of plasma and tissue lipids to the pathways of inflammation have been more recently understood. Similarly, the effects of dietary fats on blood pressure have also become more evident through observational and experimental research.

Cholesterol in the blood and tissues is derived from two sources: diet and endogenous synthesis. Dairy fat and meat are major dietary sources. Dietary cholesterol raises plasma cholesterol levels. Although both high density lipoprotein (HDL) and low density lipoprotein (LDL) fractions increase, the effect on the total/HDL ratio is still unfavorable, but small. The upper limit for dietary cholesterol intake has been prescribed, in most guidelines, to be less than 300 mg per day in healthy individuals and less than 200 mg per day in those with CHD. However, as endogenous synthesis is sufficient to meet the physiological needs, there is no requirement for dietary cholesterol and it is advisable to keep the intake as low as possible. If intake of dairy fat and meat are controlled, then there needs to be no severe restriction of egg yolk intake, although some limitation remains prudent.

Saturated Fatty Acids (SFA)

The relationship of dietary saturated fat to plasma cholesterol levels and to CHD was graphically demonstrated by the Seven Countries Study involving 16 cohorts, in which saturated fat intake explained up to 73% of the total variance in CHD across these cohorts. In the Nurses Health Study, the effect of SFAs was much more modest, especially if saturates were replaced by carbohydrates. The most effective replacement for

SFAs, in terms of CHD prevention, is by polyunsaturated fatty acids (PUFAs) and monounsaturated fatty acids (MUFAs). This agrees with the outcome of large randomized clinical trials, in which replacement of saturated and trans-fats by polyunsaturated vegetable oils effectively lowered CHD risk. Replacement of SFA with either polyunsaturated or monounsaturated fat was shown to lower both LDL and HDL cholesterol. But substitution of SFAs with higher carbohydrate intake especially refined carbohydrates increased the risk for atherogenic dyslipidemia associated with insulin resistance and obesity.

Trans-Fatty Acids (t-FAs)

t-FAs are geometrical isomers of unsaturated fatty acids that assume a SFA-like configuration. Partial hydrogenation, the process used to create t-FA, also removes essential fatty acids such as linoleic acid (LA) and alpha linolenic acid (ALNA). Metabolic studies have demonstrated that t-FAs render the plasma lipid profile even more atherogenic than SFA, by not only elevating LDL cholesterol to similar levels but also decreasing HDL cholesterol. As a result, the ratio of LDL cholesterol to HDL cholesterol is significantly higher with a t-FA diet (2.58) than with a SFA diet (2.34) or an oleic acid diet (2.02). This greatly enhances the risk of CHD. In controlled trials, each 1% energy replacement of t-FAs with SFAs, MUFAs, or PUFAs, respectively, decreased the total cholesterol/HDL cholesterol ratio by 0.31, 0.54, and 0.67. In prospective cohort studies, each 2% energy replacements of t-FAs with SFAs, MUFA, or PUFA lowered CHD risk by 17%, 21%, and 24%, respectively. Therefore, risk of developing CHD depends on the t-FAs content of the oil or food and also on the fatty acid composition of the substituted oil or fat. Controlled and observational studies have reported that for every 2% increase in energy from t-FAs, there was a 32% higher risk of MI or CHD death. Evidence that intake of t-FA increases the risk of CHD initially became available from large population based cohort studies in USA and in an elderly Dutch population. Eliminating t-FAs from the diet would be an important public health strategy to prevent CHD. Since these are commercially introduced agents into the diet, policy measures related to the food industry practices would be required along with public education. t-FAs have been eliminated from retail fats and spreads in many parts of the world, but deep-fat fried fast foods and baked goods are a major and increasing source. In the USA, New York City has an ongoing successful program to phase out t-FAs in restaurant foods. Other US states and some countries are implementing or initiating such efforts to help their citizens reduce their CHD risk.

MUFA

The only nutritionally important MUFA is oleic acid, which is abundant in olive and canola oils and also in nuts. The epidemiological evidence related to MUFA and CHD is derived from studies on the Mediterranean diet (detailed below), as well as from the Nurses Health Study and other similar studies in USA.

PUFA

PUFA are categorized as n-6 PUFA (mainly derived from linoleic acid) and n-3 PUFA (mainly present in fatty fish and also derived from alpha-linolenic acid). Clinical trials, in which n-6 PUFA (containing linoleic acid) were substituted for SFA showed a greater impact on reduction of both plasma cholesterol and CHD risk, in contrast to trials where low-fat diets, were employed. Much of the epidemiological evidence related to n-3 PUFAs is derived from the study of fish consumption in populations or interventions involving fish diets in clinical trials. Fish oils were, however, used in a large clinical trial of 11 300 survivors of MI. After 3.5 years of follow-up, the fish oil group (1 g per day) had a statistically significant 20% reduction in total mortality, 30% reduction in cardiovascular death, and 45% decrease in sudden death. The results of randomized clinical trials indicate that consumption of <1 g per day of n-3 fatty acids eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) present in fish or fish oil is protective for cardiovascular diseases. At higher doses (>3 g per day) they likely decrease plasma triacylglycerols, blood pressure, platelet aggregation, and inflammation.

The Lyon Heart Study, in France, incorporated an n-3 fatty acid (alpha-linolenic acid) into a diet, altered to develop a 'Mediterranean diet' intervention. In the experimental group, plasma ALNA and EPA increased significantly and the trial reported a 70% reduction in cardiovascular mortality at 5 years. Total and LDL cholesterol were identical in the experimental and control groups, suggesting that thrombotic and perhaps arrhythmic events may have been favorably influenced by n-3 PUFA. Because the diet altered many other variables, such as fiber and antioxidants (by increasing fruit and vegetable consumption), direct attribution of benefits to n-3 PUFA becomes difficult to establish.

The balance of omega-6/omega-3 fatty acids has been found to be an important determinant in reducing the risk for CHD. In the Lyon Heart Study, the ratio of 4/1 of LA/ALA led to a 70% decrease in total mortality at the end of 2 years. The proportions of SFA, MUFA, and PFA as constituents of total fat intake and total energy consumption have engaged active attention, in view of the strong relationship of these fatty acids to the risk of CHD. The reduction of SFA in the diet has been widely recommended, but its replacement has been an area of debate, as to whether the place of reduced SFAs should be taken by MUFA, PUFA, or carbohydrate. Both MUFA and PUFA improve the lipoprotein profile, although PUFAs are somewhat more effective. In view of this, several recent dietary recommendations suggested that SFA should be kept below 10% of daily energy intake (preferably reduced to 7–8%), MUFA should be increased to 13–15%, and PUFA raised to 7–10% of daily energy, with the total fat contributing to less than 30% of all calories consumed. These may need to be adjusted for populations who consume less quantities of total fat, so as to ensure an adequate intake of MUFA and PUFA even under those circumstances. The emphasis is now shifting from the quantity of fat to the quality of fat, with growing evidence that even diets with 30–35% fat intake may be protective if the type of fats consumed is mostly from the MUFA and PUFA categories. Enhancing the nutritional quality of dietary fat consumption, to provide greater cardiovascular

protection, may be attempted by decreasing the sources of saturated fats and eliminating t-FAs in the diet, increasing the consumption of foods containing unsaturated fatty acids (both MUFA and PUFA), and decreasing dietary cholesterol consumption.

Carbohydrates

High-carbohydrate diets appear to reduce HDL cholesterol levels and increase the fraction of small dense LDL, both of which may impact adversely on vascular disease. This dyslipidemic pattern is consistent with the elevation of plasma triglycerides and is typical of the 'metabolic syndrome.' Carbohydrate diets with high-glycemic index might adversely impact on glucose control, with associated changes in plasma lipids, and have been linked to an increased risk of CHD. However, a diet with moderately restricted carbohydrate intake but rich in vegetable fat and vegetable protein improves lipid profile and may lower CHD risk. A recent review indicated that restriction of sugars and carbohydrates having high glycemic index are important for overall heart disease reduction. The nature of carbohydrate is of utmost important in relation to the reduction of CHD. Nonstarch polysaccharides present in intact fruits, legumes, and whole grains reduce total and LDL cholesterol and also help to improve glycemic control.

Fiber

Most soluble fibers reduce plasma total and LDL cholesterol concentrations, as reported by several trials. Fiber consumption strongly predicts insulin levels, weight gain, and cardiovascular risk factors like blood pressure, plasma triglycerides, LDL and HDL cholesterol, and fibrinogen. Several large cohort studies in the USA, Finland, and Norway have all reported that subjects consuming relatively large amounts of whole grain cereals have significantly lower rates of CHD. A recent review had indicated that consumption of soluble fibers reduces blood cholesterol as well as the postprandial blood glucose response. It was found that consumption of insoluble fiber from whole grains like nuts, legumes, and other edible seeds were associated with reduced risk of developing CHD.

Antioxidants

Though several cohort studies showed significant reductions in the incidence of cardiac events in men and women taking high-dose vitamin E supplements, large clinical trials failed to demonstrate a cardioprotective effect of vitamin E supplements. Beta-carotene supplements too did not provide protection against CHD and, in some trials, appeared to increase the risk. Observational studies have reported inverse associations between the frequency of CHD and dietary intake of antioxidant vitamins. Vitamin E and vitamin C in combination have shown a long-term antiatherogenic effects but these are beneficial only for people who have antioxidant deficiency or are exposed to high levels of oxidative stress such as smokers, diabetics, and elderly patients.

Folate

The relationship of folate to CVD has been mostly explored through its effect on homocysteine, which has been incriminated as an independent risk factor for CHD. Reduced plasma folate has been strongly associated with plasma elevated plasma homocysteine levels and folate supplementation has been demonstrated to decrease those levels. Data from the Nurses' Health Study, in USA, showed that folate and vitamin B6, from diet and supplements, conferred protection against CHD (fatal and nonfatal events combined) and suggested a role for their increased intake as an intervention for primary prevention of CHD. However, recommendations related to folate supplementation cannot be currently advocated as the results from clinical trials so far provide insufficient evidence. Meanwhile, dietary intake of folate through natural food sources may be encouraged, especially in individuals at a high risk of arterial or venous thrombosis and elevated plasma homocysteine levels.

Flavonoids and Other Phytochemicals

Flavonoids are polyphenolic antioxidants, which occur in a variety of foods of vegetable origin, such as tea, onions, and apples. Data from several prospective studies indicate an inverse association of dietary flavonoids with CHD. The role of these and other phytochemicals (such as plant stanols and sterols), in relation to CHD, needs to be elucidated further. Recent evidence indicates that consumption of foods rich in sterols and stanols help reduce the LDL cholesterol levels with beneficial effects on apolipoprotein apoB/apoAI ratio, HDL cholesterol, and triglycerides.

Sodium and Potassium

HBP is a major risk factor for CHD, accounting for almost half of all CHD events. The relative risk of CHD, for both systolic and diastolic blood pressures, operates in a continuum of increasing risk for rising pressure but the absolute risk of CHD is considerably modified by coexisting risk factors (such as blood lipids and diabetes), many of which are also influenced by diet. A cohort study in Finland observed a 51% greater risk of CHD mortality with a 100 mmol increase in 24 h urinary sodium excretion. Several clinical trials have convincingly demonstrated the ability of reduced sodium or salt diets to lower blood pressure. A meta-analysis of long-term trials suggests that reducing daily salt intake from 12 to 3 g per day is likely to reduce CHD by 25% (and strokes by 33%). Even more modest reductions in population salt intake would have substantial benefits (10% lower CHD for a 3 g salt reduction). In addition, recent follow-up studies have reported up to 30% reduction in cardiovascular events among those consuming less salt. Modeled estimates from the US, Canada, and Australia further indicate the huge life-saving benefits as well as healthcare cost saving potential of modest population wide salt reduction programs. Because salt intakes are high worldwide, influential health organizations such as the WHO have recommended reducing it to <5 g per day to improve population heart health. The benefits of dietary potassium, in lowering blood pressure, have been well demonstrated but

specific effects on CHD risk have not been well studied. Substitution of the salt used in domestic cooking with a low sodium high potassium alternative lowered average systolic blood pressure by 5.4 mmHg in China whereas replacement of usual table salt by low sodium potassium-enriched Pansalt contributed considerably to CHD risk reduction in Finland. Keeping the dietary sodium–potassium ratio at a low level is essential, to avoid hypertension.

Calcium

Although protective against osteoporosis, calcium supplements accelerate vascular calcification and increase mortality in patients with renal failure. The Women's Health Initiative is the largest trial of vitamin D supplementation to date and has shown no effect of vitamin D plus calcium supplementation on the risk of CHD events. The pooled analysis of randomized trials have shown that calcium supplements (without coadministered Vitamin D) were associated with approximately 30% increase in the incidence of MI whereas, there was smaller and nonsignificant association with stroke and mortality.

Food Items

Fruits and Vegetables

A systematic review reported that nine of 10 ecological studies, two of three cases–control studies and six of 16 cohort studies found a significant protective association for CHD with consumption of fruit and vegetables or surrogate nutrients. In a 12-year follow-up of 15 220 male physicians in US, men who consumed at least 2.5 servings of vegetables per day were observed to have a 33% lower risk for CHD, compared with men in the lowest category (<1 serving per day). A follow-up study of NHANES, the large national survey of USA, also reported a coronary protective effect of regular fruit and vegetable intake. Persons who consumed fruits and vegetables three or more times a day were at 24% lower risk than those who consumed less than once a day. The INTERHEART also reported low consumption of fruit and vegetables to be a major risk factor for CHD, across all regions.

Fish

In the UK Diet and Reinfarction trial (DART), 2 year mortality was reduced by 29% in survivors of a first MI, in persons receiving advice to consume fatty fish at least twice a week. A meta-analysis of 13 large cohort studies suggests a protective effect of fish intake, against CHD. Compared with those who never consumed fish or did so less than once a month, persons who ate fish had a lower risk of CHD (38% lower for five or more times a week; 23% lower for two to four times a week; 15% lower for once a week; 11% lower for one to three times a month). Each 20 g per day increase in fish consumption was related to a 7% lower risk of CHD. However, the Diet and Angina Randomized Trial (DART 2) demonstrated that advice to those with stable angina to eat fatty fish did not reduce mortality, and taking fish oil capsules was associated with a

higher risk of cardiac and sudden death. The apparently conflicting results of the two trials was attributed to the differential actions of n-3 fatty acids in acute and chronic conditions, and different effects of consuming fish and taking fish oil capsules.

Nuts

Several large epidemiological studies have demonstrated that frequent consumption of nuts was associated with decreased risk of CHD, the best known among them being the Adventist Health Study. The extent of risk reduction ranged from 18% to 57%, for subjects who consumed nuts more than five times/week compared to those who never consumed nuts. An inverse dose-response relationship was demonstrated between the frequency of nut consumption and the risk of CHD, in men as well as in women. Most of these studies considered nuts as a group, combining many types of nuts (walnuts, almonds, pistachio, pecans, macadamia nuts, and legume peanuts). A meta-analysis reported that consumption of 50–100 g (1.5–3.5 servings) of nuts five times/week as part of a heart healthy diet with total fat content (high in mono- and polyunsaturated fatty acids) of 35% of energy, significantly decreased bad cholesterol levels.

Soy

Soy is rich in isoflavones, compounds that are structurally and functionally similar to estrogen. Several animal experiments suggest that intake of these isoflavones may provide protection against CHD, but human data on efficacy and safety are still awaited. Naturally occurring isoflavones, isolated with soy protein, reduced the plasma concentrations of total and LDL cholesterol without affecting the concentrations of triglycerides or HDL cholesterol in hypercholesterolemic individuals.

Dairy Products

Dairy consumption has been correlated positively, in ecological studies, with blood cholesterol as well as coronary mortality. Milk consumption correlated positively with coronary mortality rates in 43 countries and with MI in 19 regions of Europe.

Alcohol

The relationship of alcohol to overall mortality and cardiovascular mortality has generally been J-shaped, when studied in western populations in whom the rates of atherothrombotic vascular disorders are high. The protective effect of moderate ethanol consumption is primarily mediated through its effect on the risk of CHD, as supported by more than 60 prospective studies. A consistent coronary protective effect has been observed for consumption of one to two drinks per day of an alcohol-containing beverage but heavy drinkers have higher total mortality than moderate drinkers or abstainers, as do binge drinkers.

Composite Diets and CHD

The Mediterranean Diet

The traditional Mediterranean diet has been described to have eight components: (1) high monounsaturated-to-saturated fat ratio, (2) moderate ethanol consumption, (3) high consumption of legumes, (4) high consumption of cereals (including bread), (5) high consumption of fruits, (6) high consumption of vegetables, (7) low consumption of meat and meat products, and (8) moderate consumption of milk and dairy products. Most of these features are found in many diets in that region. The characteristic component is olive oil, and many equate a Mediterranean diet with consumption of olive oil.

A secondary prevention trial of dietary intervention in survivors of a first recent MI (the Lyon Heart study), which aimed to study the cardioprotective effects of a 'Mediterranean type' of diet, actually left out its most characteristic component, olive oil. The main fat source was rapeseed oil. Vegetables and fruits were also increased in the diet. On a 4-year follow-up, the study reported a 72% reduction in cardiac death and nonfatal MI. The risk of overall mortality was lowered by 56%. Large cohort studies in Greece and in several elderly European population groups have also reported a protective effect against CHD and better overall survival in persons consuming a Mediterranean type of diet. The protection was afforded by the composite diet rather than by any single component. Improvement in metabolic syndrome and reduction of inflammatory markers has also been observed with this diet, which may explain part of the protection against CHD.

Dietary Approaches to Stop Hypertension (DASH) Diets

A composite diet, employed in the DASH trials, has been found to be very effective in reducing blood pressure in persons with clinical hypertension as well as in people with blood pressure levels below that threshold. This diet combines fruits and vegetables with food products, which are low in saturated fats. The blood pressure lowering effect is even greater when the DASH diet is modified to reduce the sodium content. Though the effects on CHD prevention have not been directly studied, the blood pressure and lipid lowering effects of the low salt-DASH diet are likely to have a substantial impact on CHD risk. Notably, the addition of exercise and weight loss to the DASH diet results in additional blood pressure lowering beyond the DASH diet alone, greater improvements in vascular and autonomic function, and reduced left ventricular mass.

Vegetarian Diets

A reduced risk of CVD has been reported in populations of vegetarians living in affluent countries and in case-control comparisons in developing countries. Long-term vegetarians have also been shown to have better antioxidant status (as measured by the plasma ascorbic acid status) and CHD risk profile than do apparently healthy omnivores. Reduced consumption of animal fat and increased consumption of fruit, vegetables, nuts, and cereals may underlie such a protective

effect. However, 'vegetarian diets' per se need not be healthful. If not well planned, they can contain a large amount of refined carbohydrates and t-FAs, although being deficient in the required levels of vegetable and fruit consumption. The composition of the vegetarian diet should, therefore, be defined in terms of its cardio protective constituents.

Prudent versus Western Patterns

In the Health professionals follow-up study in USA, a prudent diet pattern was characterized by higher intake of vegetables, fruit, legumes, whole grains, fish, and poultry whereas the western pattern was defined by higher intake of red meat, processed meat, refined grains, sweets and dessert, French fries, and high-fat dairy products. After adjustment for age and other coronary risk factors, relative risks, from the lowest to the highest quintiles of the prudent pattern score, were 1.0, 0.87, 0.79, 0.75, and 0.70, indicating a high level of protection. In contrast, the relative risks, across increasing quintiles of the western pattern, were 1.0, 1.21, 1.36, 1.40, and 1.64, indicating a mounting level of excess risk. These associations persisted in subgroup analyses according to cigarette smoking, body mass index, and parental history of MI. In the INTERHEART study, consumption of a prudent diet (high in fruits and vegetables) was associated with up to 30% reduced risk of MI worldwide.

Japanese Diet

The traditional Japanese diet has attracted much attention because of the highest life expectancy and low CHD mortality rates among the Japanese. This diet is low in fat and sugar and includes soy, seaweeds, raw fish, and a predominant use of rice. It has been high in salt, but salt consumption has recently been declining in response to Japanese Health Ministry guidelines. The Okinawa diet (from a prefecture of Japan renowned for high life expectancy) when compared to the Mediterranean and DASH diets was found to have the lowest fat, particularly saturated fats, which may likely be a contributing factor to the low CHD mortality rates in Japan.

Prevention Pathways

The powerful relationship of specific nutrients, food items, and dietary patterns to CHD has been persuasively demonstrated by observational epidemiological studies (which indicate the potential for primary prevention in populations) and by clinical trials (which demonstrate the impact on secondary prevention in individuals).

Atherosclerotic vascular diseases (especially CHD) are multifactorial in origin. Each of the risk factors operates in a continuous manner, rather than across an arbitrary threshold. When multiple risk factors coexist, the overall risk becomes multiplicative. As a result of these two phenomena, the majority of CHD events occurring in any population arise from any individuals with modest elevations of multiple risk factors rather than from the few individuals with marked elevation of a single risk factor.

These phenomena have two major implications for CHD prevention. First, it must be recognized that a successful prevention strategy must combine population-wide interventions (through policy measures and public education) with individual risk reduction approaches (usually involving counseling and clinical interventions). Second, diet is a major pathway for CHD prevention, as it influences many of the risk factors for CHD, and can have a widespread impact on populations and substantially reduce the risk in high-risk individuals. Even small changes in blood pressure, blood lipids, body weight, central obesity, blood sugar, inflammatory markers, etc., can significantly alter the CHD rates, if the changes are widespread across the population. Modest population-wide dietary changes can accomplish this, as demonstrated in Finland and Poland. At the same time, diet remains a powerful intervention to substantially reduce the risk of a CHD-related event in individuals who are at high risk due to multiple risk factors, prior vascular disease, or diabetes.

A diet, which is protective against CHD, should integrate: plenty of fruits and vegetables (400–600 g per day); a moderate amount of fish (two to three times a week); a small quantity of nuts; adequate amounts of PUFA and MUFA (together constituting approximately 75% of the daily fat intake); low levels of SFA (less than 25% of the daily fat intake); limited salt intake (preferably less than 5 g per day) and restricted use of sugar. Such diets should be culturally appropriate, economically affordable and based on locally available foods.

National policies and international trade practices must be shaped to facilitate the wide availability and uptake of such diets. Nutrition counseling, of individuals at high risk, too must adopt these principles while customizing dietary advice to specific needs of the person. CHD is eminently preventable, as evident from research and demonstrated in practice across the world. Appropriate nutrition is a major pathway for CHD prevention and must be used more widely to make CHD prevention even more effective at the global level.

See also: Carbohydrates: Requirements and Dietary Importance. Dietary Fiber: Physiological Effects and Health Outcomes. Dietary Guidelines, International Perspectives. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids. Metabolism; Hyperlipidemia: Overview. Hypertension: Dietary Factors. Nutrition Transition, Diet Change, and its Implications. Nutritional Considerations for the Management of Hypertension. Obesity: Complications. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Salt: Epidemiology. *Trans-Fatty Acids*: Health Effects, Recommendations, and Regulations

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CYSTIC FIBROSIS

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Definition and Etiology

Cystic fibrosis (CF) is a multisystem autosomal recessive disorder caused by the mutation of a single gene on the long arm of chromosome 7 that codes for the CF transmembrane regulator (CFTR). This protein regulates the passage of chloride through the membrane of secretory epithelia, the dysfunction of which results in an altered composition of epithelial secretions. Clinically, CF is characterized by chronic pulmonary infection with periods of acute exacerbation, pancreatic insufficiency, and excessive losses of sweat electrolytes. The latter forms the basis for the diagnostic test. The mutated gene was identified in 1989 and since then more than 800 CFTR mutations have been reported, the most common of these being $\Delta F508$.

Prevalence

Approximately 5% of the Caucasian North European and North American populations are carriers of the gene defect causing CF, leading to an approximate incidence of 1 in 2500 live births. This inheritance is illustrated in **Figure 1**. The incidence of CF in non-Caucasians is much lower and estimated to be approximately 1 in 100 000 in Oriental populations.

Prognosis

The median age of survival has dramatically risen from approximately 2 years in the 1940s to nearly 30 years in the 1990s. A current survival estimation following diagnosis is approximately 40 years. This improved prognosis can be attributed to a combination of factors, including aggressive

management of infections, effective antibiotics, improved nutritional management, modern physiotherapy techniques, and the centralization of treatment in specialist centers. The survival age for females with CF would appear to be less than that for males. This may be related to poorer nutritional status among female CF patients. Expert management started immediately after an early diagnosis of CF by neonatal screening results in an important beneficial effect on outcome and may be critical to the clinical course of the condition and long-term prognosis. Although optimized nutrition, antibiotics, and chest physiotherapy remain the mainstay of CF management, new approaches to treatment are being developed that may add to the traditional medical therapy for CF. As prognosis and survival improves, nutrition-related issues become more prevalent, including the effective management of pregnancy, diabetes, osteoporosis, and transplantation.

Clinical Features

The clinical features of CF are listed in **Table 1**.

Pathogenesis of Lung Disease

Pulmonary disease can be demonstrated within the first few months of life. Bacterial infection is characterized by high levels of neutrophils and mediators of infection in the form of interleukin 1, interleukin 8, and elastases. Mucous glands become dilated leading to obstruction, secondary infection, and progressive lung damage. Frequent periods of respiratory infection and exacerbation are common in CF patients with increased cough, increased sputum production, and shortness of breath. The immune response appears to be of great significance. Chronic inflammation has been cited as the cause of considerable lung damage seen in CF. Steroidal anti-inflammatory drugs have been shown to be beneficial but have nutritional side effects, such as hyperglycemia and osteoporosis. Nonsteroidal anti-inflammatory drugs, such as ibuprofen, have been used in some centers with positive results, but their long-term effect on renal function is not yet known. The impact of malnutrition on lung disease and respiratory muscle function has been extensively studied in patients with CF. Malnutrition and deterioration of lung function are interdependent. Prevention of malnutrition from the time of diagnosis is associated with better lung function and improved survival.

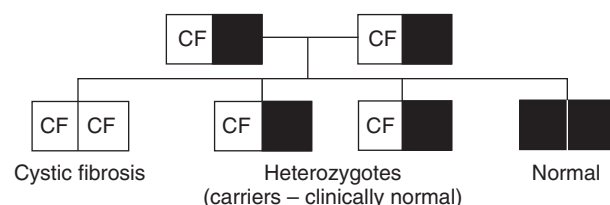


Figure 1 Mode of inheritance of CF: A Mendelian-inherited recessive characteristic. Reproduced from Figure 16.3 in Gibney MJ (ed.) (2005) *Clinical Nutrition. Nutrition Society Textbook Series*. Blackwell publishing. ISBN 0-632-05626-6, with permission from Wiley.

Table 1 Clinical features of respiratory features of CF

Respiratory features of CF	
Atelectasis	Incomplete expansion of a lung or part of a lung due to airlessness or collapse
Bronchiectasis	Chronic dilatation of the bronchi associated with coughing and expectoration of purulent mucus
Bronchitis	Inflammation of one or more bronchi
Pneumonia	Inflammation of the lungs with air spaces becoming filled with exudates
Pneumothorax	Accumulation of air in the pleural space
Gastrointestinal features of CF	
Cholelithiasis	The presence or formation of gallstones
Cirrhosis	Liver disease characterized by loss of normal liver tissue and fibrosis
Distal intestinal obstruction syndrome	Blockage of the bowel with feces, mucus, and undigested food
Gastroparesis	Paralysis of the stomach or delayed gastric emptying
Malabsorption	Impaired intestinal absorption of nutrients
Maldigestion	Impaired intestinal digestion of nutrients
Meconium ileus	Blockage of the bowel with meconium
Osteoporosis/osteopenia	Reduction in bone mass
Pancreatic insufficiency	Reduction of enzyme production from the pancreas
Portal hypertension	High pressure in the portahepatic artery
Rectal prolapse	Protrusion of the rectal mucous membrane through the anus
Splenomegaly	Enlargement of the spleen

Gastrointestinal Complications

Individuals with CF can develop a variety of gastrointestinal (GI) disorders related to the pathophysiological changes associated with CF. Pancreatic insufficiency, which is present in most CF patients, leads to many of the GI manifestations of CF, including steatorrhea, abdominal pain, distal intestinal obstruction syndrome (DIOS), and rectal prolapse. Gastroesophageal reflux occurs frequently in CF patients due to decreased lower esophageal sphincter pressure and is usually treated by proton pump inhibitors. In patients with advanced lung disease, vomiting is common after strenuous bouts of coughing and this over time may lead to decline in nutritional status. Peptic ulcer disease, pancreatitis, and intussusception also occur at varying degrees in patients with CF. Crohn's disease and celiac disease occur more frequently in the CF population than in controls; and GI tumors, although rare, have an increased incidence in CF.

Meconium ileus is the presenting complaint in up to 15% of infants with CF. This is a condition in which the small intestine is blocked with tenacious meconium and surgical intervention is required to correct it. Excessive mucus in the small bowel of patients with CF can provide a physical barrier to the absorptive surface. Undigested or unabsorbed food in association with this mucus, and possibly a reduced gut motility, can lead to a partial or complete obstruction of the GI tract in older children and adults known as meconium ileus equivalent, or more accurately DIOS. This is a condition specific to CF. The usual clinical presentation is one of abdominal pain, abdominal distension, and constipation. It can be precipitated by dehydration, change in eating habits, change in enzyme brand or dose, or immobility. DIOS is treated with a laxative regime and should have a diet and enzyme review.

CF-Related Diabetes Mellitus

Diabetes requiring insulin is the most common comorbidity in CF. The islets of Langerhans are the last cells to be damaged

in the process of fibrosis of the pancreas. The incidence of diabetes in CF has been reported to be 8–15%, but this may be underestimated due to lack of screening. It is estimated that 50% of patients older than 30 years will have some degree of glucose intolerance. The primary cause of CF-related diabetes mellitus (CFRD) is insulin deficiency secondary to pancreatic fibrosis. Diagnostic criteria for CFRD are the same as for non-CFRD. Glucose metabolism is also affected by many factors, including infection, malabsorption, abnormal intestinal transit time, and steroid use, all features of CF. Although CFRD shares many of the characteristics of both type 1 and type 2 diabetes, it is itself a distinct clinical condition. Hyperglycemia may adversely influence weight and pulmonary function; and as the age of survival increases, it may lead to the development of microvascular complications. Retrospective studies have shown that in those individuals presenting with overt diabetes mellitus, deterioration in weight and respiratory status for 2 years before diagnosis are reversed once insulin therapy is instituted. A program of multiple daily insulin injections and self-monitoring of blood glucose with the aim of normoglycemia is the preferred treatment with regular follow-up with the Endocrinology team. All patients with CF should be screened annually for CFRD using the oral glucose tolerance test. Minimal dietary restrictions are imposed on this group of patients in an attempt to maximize nutritional intake. See the Section on Dietary Management of CF.

Liver Disease

Another complication associated with increased longevity in CF is liver disease, which affects between 2% and 37% of adults with CF. The development of liver disease in patients with CF has been attributed to the blockage of small bile ductules with thick secretions and the subsequent development of progressive cholestasis, biliary fibrosis, and eventually biliary cirrhosis and portal hypertension. The persisting acidic conditions in the upper small bowel lead to bile salt precipitation and defective lipid emulsification. Unhydrolyzed fat

and other products of maldigestion may interfere with bile acid reabsorption in the terminal ileum, thereby reducing the total bile salt pool. Fecal losses of primary and secondary bile acids lead to an imbalance of bile salts, which further increases the viscosity of the already tenacious bile. Treatment with ursodeoxycholic acid has led to an improvement in bile excretion and liver function tests. Complications of liver disease, including ascites, gastric, and esophageal varices may further exacerbate a patient's nutritional status. In a small number of patients, liver failure may require liver transplantation. See the Section on Dietary Management of CF.

Nutritional Management

Aggressive nutritional management of patients with CF is key in their overall management. Nutritional management of CF involves maximizing dietary intake; minimizing malabsorption and maldigestion; monitoring vitamin intakes and serum levels; and adapting eating patterns in the event of diabetes, osteoporosis, DIOS, or liver disease. Nutritional support in the form of nocturnal gastrostomy feeding may be necessary if nutritional failure persists (body mass index (BMI) lower than 18.5 kg m^{-2}). It is well recognized that the malnutrition seen in CF patients is due to an energy imbalance caused by three main factors: decreased dietary intake, increased energy requirements, and increased energy losses. There appears to be a direct association between the degree of malnutrition and the severity of pulmonary disease, affecting overall prognosis. Many patients are capable of balancing these factors effectively and have a normal growth velocity and good nutritional status. However, as lung function

deteriorates, energy requirement increases and appetite decreases leading to a loss of energy stores and lean tissue further contributing to progressive deterioration of lung function (see Figure 2).

Decreased Dietary Intake

People with CF are advised to consume a diet high in energy with no fat restriction. Before the development of enteric-coated enzymes in the mid 1980s, patients with CF were advised to follow a low-fat diet in an attempt to minimize fat malabsorption and steatorrhea. Unfortunately, older patients continue this practice as they have developed an aversion to fatty foods after many years of avoiding them. Decreased dietary intake secondary to anorexia is common in CF patients and can become more of a problem during recurrent chest infections. There has also been an increased number of reports of eating disorders and abnormal eating behavior in the CF population. In addition, polypharmacy, repeated exacerbations of CF, organomegaly, GI problems, food intolerance, and poor social circumstances can reduce oral intake.

Increased Energy Requirements

Energy requirements are increased during periods of infection by catabolism and fever and continue to increase with advanced pulmonary disease. It has been estimated that CF patients require 120–150% of the estimated average requirement of energy. As pulmonary function deteriorates, mobility also decreases and, as a result, overall energy expenditure is reduced. Owing to the heterogeneity of CF, the energy requirements of individuals will vary and should be assessed on

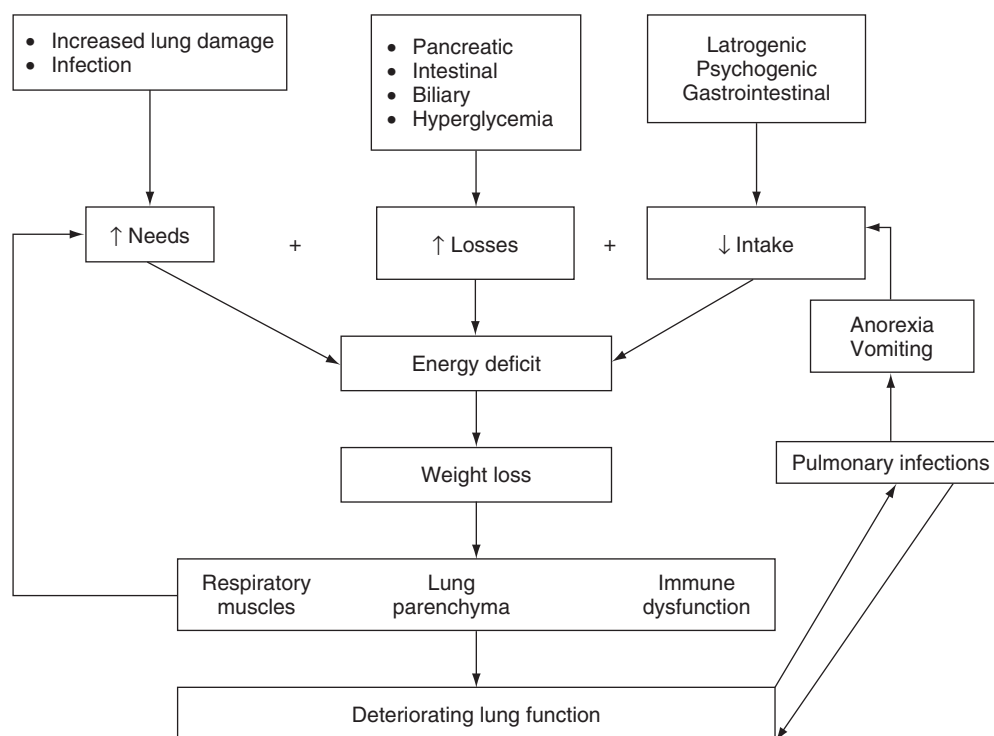


Figure 2 Interdependent factors that may give rise to progressive energy deficit as lung function deteriorates.

an individual basis. Energy losses through sputum may also be significant in a patient with a marginal energy intake. Salbutamol, often used as a bronchodilator in CF, can increase basal metabolic rate.

Increased Energy Losses

Pancreatic changes are caused by the obstruction of small ducts with thick secretions and cell debris. Functional tissue becomes replaced with fibrotic tissue leading to pancreatic exocrine insufficiency when more than 90% of the normal structure of the pancreas is lost. Pancreatic insufficiency is the most common GI manifestation in CF, occurring in at least 95% of patients. The production of pancreatic secretions, including enzymes and bicarbonate, is reduced, necessitating pancreatic enzyme replacement therapy (PERT). PERT is supplied in the form of gelatin capsules containing microspheres, which are swallowed whole with food. The capsule dissolves within the stomach and releases the microspheres, which are protected from the gastric acid by an enteric coating. Enzymes should be taken immediately before or during a meal to maximize their efficacy. The microspheres mix with the stomach contents and pass through the pylorus into the duodenum where they become activated. Microspheres should be less than 1.5 mm in diameter to ensure that they leave the stomach with food. Fibrosis of the pancreas tends to be a progressive process, so increasing amounts of oral enzyme supplements are often required as patients get older. All people with CF have some level of pancreatic dysfunction but requirements of enzymes are variable and must be assessed individually. Clinically, the aim of PERT is to correct symptomatic steatorrhea, relieve any abdominal pain, reduce the mass and frequency of stool passed, and achieve weight gain within normal limits.

The enteric coating on enzyme supplements is designed to dissolve at pH 6, the optimal pH for pancreatic enzymatic action. Owing to the reduced production of bicarbonate and the resulting lower pH of the duodenum in patients with CF, the enteric coating of the enzyme may fail to dissolve so that the enzyme does not become activated at the absorptive surface of the small bowel. Increasing the duodenal pH by taking proton pump inhibitors may improve absorption. Changing the brand of enzyme may also improve absorption as dissolution characteristics of the enteric coating and proportions of enzymes contained within the microspheres vary. Patients should be dissuaded from chewing enzymes as this breaks the enteric coating and leads to deactivation in the acid medium of the stomach. Even with maximal PERT, it has been estimated that between 10% and 20% of ingested fat will be malabsorbed. Colonic strictures known as fibrosing colonopathy (FC) in CF populations receiving high-potency enzymes with a more concentrated dose of lipase and protease per capsule have been reported. The etiology of this FC remains unclear. Recently, it has been suggested that FC may be related to the presence of methacrylic acid copolymer coating present in some preparations rather than actual enzyme strength. Some adult patients continue to take high-dose enzymes and are advised to do so within recommended levels. The working group on PERT use recommends that no more than 10 000

units of lipase per kilogram body weight should be taken per day.

Dietary Management of CF

Patients with CF are encouraged to consume a diet providing 150% of the recommended intake for age and sex. However, this is only a guideline, because in practice, the energy requirement for a patient with CF is that which maintains their ideal body weight when malabsorption has been controlled. Maximizing energy intake from everyday foods should be the initial step in the promotion of a high-energy diet. As fat is the most concentrated source of energy in the diet, liberal use of fat should be encouraged; this can best be achieved by recommending frequent consumption of high-fat meals and snacks, including confectionery, desserts, and cakes. PERT should be dosed accordingly.

Dietary Supplements

The energy intake of many patients with CF is commonly suboptimal. Many patients find it difficult to eat sufficient food daily to attain or maintain their ideal body weight. During a respiratory exacerbation of CF, energy requirements are at a maximum, but appetite is often reduced. Dietary supplements in the form of sip feeds can be a useful adjunct to a high-energy diet. Care should be taken to ensure that supplements are used in addition to a diet and not as a substitute for normal foods.

Enteral Feeding

When diet and oral dietary supplements are undesirable or ineffective and nutritional failure persists, i.e., BMI lower than 18.5 kg m^{-2} , enteral feeding should be considered. Research has demonstrated a sustained weight gain and a slowing decline in respiratory function associated with supplemental enteral feeding. Artificial nutritional support can be provided via nasogastric or gastrostomy tube depending on patient's preference. Gastrostomy feeding is becoming more popular, whether passed endoscopically or under fluoroscopic guidance. The introduction of low-profile gastrostomy feeding tubes or 'button' tubes have made this method of nutritional support more acceptable to patients. The type of feed used and the PERT given with it varies between centers. Feeds are usually administered overnight in an attempt to provide 30–50% of energy requirements and to allow for maximal oral intake during the day. Gastrostomy feeds can be used over longer periods during acute pulmonary infection, loss of appetite, or in a severely malnourished patient. Patients with a history of poor intake should be monitored for refeeding syndrome.

Specific Dietary Considerations

There are some medical complications of CF that warrant particular nutritional attention.

Liver Disease

Patients with liver disease as a complication of their CF may have ascites, gastric, or esophageal varices, all of which may affect nutritional status and options for nutritional support. Dietary management of the patient with CF and liver disease centers on maximizing energy intake and is best achieved by encouraging small, frequent, energy-dense meals, snacks, and drinks. Suboptimal oral intake can arise in patients with hepatomegaly or splenomegaly, who often have a feeling of fullness after eating, 'a condition' referred to as the 'small stomach syndrome.' The benefits of gastrostomy insertion should be carefully weighed in a patient with gastric varices or splenomegaly due to risk of bleeding. A moderate sodium restriction may alleviate ascites. If coagulation is impaired, supplementation with vitamin K may be indicated.

Treatment of liver disease in CF is with ursodeoxycholic acid, which has a positive effect on liver enzymes. Whether this improvement is associated with improvement in nutritional status is unknown.

CF-Related Diabetes Mellitus

The dietary treatment of CFRD varies from standard diabetic dietary advice. The principle of the diet centers on maintaining caloric intake while ensuring glycemic control. The treatment of CFRD should enhance rather than impair a patient's nutritional status. This is done by encouraging a high-fat diet and confining the intake of refined carbohydrate to mealtimes. Insulin doses should be increased so as to maximize the flexibility of the diet, particularly in those patients who are already nutritionally compromised. Patients taking oral nutritional supplements and/or overnight gastrostomy feeds need to have their insulin doses carefully monitored and adjusted accordingly.

Bone Disease in CF

Osteopenia and osteoporosis are now widely recognized in the CF population. There are a number of contributing factors to this early development of bone disease, including steroid usage; malabsorption of calcium and, more importantly, vitamin D; poor nutritional status; decreased levels of physical activity; and a reduced peak bone mass in CF patients compared with healthy individuals. Assessment of bone health is by dual-energy X-ray absorptiometry scanning and there are a variety of treatment options available depending on the severity of disease ranging from dietary calcium and vitamin D supplementation to the use of bisphosphonate drugs, which aim to halt the progression of bone loss and promote bone formation.

Fertility Issues

As the number of people with CF of a reproductive age increases, so does the incidence of pregnancy in this group. Although almost all males with CF are infertile owing to the absence of the vas deferens, most females are fertile. Pregnancy in women with CF requires special nutritional attention with regular monitoring, particularly with respect to adequate weight gain, and vitamin and mineral status.

Body Composition Studies in CF

Studies of body composition in CF patients have shown deficits in total body mass, lean body mass, and body fat, which affect body density. As skinfold thickness percentiles are derived from body density, it has been suggested that the assessment of the body fat content of children with CF using, or derived from, body density, such as skinfold thickness, is invalid. Muscle function indices have been shown to respond to refeeding in malnourished patients with CF before body composition or biochemical indices of protein status improved, and so appear to be sensitive markers of nutritional status.

Assessment of Nutritional Status

Malnutrition in CF remains a major clinical problem. Growth and nutritional status should be monitored at each clinic visit to ensure early detection of any deterioration and to prompt appropriate nutritional intervention. The many factors that complicate nutritional status in CF are shown in [Table 2](#).

When weight falls to a BMI of less than 18.5 kg m^{-2} , nocturnal enteral feeding should be considered. At diagnosis and when the patient shows clinical deterioration, the following should be determined: electrolytes, serum albumin and other liver function tests, oral glucose tolerance test, full blood count, serum retinol, and α -tocopherol. If there is any evidence of iron deficiency, iron status should be assessed. Other medical disorders should be considered in the evaluation of nutritional failure. These include diabetes mellitus, liver disease, Crohn's disease, celiac disease, chronic abdominal pain, DIOS, and esophagitis.

Vitamin Status in CF

At least 85% of CF patients have some level of pancreatic insufficiency leading to a degree of fat malabsorption. For this reason, unless supplemented, most patients are at risk of developing either clinical or subclinical deficiencies of the fat-soluble vitamins, vitamin A, D, E, and K. Those most at risk appear to be individuals with poorly controlled malabsorption, poor adherence to treatment, liver disease, bowel resection, or following a late diagnosis.

Table 2 Factors affecting nutritional status

- Variation in gene mutation
- Frequency of pulmonary exacerbations
- Gastroesophageal reflux
- Distal intestinal obstruction syndrome
- Pancreatitis
- Liver disease
- Diabetes mellitus
- Drug therapy
- Dietary dislikes and misconceptions
- Psychological problems/eating disorders
- Pregnancy
- Transplantation

Vitamin A

Vitamin A should be supplemented at a dose of 4000–10 000 IU day⁻¹. However, low-serum levels of retinol have been noted even at this dose. If retinol levels are persistently low despite adequate supplementation, an assessment of compliance, retinol-binding protein (RBP), and zinc levels should be checked. Special care should be given to vitamin A supplementation during pregnancy as high levels are reported to be teratogenic.

It is important to consider hepatotoxicity with large supplemental doses of vitamin A in a patient who may store vitamin A in the liver, yet shows low-serum levels of retinol, and who may display ocular signs of deficiency. The free alcohol retinol is almost entirely attached to RBP, which is synthesized in the liver. Decreased levels of RBP, which may occur in up to 25% of patients with CF, may be due to an abnormality in its production by the liver, zinc deficiency, or protein-energy malnutrition. Even with adequate vitamin supplementation and PERT, up to 20% of patients may have ocular signs of deficiency of retinol. Xerosis may improve by increasing the dose of vitamin A alone or combined with zinc. It has been suggested that there may exist a specific defect in the handling of retinol in the GI tract of people with CF unrelated to the level of fat malabsorption. A correlation has been demonstrated between low levels of vitamin A and poor lung function.

β-Carotene

β-carotene is one of the carotinoids present in plasma and a precursor of vitamin A. It is effective as an antioxidant at lower oxygen saturation states than vitamin E. It has a biological role as a lipid-soluble chain-breaking antioxidant in biomembranes. Routine supplementation with β-carotene could diminish lipid peroxidation and improve essential fatty acid status.

Vitamin D

Vitamin D deficiency may be caused by malabsorption, underexposure to sunlight, or defects in metabolism due to liver disease. Although skin exposure to sunlight is the major source of vitamin D, serum concentrations will vary between individuals depending on endogenous production in the skin. Rickets as a result of vitamin D deficiency is rare but has been described in CF patients. Osteopenia and retarded bone maturation have been reported in a number of CF patients, even with supplementation to recommended levels. Bone density has been shown to be significantly decreased in all sites compared with that of normal young adults. Other variables such as activity levels and nutritional status have not been adequately researched, although the incidence of osteoporosis was found to be higher in those patients with severe respiratory disease. To attain and maintain normal serum levels, a daily dose of 400–2000 IU is generally required in adults.

Vitamin E

Cholestasis and a reduced enterohepatic circulation of bile acids contribute to the malabsorption of fat-soluble vitamins from the small intestine. Vitamin E is highly lipophilic and its deficiency correlates with a degree of fat malabsorption.

Subclinical neuroelectrophysiological abnormalities are already present in approximately 40% of patients by 2 months of age. Neurological signs of vitamin E deficiency are responsive to supplementation if initiated early, but are irreversible if treatment starts after the neurological lesions are present. As circulating α-tocopherol is transported in the blood attached to lipid, it should be expressed as a ratio to total lipid to be correctly interpreted. Current recommendations are to monitor serum vitamin E levels annually and adjust supplementation accordingly. A daily dose of 400 IU day⁻¹ should achieve normal serum levels in adults.

Vitamin K

A review of the literature providing conflicting opinions in the area of routine supplementation of vitamin K as the prevalence of vitamin K deficiency has not been established. Theoretically, the risk factors for patients developing vitamin K deficiency are pancreatic insufficiency, severe liver disease, extensive small bowel resection, and chronic broad-spectrum antibiotic use. Monitoring the coagulation system is advised, as vitamin K estimations are not generally routinely available. It seems prudent to prescribe vitamin K supplements to patients with the above-mentioned risk factors. Vitamin K has recently been shown to play an important role in bone health. There are no specific guidelines on supplementation, but doses of 5–10 mg appear to be a prudent guide. Annual monitoring of fat-soluble vitamin levels should be carried out and doses of vitamins altered as appropriate.

Water-Soluble Vitamins

Supplementation with water-soluble vitamins is, in general, thought to be unnecessary in CF. In cases where dietary intake is poor or unbalanced, supplementation of vitamin C is advised. Supplementation with other water-soluble vitamins is not routinely recommended.

Mineral Status in CF

Fat malabsorption can lead to the formation of insoluble fatty acid complexes with minerals in the gut, leading to a reduction in their absorption. CF may also be associated with intestinal mucosal defects, which may further retard the absorption of nutrients. Suboptimal levels of zinc, selenium, manganese, and iron have all been described in CF. Routine iron supplementation is not recommended as it has been suggested that *Pseudomonas aeruginosa* grows in tissues with a high concentration of iron. In addition, levels of iron may be suppressed as a normal body response in times of infection, and attempting to correct this is potentially harmful. Sodium and chloride do not need to be supplemented unless in very hot climates or during excessive exercise.

The Oxidant/Antioxidant Imbalance in CF Patients

Patients with CF frequently exhibit increased oxygen free radical generation from activated neutrophils due to chronic lung inflammation. This, coupled with antioxidant deficiencies due to exocrine pancreatic insufficiency, results in an oxidant/

antioxidant imbalance. Consequently, free radical attack on unsaturated fatty acids of lipid structures occurs leading to lipid peroxidation. An efficient antioxidant supply is suggested to control tissue damage by restoring the oxidant/antioxidant balance.

Conclusions

There is a complex relationship between physiological, environmental, and genetic variables leading to a great variability in energy requirements among individuals with CF. Despite advances in the treatment of CF, the need for good nutritional strategies in CF will continue. Individually tailored nutritional advice for each patient with CF by a dietitian experienced in the area of CF is essential.

See also: Biochemical Indices. **Diabetes Mellitus:** Classification and Chemical Pathology; Dietary Management; Etiology and Epidemiology. **Eating Disorders:** Anorexia Nervosa; Bulimia Nervosa. **Liver Disorders:** Nutritional Management. **Malnutrition:** Secondary, Diagnosis and Management. **Nutritional Assessment:** Anthropometry; Clinical Examination. **Nutritional Support:** Adults, Enteral; Infants and Children, Parenteral. **Parenteral Nutrition.** **Vitamin A:** Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. **Vitamin D:** Physiology, Dietary Sources, and Requirements. **Vitamin K**

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CYTOKINES

Nutritional Aspects

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Chemistry and Classification

Cytokines comprise a wide range of proteins which are released mainly from cells of the immune system in response to invasion of animals by pathogens or severe injury. Cytokines induce a state of inflammation in the body and modulate the activity of the immune system. Recent research shows that cytokine production is not restricted to cells in the immune system but that fibroblasts, endothelial cells, adipocytes, and specialised tissues, such as the ovary, produce cytokines. Although largely influencing immune function, a number of cytokines act as growth factors and lead to the proliferation and differentiation of a wide range of cell populations in the body. Cytokines act generally in an autocrine, or paracrine, fashion and are active in the sub-nanomolar range. Cytokines are sub-classified into interleukins (IL), tumor necrosis factors (TNF), interferons, and colony stimulating factors. Some representative examples from the family of cytokines are detailed in **Table 1**. All cytokines influence cells of the immune system, however, only three exert metabolic effects upon the host. These are denoted as proinflammatory cytokines, interleukin 1 (IL-1), interleukin 6 (IL-6), and tumor necrosis factor- α (TNF), respectively. A summary of the cell sources, main cell targets and actions of the proinflammatory cytokines is shown in **Table 1**.

Metabolism and Metabolic Functions

Widespread metabolic changes occur as a result of cytokine production (**Figure 1**). These responses are powerful, focussed, and dangerous to both host and pathogens. A hostile environment for pathogens is created within the body by the

release of oxidant molecules (superoxide, hydrogen peroxide, perchlorous acid, nitric oxide) from phagocytes. As will be seen below (*see Role in Disease and Disease Processes*) although the inflammatory response is essential for the survival of species in their battle against invasion by pathogens, individuals within the species will succumb to the response itself. Individual characteristic, within a species, will determine the strength, duration, chronicity and appropriateness of the response, and ultimately whether the response at an individual level is beneficial, detrimental, or lethal.

Cytokines orchestrate metabolic responses to ensure that during infection nutrients are provided for the immune system as a result of wasting of peripheral tissues. Amino acids released as a consequence of increased proteolysis in muscle, skin, and bone, provide substrate for the synthesis of cells in the system. Glutamine, released from muscle, and glucose derived from increased hepatic gluconeogenesis of amino acids, are major sources of nutrition for the system. Likewise increased lipolysis in adipose tissue provides fatty acids as metabolic fuel for the body. In addition proinflammatory cytokines raise fasting plasma triacylglycerol concentrations by enhancing free fatty acid efflux from adipose tissue, raising hepatic triacylglycerol synthesis and inhibition of entry of triacylglycerol into adipose tissue by inhibition of adipose tissue lipoprotein lipase activity. The increase in plasma concentrations have a functional significance in that further fuel is provided for immune cells and plasma bacterial endotoxin is sequestered onto these lipid molecules, before elimination by the liver. Zinc, an important cofactor in DNA synthesis, is released from peripheral tissues, incorporated into the zinc transporting protein metallothionein in liver and kidney, and subsequently utilized by the immune system. A loss of appetite often occurs. All of these metabolic changes may be

Table 1 Main properties of the proinflammatory cytokines

<i>Cytokine</i>	<i>Molecular weight</i>	<i>Cell sources</i>	<i>Main cell targets</i>	<i>Main actions</i>
Interleukin-1 α Interleukin-1 β	33 000 17 500	Monocytes macrophages, astrocytes, epithelial cells, endothelium, fibroblast, dendritic cells	Thymocytes, neutrophils, T and B cells, skeletal muscle, hepatocytes	Immunoregulation, inflammation fever, anorexia, acute-phase protein synthesis, muscle proteolysis, enhanced gluconeogenesis
Interleukin-6	20 000	Macrophages, T cells, fibroblasts	T and B cells, thymocytes, hepatocytes	Acute-phase protein synthesis, immune cell differentiation
Tumor necrosis factor- α	50 000 (trimer)	Macrophages, lymphocytes	Fibroblasts, endothelium, skeletal muscle hepatocytes	AS for IL-1

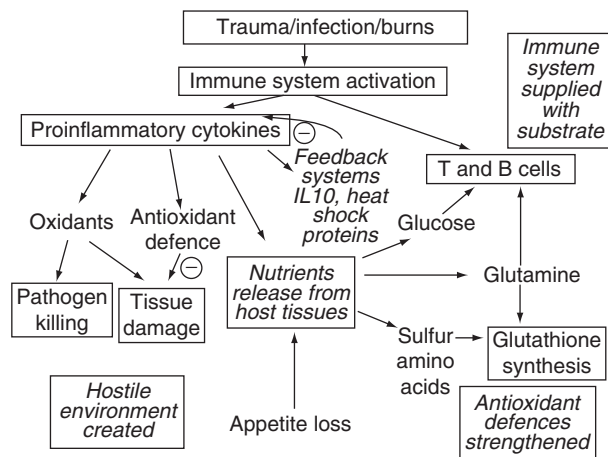


Figure 1 Interaction of proinflammatory cytokines with metabolism and key actions in the immune response.

purposeful in permitting a situation in which substrate is more closely tailored to the requirements of the immune system than would occur from the vagaries of habitual dietary intake. It is important that the immune system receives a guaranteed source of nutrition immediately the body is infected or damaged, because bacterial cells multiply at least 50 times more rapidly than T cells under favorable conditions. Under the actions of cytokines, the metabolic activity of the liver is greatly enhanced and modified. Large increases in the rates of gluconeogenesis, glycogen breakdown, and urea and fat synthesis occur. Blood glucose, urea, and triacylglycerol concentrations may rise. Paradoxically, however, metabolism of xenobiotics is decreased owing to a reduction in the activity of cytochrome P-450. The profile of export proteins synthesized by the liver is changed; synthesis of albumin is reduced, and the synthesis of a group of proteins closely associated with inflammation (acute-phase proteins) is increased. Acute-phase proteins are multifunctional and include caeruloplasmin (an antioxidant and copper transport protein), C-reactive protein (to improve macrophage activity), fibrinogen (for blood clotting), complement proteins (for enhanced phagocytosis and pathogen destruction), and metallothionein (a zinc transport protein).

The antioxidant defences of the body are strengthened by increases in the activities of superoxide dismutase, catalase, glutathione peroxidase, and reductase, and by increases in the hepatic synthesis of the reduced form of glutathione (GSH). The liver thus becomes the main focus for the synthesis of molecules for the nutrition, support and direction of the immune system, and for the protection of the body from the adverse effects of cytokine action. Indeed when the ability of the liver of patients with sepsis (a severe clinical form of inflammation induced by infection), to extract amino acids from the circulation was assessed it was found that patients who subsequently died had only half of the ability of patients who survived.

A number of molecules synthesized in enhanced amounts when cytokines are produced are part of complex feedback systems which limit cytokine production and effects (Figure 2). These include GSH and some acute-phase proteins which suppress cytokine production, and cytokine receptor antagonist

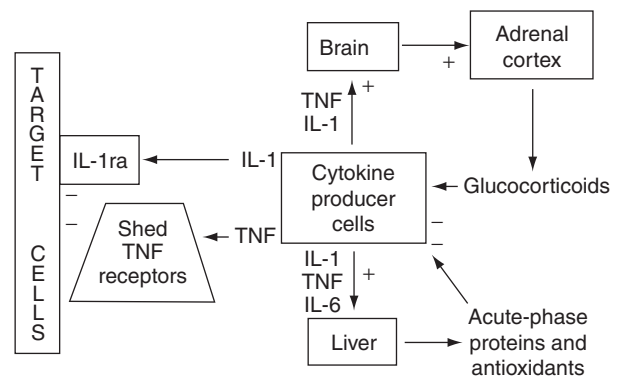


Figure 2 Feedback control systems for cytokine production.

molecules for IL-1 and TNF. The first two types of molecule are derived from liver and the last two from lymphocytes and the cellular targets for TNF, respectively. Other molecules also moderate cytokine actions. Anti-inflammatory cytokines such as IL-10 and heat shock proteins exert an anti-inflammatory influence in the latter stages of the inflammatory response. This down-regulation of inflammation, once the infectious agent has been defeated, is important for survival since the inflammatory process depletes the body. The balance between the proinflammatory and anti-inflammatory process is of key importance for survival because excessive production of IL-10 has also been associated with increased mortality.

Role in Disease and Disease Processes

Despite the importance of cytokines in protecting the host from pathogens, the molecules may have damaging and even lethal effects upon the host. Thus the response of the host to a pathogen may play as significant a part in the demise of the host as the effects of the pathogen itself. Cytokines may also play a major part in tissue damage in chronic inflammatory disease in which no infective agent is operating. Excessive or inappropriate cytokine production has been associated with increased morbidity and mortality in a wide range of diseases and conditions in which inflammation plays a part. These include diseases where the immune system is clearly interacting with invading pathogens, such as in malaria, meningitis, sepsis, and Acquired immuno deficiency syndrome (AIDS) and conditions such as asthma, inflammatory bowel disease, rheumatoid arthritis, and cancer where inflammatory disease develops without obvious involvement of pathogens. Furthermore proinflammatory cytokines may be involved in the progression of disease processes such as plaque development in atherosclerosis and demyelination in multiple sclerosis and Alzheimer's disease (Figure 3).

Damage may also be caused to the host by release of free radicals and other oxidant molecules that are released from phagocytic cells in response to the inflammatory stimulus and IL-1 and TNF. Furthermore oxidant molecules up regulate production of IL-1, TNF, and IL-8 by activation of the transcription factor, nuclear factor kappa B (NFkB). The factor is normally held quiescent in the cytoplasm owing to attachment to an inhibitory component (Ikb). In the presence of

oxidants I κ B dissociates from NF κ B, migrates to the nucleus and brings about the transcription of a large range of genes associated with the inflammatory process (Figure 4). Unfortunately the human immunodeficiency virus (HIV) has an NF κ B response element on its genome. Thus a deleterious consequence of the inflammatory response, in HIV + individuals, is increased viral replication and progression toward AIDS.

The fact that insulin resistance and disordering of lipid metabolism occur in obesity, diabetes mellitus, and during the inflammatory response has led to the investigation of the possibility that obesity exerts an inflammatory influence on

individuals. Large population studies show a strong association between indices of inflammation, abnormal lipid and carbohydrate metabolism, obesity and atherosclerosis. This association is particularly strong within populations with a high incidence of obesity, diabetes and cardiovascular disease, for example, Pima Indians and South Asians. TNF- α is produced, not only by cells of the immune system but also by adipocytes and may provide the link between inflammation, insulin sensitivity, and the diseases associated therewith (Figure 5). TNF- α results in insulin insensitivity, indirectly by stimulating stress hormone production and directly by sustained induction of SOCS-3, which has been shown to decrease insulin-induced insulin receptor substrate 1 (IRS1) tyrosine phosphorylation and its association with the p85, regulatory subunit of phosphatidylinositol-3 kinase; and by negative regulation of PPAR gamma, an important insulin-sensitizing nuclear receptor. Adipose tissue produces a wide range of cytokines including TNF- α and leptin. Production of the latter relates positively to adipose tissue mass and through its actions on immune function exerts a proinflammatory influence. It is unclear whether chronic inflammation is a trigger for chronic insulin insensitivity and conditions associated therewith, or whether the reverse is the case. Evidence at present favors the former interpretation of the data.

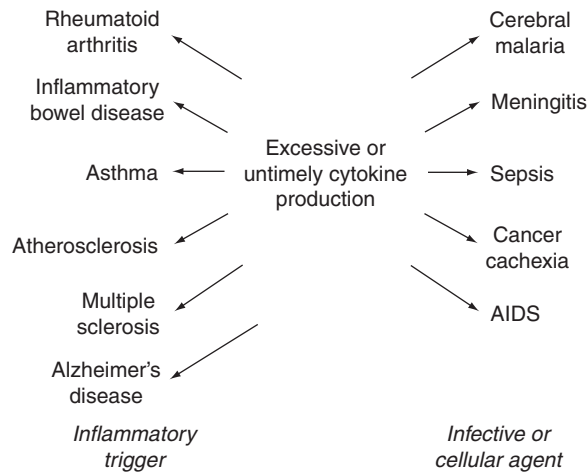


Figure 3 Adverse effects of cytokines in diseases and pathological processes.

Influence of Genetic Factors on Inflammatory Processes

Genetic factors play a part in the propensity of individuals to produce damaging or life-threatening amounts of cytokines

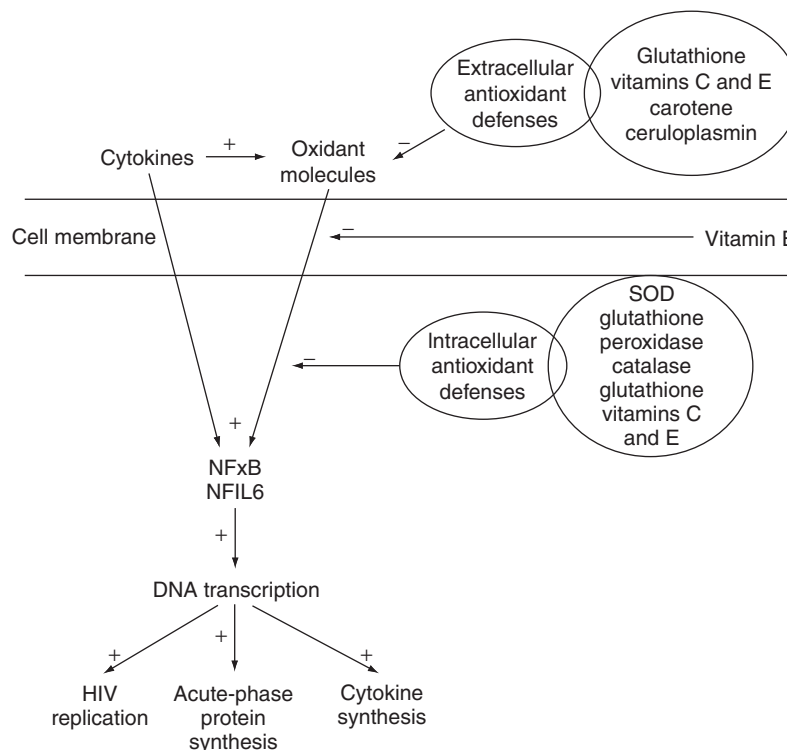


Figure 4 Role of antioxidants in influencing transcription factor activation.

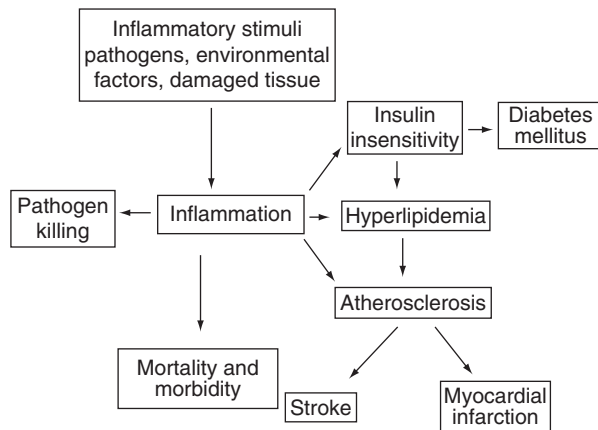


Figure 5 Beneficial and adverse effects of inflammation stimulated by cytokines during disease processes.

during inflammation. Males and postmenopausal females each possess a genetically determined propensity to produce high, medium, or low levels of cytokines in response to stimuli. Single base changes in the promoter regions of cytokine genes (single nucleotide polymorphisms (SNP)) result in these different levels of production. In the case of TNF- α , production of the cytokine is influenced by SNPs in the TNF- α (TNF2; TNF- α -308) and TNF- β (TNFB2; Lymphotoxin- α + 252) genes. Individuals with the TNF2 (– 308A) or TNFB2 (+ 252A) alleles produce higher amounts of TNF- α . In premenopausal women the capacity to produce cytokines is influenced by the hormones of the estrous cycle. Although the capacity for genetically determined levels of cytokines produces no apparent harm in healthy subjects, in disease genetics it has an impact on morbidity and mortality. Studies in the Gambia showed that subjects who were homozygous for the TNF2 (– 302A) allele had seven times the rate of death or serious neural symptoms than of subjects with one or no copy of the allele. Likewise, in patients with severe sepsis, possession of a TNFB22 (LT- α + 252AA) genotype resulted in 72% mortality in men compared with 42% mortality in men with a TNFB11 (LT- α + 252GG) genotype. Women were less severely affected by this genotypic influence. There is controversy about the reason for the retention of this lethal characteristic within the gene pool of the population. It is possible that rather as is the case with sickle cell anemia trait in heterozygotes the presence of the genetic characteristic gives an immunological advantage. Homozygotes who are less numerous than heterozygotes, pay the price for the advantageous retention of the genetic characteristic within the population.

Influence of Aging

In general, the metabolic and structural changes that occur as animals age and enter a state of senescence closely resemble those seen during long term inflammatory stress, for example, a loss of skeletal muscle, reduction in T lymphocyte function, decreased serum albumin concentrations, raised plasma acute phase proteins and, fasting plasma triacylglycerol concentrations. Furthermore it is interesting to note that in longevity studies an SNP which results in raised production of IL-6 is

rarer in the older than younger subjects. An SNP which results in raised IL-10 production is more common in nonagenarians than in younger subjects and conversely. Thus inflammation, although an essential component of the ability of the body to combat pathogens, is inimical with longevity.

Influence of Nutrients on Cytokine Biology

Proinflammatory cytokines exert widespread effects on metabolism, involving alterations in lipid, carbohydrate, and protein metabolism. In addition there are substantial changes in micronutrient metabolism. A number of intracellular signaling pathways are activated by the actions of cytokines on target cells; these include prostaglandins (PG) and leukotrienes (LT), cyclic AMP and protein kinase C (PKC). There are thus many levels at which nutrient intake can modify the intensity and characteristics of the response to inflammatory stimuli. The ability of nutrients to modify inflammation has been used in the treatment of diseases with an inflammatory basis. The interaction between nutritional status and inflammation is also important in public health by determining the effects of infection on growth and well-being of populations with a poor nutrient intake.

The earliest indications that nutritional status could affect cytokine biology came from studies on malnourished hospital patients. White blood cells from the patients had a reduced capacity to produce cytokines. The high mortality rates in these patients highlighted the importance of cytokines in the process of recovery from injury and infection. Protein supplements improved cytokine production and decreased the mortality rate. Because these observations were made a large number of studies have been conducted in animals and human volunteers which show that fats, amino acids, and micronutrients change the ability of mammals to produce and respond to IL-1, IL-6, and TNF (Figure 6). The figure shows whether a change in the intake of a nutrient, or nutrient status, alters cytokine production, or the response of target tissues to the actions of cytokines.

Influence of Fats on Cytokine Production and Effects

Dietary fats can be divided into four main types. Some are rich in *n*-6 polyunsaturated fatty acids (PUFAs); fats in this group include corn, sunflower, and safflower oils. Some are rich in *n*-3 PUFAs; these include fats from marine sources. Some are rich in monounsaturated fatty acids; these include olive oil and butter. Some fats are characterized by a high content of saturated fatty acids, usually accompanied by low concentrations of PUFAs; coconut oil, butter, suet, and lard fall into this category.

The production and actions of proinflammatory cytokines are profoundly influenced by dietary fat intake. There are a number of levels at which fats may modify cytokine biology. Most relate to the ability of fats to change the fatty acid composition of membrane phospholipids. Subsequently, membrane fluidity may be changed, the types and amounts of prostaglandins and leukotrienes produced during inflammation may be altered and the synthesis of a number of other

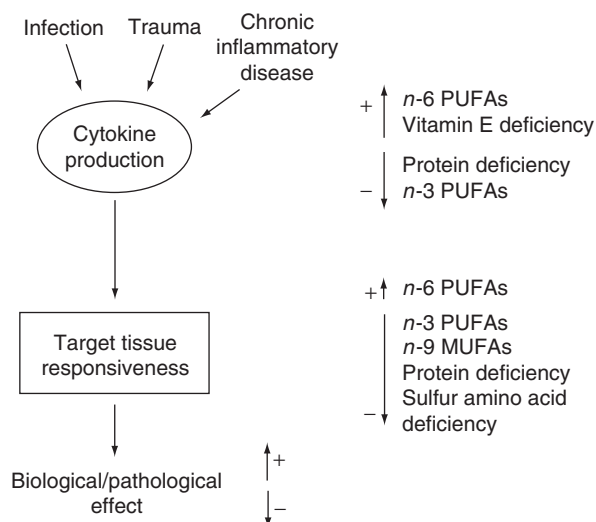


Figure 6 Summary of the influence of nutrients on cytokine production and actions following inflammatory stimuli.

cellular mediators which arise from phospholipids (platelet activating factor, diacylglycerol, ceramide) may also be changed. As a result of these changes the binding of cytokines to target tissues and the intensity of the inflammatory response may be altered.

The unsaturated fatty acids compete, with each other, for inclusion into the sn1 and sn2 positions of phospholipids, particularly the sn2 position which is acted upon by phospholipase A2. Normally arachidonic acid (AA C20:4 *n*-6) is released from this position and provides the parent compound for prostaglandins and leukotrienes. However the long-chain PUFA eicosapentaenoic acid (EPA C20:5 *n*-3) may compete with AA for insertion at sn2. Prostaglandins and leukotrienes with a lower bioactivity may result. This biological effect may account in part for the anti-inflammatory effects of fish oil. Many animal studies indicate that fats rich in *n*-6 PUFAs exert a proinflammatory influence whereas fats rich in monounsaturated fatty acids or *n*-3 PUFA have the opposite influence. In human studies however evidence for the influence of *n*-6 PUFA or monounsaturated fatty acids is not so clear cut. It has been postulated that the large increase in inflammatory disease that has occurred in the last 50 years, in industrialized countries, is owing to a major increase in the intake of *n*-6 PUFAs during that time (from approximately 5–7% of dietary energy). In a study on over 850 free-living US citizens it was found that plasma concentrations of TNF- α receptor subunits correlated positively with *n*-6 PUFA intake, and inversely with *n*-3 PUFA intake. It has also been postulated that the lower levels of inflammatory disease associated with the habitual consumption of a Mediterranean diet is due in part to high intakes of monounsaturated fatty acids. The evidence for *n*-3 PUFAs producing an anti-inflammatory effect in humans is however much stronger. Furthermore *n*-3 PUFAs have been shown to produce beneficial effects in inflammatory disease. In over 12 double-blind randomized controlled clinical trials, fish oil produced significant clinical benefit in patients with rheumatoid arthritis. A number of trials also report beneficial effects of fish oil in the treatment of Crohn's

disease. The precise mechanisms for these effects is unclear. A number of studies have demonstrated the ability of fish oil to reduce proinflammatory cytokine production and to alter the production of eicosanoids. However, recent studies have indicated a genomic influence on the ability of fish oil to reduce TNF production, thus indicating that fish oil may not be universally effective as an anti-inflammatory agent. In the GISSI trial fish oil supplements were shown to reduce the chance of stroke or a second myocardial infarct by 15%. As inflammation plays a role in atherosclerosis it is interesting to note that a trial of fish oil on patients with severe atherosclerosis showed that a period of seven weeks of receiving a supplement of 6 g d⁻¹ of fish oil significantly reduced macrophage activity in plaques.

Modulation of Cytokine Biology by Amino Acid and Protein Intake

Substantial increases occur in protein synthesis as the result of infection. It has been estimated that approximately 45 g protein is required to produce and maintain the increased quantities of white blood cells and acute phase proteins in an infected individual. This demand will have a considerable impact on the availability of amino acids for other processes in the body that involve protein synthesis. The inhibitory effect of infection upon growth, pregnancy, and lactation is well recognized. Output of amino acids from skeletal muscle, skin, and bone provide substrate for the synthesis of cells and proteins associated with the response to infection and trauma, as indicated earlier. However the supply may not always match demand, as is evident from the decrease in plasma concentrations of a number of amino acids. In particular, reductions occur in the concentrations of a metabolically related group of amino acids, including glycine, serine, and taurine. All three are metabolically related with the sulfur amino acids. Glycine and serine, together with the sulfur amino acids, are found in high concentrations in many compounds associated with the immune and inflammatory response, most notably comprising 66% of glutathione, 56% of metallothionein, and up to 25% of many acute-phase proteins. Experimental studies have shown that the production of cytokines, acute phase proteins, and glutathione is influenced by the adequacy of both protein and sulfur amino acid intake. The partitioning of cysteine into glutathione and proteins in the liver may change if dietary sulfur amino acid intake becomes inadequate. This phenomenon is owing to the biochemical properties of rate limiting enzymes in both pathways. Although the *K*_m for γ -glutamyl cysteine synthetase (rate limiting for GSH synthesis) is 0.35 mM that for amino acid activating enzymes (rate limiting for protein synthesis) is only 0.003 mM. This biochemical characteristic means that the GSH synthesis will fall below maximal rates at much higher intracellular cysteine concentrations than protein synthesis. Thus at low sulfur amino acid intakes antioxidant defences will become compromised. Low concentrations of GSH in tissues may have implications for the extent of inflammatory processes in the individual. In animal studies, decreased lung GSH concentrations are associated with accumulation of inflammatory cells in tissues. In studies on HIV patients given *N*-acetyl

cysteine, to improve GSH status, a decrease in plasma IL-6 concentrations has been noted indicating a reduction in inflammation. In view of the effects of NFkB activation on HIV replication it is interesting to note that the drug also brought about a reduction in HIV mRNA levels.

Modulation of Cytokine Biology by Micronutrients

Micronutrients play widespread and complex roles in the response to infection and trauma. They are incorporated into substances that are synthesized in increased amounts during the response and into components of antioxidant defense, and also modulate immune function. Trace elements are present in several acute-phase proteins and enzymes associated with antioxidant defense (Figure 4). These proteins include metallothionein (Zn), caeruloplasmin (Cu), superoxide dismutases (Mn, Cu, Zn), and glutathione peroxidase (Se). Deficiencies in copper impair the ability of rats to increase superoxide dismutase and caeruloplasmin activities in response to inflammatory agents. Deficiencies in zinc impair the ability to increase metallothionein synthesis; furthermore, zinc deficiency has potent suppressive effects upon lymphocyte proliferation. Iron status may influence inflammation and immune function in a number of ways. Normally iron is tightly bound to transport proteins such as transferrin and ferritin. However, following tissue damage and infections, such as malaria which may destroy red blood cells, free iron may be released and exert a proinflammatory effect by catalyzing free radical production. The latter effect may activate NFkB and up-regulate cytokine production. Indeed, iron dextran infusion has been shown to exacerbate inflammatory symptoms in rheumatoid arthritis. Desferrioximine, an iron chelator, suppresses TNF and IL-1 production by rodent macrophages. Iron deficiency also decreases the ability of such cells to produce cytokines. Impairment of immunological defense is commonly found in iron-deficient animals and human populations including defects in T cell proliferation and in the ability of macrophages to engulf and kill bacteria. The latter defect may relate to the role of iron as part of the NADPH oxidase complex that is responsible for the respiratory burst and generation of hydroxyl radicals that kill bacteria. Myeloperoxidase activity which generates hypochlorous acid for bacterial killing is also a haemoprotein which is decreased in activity by iron deficiency.

Vitamins also exert a number of effects upon cytokine biology. These effects may relate to the roles which some of these nutrients play as antioxidants and growth factors (see Figure 4). Rats deficient in vitamin E exhibit an enhanced inflammatory response to endotoxin; addition of the vitamin to the diet will suppress this effect. In healthy subjects and smokers a daily dose of 600 IU of vitamin E, for 4 weeks, reduced the ability of white blood cells to produce TNF and IL-1. Cigarette smoking enhances cytokine production and raises acute-phase protein concentrations. The extent of the elevation is inversely related to vitamin E status. Strenuous exercise results in a small increase in plasma concentrations of IL-1 and IL-6; vitamin E supplementation will prevent this effect.

Vitamin A status also influences cytokine production, although the mechanism underlying the effect is unclear. Macrophages taken from Indian children who had received a supplement of 100 000 IU of retinol produced seven times the quantity of IL-1 produced by cells of children who had not received supplementation. The effect may be more pharmacological than nutritional in nature. Mice given vitamin A, at a dose that was 16 times their requirement, had macrophages which produced twice as much IL-1 upon stimulation than cells from unsupplemented animals.

Hormone-like properties have been attributed to vitamin D in relation to its effects on calcium. It is now apparent that endocrine effects of the vitamin extend to immune function. Macrophages treated with 1,25-dihydroxyvitamin D₃ produce increased amounts of TNF and were more effective at killing *Mycobacterium avium* than untreated cells.

Vitamin B₆ supplementation has been found to increase lymphocyte proliferation and production of IL-2 in elderly subjects. In the National Health and Nutrition Examination Survey (NHANES) study, on over 2600 subjects, vitamin B₆ intake was found to be inversely related to plasma C-reactive protein concentrations indicating an anti-inflammatory role for the vitamin. Little is known of the effects of other water soluble vitamins on cytokine biology. Although no clear effects of vitamin C status on proinflammatory cytokine production have been reported, doses of the vitamin reduce the incidence of respiratory infections in ultra marathon runners. In a study on cystic fibrosis patients a negative correlation was found between markers of inflammation and plasma vitamin C. Whether this finding indicates that inflammation depletes plasma vitamin C concentrations or that vitamin C has an anti-inflammatory role is unclear.

Conclusions

The objective of the response of the body to infection and trauma is to disadvantage and destroy invading organisms, but at the same time to protect healthy tissues from the damaging influence of compounds produced during the response. Cytokines play a central role in the protection of the animal from damage during the response. The close interrelationship between proinflammatory cytokines, oxidant molecules, and antioxidant defences gives a biological advantage to the host (Figure 7).

The essence of survival of an individual or species lies in the ability to prioritize physiological processes, particularly those processes which exert a large metabolic demand. Thus at various times throughout the life cycle mammals will focus metabolic processes upon achieving growth, the construction of placenta and fetus, the synthesis of milk components, or the repulsion of invasion by pathogens. For the infected individual, the marshaling of resources to combat the invading pathogen must assume a priority over all other physiological events. These other physiological processes can continue once the invasion has been repulsed and the damage done by the invader repaired.

The production of cytokines and other molecules associated with the inflammatory process carries risks of damage to the host as well as a survival advantage. The risk to the host is

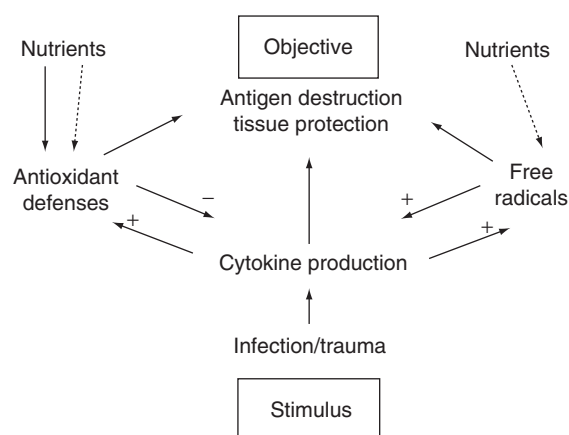


Figure 7 Interactions between nutrients, anti-oxidant defences, oxidant stress and cytokine production in disease.

minimized by a sophisticated range of feedback control systems and synthesis of substances which protect the host. As discussed above, nutrient intake modulates cytokine biology and the control and protective systems. A wide range of nutrients modulate cytokine biology at the level of production and sensitivity of target tissues (see [Figure 6](#)). As a consequence of the modulation, the extent of depletion of nutrient stores and the risk of damage during the inflammatory response will be changed. The extent of tissue depletion and risk to the host will thus range from mild and transient in nature, to severe, chronic, or lethal in effect.

See also: Adipose Tissue: Structure, Function and Metabolism. Aging. Amino Acids: Metabolism. Antioxidants. Dietary Modulation of Inflammation. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids. Hyperlipidemia: Overview. Nutrition and HIV/AIDS. Nutrient–Gene Interactions: Health Implications. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Parasitism

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Edited by
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Encyclopedia of Human Nutrition

Third Edition



ENCYCLOPEDIA OF HUMAN NUTRITION

THIRD EDITION

ENCYCLOPEDIA OF HUMAN NUTRITION

THIRD EDITION

EDITOR-IN-CHIEF

Benjamin Caballero

Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

VOLUME 2



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Dr. Caballero is a recognized expert on the nutritional needs of children and adults, and on nutrient requirements in undernourished populations. For the past 10 years, he has focused on the problem of childhood obesity in the US and in developing countries, and explored the impact of dietary transition and globalization on health indicators. He is an active participant in key scientific committees advising the US government on issues of diet and health, including the Dietary Reference Intakes (DRI) Committee; the Expert Panel on Macronutrient Requirements; and the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences. He was a member of the Dietary Guidelines for Americans Advisory Committee, and is currently a member of the Scientific Advisory Board of the Food and Drug Administration (FDA) and of the International Life Sciences Institute (ILSI).

Dr. Caballero is an active leader in the area of global health, specifically on diet, lifestyle, and disease risk. He is Chairman of the Board of the Pan American Health and Education Foundation, in Washington, DC, USA and member of the Board of Directors of the International Nutrition Foundation, Boston, MA, USA. Recent awards include the Ancel Keys Prize for achievements in international public health and the Thompson–Beaudette Lectureship from Rutgers University. In 2011, he was named to the Spanish Academy of Nutritional Sciences.

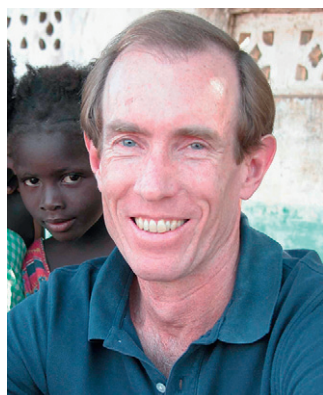
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Andrew Prentice studied biochemistry before a PhD in Nutritional Science at Darwin College, University of Cambridge. Animal and laboratory work for his thesis on 'The Biochemical Effects of Riboflavin Deficiency' was conducted at the Dunn Nutrition Unit. His early post-graduate studies were conducted in The Gambia with a focus on malnutrition in mothers and babies. He subsequently headed the Regulation of Human Energy Metabolism Group at the Dunn Clinical Nutrition Centre, Addenbrookes Hospital, Cambridge, UK, conducting detailed metabolic studies using whole-body calorimetry and stable isotopic tracer methods. In 1999, Professor Prentice established the MRC International Nutrition Group at the London School of Hygiene & Tropical Medicine inheriting the permanent field laboratories at MRC Keneba in rural Gambia. His team now researches key areas of diet-disease interactions in The Gambia, Tanzania, and Kenya, with the ultimate aim of improving nutritional interventions in poor populations.

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PREFACE

By the turn of the twentieth century, nutrition science had completed a slow but remarkable historical transition, from a discipline focused on preventing nutrient deficiencies (and hence focused on identifying minimum nutrient needs) to one aimed at reducing disease risk and optimizing health, seeking to define an elusive optimal diet. But progress in our knowledge has not yet caught-up with that transition in focus, and our understanding of how diet constituents affect long-term disease risks is still not on a par with our knowledge of essential nutrients, their metabolism, and required intake levels. One reason is that the experiments needed to unravel diet–health interrelationships are more complex, costly, and in some cases unfeasible, compared with the classical studies that identified vitamins and other essential nutrients. Another reason is that, although the discovery of essential nutrients was based on a strong, unifying scientific paradigm (the concept of a compound essential for human life but which humans are unable to make), there is no single or unifying paradigm from which to explore diet–health relationships. In addition, our ability to timely process and integrate scientific discoveries is now continuously challenged by the massive volume of information of the digital era.

In that context, the need to provide accurate, succinct, and up-to-date information on a wide range of topics is more important than ever, and is the aim of this Encyclopedia. Currently, nutrition research and practice is fundamentally a multidisciplinary endeavor, so we aim to offer scientific information to a wide audience of researchers and professionals. In addition, the information revolution of the internet has

changed the consumer from a passive recipient of advice to an active participant in decisions involving health and related issues. Thus, although this work is not specifically targeted to the general public, we hope that the educated readers with a minimum scientific background should also be able to obtain from this book useful (and reliable) information on their topic of interest.

This third edition builds on the success of the previous one. We have included new articles or made extensive updates when needed, while keeping the proven core of established knowledge. The comprehensive index and extensive cross-referencing will allow readers to quickly identify specific topics, and to move deeper into related areas if desired.

We have a great debt of gratitude to the hundreds of authors who contributed to the large body of knowledge represented here. In turn, authors benefited from the valuable feedback of our distinguished Editorial Advisory Board. Of course, as editors we are ultimately responsible for the content, particularly for any errors. Finally, both the print and electronic version have the unmistakable production quality of the Major Reference Works division of Elsevier, and this is the result of the unrelenting enthusiasm and hard work of our editorial team.

We hope this work will be a valuable addition to the knowledge base of any person interested in the critical area of nutrition, diet, and human health.

Benjamin Caballero
Editor-in-Chief

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GUIDE TO USE OF THE ENCYCLOPEDIA

Structure of the Encyclopedia

The Encyclopedia is arranged as a series of entries in alphabetical order. Some entries comprise a single article, whilst entries on more diverse subjects consist of several articles that deal with various aspects of the topic. In the latter case the articles are arranged in a logical sequence within an entry.

To help you realize the full potential of the material in the Encyclopedia we have provided three features to help you find the topic of your choice.

Contents Lists

Your first point of reference will probably be the contents list. The complete contents list appearing in each volume will provide you with the volume number and page number of the entry. On the opening page of an entry a mini-contents list is provided so that the full details of the articles within the entry are immediately available.

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All of the articles in the Encyclopedia have been cross referenced. The cross references, appear at the end the articles and they link together related articles.

Example

The following list of cross references appear at the end of the entry entitled Nutritional Assessment: Clinical examination (00199).

See also: Dietary Intake Measurement: Methodology (00075). Energy Expenditure: Doubly Labeled Water (00093); Indirect Calorimetry (00092). Nutritional Assessment: Anthropometry (00197); Biochemical Indices (00198)

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DEHYDRATION

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Glossary

Hypertonic Increased concentration of solutes (most typically sodium) in the blood.

Hypo/hyperkalemia Low/high plasma potassium concentration.

Hypo/hyponatremia Low/high plasma sodium concentration.

Hypovolemia Reduced blood cardiac output, usually resulting from dehydration.

Osmolality A measure of solute content in the blood. Dehydration tends to raise blood osmolality.

An adequate water content is essential to maintain cellular homeostasis. Since there is a continuous turnover of body water, dehydration occurs when the water output (losses) is higher than the input (intake). Water is lost from the body primarily in urine, but also in feces, sweat, and evaporation from exhaled air. Dehydration most commonly occurs secondary to excess losses due to gastrointestinal illness (e.g., diarrhea) or impaired kidney function. It can also develop as a side effect of drugs, such as diuretics. Dehydration alters circulatory hemodynamics, impairs the heat transfer and thus may affect the core body temperature, and results in electrolyte and acid–base imbalances. Management of dehydration depends on the severity and on concurrent medical conditions. In mild-to-moderate cases, oral rehydration with adequate fluid solutions may be sufficient. In severe cases, or when gastrointestinal function is severely impaired, intravenous fluids may be required, along with appropriate electrolyte replacement.

Physiological Functions of Water

After oxygen, water is the most essential nutrient needed to sustain human life. In healthy individuals, water comprises between 45% and 70% of total body weight and is responsible for connecting the diverse physiological functions of the body (Table 1).

Water is necessary to maintain homeostasis of the internal environment. The most obvious roles of water in the human body are to provide an aqueous medium for transport of material in blood, to dissolve and pass nutrients between blood and cells, to serve as a medium for intracellular reactions, and to transfer metabolic products for redistribution or excretion via urine. Since both the quantity of reactants and the volume of fluid in which they are dissolved influence chemical reaction rates, imbalances in hydration status can alter cellular and tissue function.

Dehydration also adversely affects the body's ability to regulate temperature. Energy transformations during digestion, absorption, and metabolism as well as muscular

Table 1 Major physiological functions of water

Function	Example
Waste product removal	Urea excretion by kidneys
Solvent for chemical reactions	Glycolysis in the cell cytosol
Transport medium	Blood
Lubrication	Synovial fluid of joints
Shock absorber	Disks between vertebrae of spinal column
Temperature regulation	Evaporative sweat loss

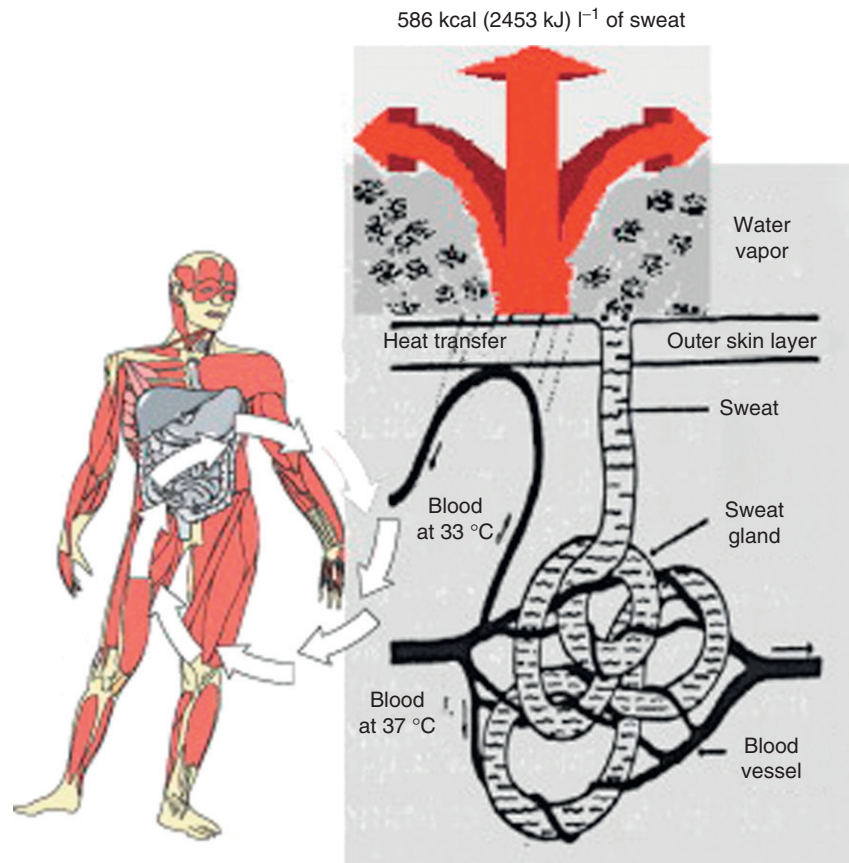


Figure 1 Metabolic heat transfer to the skin and dissipation of heat by evaporation of sweat. The body has more than 2 million sweat glands that secrete sweat to the surface of the skin. Blood-perfusing skin capillary beds transfer heat by convection to the surface of the skin. Heat is dissipated by vaporizing the water in sweat. The heat of vaporization of water at 20 °C is 586 kcal l⁻¹ (2453 kJ).

contraction generate heat. The heat released from the digestion of a mixed meal (thermic effect of food) equals 10–15% of the caloric content of the food ingested. Muscular contraction is dependent on the transformation of chemical energy (adenosine triphosphate, ATP) to mechanical energy. Nearly three-quarters of the energy used for muscular contraction is released as heat. Unless localized heat production from metabolism and muscular contraction is dissipated, the heat burden can be structurally damaging to enzymes or other proteins. Water absorbs heat produced at the cellular level and transfers it to the surface of the skin, where it can be dissipated to the external environment (**Figure 1**).

The evaporative dissipation of heat through sweating is a two-phase, water-dependent mechanism. Water is removed from capillary blood perfusing sweat glands to produce a thin layer of sweat over the surface of the skin. Simultaneously, the water component of blood carries heat produced from cellular metabolic processes to capillary beds located near the surface of the skin. Heat is transferred by conduction to the skin surface, where it vaporizes sweat coating the skin, thus transferring body heat to the external environment. The heat of vaporization of water is 586 kcal l⁻¹ (2453 kJ l⁻¹) at 20 °C. Approximately 500 ml of sweat is lost per day under average ambient environmental conditions. Such obligatory water loss

occurs without visible or tactile sensations and is termed 'insensible' sweat. However, given a sufficient thermal challenge, humans are capable of producing approximately 10 l of 'sensible' sweat per day. Theoretically, if the entire 10 l of sweat was evaporated, more than 5000 kcal (20 930 kJ) of heat per day would be dissipated via the sweating mechanism. Humidity of the air and sweat that drips from the surface of the skin considerably reduce the potential for evaporative heat dissipation; therefore, actual evaporative cooling is usually less than the theoretical maximum. As water is the main component of sweat, it is not surprising that dehydration affects the sweat response. The relationship between body water loss by dehydration and the rate of sweating achievable during exercise is shown in **Figure 2**, which illustrates that dehydration reduces sweating rate at any given level of thermal drive (core temperature) during exercise. A diminished sweating response can lead to a dangerous heat buildup unless thermal strain is curtailed by other mechanisms.

Development of Dehydration

In physiological terms, dehydration is the process of progressing from the euhydrated (normally hydrated) to

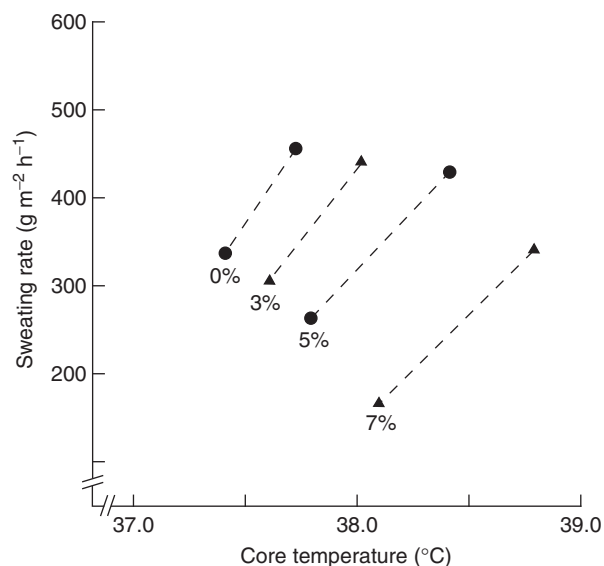


Figure 2 The influence of water loss by dehydration (hypohydration) on the sweating response to exercise following normal hydration (0%) and dehydration equal to 3%, 5%, and 7% of the body weight. The primary stimulus for sweating is the increase in core temperature (thermal drive). Note that dehydration reduces the sweating rate at any given level of thermal drive. Hypohydration compromises exercise by reducing sweat rate and evaporative cooling and increasing body core temperature. Reproduced from Sawka MN, Young AJ, Francesconi RP, *et al.* (1985) Thermoregulatory and blood responses during exercise at graded hypohydration levels. *Journal of Applied Physiology* 59: 1394–1401, with permission from APS.

hypohydrated (less water than normal) state. In actual usage, dehydration means losing body water faster than it is replaced. The resultant condition is commonly referred to as the 'dehydrated state' and is associated with hypovolemia (low blood volume).

Contributing Factors

Water is lost through a variety of avenues, including urine, feces, breath, and sweat. In illness or disease, excessive diuresis, diarrhea, and/or vomiting are the main pathways of water loss. During exercise or heat exposure, sweating is the primary mechanism for dehydration. Significant water loss may be stimulated by cold- or altitude-induced diuresis. Additionally, some prescription drugs and over-the-counter herbal products have diuretic effects that exacerbate water loss. Under normal conditions, the body regulates its water contents tightly over a 24-h period (approximately ± 200 ml); however, over short periods, water loss can significantly exceed water gain (Figure 3).

Body Fluid Balance

Body water losses are rapidly reflected in blood. Volume and electrolyte changes in response to decreased blood water content (increased osmolality) trigger the hypothalamus to stimulate antidiuretic hormone (ADH) release from the posterior lobe of the pituitary gland. ADH acts on the kidney

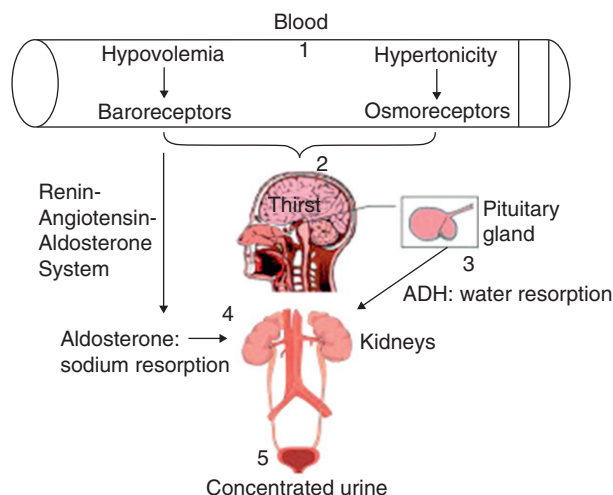


Figure 3 Water and sodium physiology: mechanisms controlling body water gain and loss. As water is lost from the body via sweat, urine, respiration, and feces: (1) Plasma osmolality increases and plasma volume decreases with water loss. (2) The increase in osmolality acts on the 'thirst center' in the hypothalamus to secrete ADH and stimulates the conscious desire for water. (3) The release of ADH from the pituitary gland increases tubular resorption of water by the kidney. (4) Aldosterone is formed via a series of reactions involving renin, which is released from the adrenal cortex in response to decreased blood pressure, and a plasma protein, angiotensinogen. Aldosterone promotes sodium resorption by the kidney to maintain plasma volume. (5) These events conserve water and result in the production of concentrated urine.

to increase tubular water resorption and maintain plasma volume. Decreased plasma volume also results in a complex series of events resulting in the release of renin from the kidneys and the subsequent formation of angiotensin II and the mineralocorticoid, aldosterone. Angiotensin II is a potent vasoconstrictor and stimulator of thirst. Aldosterone promotes sodium resorption, which allows the blood to retain more water. The net result of these regulatory mechanisms is concentrated urine and maintenance of the plasma volume, provided that exogenous fluid intake increases proportionally. If fluid intake is not increased, dehydration will still result.

Thirst

Thirst is not a good short-term regulator of fluid balance. Humans frequently lose up to 2% of their body weight as water before the thirst mechanism is activated. The actual point at which the thirst mechanism is activated varies considerably between individuals. Some athletes are closely attuned to their anticipated fluid needs and develop the habit of drinking before they become dehydrated. However, the majority of individuals do not feel compelled to drink until they have become moderately dehydrated, even though fluids may be available. These individuals are called 'voluntary dehydrators.' Voluntary dehydrators frequently replace only approximately two-thirds of their short-term fluid losses.

Pathophysiology of Dehydration

Dehydration and Human Performance

Natives of desert regions have, over the years, become habituated to being chronically dehydrated. A study of the desert inhabitants found that they had a curtailed thirst drive that was associated with the excretion of low volumes of concentrated urine and a high incidence of kidney disease (kidney stones). When additional water intake (approximately twice normal) was ingested in a subsample of this population, they were able to exercise 10% longer in the desert environment, presumably due to improved thermoregulation. The results of this and other studies illustrate that humans probably do not adapt to dehydration but can become used to a mild chronic dehydration due to inadequate fluid intake. This is not a true physiological adaptation since there are negative health and performance effects associated with chronic dehydration.

Body Water Deficits

When fluid intakes are insufficient to maintain the normal body water content (approximately 60% for males and 50–55% for females), deficits arise in all fluid compartments, with the reduction in plasma volume being of particular concern. Dehydration decreases plasma volume and increases tonicity. Plasma hypertonicity signals the circulatory system to conserve plasma volume for internal organs at the expense of skin blood flow. Reduction in skin blood flow decreases evaporative cooling. Additionally, decreased plasma volume reduces stroke volume and cardiac output, which impairs cooling capacity and exercise performance. The effects of dehydration on the heart rate, body temperature, and endurance are shown in Figure 4. Consuming water to replace sweat loss while cycling for 6 h at 55% $\dot{V}O_2$ max in the heat was associated with lower heart rates and core temperatures compared to a trial in which no water was ingested. The increase in heart rate while cycling without water replacement is a compensatory mechanism to maintain cardiac output in response to reduced plasma volume. Elevated core temperatures in cyclists not consuming water resulted from reduced skin blood flow and ultimately forced the cessation of exercise.

Dehydration and Heat Illness

If water loss due to sweating is not replaced during exercise, plasma volume and sweat rate will be decreased (Figure 2). The combination of reduced peripheral blood flow for heat exchange and reduced sweat volume for evaporative cooling leads to an overall reduction in the ability to dissipate heat.

The consequence of impaired heat dissipation is hyperthermia. Without evaporative cooling, human core temperatures can elevate 5°C h^{-1} during moderate intensity work. The heat production is proportional to the intensity and duration of work, ranging from 75 kcal h^{-1} (314 kJ) at rest to 300 kcal h^{-1} (1256 kJ) during moderate exercise and 600 kcal h^{-1} (2512 kJ) for maximal sustained work. Brief periods of intense exercise can generate heat at the rate of 900 kcal h^{-1} (3768 kJ).

Hyperthermia can lead to serious or even life-threatening heat injury if left unchecked. Heat injury can result if the rate of

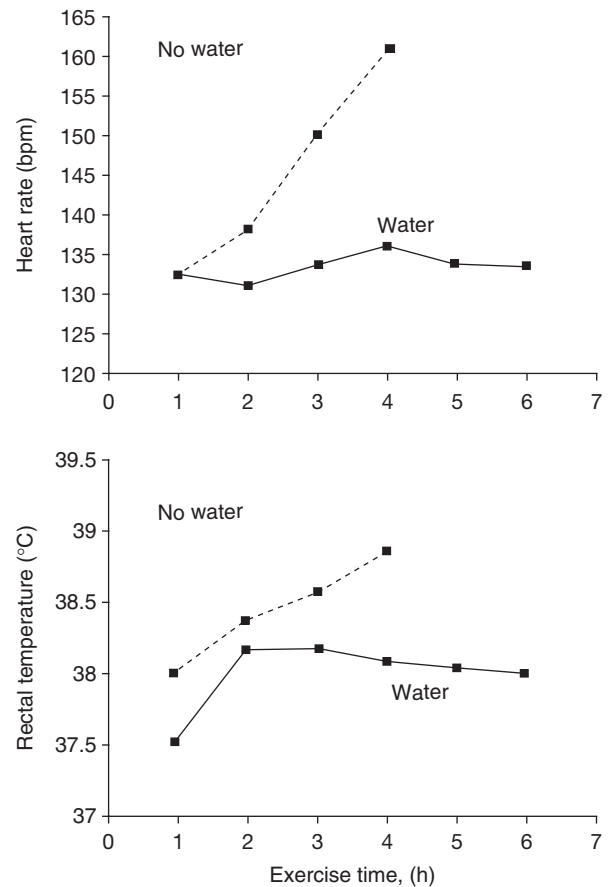


Figure 4 Consequences of consuming or not consuming water during cycle ergometer work (50% $\dot{V}O_2$ max, 30°C , 50% relative humidity). Subjects drank water to replace that lost during exercise (water group) or did not drink during exercise (no water group). Note that the no water group could not exercise as long as the water group. Adapted from Barr SI, Costill DL and Fink WJ (1991) Fluid replacement during prolonged exercise: Effects of water, saline, or no fluid. *Medicine and Science in Sports and Exercise* 23: 811–817, with permission from LWW.

heat production is greater than the rate of cooling. When fluid losses are not replaced during activity, heat dissipation mechanisms are compromised. The buildup of heat in blood and tissues adversely affects various physiological systems. Minor heat injury syndromes include prickly heat (skin rash resulting from plugged sweat glands), heat syncope (light headedness due to pooling of blood in the extremities), and heat cramps (muscle cramps related to electrolyte loss). These heat illnesses are of concern but not life-threatening. Major hyperthermia syndromes involving dehydration are heat exhaustion and heat stroke. Conversely, overhydration (replacing large volumes of fluid loss) without sodium provision can, in certain instances, lead to an overdilution of blood sodium (hyponatremia). The symptoms of hyponatremia are similar to those of other forms of heat illness, but the treatment is critically different.

Heat Exhaustion

Two types of heat exhaustion can be distinguished: water depletion (inadequate consumption of water) and salt depletion

(loss of large volumes of sweat that are replaced without adequate sodium intake). Heat exhaustion usually occurs on a continuum somewhere between these two extremes. During heat exhaustion, thermoregulatory mechanisms cannot dissipate heat effectively, primarily because of reduced skin blood flow. People who are unacclimatized to the heat or not in good physical condition are more susceptible to heat exhaustion. Symptoms vary but usually include a temperature of less than 39.5 °C, malaise, weakness, fatigue, headaches, anorexia, nausea, vomiting, diarrhea, and muscle cramps. Although irritability, anxiety, and impaired judgment may be present, the

subject is usually alert and capable of responding to questions. If left untreated, heat exhaustion can progress to heat stroke.

Heat Stroke

Heat stroke is less common than heat exhaustion but is much more serious. Heat stroke is a life-threatening disorder that requires immediate medical treatment. Two forms of heat stroke are generally classified as exertional or classical. Exertional heat stroke generally occurs in young subjects working too hard for too long in the heat. Classical heat stroke is associated with environmental heat waves and primarily afflicts the very young, very old, poor, and debilitated. The pathophysiology of heat stroke involves failure of the body's thermoregulatory mechanisms following a severe heat overload. As core temperature elevates, cell function deteriorates culminating in massive cell damage. Dehydration is often a contributing factor to heat stroke, but the basic pathophysiology is uncontrolled heat overload. Core temperature is higher than that seen in heat exhaustion, generally 41 or 42 °C. Core temperatures higher than 39.5 °C reduce the function of motor centers in the brain and subsequently the ability to recruit motor units required for muscular activity. Exertional heat stroke is characterized by cessation of sweating, hot and dry skin, physical deterioration, confusion, collapse, and seizure. Rhabdomyolysis (muscle fiber destruction) may result from exertional heat stroke. In one reported case, an accelerated rhabdomyolysis resulting from exertional heat stroke occurred during an 8-km fun run when the ambient temperature was higher than 37 °C. This unfortunate runner collapsed with a rectal temperature of 42 °C and suffered acute renal failure as a consequence of an impaired immune system, infection, and decreased clotting ability. He eventually recovered, but it was necessary to amputate one of his legs that became infected following the rhabdomyolysis.

Other Consequences of Dehydration

There are other physiological consequences of dehydration that are not as serious as heat illness but can contribute to decreased performance capacity. Dehydration impairs thermoregulation in both hot and cold environments. The metabolic heat production in the cold may be less efficient in

Table 2 Types of dehydration

Mild hypovolemia

Fluid intake is insufficient to meet needs, 2–5% body weight loss, yellow urine, dry lips, reduced skin elasticity

Many people are chronically hypovolemic in outdoor environments
Simply need to be reminded to drink, easily corrected by fluids and consuming food

Hypertonic dehydration (hypernatremic dehydration)

Body water losses greater than sodium losses, elevated blood osmolality and hypernatremia

May be accompanied by fever, profuse sweating, and/or evaporative water loss

Acute weight loss; person eats but does not drink

Treatment is provided by additional fluids (water is best)

Isotonic dehydration

Body loses equal amounts of water and sodium from routes other than sweating

Gastrointestinal fluid loss – vomiting, diarrhea

Blood electrolytes normal

Acute weight loss, tachycardia, orthostatic hypotension

Treat by replacing lost fluid and electrolytes or hypotonic dehydration may develop

Hypotonic dehydration (dilutional hyponatremia)

Can develop when isotonic dehydration is treated with only water

Hypotonic dehydration can occur anytime the body sodium loss exceeds water loss (e.g., sodium-restricted diets, diuretic use, overzealous hydrators, fluid replacement with only water following repeated vomiting and diarrhea)

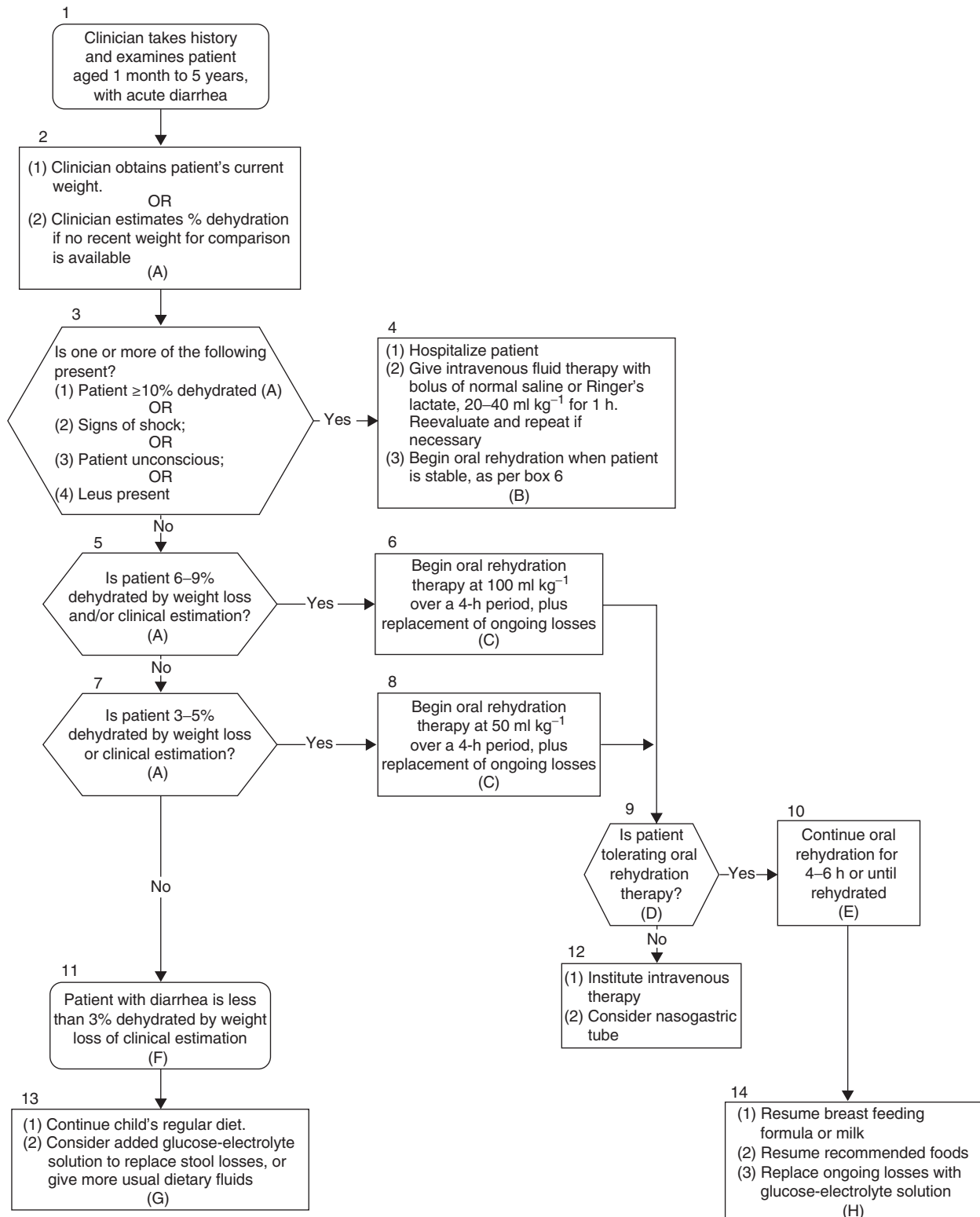
Table 3 Guidelines for the assessment of dehydration^a

Variable	Dehydration		
	Mild, 3–5%	Moderate, 6–9%	Severe, > 9%
Blood pressure	Normal	Normal	Normal to reduced
Quality of pulses	Normal	Normal or slightly decreased	Moderately decreased
Heart rate	Normal	Increased	Increased, severe cases bradycardia
Skin turgor	Normal	Decreased	Decreased
Fontanel	Normal	Sunken	Sunken
Mucous membranes	Slightly dry	Dry	Dry
Eyes	Normal	Sunken orbits	Deeply sunken orbits
Extremities	Warm, normal capillary refill	Delayed capillary refill	Cool, mottled
Mental status	Normal	Normal to listless	Normal to lethargic or comatose
Urine output	Slightly decreased	< 1 ml kg ⁻¹ h ⁻¹	<< 1 ml kg ⁻¹ h ⁻¹
Thirst	Slightly increased	Moderately increased	Very thirsty or too lethargic to indicate

^aThe percentages of body weight loss and their corresponding categorization sometimes vary depending on the author.

dehydrated individuals. The mechanism is not well understood, but it may involve a concomitant reduction in energy intake, decrease in resting metabolic rate, impaired shivering

response, impaired vasodilation/constriction response, or a combination of these factors. Dehydration also blunts appetite, which in turn may elicit energy and thermoregulatory defects.



Management of Dehydration

Identifying Types of Dehydration

Although prevention is the best management strategy for dehydration, water imbalances may also be treated after the recognition of dehydration by replacement of appropriate fluids, such as water or water containing electrolytes. Dehydration usually occurs along a continuum of fluid and electrolyte loss. The ratio of water to electrolyte loss determines the type of dehydration present. A convenient classification of types of dehydration, their characteristics, and how to treat them is shown in [Table 2](#).

It should be noted that hypotonic dehydration is the result of treating isotonic dehydration with nonelectrolyte-containing fluids and can lead to a potentially dangerous condition known as hyponatraemia (low blood sodium). This may be a particular problem in the case of 'overzealous hydrators' such as athletes who overcompensate for sweat losses. Hypotonic dehydration is also seen in infants and children who may be afflicted with gastrointestinal disturbances such as diarrhea, stomach flu, or acute gastroenteritis if water alone is used to hydrate. The American Academy of Pediatrics has published guidelines for oral rehydration therapy of infants and children younger than 5 years old with acute gastroenteritis. [Table 3](#) gives the Academy's guidelines for the assessment of dehydration. An algorithm for the treatment and hydration of children with acute diarrhea is shown in [Figure 5](#).

Clinical standards for assessing dehydration in adults have not been well established. Measurements of plasma osmolality and urine-specific gravity and osmolality can be used to assess the relative dehydration if baseline (euhydrated) values are known. However, due to significant interindividual variation, the use of absolute specific gravity and osmolality values for the diagnosis of dehydration remains questionable. Monitoring the urine color has been suggested as a non-invasive way to measure hydration status, where light, pale yellow urine generally indicates a favorable hydration status. Assessing urine color may be a simple method to assess hydration status, but it can also be artificially influenced by dietary intake (i.e., nutritional supplements). An acute change

in body weight is the most common and practical method used to assess hydration status. It is assumed that the short-term body weight loss is primarily the result of water loss.

Treating Different Types of Dehydration

In the majority of simple, nonsevere dehydration cases, plain water is an adequate rehydration solution. However, there are instances (e.g., children younger than 5 years of age dehydrated by vomiting and diarrhea) when water containing sodium and potassium is the proper hydrating agent. The most effective way of preventing and treating mild to moderate dehydration in infants and children with acute diarrhea is the oral administration of oral rehydration solutions (ORSs). There are a number of commercially available ORSs. These solutions are designed to replace fluid and electrolytes when both the water and food intake have been restricted or compromised by diarrheal disease. The World Health Organization recommends the ORS shown in [Table 4](#) for individuals afflicted with diarrheal disease and vomiting. Oral modes of fluid and electrolyte administration are always preferred in mild (3–5%) to moderate (6–9%) dehydration; however, intravenous fluids may be required in cases of severe dehydration (>9%) and vomiting or if the patient is in a comatose state. When i.v. fluids are administered, 0.45% saline with 5% dextrose is an effective hydrating agent.

In most instances involving heavy sweating, plain water containing 1.25 g of NaCl per liter is a suitable rehydration solution. Increasing the concentration of NaCl to 5 or 6 g l⁻¹

Table 4 Composition of recommended WHO/UNICEF oral rehydration solution

<i>Solute</i>	<i>Content (mmol l⁻¹)</i>
Glucose	75
Sodium	75
Chloride	65
Potassium	20
Citrate	10
Total osmolality	245

Figure 5 Algorithm for children with dehydration from acute diarrheal disease. The letters at the bottom of the decision boxes refer to the following: (a) See [Table 3](#) for guidance in the assessment of the degree of dehydration. (b) Restoration of cardiovascular stability is critical and is accomplished by giving bolus i.v. therapy. In the patient who does not respond, consider the possibility of an underlying disorder. When the patient is in a stable condition and has achieved satisfactory mental status, oral rehydration therapy (ORT) can be implemented. (c) Solutions containing 45–90 mmol l⁻¹ sodium should be given in a volume of 100 ml kg⁻¹ for moderate dehydration and 50 ml kg⁻¹ for mild dehydration. Giving the child these volumes requires patience and persistence, and progress must be monitored frequently. (d) Intractable, severe vomiting, unconsciousness, and ileus are contraindications to ORT. Persistent refusal to drink may require a trial of i.v. therapy. (e) The rehydration phase usually can be completed in 4 h; reevaluation should occur every 1 or 2 h. See referenced text for guidance to decide when rehydration has been achieved. (f) The type and intensity of therapy will vary with the individual clinical situation. (g) Often, a child has diarrhea but remains adequately hydrated. The parent can be reassured but should be taught to assess hydration and to identify a worsening condition. If the stool output remains modest, ORT may not be required if early, age-appropriate feeding is instituted and increased consumption of usual dietary fluids is encouraged. More significant stool losses can be replaced with an oral rehydrating solution at the rate of 10 ml kg⁻¹ for each stool. (h) Breast-feeding should be resumed. Nonlactose formula, milk-based formula, or milk may be given, although a small percentage of children will not tolerate lactose-containing fluids. Lactose-containing solutions seem to be tolerated better when combined with complex carbohydrates in weaned children. Children who are eating foods may resume eating, although certain foods are tolerated better than others. Recommended foods include complex carbohydrates (rice, wheat, potatoes, bread, and cereals), lean meats, yogurt, fruit, and vegetables. Avoid fatty foods and foods high in simple sugars (including juices and soft drinks). Supplement feeding with an oral electrolyte solution, 10 ml kg⁻¹ for each diarrheal stool and the estimated amount vomited for each emesis.

may promote the rate of rehydration but may not be palatable for some individuals. Most commercial sports drinks contain 1.2–1.8 g NaCl per liter and are also good rehydration solutions, especially when both fluid and electrolytes have been lost through sweating. Fruit juices can also provide fluid, energy, and electrolytes (e.g., fresh orange juice contains approximately 10 mg of sodium and 2000 mg of potassium per liter) but may be too concentrated and delay gastric emptying. Diluting fruit juices 1:3 with water may yield a more appropriate rehydration solution. The inclusion of carbohydrate in the rehydration solution provides energy for the intestinal sodium pump, which facilitates sodium transport across the intestinal cell wall into the blood, where it in turn exerts a positive osmotic effect on water absorption from the gut. Glucose and electrolyte sports beverages are useful rehydration solutions for sporting activities but are not a good choice for children with diarrhea because these beverages have lower electrolyte and higher carbohydrate concentrations than recommended.

Groups at Risk for Dehydration

Predisposing Factors for Heat Illness

Certain segments of the population are at greater risk for dehydration and subsequent heat illness than others (Table 5). The predisposing factors for dehydration and heat illness in these populations are obesity (extra exertion, heat production, and sweating are required to move a larger mass), insufficient heat acclimation (associated with reduced sweating and evaporative cooling and increased cardiovascular and renal stress), socioeconomic barriers to cooling methods (fans, air

conditioners, etc.), pyrexial illness (fever), drug and alcohol abuse (interferes with fluid balance and thermoregulation), physical work in environments that contribute to dehydration (heat: sweating; cold: respiratory water loss and diuresis; altitude: respiratory water loss and diuresis), and athletic competition and training (if athletes do not replace sweat loss). Even athletes who make a conscious attempt to drink during exercise only ingest approximately 300–500 ml fluid per hour; fluid loss through sweating ($500\text{--}1000\text{ ml h}^{-1}$) can easily surpass this intake of fluid.

Elderly and Children

The very young and the very old are two populations especially susceptible to dehydration. Children have less surface area-to-mass ratio for evaporative cooling and are less inclined to replace fluids; thus, they are less efficient thermoregulators than adults when exposed to high environmental temperatures. In the US, approximately 9% of all hospitalizations of children younger than 5 years of age are due to diarrhea.

Aging is associated with decreased thirst, sweating, and renal responses that place the elderly at high risk during periods of extreme shifts in environmental temperature. Dehydration is a common cause for hospitalization and death in the aged population. Statistics from a 1991 US survey of Medicare recipients revealed that almost half of the Medicare beneficiaries hospitalized for dehydration died within a year of admission. Older men and women may have a higher osmotic operating point (the point at which the thirst sensation is triggered), which may contribute to hypovolemia. Certain behavioral factors may also influence drinking patterns in older adults who may wish to avoid the physical difficulty associated with trips to the bathroom. Besides contributing to an increased risk for hyperthermia, dehydration also alters the effective dosage of medications through plasma volume changes, leading to further medical complications in the elderly. Dehydration in the elderly often accompanies or results from clinical conditions and/or medications.

Prevention of Dehydration

Dehydration resulting from nondisease causes can be easily prevented provided that people are inclined to drink and have

Table 5 Dehydration and heat illness: Populations at risk

Elderly
Poor
Young children
Obese people
Alcoholics
People afflicted with respiratory, cardiovascular, cerebrovascular, renal, or diarrheal disease
Athletes and outdoor workers

Table 6 Fluid replacement: Summary of recommendations of the American College of Sports Medicine

It is recommended that individuals consume a nutritionally balanced diet and drink adequate fluids during the 24-h period before an event, especially during the period that includes the meal before exercise, to promote proper hydration before exercise or competition.
It is recommended that individuals drink approximately 500 ml of fluid approximately 2 h before exercise to promote adequate hydration and allow time for excretion of excess ingested water.
During exercise, athletes should start drinking early and at regular intervals in an attempt to consume fluids at a rate sufficient to replace all the water lost through sweating, or consume the maximal amount that can be tolerated.
During exercise lasting less than 1 h, there is little evidence of physiological or physical performance differences between consuming a carbohydrate–electrolyte drink and plain water.
Inclusion of sodium ($0.5\text{--}0.7\text{ g l}^{-1}$ of water) in the rehydration solution ingested during exercise lasting longer than 1 h is recommended since it may be advantageous in enhancing palatability, promoting fluid retention, and possibly preventing hyponatremia in certain individuals who drink excessive quantities of fluid. There is little physiological basis for the presence of sodium in an oral rehydration solution for enhancing intestinal water absorption as long as sodium is sufficiently available from the previous meal.

Source: Reproduced from the American College of Sports Medicine (1996) Position stand on exercise and fluid replacement. *Medicine and Science in Sports and Exercise* 28: i–vii, with permission from LWW.

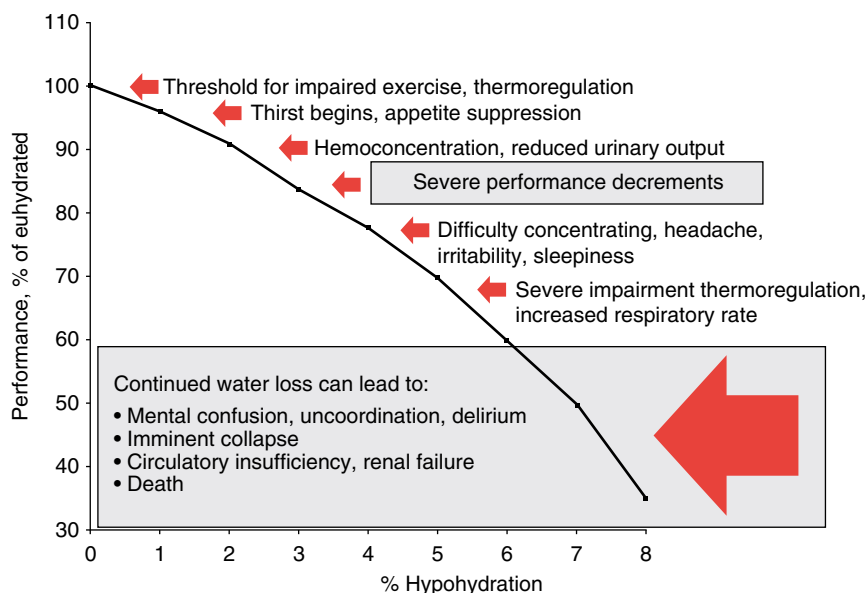


Figure 6 Progressive physiological effects of dehydration on physical performance and pathophysiology of hypohydration. The onset, magnitude, and severity depend on the workload, level of physical fitness, ambient temperature, relative humidity, and degree of heat accumulation of the individual. Reproduced with permission from Askew EW (1996) Water. In: Ziegler EE and Filer Jr LJ (eds.) *Present Knowledge in Nutrition*, pp. 98–108. Washington, DC: ILSI Press.

access to cool, safe sources of fluids. Drink flavoring, beverage temperature, and sodium chloride content are important promoters of fluid intake in active children. Education of athletic coaches, the general public, and health care providers is necessary to increase awareness of the importance of proper hydration. The American College of Sports Medicine has issued a set of guidelines for fluid replacement (**Table 6**).

Simple methods, such as recording body weight before and after exercise to determine fluid loss and observing the color of urine or the turgidity of skin, can be useful for monitoring hydration status. The simplest insurance against dehydration is to consume fluids before and during physical activity or heat exposure to match water loss. The amount of fluid needed to maintain a favorable hydration status is variable between individuals but often necessitates drinking in the absence of thirst. Excess fluid consumption is rarely a problem. However, caution should be used to avoid dilutional hyponatremia from overzealous hydration. Humans can acclimate to work in a hot environment and enhance their ability to thermoregulate and conserve fluid, but they cannot adapt to dehydration. Acute dehydration can decrease physical performance and thermoregulation ability and increase the risk of heat illness. Chronic dehydration can reduce the metabolic and thermoregulatory efficiency and increase predisposition to kidney disease. The deleterious effects of dehydration on physiological function are summarized in **Figure 6**.

See also: Children: Nutritional Requirements. Diarrheal Diseases. Electrolytes: Acid–Base Balance. Older People: Nutritional Requirements. Sodium: Physiology. Thirst Physiology

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DENTAL DISEASE

Etiology and Epidemiology

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Glossary

DMFT It is a measure of total dental decay experience in older children and adults, measured as the number of decayed, missing, or filled permanent teeth.

dmft It is a measure of total dental decay experience in children, measured as the number of decayed, missing or filled primary (milk) teeth.

Enamel Defects

The structural integrity of the hard tissues in the mouth is influenced by the nutritional status of the mother during fetal development and of the child during the early years when the permanent teeth are being formed (especially adequate availability of calcium and vitamin D). Excessive intake of fluoride during the period when the enamel of the primary or secondary teeth is being formed will lead to the characteristic mottling defect of fluorosis. Except in rare circumstances, when the intake of fluoride is very high, this condition is usually minor, with no functional significance and only visible to professional inspection.

Dental Caries, Erosion, and Gum Disease

Once the teeth have erupted, they are subjected to three main conditions, all of which may threaten their survival: dental caries, tooth wear, and gum disease. Inappropriate dietary habits are a necessary contributing factor to dental caries development and may also contribute to tooth wear as one cause of acid erosion. In contrast, diet has no effect on the common forms of gum disease.

Etiology of Caries

The causes of dental caries, and factors influencing the process have been the subject of research for more than 100 years. The importance of oral bacteria was discovered well before the specific influence of sugars derived from the diet became known around 1950. Although the protective effect of fluoride has also been known for more than 50 years the mechanism of this effect is still a subject of debate.

Different approaches have been used to try to understand the caries process. Experimental studies have either induced clinically apparent caries or attempted to model the early

stages of caries. Ethical limitations on studies that might cause caries in humans, and increasing resistance to animal experimentation, have recently stimulated a great deal of imaginative work with laboratory modeling.

Direct studies of caries induction are rarely conducted nowadays. But in the past, important evidence in this field has been drawn from experiments in which caries is induced in laboratory animals and, in one important instance, from a similar experiment in human subjects. The animal experiments are now regarded with some suspicion, because the information gained is not readily interpreted in terms of human risk. The animals used have appreciable differences from their human counterparts in the structure of their teeth, their way of eating, and other factors such as saliva and oral bacterial populations. These animal experiments have been useful, however, in establishing that the majority of carbohydrates are capable of inducing caries under appropriate conditions.

A key human experiment, the Vipeholm study, was conducted in the 1950s, before it was entirely clear that sugar is capable of causing caries. It was important in that it demonstrated conclusively that consumption of a large amount of sugar would not necessarily result in a discernable influence on caries risk, provided it is eaten at meal times; whereas frequent consumption of quite small amounts of sugar had a marked influence. Subjects given 340 g per day of sugar at meal times showed no increase in caries incidence, whereas subjects given 50 or 100 g between meals showed an increase. Typical European intakes of sugar are less than 100 g per day. The subjects in this study had little or no oral hygiene and no access to fluoride.

Most dental practitioners recommend reducing the frequency of consumption of cariogenic carbohydrates, which include simple sugars. Thus, the current fashion of eating and drinking perpetually, and sipping sugar-containing drinks from a can over long periods, seems designed to cause caries, and cannot be recommended.

Research on the causes of dental caries has faced a number of questions. These include: the reasons for the large differences in the disease experience of individuals within the same population or even family group; why the prevalence and severity of the disease is so different in different populations; and why these can change so dramatically with time. Entirely satisfactory answers to these questions are still being sought but much has been learned over the last hundred years about the contributing factors and protective measures that determine the likelihood of this disease developing. This knowledge has been synthesized into the currently held view that clinically significant caries will only develop when a number of circumstances occur simultaneously. Inappropriate dietary habits (frequent consumption of sugars or starches) will allow the selective proliferation of bacteria attached to the tooth surface that are capable of metabolizing sugars to organic acids (especially lactic acid). These acids will facilitate the dissolution of the tooth enamel whenever the production of acids is sufficient to lower the local pH below a critical level. The presence of saliva, or of other components of the food matrix, will influence the pH attained and also the rate at which mineral is lost from the tooth surface.

Dental caries is not, however, a simple unidirectional process of demineralization. Some tooth mineral may be removed at almost every eating or drinking occasion but this loss will generally be made good by the subsequent accretion of mineral from saliva. Thus the development of a cavity arises only when the balance of repeated cycles of demineralization and remineralization results in localized overall mineral loss. It is for this reason that caries is most likely to occur at sites (for example between two closely abutting teeth) where food residues are likely to be trapped and access for saliva is limited.

The presence of fluoride not only radically alters both demineralization and remineralization, but also may inhibit the activity of the acid-generating bacteria. To date, the most effective preventative approaches to reduce dental caries incidence have involved the use of fluoride either (at low concentrations) in community water supplies or (at higher concentrations) in toothpaste.

Caries-Causing Bacteria

The surfaces of all teeth are normally covered with a biofilm (plaque) composed of a range of bacterial species embedded in a sticky organic material produced by the metabolic activity of specialized bacteria. Colonization of the surfaces of the teeth starts as soon as they erupt in a baby's mouth (from approximately 6 months of age) and continues throughout life. There is evidence to suggest that initial colonization of a baby's teeth with cariogenic bacteria may arise by infection from the mother's mouth. The common practice of sampling the food in a baby's dish, to check that it is not too hot, using the same spoon that is to be used to feed the baby, may be a particularly effective way of transferring bacteria from carer to baby. Brushing the teeth with a toothbrush will remove part, but not all of this film and its accompanying bacterial population. Many of the bacteria present are harmless but a number of species are capable of both converting carbohydrates to acids (acidogenic) and also of continuing to be metabolically

active when the local pH has become too acidic for most bacteria to tolerate. It is these bacteria that cause caries.

Fermentable Carbohydrate

Acidogenic bacteria metabolize (ferment) simple sugars (glucose, fructose, sucrose, lactose, maltose) to acids. Sugars may be present as a result of their direct consumption or as a result of the breakdown of starches within the mouth by the enzyme salivary amylase. Thus a substantial proportion of a typical diet will contain a source of fermentable carbohydrate and many, if not all, eating and drinking occasions will give these bacteria one of these metabolic precursors. The more frequently an individual consumes carbohydrate the more the acidogenic bacteria thrive and other, less acid-tolerant, bacteria are disadvantaged.

A wide variety of foods contain carbohydrate that is capable of giving rise to acids as a result of bacterial metabolism (fermentation) within dental plaque. Of the common dietary sugars, sucrose, fructose, and glucose are found in fruit and fruit juices, soft drinks, jams, honey, chocolate, and other confectionery and in an immense variety of composite foods and drinks. Lactose arises naturally in milk and milk products but is also widely used as an ingredient in its own right in the food industry.

Starches are also termed fermentable carbohydrates because they are partially broken down during chewing by amylase in saliva to maltose and glucose. Residues of starchy foods are frequently caught between the teeth and in the fissures of molar teeth, where they may be broken down to sugars and acids over long periods. Measurements of the pH of plaque following ingestion of starches have suggested that the depression of pH may be as great and lasts even longer than for some sources of sugars, such as drinks, because of slow clearance. Highly processed starchy products, such as heat- and pressure-processed extruded snacks, are likely to be more readily converted to sugars than less processed starchy foods, such as bread.

Clearly individual dietary choices and eating habits may influence the risk of caries. The physical characteristics of fermentable carbohydrates, will alter the rate at which they are cleared from specific sites in the dentition. Foods that are inclined to remain for long periods in stagnation sites, such as toffees or raisins, are likely to give rise to a greater local fall in pH than those that are rapidly cleared, such as chocolate. Clearance rates are also influenced by the increase in salivary flow that eating, or drinking, stimulates. When saliva flow is greater, for example after consuming a strongly flavored food, clearance will be faster and demineralization is likely to be less than after consuming a bland food.

Susceptible Sites

Dental caries is more likely to occur at stagnation sites between teeth and in the fissures of molar teeth. Plaque will accumulate in these sites, where it is less likely to be disturbed by toothbrushing. At the same time, the protective buffering of saliva, and the remineralization that arises from its mineral content, are attenuated by the inaccessibility of such sites,

whereas food debris is retained for longer periods. The reduction in salivary flow during sleep makes food debris remaining in these sites at night particularly damaging to the teeth.

Experimental Models of the Caries Process

Because direct manipulation of the caries process in human subjects is now impossible for ethical reasons, a number of techniques have been developed that provide insights without risking clinical damage to the teeth of experimental subjects. Much of the earlier work relied on measurements of the change in plaque pH that followed a single consumption episode of a food or drink containing a source of fermentable carbohydrate. This approach provides an indication of the cariogenic potential of these exposures, especially the fundamental question of whether pH falls to a level expected to give rise to demineralization of the tooth enamel. Plaque pH measurements have thus been used to assess whether a food or drink product may be considered safe for teeth. But this technique does not provide any information on the influence of repair processes that follow exposure to a demineralizing challenge.

Approaches that provide an insight into the balance of demineralization and remineralization episodes over a period of time and with naturalistic eating and drinking circumstances have now become more commonly used. These involve placing of enamel samples within a subject's dentition and carefully assessing any changes in the surface of this sample over a period of time. Particular cariogenic challenges can be applied, but because they are continued for only a limited period of time, the subjects own teeth will not be appreciably affected. In many cases the enamel sample is not cleaned with fluoride toothpaste whereas the subjects own teeth are so protected (the additional enamel sample being removed while the teeth are brushed).

These models have provided useful information, not only on the relative cariogenic potential of different foods and drinks, but also on the protective effects of fluoride toothpaste and the influence of stagnation sites on caries risk with different dietary practices. Useful indications of answers to important public health questions are beginning to emerge from this kind of research, such as the number of exposures to fermentable carbohydrate that can be tolerated without appreciable risk to the teeth, and the influence of fluoride toothpaste use on this number. Current data suggest that more than five exposures per day can be tolerated when fluoride toothpaste is used but less than three when it is not.

Etiology of Tooth Wear

The enamel surfaces of the crowns of the teeth may also be damaged by wear arising from abrasion, attrition, or erosion. Abrasion can arise from the action of rubbing a hard substance across the surface of the teeth, for example when brushing with toothpaste too vigorously with a hard toothbrush. Attrition involves one tooth surface wearing down by contact with another. A third form of wear involves the direct erosive action of acids present in foods (such as yoghurt or pickles) or drinks

(especially citrus fruit juices). No bacterial metabolism is required for these processes to occur. It is unclear, however, whether the apparent increase in prevalence of clinically observed erosion of the teeth is the result of dietary habits or some other factor. Only recently have dental health surveys assessed this problem specifically, so the possibility exists that it has been noticed more, rather than actually occurring more, in these later surveys. It is also not always possible to distinguish acid erosion from other causes of tooth wear, such as over-vigorous tooth brushing. In addition, a common source of acid erosion is not dietary but arises from the regurgitation of the extremely acidic contents of the stomach. This is often seen in young children and, in adults, may be a presenting symptom of bulimia nervosa as a result of repeated vomiting.

Etiology of Gum Disease

Gum disease arises as a result of bacterial infection of the gums, especially at the tooth margin. It is often assumed that excessive accumulation of plaque, arising from inappropriate dietary habits, is a factor in this condition but there is little evidence for any material influence of diet. The milder forms of gum disease are extremely common in all populations. More severe disease is the most frequent cause of tooth loss in older people. The best form of protection from gum disease is regular toothbrushing.

Protection from and Prevention of Dental Caries

Variations among individuals, and with time, will arise as a result of differences in acid generation from sugars at different localities within the dentition. These variations may be influenced by changing dietary habits and the extent of colonization of the relevant tooth surface by acidogenic bacteria. A common habit of swishing drinks and soft foods between the teeth is likely to be unhelpful. They may also be affected by changes in saliva flow, for example as a result of the use of certain medications or radiotherapy.

These factors may provide a reasonable explanation for many of the differences in caries experience observed between individuals and populations, and between different locations within an individual's dentition. They do not explain the dramatic reduction in caries prevalence seen throughout the developed world in the last 30 years. There is no doubt that the cause of this improvement has been the introduction of fluoride toothpaste.

Fluoride

Fluoride has provided the great success story in dental public health in the last 30 years. There are two main routes of delivery: water and toothpaste. Both are, in effect, dietary modifications, because it has been established that the use of fluoride toothpaste has both a topical and systemic impact.

The observation that tooth decay was less common in communities whose water supply naturally contained low concentrations of fluoride, led to the introduction of

appropriate concentrations of fluoride into many public water supplies that did not naturally contain it. Dental caries prevalence appeared to fall by between 20% and 50% as a result of this simple public health measure.

There have been many thorough studies of the general health of populations receiving fluoridated water without any credible evidence emerging of adverse effects at the level used for caries prevention as opposed to a few areas where the fluoride content of the water is naturally very much higher. Despite vocal opposition to what is seen by some as compulsory medication of the population, many countries (for example, the USA and Ireland) still use this approach widely. Some, however, such as the Netherlands, have discontinued the practice.

Even greater improvements in dental health have followed the introduction of fluoridated toothpastes. The benefit seen at the population level from this innovation have been far greater than predicted by the controlled clinical trials that preceded their widespread sale to the public. Improvements of greater than 60% have been common. Interestingly, caries rates in the Netherlands continued to fall after the discontinuation of fluoridated water supplies, probably as a result of intense dental health education of the population on the value of regular brushing with fluoride toothpaste. In contrast, the abandonment of water fluoridation in the UK region of Anglesey was followed by a sharp rise in caries incidence.

A Practical Approach to the Prevention of Caries

The success of fluoride toothpaste in preventing dental caries has resulted in a change in professional approaches to prevention. Instead of focusing simply on attempts to reverse the main causative factors, attention is now centered on exploiting protective influences. The interaction of the three main causative factors is illustrated in **Figure 1**. Numerous attempts

to change the impact of any of these influences on caries have proved ineffective, except perhaps, under the most extreme situations, such as during war time.

In contrast, exploitation of the protective potential of fluoride, toothbrushing and salivary stimulation have proved successful. **Figure 2** illustrates the role of these factors in comparison with the pervasive challenge of diet. Where a tooth site is shielded from saliva (stagnation site) and the availability of fluoride and oral hygiene are insufficient it is likely that dietary modification of sufficient magnitude might exert some influence on the final outcome. But where these protective factors are adequate it is highly unlikely that dietary variations will exert any material effect. These predictions are borne out by epidemiological observations. The majority of attempts to prevent caries by dietary changes have proved unsuccessful and in most developed

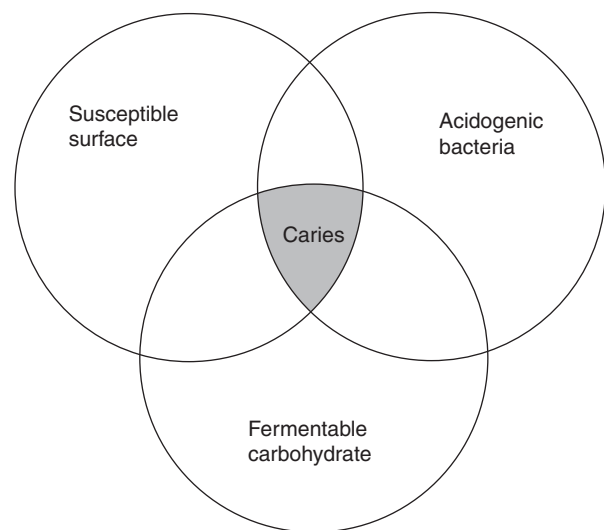


Figure 1 Interacting factors causing tooth decay.

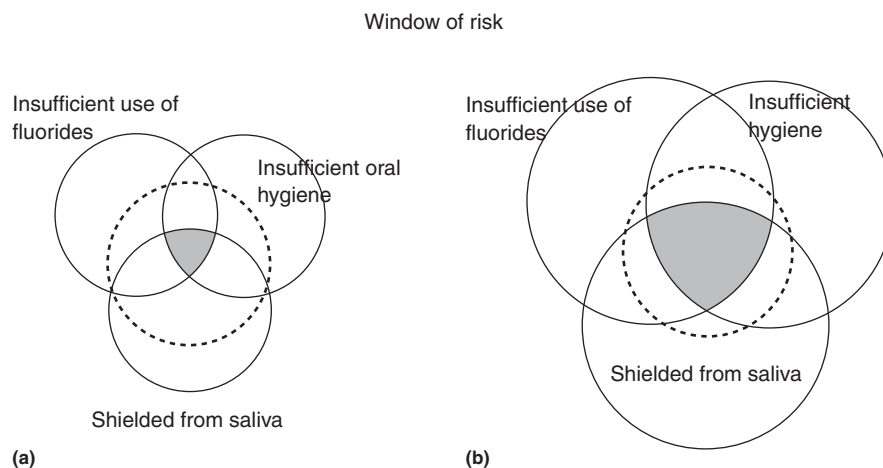


Figure 2 A new model to explain and guide caries prevention. The local factors: insufficient use of fluorides, insufficient oral hygiene and protection from saliva form a 'window of risk' through which the circle of cariogenic food (shown dashed) can be seen in the background. (a) In this example it is clear that it would be an impossible task to reduce the circle food to such an extent that the window is not completely filled (less caries risk). (b) If oral hygiene and, concomitantly, fluoride supply is neglected (large window of risk) reduction in the burden of cariogenic food could reduce the caries risk.

countries, where fluoride use is adequate, wide variations in dietary exposure to fermentable carbohydrates between individuals are not accompanied by predictable differences in caries experience.

Epidemiology

Studies of Risk Factors

A large number of observational epidemiological studies have been conducted that have attempted to show associations between caries experience and one, or several, of the known risk factors. The large majority of these studies have been of poor design, examining insufficient numbers of subjects, and ignoring important confounding influences. Many have been cross-sectional in design and have sought to draw conclusions on the causes of caries from assessments of the dietary and other habits of subjects at the same time as measuring their caries experience. Such a study design is somewhat unsatisfactory for this purpose as it gives no information about past exposure.

The few longitudinal studies in the scientific literature are equally weakened by poor data on dietary habits and, in some cases, idiosyncrasies in caries assessments. Taken together these studies provide scant evidence on the relative importance of the different etiological factors. It is fortunate that more convincing evidence is available from experimental studies.

National Trends in Caries Prevalence

Data on dental caries within populations are nowadays more reliable as they are collected to internationally recognized standards. Surveys of 12-year-old children are carried out

periodically in most countries, and the data are collated by the World Health Organization (WHO) (see [Table 1](#)). However, many of these data sets do not refer to nationally representative samples but to selected population subgroups. None the less, they provide a useful insight into the wide variations in caries prevalence and its alteration with time. In contrast, data for adults are scarce.

The general picture emerging from the repetition of these national surveys is clear. In many countries the prevalence of caries is falling, often dramatically. In poorer countries this is

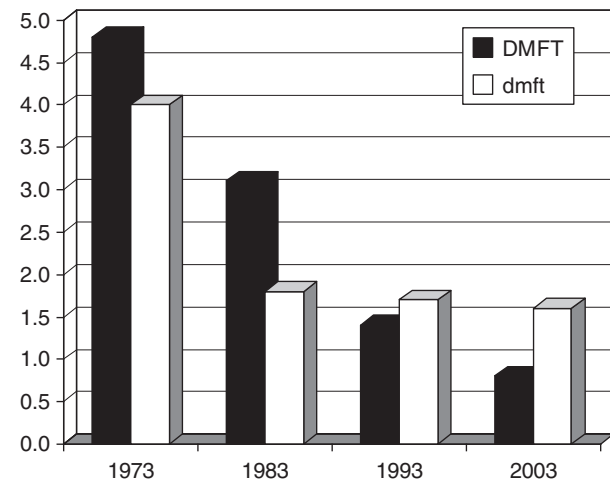


Figure 3 Decay experience of British children at 10-yearly national surveys. Filled bars: mean number decayed, missing, and filled permanent teeth in 12-year-old children. Open bars: mean number of decayed, missing, and filled primary teeth in 5-year-old children.

Table 1 Examples of the range of caries prevalence within and between regions. The table shows the mean number of teeth with decay experience DMFT in 12 year-old children, taken from recent large population surveys listed in the WHO Oral Health Country/Area Programme

WHO region	Lowest DMFT	Country	Year of survey	Highest DMFT	Country	Year of survey
Europe	0.7	Germany Denmark UK	2005 2008 2005	3.4	Latvia	2004
Americas	0.6	Trinidad and Tobago	2004	5.2	Guatemala	2002
Africa	0.4	Ghana	2000	4.4	Gabon	2000
Southeast Asia	0.5	Nepal	2004	3.9	India	2003
Eastern Mediterranean	0.4	Egypt	2002	2.6	Kuwait	2000
Western Pacific	0.8	Hong Kong	2001	2.9	Philippines	2006

Note: These surveys are not necessarily representative of the whole population cited. Since dental caries prevalence is generally falling throughout the world, data has only been cited from surveys taken after 2000.

not often the case, and even within the richest countries, the dental health experience of the economically disadvantaged is significantly poorer than that of those with a higher socio-economic position. In many countries there is evidence that inequalities in dental health between the rich and the poor have widened.

Attempts to account for these trends are hampered by the unreliability of data on factors likely to attenuate caries risk. All assessments of these factors rely on people (often children) accurately remembering and reporting aspects of their everyday behavior, such as whether they clean their teeth and how often.

Fluoride Toothpaste

The effect, at a population level, of the introduction and widespread availability of fluoride toothpaste is clear. **Figure 3** shows the fall in caries in 5 and 12 year old children in the UK seen in successive nationally representative surveys. A similar picture has been seen in Denmark (**Figure 4**). Fluoride toothpaste was introduced on to the UK market around 1976 and rapidly became universal. The falls in caries prevalence seen at the next survey date (1983) exceeded expectations

based on earlier clinical trials, and led many experts to predict that no further fall would occur. In the event an even greater decline was seen among 12 year olds at the next decennial survey (1993) and a further substantial improvement was recorded in 2003. The caries prevalence among 5 year olds appeared to have reached a plateau by 1993 but modest falls were recorded in subsequent surveys. This disappointing result for the younger age group may be the result of inadequate knowledge among some carers, especially in the socio-economically deprived sectors of the population, of the importance of early introduction of the use of fluoride toothpaste. Regrettably, self-reported use of fluoride toothpaste (almost certainly an over-estimate of actual use) is still not universal, even among children in comfortable socio-economic conditions.

The variation in caries experience with family income is illustrated for the UK in **Figure 5**. A clear gradient exists, with the poorest dental health seen in the lowest income families. Trend data indicate that the greatest improvements have occurred among higher income families and the least among those at the other end of the socio-economic scale. The reasons for these differences are not entirely clear but oral hygiene and use of fluoride toothpaste appear to be one factor.

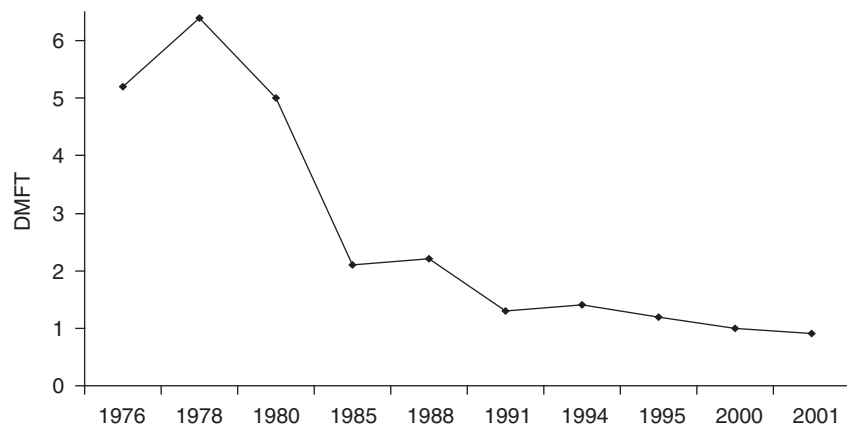


Figure 4 Change in caries experience of 12 year-old children in Denmark. Decayed, missing or filled permanent teeth (DMFT).

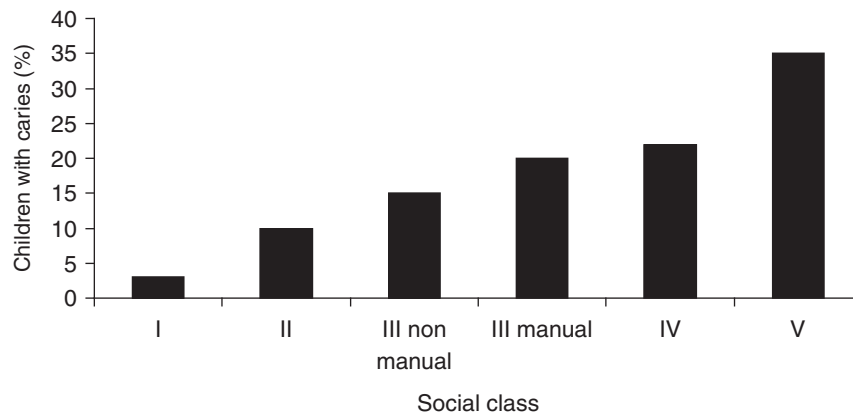


Figure 5 Percentage of children aged 1½–4½ years with caries across social class groups in the UK, calculated from Hinds and Gregroy (1995). Social Class I has the highest income, V the lowest.

Evidence of gum disease (an indicator of oral hygiene) is more common among poorer children.

Diet

Experts have pointed to changes in caries prevalence following dramatic changes in food supply as evidence of the practical utility of dietary manipulation as a means of reducing the remaining burden of this disease. These have all occurred before the advent of widespread use of fluoride. When the food supply of fermentable carbohydrates is severely restricted, changes in frequency of consumption may occur to a degree sufficient to alter caries risk when fluoride is not used. Where fluoride toothpaste and oral hygiene are adequate, even such extreme changes in diet are unlikely to alter caries experience materially. In addition, attempts to use dietary manipulation to reduce the risk of caries in free-living populations have proved disappointing.

Other Factors Affecting the Epidemiology of Caries

The influence of other factors that might be expected to have a bearing on caries experience has proved difficult to establish for practical reasons. These include the susceptibility of particular sites within the dentition of an individual's mouth, and local salivary flow rates. Both of these factors are known to be strongly influenced by genetic inheritance. The morphology of the teeth and, especially, the depth and shape of the fissures on the surface of the molar teeth are strongly inherited. It is generally difficult to predict, in advance of caries developing, which sites will be particularly susceptible. But one successful preventative approach has been to identify children with deep fissures in their molar teeth at an early age and offer prophylactic treatment in the form of sealants. This addresses the most common site of early childhood caries (the molars) and those children most at risk because of unfavorable tooth morphology.

Salivary flow rate, both at rest and when stimulated by eating or drinking, is also known to be a crucial influence on risk. Patients who have had a salivary duct removed for any reason have a far higher risk of caries than those with normal function. Some people have low saliva flow rates and, again, have a greater risk. Older people are inclined to suffer from a dry mouth and a number of medications reduce saliva flow.

Epidemiological studies to assess the importance of saliva flow rates in altering the risk of caries have not been carried

out because of the practical difficulty of measuring this factor. But the stimulation of saliva flow by chewing has been successfully exploited to reduce caries risk in experimental studies using chewing gum (usually sugar-free). Reductions in caries incidence were seen when subjects were encouraged to chew the gum, especially between and immediately after meals, while continuing their normal regular oral hygiene practices. Convincing evidence of any effect at the population level, however, is awaited.

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DIABETES MELLITUS

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Classification and Chemical Pathology

Dietary Management

Etiology and Epidemiology

Classification and Chemical Pathology

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Glossary

Autoimmune The body's development of intolerance of the antigens of its own cells.

Gluconeogenesis The formation of glucose from noncarbohydrate sources such as amino acids.

Glycosuria The abnormal presence of sugar in the urine.

HLA antigen Gene product of the major histocompatibility complex.

Uremia Accumulation of urinary waste products in the blood.

Introduction

Diabetes mellitus is a common, serious metabolic disorder with diverse causes and multiple complications. In this article, the definition and classification will be discussed and diagnostic criteria outlined. Subsequently, an overview of the physiology of normal blood glucose homeostasis and normal insulin action will lead to consideration of the pathophysiologic events that occur in uncontrolled diabetes.

Definition

Diabetes mellitus is a chronic disorder that results from a deficiency of the hormone insulin. This occurs either because of an absolute decrease in the amount of insulin produced by the β cells of the islets of Langerhans in the pancreas, or because of a relative deficiency of insulin in patients whose tissues are resistant to the hormone. The hallmark of untreated diabetes mellitus is elevated blood glucose concentrations. Frequently, there are associated disturbances of fat and protein metabolism, and perturbations in multiple other hormones. In addition to reversible acute metabolic abnormalities resulting from inadequate effects of insulin, long-term diabetes often is characterized by the development of irreversible complications that include damage to the kidney, retina, nervous system, and both large and small blood vessels.

The diagnosis of diabetes mellitus is based on the existence of hyperglycemia alone and does not require the presence of any of the associated metabolic or systemic complications. Although patients with diabetes mellitus exhibit a characteristic

pattern of metabolic abnormalities and long-term complications, this disease state results from multiple underlying causes that include genetic as well as environmental influences, and certain pancreatic or hormonal conditions.

Diagnostic Criteria in the NonPregnant Patient

Random Plasma Glucose Determination

In the presence of symptoms of hyperglycemia, a random plasma glucose $> 11.1 \text{ mmol l}^{-1}$ (200 mg dl^{-1}) is consistent with a diagnosis of diabetes, in an ambulatory patient. Classic symptoms of hyperglycemia include thirst, polydipsia, polyuria, and unexplained weight loss. If there is moderately elevated glucose and an absence of symptoms, a second plasma glucose determination $> 11.1 \text{ mmol l}^{-1}$ (200 mg dl^{-1}) made at a separate time similarly supports the diagnosis of diabetes.

Fasting Plasma Glucose Determination

The diagnosis of diabetes mellitus can be made when fasting plasma glucose concentrations are significantly elevated ($> 7.0 \text{ mmol l}^{-1}$, or 126 mg dl^{-1}) on at least two occasions. Fasting glucose below 7.0 mmol l^{-1} , but above 5.6 mmol l^{-1} (100 mg dl^{-1}), whereas not normal, does not meet the criteria for definite diagnosis and is classified as impaired fasting glucose. Although not absolutely predictive of diabetes, individuals with impaired fasting glucose progress to overt diabetes at a rate of approximately 5% per year. Plasma or serum measurements are generally more reliable than whole blood glucose determinations (in which the normal range is lower),

because they are independent of the hematocrit and are appropriate for accurate, automated analysis. For accurate measurement, blood samples must be put into tubes containing sodium fluoride (which prevents glycolysis) or be centrifuged within 30 min to remove cells. Fasting plasma glucose has traditionally been considered the desired method to diagnose diabetes, because of its simplicity and reproducibility.

Oral Glucose Tolerance Test

In the absence of an obvious elevation in fasting or random plasma glucose levels, the diagnosis of diabetes mellitus can be made with an oral glucose tolerance test (OGTT). This involves, for the nonpregnant adult, the ingestion of a solution containing 75 g of glucose over 5 min, with a measurement of baseline and 2 h plasma glucose. The criteria used to diagnose diabetes are listed in **Table 1**. The diagnosis can be made if the fasting glucose exceeds 7.0 mmol l^{-1} (125 mg dl^{-1}) or the 2 h value exceeds 11.1 mmol l^{-1} (200 mg dl^{-1}). Persons with impaired glucose tolerance have normal fasting values but 2 h postglucose load values above 7.8 mmol l^{-1} (140 mg dl^{-1}). They progress to overt diabetes at a higher rate than individuals with normal glucose tolerance. The presence of either impaired glucose tolerance, impaired fasting glucose, or both of these abnormalities therefore often is referred to as prediabetes.

The standard OGTT must be performed under certain conditions for the above thresholds to apply. Subjects need to ingest at least 200 g carbohydrate per day during the 3 days preceding the test, fast overnight ($> 8 \text{ h}$), not smoke on the day of the test, and have the test performed in the morning. Because glucose tolerance is reduced by bed-rest and stressors such as recent surgery or burn injury, subjects must be ambulatory and have been so for at least a month before the test. Despite this standardization, results are not always precisely reproducible, even in the same person, which may relate in part to variable rates of absorption of glucose from the small intestine. For this reason, elevated fasting glucose is a more reliable diagnostic criterion.

In children, if an OGTT is performed, the amount of glucose to be ingested should be determined by body weight, that is, 1.75 g per kg ideal body weight.

Glycosylated Hemoglobin

Glycohemoglobin is formed when a ketoamine reaction occurs between glucose and the *N*-terminal amino acid of the β

chain of hemoglobin. The amount of glycohemoglobin generated is proportional to the mean blood glucose during the 8–10 weeks before the test. Thus, the glycohemoglobin level is a useful indicator of long-term blood glucose control. A recent recommendation from an international committee, and adopted by the American Diabetes Association in 2010, is that hemoglobin A1c $> 6.5\%$ can be considered diagnostic of diabetes. Previous critics of this test are concerned that the normal range is broad, the test is not well standardized between laboratories, and it can be affected by conditions that alter the lifespan of the red blood cell. However, in recent years increasing standardization of the methodology used in large clinical trials, and understanding of good correlation between even mildly abnormal glycohemoglobin concentrations and risk of retinopathy has led to acceptance of this test. The ease of sample collection (nonfasting state), low biologic variability and low preanalytic instability also favor use of this test. Persons with hemoglobin A1c in the range 5.7–6.4% are to be considered ‘at high risk’ of conversion to diabetes.

Glycosuria

Glycosuria may indicate the presence of diabetes, but it is not diagnostic, nor does the absence of glycosuria exclude diabetes. In individuals with a low renal threshold, glucose may be present in the urine in the absence of hyperglycemia. Such ‘renal glycosuria’ is particularly common during the later stages of pregnancy and in some renal tubular disorders. The excretion of other sugars such as lactose (more common during pregnancy), or fructose, galactose or xylose (persons with inborn errors of metabolism) can yield false positive results through cross reactivity in the testing method unless glucose-specific test strips are used. In patients with compromised renal perfusion or function, glycosuria may be absent in spite of significant hyperglycemia.

Diagnostic Criteria for Diabetes in Pregnant Women

Some controversy exists with regard to proper methods of diagnosis of diabetes in pregnancy. The Fifth International Workshop-Conference on gestational diabetes recommends screening most pregnant women between 24 and 28 weeks with a 50-g oral glucose load test. No special preparations are required for this test, and fasting is unnecessary. Blood glucose is measured once only, after 1 h. Women with values above 7.8 mmol l^{-1} (140 mg dl^{-1}) are evaluated with a full OGTT with a glucose load of 100 g. Clearly, use of a lower threshold as some practitioners do (e.g., 7.2 mmol l^{-1} , 130 mg dl^{-1}) minimizes the occurrence of false negative tests. The criteria for diagnosis of gestational diabetes are listed in **Table 2**. However, the risk of gestational diabetes is considered to be low in certain groups of women such that no screening is warranted. A woman at high risk of gestational diabetes (prior gestational diabetes, strong family history of diabetes, or obesity) should be screened with a glucose challenge as early as is feasible during the pregnancy.

The World Health Organization (WHO) has traditionally proposed that the test and criteria for gestational diabetes should be the same as that for nonpregnant adults, with the

Table 1 Oral glucose tolerance test criteria for diabetes (75 g glucose load)

	<i>Venous plasma glucose</i>	
	<i>(mmol l⁻¹)</i>	<i>(mg dl⁻¹)</i>
Diabetes		
Fasting	> 7.0	> 126
2 h	> 11.1	> 200
Impaired glucose tolerance		
2 h	$7.8\text{--}11.1$	$140\text{--}200$
Impaired fasting glucose		
Fasting (ADA)	$5.6\text{--}6.9$	$100\text{--}125$
(WHO)	$6.1\text{--}6.9$	$110\text{--}125$
2 h	< 7.8	< 140

Table 2 Criteria for the diagnosis of gestational diabetes (100 g glucose load)

Criteria	Venous plasma glucose	
	(mmol l ⁻¹)	(mg dl ⁻¹)
Fasting	> 5.3	95
1 h	> 10.0	180
2 h	> 8.6	155
3 h	> 7.8	140

Table 3 Oral glucose tolerance test Criteria for diabetes in pregnancy, proposed by IADPSG^a (recommended by ADA, 2011) (75 g glucose load)

Criteria	Venous plasma glucose	
	(mmol l ⁻¹)	(mg dl ⁻¹)
Gestational Diabetes		
Fasting	> 5.1	> 92
1 h	> 10.0	> 180
2 h	> 8.5	> 153

^aIADPSG, International Association of Diabetes in Pregnancy Study Group, 2010.

exception that individuals fitting the category of impaired glucose tolerance or impaired fasting glucose be treated the same as patients with diabetes (Table 1).

In 2010, the International Association of Diabetes and Pregnancy Study Groups made some additional recommendations based on inferences that were drawn from a large observational study (the HAPO study) of pregnancies in non-diabetic (by WHO criteria) women whose glucose tolerance data were linked with clinical fetal outcomes. These newest recommendations for diagnosis of gestational diabetes include an assessment of glycemia at the first prenatal visit in all women by measuring either a fasting or random plasma glucose, or hemoglobin A1c. Screening only high-risk women at this time is also considered appropriate, since generalized testing has not been shown to be beneficial before 24–28 weeks. The diagnosis of frank diabetes (not gestational diabetes) can be made in women with a random but confirmed blood sugar ≥ 11.1 mmol l⁻¹ (200 mg dl⁻¹), a fasting plasma glucose ≥ 7.0 mmol l⁻¹ (126 mg dl⁻¹), or hemoglobin A1c $\geq 6.5\%$. This group recommended adoption of the WHO 75 g OGTT at 24–28 weeks in all women without an earlier diagnosis, and that the screening 50 g test be abandoned. Gestational diabetes can be diagnosed any time with a glucose tolerance test (Table 3). The newly recommended diagnostic criteria for gestational diabetes are slightly different from the nonpregnant population.

Classification

A new classification system for diabetes mellitus was developed in 1997, which divides patients into four major groups and a number of subgroups, as shown in Table 4. It is probable that these categories will be further refined as knowledge of the underlying etiologies of various forms of diabetes progresses.

Table 4 Classification of diabetes mellitus

- I. Type 1 diabetes (formerly designated insulin-dependent diabetes or IDDM)
 - A. Autoimmune
 - B. Idiopathic
- II. Type 2 diabetes (formerly designated noninsulin-dependent diabetes)
- III. Secondary diabetes
 - A. Genetic defects of β cell function (e.g., maturity onset diabetes of the young)
 - B. Genetic defects of insulin action pathway
 - C. Exocrine pancreatic disease
 - D. Endocrinopathies (e.g., Cushing's syndrome, acromegaly)
 - E. Drugs or chemicals
 - F. Infections (e.g., congenital rubella)
 - G. Other genetic syndromes (e.g., Down, Klinefelter syndromes)
- IV. Gestational diabetes

Classification proposed by the Expert Committee on the Diagnosis and the Classification of Diabetes Mellitus under the sponsorship of the American Diabetes Association (Diabetes Care 27: S5–S10, 2004).

Type 1 Diabetes Mellitus

This form of diabetes is defined by insulin deficiency due to destruction of the β cells of the pancreas. It was formerly designated 'insulin-dependent diabetes,' but efforts are being made to eliminate this name because many patients with other types of diabetes also require insulin for adequate control. The predominant cause is believed to be an autoimmune attack against the insulin-producing β cells within the islets of Langerhans (diabetes Type 1A). At the time of diagnosis, most patients demonstrate antibodies to certain pancreatic auto-antigens, which include antibodies to islet cell cytoplasmic components (ICA), glutamic acid decarboxylase (GAD), insulin, and tyrosine phosphatases IA-2 and IA-2 β . Such auto-antibodies, when present, help to confirm the diagnosis. This disease also has strong human leukocyte antigen (HLA) antigen associations, which may either predispose to or protect from the development of diabetes. In a minor subset of patients classified as idiopathic Type 1 diabetes (Type 1B), the presentation and clinical course is similar to autoimmune Type 1A diabetes, but all tests for autoimmune markers are negative. Viral infections are also considered to underlie some causes of Type 1 diabetes.

Early Diagnosis of Autoimmune Diabetes

Type 1 diabetes has a variable presymptomatic phase that may extend for several years, during which time it is possible to make a diagnosis. This form of diagnostic testing is reserved for research purposes, because the disease is not sufficiently common to warrant widespread screening strategies, and because practical methods for preventing the progression to overt diabetes are not available. As Type 1 diabetes is occasionally familial, screening of individuals with strong family histories can be performed by measuring levels of the specific pancreatic auto-antigens described above; subjects with high titers of antibodies who possess unfavorable HLA subtypes, indicating significant risk of later development of diabetes, may then undergo intravenous glucose tolerance testing with quantitation of the

insulin response. Diminution of the early phases of insulin release can be seen even years before the onset of symptoms of disease. Such diagnosis is important currently only to enable participation in clinical trials of diabetes prevention.

Type 2 Diabetes Mellitus

This is a heterogeneous disorder in which there is both resistance to the action of insulin and relative insulin insufficiency. In contrast to Type 1 diabetes, endogenous insulin secretion is at least partially preserved and thus most patients are not insulin-dependent for acute survival (hence the former name, noninsulin-dependent diabetes, NIDDM). The circulating insulin levels usually are adequate to protect these patients from ketosis, except during periods of extreme stress. Some patients in this category can be treated with oral agents (sulphonylureas, metformin, thiazolidinediones, and dipeptidyl peptidase IV inhibitors), but many are managed with insulin because their pancreases are unable to produce sufficient insulin to overcome their tissue insulin resistance. Obesity is a frequent contributing factor to the insulin resistance in this disorder.

Occasionally, it is difficult to determine whether a patient has Type 1 or Type 2 diabetes. This is particularly likely in a nonobese person above the age of 35, who has never had significant ketosis but who has been treated with insulin. Unfortunately, there is no completely reliable diagnostic test. Measurement of autoantibodies in such persons may not be helpful, because patients with Type 1 diabetes may lose these markers with time. Several studies have shown that the plasma C-peptide level is a good discriminator between the two forms of diabetes. C-peptide is released during processing of proinsulin to insulin and, thus, is an indicator of endogenous insulin secretion. Values above 0.6 nmol l^{-1} , either basal or following provocation with a 1 mg glucagon stimulus, indicate sufficient residual insulin secretion for a person to be considered in the Type 2 diabetes class.

Secondary Diabetes Mellitus/Other Specific Types

This broad category includes multiple disorders that are associated with either extensive pancreatic destruction or significant insulin resistance. Secondary diabetes as a consequence of decreased insulin production can occur following pancreatectomy, chronic pancreatitis, cystic fibrosis, or hemochromatosis. In the absence of pancreatic damage, secondary diabetes can result from extreme insulin resistance induced by glucocorticoids (Cushing's syndrome), growth hormone (acromegaly), adrenergic hormones (phaeochromocytoma), other medical conditions such as uremia, hepatic cirrhosis, or polycystic ovary syndrome, or medications (exogenous glucocorticoids or certain diuretics).

Included in this category of secondary diabetes are patients who have diabetes resulting from the effects of mutations in a single gene and thus are classified as having monogenic diabetes. There now are more than two dozen distinct genes for which mutations have been shown to cause diabetes. The most common include six different genes that cause various forms of the syndrome designated 'maturity onset diabetes of the young,

or MODY'. This syndrome was first characterized clinically as a form of diabetes with autosomal dominant inheritance, onset early in life (often under age 25 or even in early childhood), and a clinical course more like Type 2 than Type 1 diabetes. One of the MODY genes encodes the enzyme glucokinase, which has a role in sensing of glucose by the β cell, and the other MODY genes code for transcription factors thought to be important for β cell growth and development. The other known monogenic causes of diabetes are rare and include various inborn errors of metabolism that often cause resistance to insulin (e.g., insulin receptor mutations, LMNA mutations with lipodystrophy, or Type 1 glycogen storage disease). Other genetic causes of secondary diabetes include chromosomal abnormalities, such as Down and Turner syndrome, and muscle diseases (e.g., myotonic dystrophy). In addition, multiple recognized genetic variants are associated with a higher frequency of the development of diabetes; however, the search for a common genetic underpinning of typical Type 2 diabetes is still ongoing.

Gestational Diabetes Mellitus

This disorder, which is defined as hyperglycemia first detected during pregnancy, occurs in 2–5% of pregnant women. Often one cannot determine whether glucose intolerance antedated the pregnancy, or whether hyperglycemia was provoked by the hormonal milieu associated with pregnancy. Hyperglycemia remits postpartum in 90% of women with gestational diabetes, but these women are at increased risk for subsequent development of diabetes, which is usually Type 2. In women who have documented gestational diabetes, a follow-up glucose tolerance test should be performed 6 weeks postpartum unless overt diabetes is evident, and screening with a fasting blood glucose or hemoglobin A1c level should be performed at least every 3 years thereafter.

Other Abnormalities of Glucose Tolerance

Impaired Glucose Tolerance

This is a condition defined by oral glucose tolerance testing and includes nonpregnant individuals with normal fasting blood glucose but modestly elevated postprandial glucose. Persons with impaired glucose tolerance are at a high risk for subsequent development of diabetes, usually Type 2, approximately 5% per year. Thus impaired glucose tolerance is a stage in the evolution of diabetes. Until overt diabetes develops, persons with impaired glucose tolerance are not believed to have elevated risk of microvascular complications of diabetes. However, impaired glucose tolerance is associated with an increased risk of cardiovascular disease.

Impaired Fasting Glucose

Some patients will have mildly abnormal elevations in fasting plasma glucose, even though 2 h postglucose challenge values are normal. These persons also are at increased risk of developing diabetes and therefore designated as having prediabetes, although diabetes incidence rates are highly variable between different populations. Fasting glucose is defined as impaired in the range $5.6\text{--}7.0 \text{ mM}$ ($100\text{--}126 \text{ mg dl}^{-1}$). Until recently, impaired fasting glucose was defined as $6.1\text{--}7.0 \text{ mM}$

(110–126 mg dl⁻¹), and the change was recommended by the American Diabetes Association to align better with the category of impaired glucose tolerance above. However, persons in this category who have normal postprandial glycemia, or normal 2 h postchallenge glucose values, have a lower risk of cardiovascular disease than persons with impaired glucose tolerance.

Stress Hyperglycemia

This denotes an individual who is frankly hyperglycemic (>7.8 mmol l⁻¹, 200 mg dl⁻¹) under conditions of intercurrent illness or during treatment with medications that provoke diabetes. Such persons may revert to normal glucose tolerance following removal of the stress. Although not an official category of diabetes, such abnormal glucose values in hospitalized patients cannot be ignored, although good strong evidence has emerged that aggressive treatment all the way to normoglycemia may increase mortality in some groups of patients. The risk of wound infections can be reduced by achieving at least moderate control of hyperglycemia. Precipitants of stress hyperglycemia are listed in Table 5.

Pathophysiology of Diabetes

Physiology of Normal Blood Glucose Regulation

The metabolic fate of ingested glucose is determined by the interplay of multiple hormones. Insulin is of major importance in this homeostasis, but glucagon, glucocorticoids, catecholamines, and growth hormone also have significant effects that are interactive with insulin. Glucose ingested with a meal or derived from the digestion of other dietary carbohydrates is rapidly absorbed by the small intestine. It is carried first to the liver by the portal vein, where a substantial portion (30–70%) is removed; the remainder enters the peripheral

circulation, where regulated insulin secretion and target tissue responses to insulin contribute to glucose clearance and control of blood glucose levels (Figure 1).

Following a meal, insulin is secreted from pancreatic β cells in response to increased circulating glucose concentrations. This direct effect of glucose on β cells is augmented by neural (vagal) and hormonal factors of intestinal origin (e.g., glucose-dependent insulinotropic peptide, cholecystokinin, and glucagon-like peptide 1), such that the insulin secretory response to oral glucose greatly exceeds the response to an equivalent intravenous glucose infusion.

The overall effect of the increase in insulin levels in parallel with increased glucose entry to the circulation is promotion of the net removal of glucose by the liver and stimulation of glucose transport into muscle and adipose tissue, where it is consumed as a metabolic fuel or stored. Insulin also inhibits the catabolism of the alternative energy sources, fat, and protein. This is an appropriate response to the abundance of circulating nutrients that occurs after meals. During fasting, insulin levels are low, these processes are reversed, and stored fuel is made available to all tissues.

Liver

Glucose enters the liver by facilitated (carrier-mediated) diffusion driven by the concentration gradient that exists in the fed state. A portion of the glucose taken up by the liver is metabolized via glycolytic pathways to produce ATP. A substantial amount is transformed into glycogen and stored. The maximal storage capacity of the liver is about 100 g glycogen (400 kcal). The specific molecular effects of insulin in the fed state lead to altered activities of enzymes that trap glucose inside the hepatocyte, promote glycolysis, and enhance glycogen synthesis (see Figure 1). Insulin also inhibits enzymes important for both glycogenolysis and gluconeogenesis and thus shuts off hepatic glucose production. A portion of the glucose entering the liver is converted into triglyceride and exported to the adipocyte for storage.

Skeletal muscle

In skeletal muscle, insulin directly stimulates glucose uptake, which is the rate-limiting step for muscle clearance of glucose. This appears to occur predominantly by causing the rapid translocation of glucose transporters (in particular the Glut 4 transporter) from a sequestered intracellular site to the muscle cell surface. Insulin also stimulates glycolysis and the net formation of glycogen in muscle. Even at low-insulin concentrations, a rise in ambient glucose stimulates substantial glucose clearance by muscle, probably via the Glut 1 transporter. Glycogen stores in muscle (500–600 g glycogen in a 70 kg human) serve as a rapidly mobilized energy source during exercise, but do not directly support blood glucose concentrations in the fasted state, because muscle lacks the enzyme glucose-6-phosphatase which is needed for release of free glucose to the circulation. Insulin-stimulated amino acid entry into muscle enhances insulin stimulatory effects on protein synthesis and decreases the availability of circulating amino acids as substrates for hepatic gluconeogenesis. Muscle proteolysis, which yields amino acid precursors that contribute to hepatic gluconeogenesis in the fasted state, is inhibited by insulin.

Table 5 Risk factors for the development of stress hyperglycemia in critical illness

Factor	Major mechanism
Preexisting diabetes mellitus	Insulin deficiency (relative or absolute)
Infusion of catecholamine pressors	Insulin resistance
Glucocorticoid therapy	Insulin resistance
Obesity	Insulin resistance
Increasing APACHE ^a score	Higher counterregulatory hormone levels
Older age	Insulin deficiency
Excessive dextrose administration	Glucose removal rates overwhelmed in the face of ongoing hepatic glucose production
Pancreatitis (acute and chronic)	Insulin deficiency
Sepsis	Insulin resistance
Hypothermia	Insulin deficiency
Hypoxemia	Insulin deficiency
Uremia	Insulin resistance
Cirrhosis	Insulin resistance

^aAPACHE, Acute Physiology and Chronic Health Evaluation.

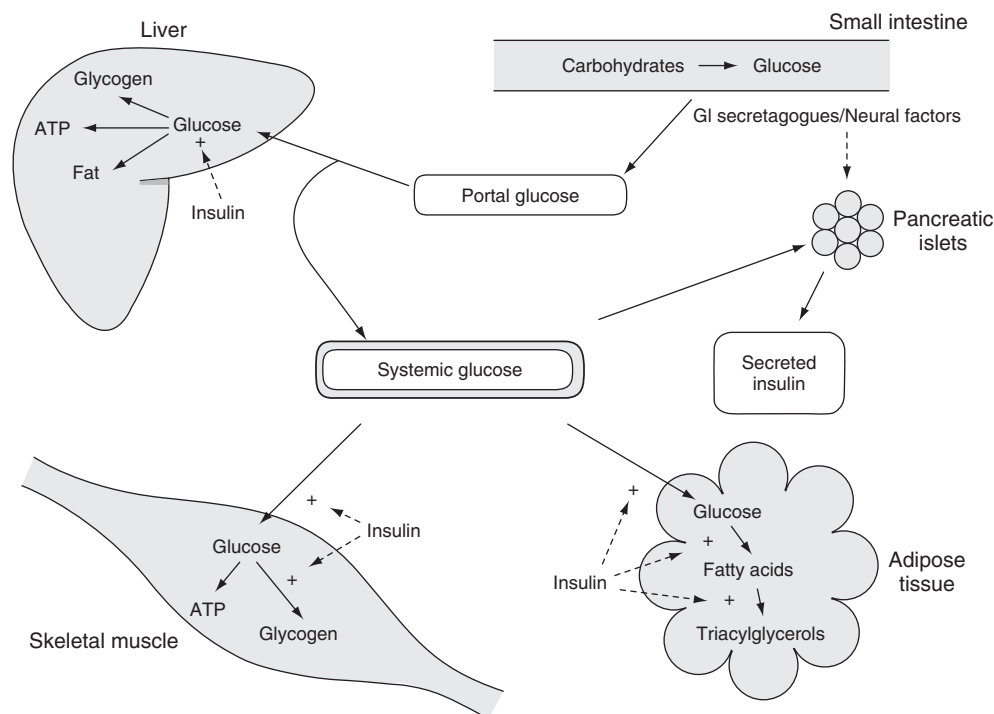


Figure 1 Insulin effects on glucose homeostasis in the fed state.

Adipose Tissue

In adipose tissue, insulin stimulates glucose uptake via the Glut 4 transporter, providing substrate for energy generation and glycerol synthesis. Even more important effects of insulin in adipose tissue are inhibition of lipolysis and stimulation of free fatty acid (FFA) uptake and triglyceride synthesis. This limits the availability of fat-derived fuels for other tissues and indirectly contributes to the lowering of blood glucose by favoring glucose utilization in multiple tissues.

From this brief overview, it can be seen that the rise in insulin following a meal has multiple tissue-specific actions that serve to lower blood glucose, prevent hyperglycemia, and inhibit the mobilization of alternative metabolic fuels. Many of the metabolic abnormalities that develop acutely in uncontrolled diabetes can be explained by the loss of these actions of insulin.

Pathophysiology of Uncontrolled Diabetes

Uncontrolled diabetes mellitus occurs when circulating insulin levels are inadequate to lower elevated blood glucose concentrations. This condition includes a spectrum of metabolic abnormalities that range from the effects of mild insulin deficiency, that is, hyperglycemia, to the effects of marked and prolonged insulinopenia, that is, ketoacidosis and fluid and electrolyte depletion. Diabetic ketoacidosis, which is the most severe acute manifestation of insulin deficiency, occurs in patients with Type 1 diabetes, those with severe pancreatic disease of other etiologies, and a subset of patients with poorly controlled Type 2 diabetes. In persons without absolute insulin deficiency, although the combination of significant insulin resistance and relatively low levels of insulin can result in

significant hyperglycemia, ketone body production sufficient to cause ketosis and metabolic acidosis usually does not occur. Even low levels of insulin, such as are typically present in Type 2 diabetes, suffice to restrain lipolysis and limit the availability of FFA precursors for ketone body formation. Otherwise, many of the derangements seen in uncontrolled diabetes are common to all forms of diabetes.

The pathophysiologic events that affect blood glucose levels in states of mild-to-moderate insulin deficiency are in two broad categories. First, the normal pathways for glucose clearance after a meal are ineffective; second, body fuel stores are broken down with release of other substrates that lead to inappropriate synthesis of more glucose. These events are brought about by insulinopenia and often are further promoted by the relative abundance of the counter-regulatory hormones, glucagon, catecholamines, and, to a lesser extent, cortisol and growth hormone. In addition, hyperglycemia itself further inhibits pancreatic β cell insulin secretion, compounding the problem ('glucose toxicity').

Following the ingestion of a meal, a substantial portion of the glucose absorbed into the portal circulation is removed by the liver, where it is stored as glycogen, converted to lipid, or consumed via energy-generating pathways. Each of these processes is decreased by insulin deficiency, resulting in increased entry of absorbed glucose to the systemic circulation. Skeletal muscle represents the main tissue site for removal of circulating blood glucose following a meal. In diabetes, insulin deficiency leads to a marked decrease in activity of the Glut 4 glucose transporter largely as a consequence of decreased insulin-stimulated Glut 4 localization to the surface membranes. This decreases the normal postmeal flux of glucose into skeletal muscle. In addition, glucose that does enter

muscle is metabolized inefficiently in the absence of insulin. Other insulin-sensitive tissues such as adipose tissue and myocardium are affected in a similar manner, with consequent reduction both in glucose uptake and metabolism, although their contribution to glucose clearance is quantitatively less than that of muscle.

In postabsorptive or fasted states, hyperglycemia in uncontrolled diabetes does not resolve and often worsens (Figure 2). Abnormally, low-insulin concentrations lead to an exaggeration of metabolic responses that normally serve to protect against the development of hypoglycemia during fasting. These responses to low insulin and elevated counter-regulatory hormones include, initially, the conversion of stored glycogen to glucose. Simultaneously, the hepatic enzymes involved in gluconeogenesis are activated, which results in glucose production from such carbon sources as lactate and pyruvate (byproducts of muscle glycolysis), amino acids (from muscle protein breakdown), and glycerol (derived from adipocyte triglyceride stores). With persistent insulin deficiency, glycogen stores are depleted, and hepatic gluconeogenesis becomes the most important contributor to the increasing hyperglycemia. Meanwhile, body stores of protein and fat are being depleted in the futile synthesis of new glucose that cannot be used efficiently and serves to aggravate the existing hyperglycemia.

Excessive glucose accumulation in the circulation and in the extracellular space leads to the movement of water out of cells to maintain osmotic balance, causing intracellular dehydration. The high filtered load of glucose at the renal glomerulus overwhelms the reabsorptive capacity of the renal tubule, and an osmotic diuresis results. Ultimately, this leads not only to water loss along with the glucose, but also excess

excretion of potassium, sodium, magnesium, calcium, and phosphate in the urine. The magnitude of the total body electrolyte loss depends on the duration and severity of the hyperglycemia. The main symptoms with moderate insulin deficiency are polyuria, and consequent thirst and polydipsia. With more severe and prolonged insulin deficiency, loss of large quantities of glucose in the urine can lead to weight loss. If hyperosmolality is not compensated by an adequate increase in water intake, patients can develop altered mental status and obtundation. In elderly patients with Type 2 diabetes, this sequence can lead to the life-threatening state of nonketotic hyperosmolar coma.

In Type 1 diabetes, the clinical picture of poor control differs from that described above in that insulin deficiency is more severe (see Figure 3). Glucose uptake by muscle is diminished, and glucose production by the liver is augmented. However, marked insulinopenia also leads to rapid, uncontrolled lipolysis. Triglyceride breakdown results in accelerated release of FFA and glycerol. The increased delivery of glycerol from adipose tissue to the liver further promotes hepatic gluconeogenesis. In the absence of insulin, the liberated FFA are taken up by the liver and converted at an accelerated rate to ketone bodies (β -hydroxybutyric acid, acetoacetic acid, and acetone).

In the fasting state in nondiabetic individuals, ketone bodies are metabolized under the influence of even low levels of insulin as a source of energy, particularly in skeletal and cardiac muscle. In extreme insulin deficiency states, ketone body utilization is inhibited at the same time that synthesis is increased. With increasing duration of insulinopenia, the ketoacid levels in the bloodstream rise. Ketones, like glucose,

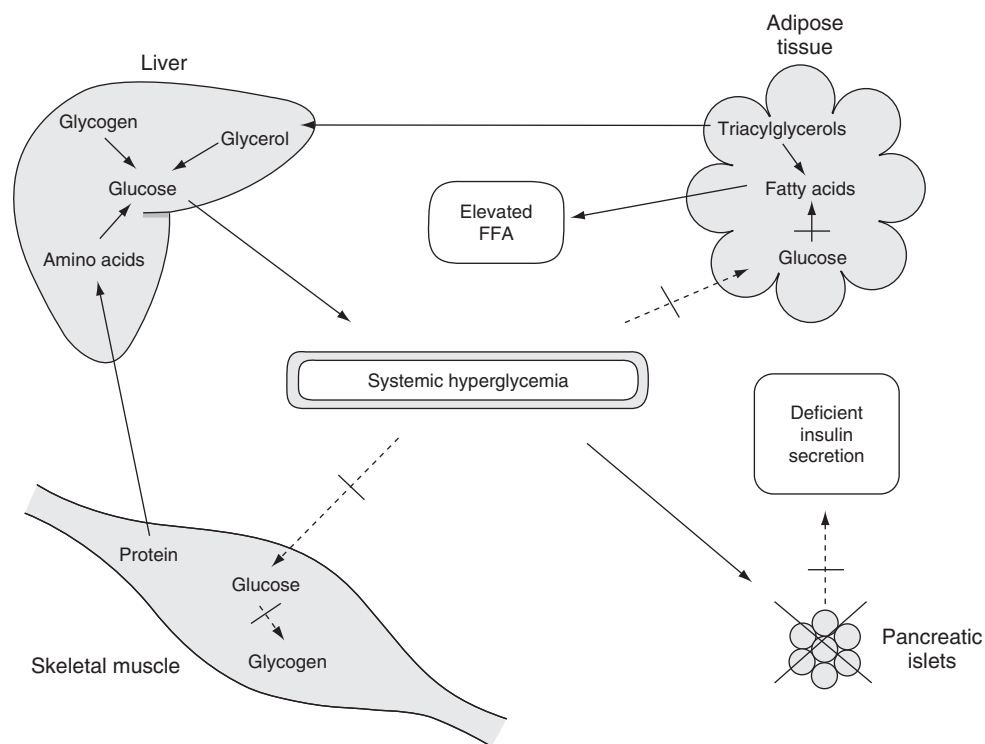


Figure 2 Metabolic events leading to hyperglycemia in the postabsorptive state in uncontrolled diabetes mellitus.

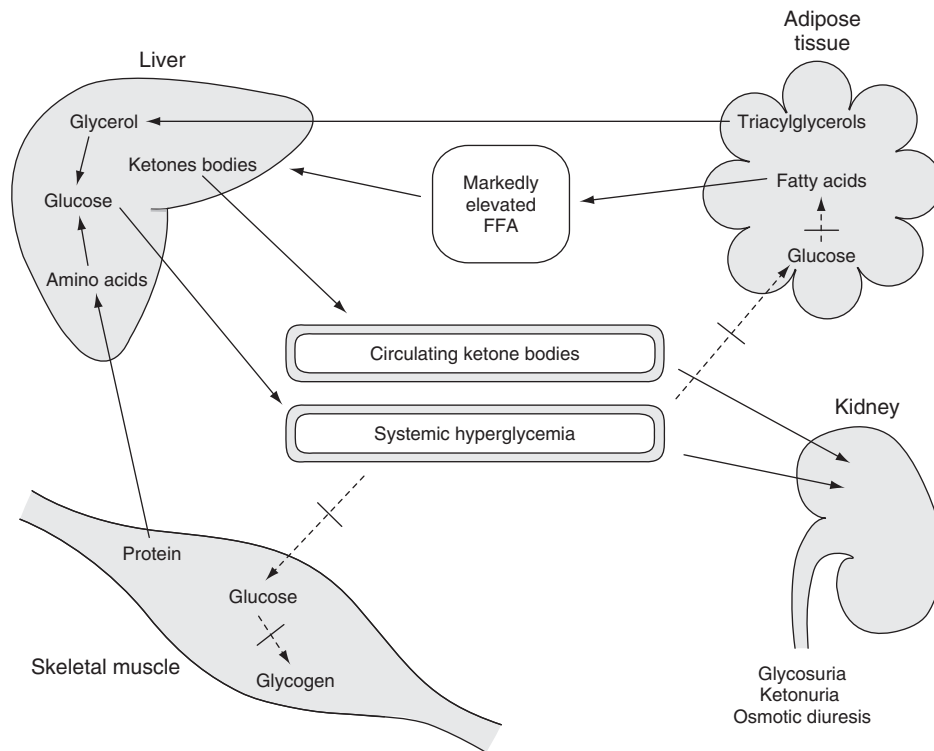


Figure 3 Metabolic events leading to the development of ketoacidosis in uncontrolled diabetes mellitus.

spill into the urine, either as free acids or, depending on the pH, as sodium or potassium salts, worsening the osmotic diuresis and electrolyte deficiency. Eventually, the blood buffering capacity for acid is overwhelmed and systemic acidemia occurs. Acidemia has a deleterious effect on all cell membranes and many cellular functions and, when severe, can cause arrhythmias, cardiac depression, and vascular collapse. In combination with the above-described hyperosmolarity and dehydration, diabetic ketoacidosis is a life-threatening situation.

In summary, poor control can lead to dangerous metabolic consequences and, occasionally, death. A primary goal of therapy is insulin replacement, which is needed to reverse the production of glucose and ketoacids by the liver, to promote muscle glucose and ketone body uptake and to inhibit further breakdown of fat and protein. An equally important goal of therapy should be the replenishment of lost extracellular and intracellular fluids and electrolytes.

See also: Carbohydrates: Regulation of Metabolism. Glucose: Metabolism and Maintenance of Blood Glucose Level

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Dietary Management

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The successful treatment of diabetes mellitus starts with a sound diet, although the specifics of the diet vary depending on the type of diabetes being treated and the individual circumstances. Individualization is the hallmark of medical nutrition therapy in diabetes. Because approximately 90–95% of all people with diabetes have type 2, and approximately 80% of them are either overweight or obese, weight reduction is often the main therapeutic goal. Many individuals also require treatment of comorbidities such as hypertension or dyslipidemia. People with type 1 diabetes, on the contrary, usually require far more attention to exact the quantity and type of carbohydrate they ingest and how their food intake matches their insulin dosages and physical activity level. In all cases, education of the patient by a trained nutritionist is essential. Diabetes is rarely well controlled unless patients have at least a basic understanding of what they should eat and why.

Overall Objectives in the Management of Diabetes

Control of Blood Glucose Level

A first and basic goal of diabetes care is to eliminate the symptoms of hyperglycemia. Treatment is inadequate if the person remains polyuric, thirsty, or continues to lose weight from hyperglycemia. To cause symptoms, however, hyperglycemia usually must average $>10\text{--}11\text{ mmol l}^{-1}$ ($180\text{--}200\text{ mg dl}^{-1}$). As blood glucose above 7 mmol l^{-1} (125 mg dl^{-1}) range is distinctly abnormal and results in long-term diabetic complications, freedom from symptoms is only the beginning of adequate therapy.

Reasonable evidence exists that better control of blood glucose concentration reduces the risk of developing long-term complications from diabetes. This is especially true of microvascular complications such as retinopathy (eye disease), nephropathy (kidney disease), and neuropathy (nerve damage) in both type 1 and type 2 diabetes. Control of blood glucose also reduces the risk of macrovascular disease (heart disease, stroke, and peripheral vascular disease), although the contribution of blood glucose to these complications is less strong.

Carbohydrate ingestion (rather than fat or protein) is the main determinant of postmeal blood glucose level. Dietary intake, oral medications, exogenous insulin therapy, physical exercise, and psychosocial factors (i.e., stress) all contribute to blood glucose levels in the person with diabetes and must be understood when establishing and implementing medical nutrition therapy.

To determine the efficacy of treating glycemia, blood glucose must be monitored. There are two ways to assess glucose control: self-monitoring of blood glucose (SMBG) and laboratory monitoring of hemoglobin A1c (HbA1c). SMBG,

done by obtaining a drop of blood and using a small hand-held meter, measures the blood glucose at the time the measurement is taken. It may be done as often as 6–8 times per day or as infrequently as several times per week. The HbA1c is a laboratory test that reflects glycemic control during the previous 60–90 days and should be done every 3–6 months. Target HbA1c is generally considered to be $<6.5\text{--}7\%$ when the upper limit of normal is $<5.7\%$.

Prevention or Control of Comorbidities

Morbidity and mortality among people with diabetes are rarely due to acute hyperglycemia or diabetic ketoacidosis. Rather, the long-term complications are either specific to diabetes (e.g., diabetic retinopathy or nephropathy) or accelerated by diabetes (e.g., atherosclerosis). Diabetes significantly increases the risk of coronary artery, cerebrovascular, and peripheral vascular disease, with these cardiovascular complications being present in up to 80% of persons with diabetes and the primary cause of death. Prudent dietary management of diabetes therefore often requires concurrent dietary management of cardiovascular disease risk factors such as hypertension and dyslipidemia. For example, all people with diabetes should be on a ‘heart-healthy’ diet to minimize the risk of atherosclerosis. For those with a greater risk for hypertension, even before the first clinical sign, preventative dietary methods (i.e., low-salt diet and weight-loss diet) should be stressed.

Minimum Intrusion on Quality of Life

To people with diabetes, the ‘diabetic diet’ can be a intimidating, often made worse by the way it is presented. Many modern dietitians refuse even to use the word ‘diet’ because of its potential negative connotations, preferring instead ‘nutrition plan’ or ‘medical nutrition therapy.’ Most patients will not totally abandon their lifetime dietary habits, such as forgoing favorite ethnic flavors and socially accepted foods. Rather, the prescribed diet that intrudes least on a person’s quality of life is the most successful nutrition plan. Expert professionals can identify exactly what changes are required and what favorite dishes, spices, or food groups can be built into a good nutrition prescription.

Dietary Approaches to Diabetes

Principles of Dietary Management of Diabetes

Assessment

The first step for planning an appropriate nutrition plan is a full assessment of the diabetic patient. Every individual is

unique and has different needs and circumstances, therefore a deep understanding and assessment is key to a more successful planning and individualized. Topics covered in the nutritional assessment are included in **Table 1**.

Individualization

Individualization is a cardinal principle of medical nutrition therapy for diabetes, facilitating individual lifestyle and behavior changes that will lead to improved metabolic control. As no one diet fits all, a standard printed diabetic diet is inadequate. Rather, people with diabetes need to consult a person trained in dietetics, who is able to develop and teach an individualized nutritional prescription. **Table 2** indicates the range of goals that may need accommodation among different people with diabetes.

Developing the Diabetes Nutrition Plan

With the emphasis on individualization, the meal plan is driven by the diagnosis, pharmacologic treatment, lifestyle, and treatment goals. Important consideration is given to dietary preferences, socioeconomic factors, and the patient's ability to understand and implement instructions. Some patients will need instruction on fine points such as carbohydrate counting and nutrition label reading; others will benefit from

the crudest of prescriptions, such as advice to stop buying concentrated sweets or frequenting fast-food restaurants.

Total Energy Intake

The total energy requirement to maintain constant body weight may be calculated using the Mifflin–St Jeor equation, taking into consideration the patient's activity level. The weight-maintaining requirement is then adjusted according to the therapeutic objective – to accomplish weight loss, maintenance of weight, or weight gain. Examples of how to make these calculations are shown in **Table 3**. Specific conditions such as childhood growth and development, pregnancy, malabsorption, or existing nutritional deficiencies are beyond the scope of this article.

Distribution of Energy Intake

Nutrition recommendations for macronutrient distribution are consistent with all persons to follow a healthy, well-balanced diet. Consumption of macronutrients based on the Dietary Reference Intakes (DRIs) for healthy adults should be encouraged. Any ideal percentage of energy from macronutrients for persons with diabetes has not been strongly supported by research. However, the distribution of carbohydrate, protein, and fat into the total energy target may

Table 1 The nutritional assessment

Diet history/nutrition information – can be obtained using dietary assessment tools such as 24-h recalls, food records, food frequency questionnaires, or dietary intake interviews

Meal patterns: Usual distribution of meals and snacks throughout the day, including variations from day to day, weekdays versus weekends, skipped meals, and external influences such as work, school, travel, vacations, and holidays

Food choices: Types and amount of food consumed at meals and snacks

Nutritional adequacy: Dietary excess or deficiency; also considers overall dietary balance

Beliefs or misconceptions: Fears or misconceptions of a 'strict' diabetic diet or about certain foods; can also include certain religious beliefs or ethnic beliefs about foods

Personal information

Age, gender, socioeconomic status, ethnicity, occupation, education, and literacy level

Ability and willingness to change (stages of change)

Emotional and mental state if distressed by a new diagnosis of diabetes or other health complications related to diabetes

External stressors that may interfere with compliance

Smoking or illicit drug history

Exercise or activity schedule

Clinical information

Type of diabetes and treatment, such as with insulin, oral hypoglycemic drugs, or diet alone

Physical activity, body weight, and blood pressure

Laboratory results, A1C, and lipid profile

Other medical conditions

Education

Diabetes education should be an ongoing interactive process between patient and health professional, and cannot be given in a single session

Individualism is key to successful nutritional management

Most important aspect is to match the type and level of information to individual needs and abilities

Important to provide written information summarizing key messages that patient can take home and refer to later

Follow-up and monitoring progress

Follow-up and review of progress essential

Frequency will depend on type of treatment, glycemic control, and patient's ability to meet goals

Consider if specific dietary targets have been achieved or reasons why targets have not been met and what barriers need to be overcome

Consider acceptability of dietary changes and impact on patient's quality of life

Clinical picture should examine glycemic control, lipid profiles, weight changes, and blood pressure

Table 2 Cases illustrating the variable clinical issues affecting people with diabetes and the resulting diversity of their nutritional needs

Type of diabetes	Type 1	Type 1	Type 2	Type 2
Age (years)	14	38	56	76
Duration of DM (years)	6	26	6	6
BMI	18	23	27	34
Physical activity	Vigorous	Moderate	Mild	Minimal
Prone to hypoglycemia	Yes	Yes	Yes	No
Prone to hyperglycemia	Yes	Yes	Yes	Yes
Blood lipids	Normal	Normal	High LDLcholesterol	High TG and low HDL
Blood pressure	Normal	High	Normal	High
Type of diabetes	Type 1	Type 1	Type 2	Type 2
Dietary preferences	Likes sweets and snacks	Healthy, little carbohydrate awareness	Spicy foods and irregular meals	Fried foods and sweets
Pharmacologic therapy	Multiple-dose insulin	Multiple-dose insulin	Oral agents plus insulin	Oral agents
Life expectancy without diabetes	66 years more	44 years more	26 years more	8 years more
Major nutritional considerations	Adequate caloric intake for growth (see Table 3)	Stabilize carbohydrate intake, count carbohydrates	Mildly hypocaloric (see Table 3)	Low salt, high vegetable for hypertension (DASH diet)
	Recognize carbohydrate portions, regularize carbohydrate intake	Low salt, high vegetable for hypertension (DASH diet)	Hypolipemic (low saturated fat)	Hypolipemic diet (low saturated fat)
	Avoid excess concentrated sweets		Regularity of meals, consistency of carbohydrate and fat intake	Moderately hypocaloric
	Learn factors causing hypoglycemia			Control of dietary carbohydrate, especially high-energy concentrated sweets
	Healthy heart diet			

BMI, body mass index; DM, diabetes mellitus; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

depend on individual needs and therapeutic objectives, such as progressive renal disease which may require more restricted protein intake or for type 2 diabetics on a low-carbohydrate diet for weight loss. The Recommended Dietary Allowance (RDA) for digestible carbohydrate is 130 g day⁻¹ for adults. This is based on the average amount of glucose utilized by the brain. However, the average median intake is 220–330 g day⁻¹ for men and 180–230 g day⁻¹ for women in the USA. Specifics of the DRIs and RDA for major macronutrients are depicted in [Table 4](#) and should be used as a guide, as stated before, customized to the individual and their individual therapeutic needs.

Even these broad guidelines are the subject of considerable controversy, with some experts recommending a lower carbohydrate and higher fat intake, particularly of mono-unsaturates. A common mistake is for patients to think they have a diet low in both carbohydrate and fat without being low in total energy intake. One must also take into consideration that carbohydrates are an important source of fiber, vitamins, minerals, and energy, and fat an important source of fat-soluble vitamins and essential fatty acids. This is particularly true for ω -3 fatty acids, found in fatty fish, such as salmon. Fat and protein intake also have satiating effects that can help with the spreading of the meals during the day. Distribution of energy intake through the day may vary. Insulin-

Table 3 Sample calculations of energy requirement in differing circumstances using the Mifflin–St Jeor formula to determine caloric requirements for children and adults

Caloric requirements = basal metabolic rate \times activity factor \times injury factor

Basal metabolic rate (BMR)

For men: BMR = 10 \times weight (kg) + 6.25 \times height (cm) – 5 \times age (years) + 5

For women: BMR = 10 \times weight (kg) + 6.25 \times height (cm) – 5 \times age (years) – 161

Multiply by the following factors:

Activity factors

1. Sedentary (little or no exercise): BMR \times 1.2

2. Lightly active (light exercise/sports 1–3 days week⁻¹): BMR \times 1.375

3. Moderately active (moderate exercise/sports 3–5 days week⁻¹): BMR \times 1.55

4. Very active (hard exercise/sports 6–7 days week⁻¹): BMR \times 1.725

5. Extra active (very hard daily exercise/sports and physical job or 2x day training): BMR \times 1.9

For weight loss, use the above calculated formula for caloric requirements and subtract by 500 calories:

Caloric requirements – 500 calories per day = modified calorie requirements

This is for a recommended 0.5–1 pound of weight loss per week

Table 4 Dietary reference intakes for macronutrients: carbohydrates, protein, and fat

<i>Nutrient</i>	<i>Function</i>	<i>Life stage group (years)</i>	<i>Recommended Dietary Allowance/adequate intake (g day⁻¹)</i>	<i>Acceptable Macronutrient Distribution Range (AMDR) (expressed in percentage of total energy intake)</i>	<i>Selected food sources</i>
Total fiber	Improves laxation, reduces risk of coronary heart disease, and assists in maintaining normal blood glucose levels	9 to >70	130	45–65	Includes dietary fiber naturally present in grains (such as found in oats, wheat, or unmilled rice) and functional fiber synthesized or isolated from plants or animals and shown to be of benefit to health.
		Females	130	45–65	
		9 to >70			
		Males			
		9–13	31		
		14–50	38		
		50 to >70	30		
		Females	26		
		9–18	25		
		19–50	21		
Protein and amino acids	Serves as the major structural component of all cells in the body, and functions as enzymes, in membranes, as transport carriers, and as some hormones. Broken down protein into amino acids become the building blocks with nine of the amino acids that must be provided in the diet—termed indispensable amino acids.	50 to >70		25–35	Proteins from animal sources, such as meat, poultry, fish, eggs, milk, and cheese and yogurt, provide all nine indispensable amino acids in adequate amounts (called ‘complete’ proteins). Proteins from plants, legumes, grains, nuts, seeds, and vegetables tend to be deficient in one or more of the indispensable amino acids (‘incomplete’ proteins). Can be ‘complete’ by combining incomplete sources together which lack the different indispensable amino acids.
		9–18		20–35	
		19 to >70		25–35	
		Females		20–35	
		9–18			
		19 to >70			
		Males			
		9–13	34	10–30	
		14–18	52	10–30	
		19 to >70	56	10–35	
Protein and amino acids	Serves as the major structural component of all cells in the body, and functions as enzymes, in membranes, as transport carriers, and as some hormones. Broken down protein into amino acids become the building blocks with nine of the amino acids that must be provided in the diet—termed indispensable amino acids.	Females	34	10–30	Proteins from animal sources, such as meat, poultry, fish, eggs, milk, and cheese and yogurt, provide all nine indispensable amino acids in adequate amounts (called ‘complete’ proteins). Proteins from plants, legumes, grains, nuts, seeds, and vegetables tend to be deficient in one or more of the indispensable amino acids (‘incomplete’ proteins). Can be ‘complete’ by combining incomplete sources together which lack the different indispensable amino acids.
		9–13	46	10–30	
		14–18	46	10–35	
		19 to >70			
		Males			
		9–13	34	10–30	

requiring diabetic patients, for example, may need a more evenly distributed energy intake, even including a bedtime snack to avoid hypoglycemia. For those on fixed insulin doses, consistent carbohydrate intake may provide easier blood glucose control, but for those on intensive insulin regimens or insulin pump therapy, variable carbohydrate intake can be based on insulin-to-carbohydrate ratios. This would not necessarily be indicated for someone with type 2 diabetes trying to lose weight, although weight-reducing diets are generally considered more effective if the total energy intake is spread more or less evenly throughout the day so that the patient does not build up a hunger and gorge late in the day. One report on Muslims observing daytime fasting during Ramadan found that more than half did not lose weight, suggesting a major redistribution of caloric intake to nighttime hours. A significant increase in hypoglycemia occurred during the days of Ramadan. Reduced energy intake for prolonged periods is most dangerous for patients taking insulin, but it may also be significant in those taking oral hypoglycemic agents such as sulfonylureas.

The Utility of Exchange Lists

There has been a shift on the part of patients and most health professionals away from the use of formal 'exchange lists' for meal planning. The traditional exchange lists which estimate not only carbohydrate but also certain proportions of fat and protein in similar foods are no longer emphasized. Carbohydrate counting and calculations using food labels give the patient more freedom and flexibility with more choices. Food labels make the calculation of specific fat and carbohydrate content easier. The trend, therefore, is to emphasize the total carbohydrate in gram amounts or by carbohydrate 'choices,' where one choice is equal to 15 g of carbohydrate. Examples of 15-g carbohydrate choice include a slice of bread, one-third cup of pasta or rice, or a small apple. Fat intake should also be addressed with more emphasis on the types of fats, saturated versus mono- and polyunsaturated. This shift in teaching allows for more emphasis on specific carbohydrate and fat awareness rather than lumping mixed foods together in exchanges.

Gastroparesis

An extremely difficult clinical challenge is posed by the patient with diabetic gastroparesis. This condition, a severe autonomic neuropathy reducing gastric motility and gastric emptying time, can sometimes be difficult to diagnose by standardized testing, such as gastric emptying studies. Gastroparesis typically causes early satiety, nausea, vomiting, and abdominal pain, with markedly variable food ingestion. Along with pharmacologic management and good glycemic control, the dietary prescription should include small, frequent feeding as tolerated, but the condition can progress to the point that any oral intake is difficult, and tube feeding or a gastrostomy is required. Fortunately, diabetic gastroparesis tends to wax and wane in severity.

Glycemic Control and Weight Gain

Research studies have repeatedly found that when a patient with poor glycemic control achieves improved glucose levels, there is a strong, almost inevitable, tendency to gain weight.

This may simply be due to the retention of energy that was previously lost in the urine as glucosuria, but the patient should be warned of the likelihood of gaining weight when poor diabetic control is adequately treated. As for quitting smoking, the health benefit of glycemic control far outweighs the risk of weight gain with smoking cessation.

Nutritional Instruction

To achieve successful nutritional outcomes, there are several key educational factors that must be considered. Basic survival skills for the patient include understanding the relationship of food to insulin and activity, reinforcement of maintaining or achieving a healthy weight, emphasizing the importance of good nutrition in the control of blood glucose and lipid levels, and understanding the types of nutrients, their functions, relation to insulin, and effect on blood glucose through in-depth education and counseling. Meal planning includes types and amounts of food, incorporating dietary fiber, understanding proper serving sizes, management of eating out and special occasions, as well as incorporating favorite recipes. There also needs to be instruction on modifications of food intake during brief illnesses and changes in food intake based on activity level. For those requiring a more comprehensive approach, carbohydrate counting, nutrition label reading, and self-monitoring blood glucose levels are also vital factors. As mentioned earlier, the key to success is individualizing the instruction to the patients' needs and lifestyle.

Special Aspects: Type 2 Diabetes

There are two pathophysiologic mechanisms underlying type 2 diabetes: the body's cells are resistant to the action of insulin (insulin resistance), and the pancreas is unable to secrete enough insulin to overcome that resistance (relative insulin insufficiency). Although it is not entirely clear which process occurs first, and the balance of the two processes may vary from individual to individual, the most common cause of insulin resistance is overweight or obesity usually in the setting of a strong family history of type 2 diabetes. Unfortunately, much evidence has shown that people of Asian ethnicity are especially prone to obesity-related type 2 diabetes even when their body weight, by Western standards, is normal. Japanese Americans, for example, show an increased risk of diabetes if their body mass index (BMI) increases to only 24. This excessive risk with even mild degrees of excess body weight may explain the marked rise in diabetes when previously undernourished populations begin to have adequate nutrition. In this context, diabetes has been described as a disease of prosperity.

Major Objectives

Approximately 90–95% of all people with diabetes have type 2, and the major increase in the prevalence of diabetes in recent years is almost entirely accounted for by trends toward increasing body weight. It cannot be over-emphasized that medical nutrition therapy of type 2 diabetes should address normalization of body weight. In most cases, the focus is on reducing dietary intake of saturated fat and increasing energy expenditure through exercise. By reducing body weight, insulin resistance is reduced, making

the patient's endogenous insulin more effective. Given that the majority of people with type 2 diabetes die of cardiovascular causes, the second emphasis of medical nutrition therapy for type 2 diabetes must address dyslipidemia and blood pressure.

Hypoenergetic diets are remarkably effective in controlling hyperglycemia. Indeed, blood glucose levels improve, often dramatically, as soon as a low-energy diet is started, apparently by reducing hepatic glucose production. The correction of insulin resistance is more closely related to actual weight loss, which takes much longer. The best strategy for accomplishing and maintaining weight loss is unclear and may vary from person to person depending on the different factors involved, such as willingness to change and other lifestyle behaviors. Dosages of antidiabetic drugs may have to be altered as the person loses weight.

Persistent insulin resistance in type 2 diabetes, together with deteriorating pancreatic insulin secretion over time, results in many people with type 2 diabetes eventually requiring exogenous insulin therapy. This does not change the diagnosis to type 1 diabetes, which is a disease of entirely different pathogenesis. Because of the insulin resistance, people with type 2 diabetes taking insulin often need high doses, often 50–100 units per day or higher. Insulin requirements will predictably be less when energy intake is reduced.

Recently, in Western societies, many overweight teenagers have presented with type 2 diabetes. It can no longer be assumed that children with diabetes have type 1. Indeed, some reports find that many more teenagers with diabetes have type 2, a marked shift from prior years, particularly among ethnic minorities. Furthermore, nutrition therapy for children with diabetes must be designed with a clear understanding of what type of diabetes they have. In cases of obesity-related type 2, calorie restriction may be indicated.

Coexisting Risk Factors

Obesity, dyslipidemia, and hypertension are especially prevalent in type 2 diabetes. The constellation of comorbidities has been called metabolic syndrome, 'syndrome X,' or the insulin-resistance syndrome (Table 5) and is associated with increased risk of future cardiovascular disease. Of note, the definition of elevated waist circumference varies by population and organization; non-Caucasian populations often have lower cutoffs than those shown below. Some investigators believe that insulin resistance is the primary underlying defect in metabolic syndrome. Whatever the pathophysiologic mechanisms, it is clear that dyslipidemia and hypertension must be diagnosed and aggressively treated if present. In fact, most evidence suggests that the management of coexisting risk factors, particularly hypertension, dyslipidemia, and smoking, is more important than the treatment of hyperglycemia in preventing cardiovascular morbidity and mortality.

Special Aspects: Type 1 Diabetes

With type 1 diabetes, there is essentially no endogenous insulin secretion remaining, due to autoimmune destruction of the insulin-producing β cells of the pancreas. The inability to produce this essential hormone necessitates lifelong injection of insulin, often multiple times daily. Furthermore, the replacement of a finely tuned normal insulin secretory

Table 5 The metabolic syndrome

Three or more of the following components

Central obesity as measured by waist circumference
Men: > 102 cm (40 in)
Women: > 88 cm (35 in)
Fasting blood triglycerides ≥ 1.69 mmol l ⁻¹ (150 mg dl ⁻¹) or on drug therapy
Blood HDL cholesterol
Men: < 1.04 mmol l ⁻¹ (40 mg dl ⁻¹) or on drug therapy
Women: < 1.29 mmol l ⁻¹ (50 mg dl ⁻¹) or on drug therapy
Blood pressure $\geq 130/85$ mmHg or on drug therapy
Fasting glucose ≥ 6.1 mmol l ⁻¹ (110 mg dl ⁻¹) or on drug therapy

mechanism, which continually provides insulin 'on demand' precisely in response to the degree of hyperglycemia, cannot be well reproduced by injections, explaining the glycemic lability of type 1 diabetes.

Major Objectives

Generally, the treatment objective in type 1 diabetes is stabilization of glycemic control in an acceptable range, control of other risk factors, and thus avoidance of long-term complications. This requires close attention not only to diet but also to its interrelationships with insulin dose and timing, physical activity, stress, and other psychosocial factors. In fact, despite the best efforts, almost all people with type 1 diabetes are prone to wide swings of blood glucose, sometimes from 2.8 to 17 mmol l⁻¹ (50–300 mg dl⁻¹) or more during a day.

To control the intrinsic 'brittleness' of type 1 diabetes, the individual needs to learn to stabilize dietary intake, making it as reproducible as possible. If carbohydrate, in particular, varies significantly from day to day and meal to meal, the person must learn to adjust insulin doses to match the changed intake. Carbohydrate counting helps stabilization of the diet or adjustment in insulin doses. It is useful for the nutritionist to understand the various insulin regimens that people with type 1 diabetes are given. Several different typical regimens, with comments on the dietary implications, are shown in Figure 1.

In addition to carbohydrate awareness, dietary fat intake should be taken into consideration. Dietary fat is often the main determinant of serum lipids and contributes significantly to total energy intake and thus body weight. It also delays gastric emptying, prolonging the glycemic response to dietary carbohydrate.

Very few people continue to measure and weigh food, but weighing is a useful tool during the instruction phase. Ultimately, people with type 1 diabetes should become proficient in estimating the carbohydrate content of food so that their food selection becomes habitual.

Energy intake distribution will depend on the type of insulin, the number of injections, and the aggressiveness of glycemic targets. Often, small changes in food ingestion can make a significant difference. If, for example, a patient tends to develop hypoglycemia at approximately noon, the skillful dietitian can either emphasize the necessity of eating lunch regularly before noon or suggest the patient consume some of the lunch carbohydrates as an 11 a.m. snack. These changes may eliminate the need to change

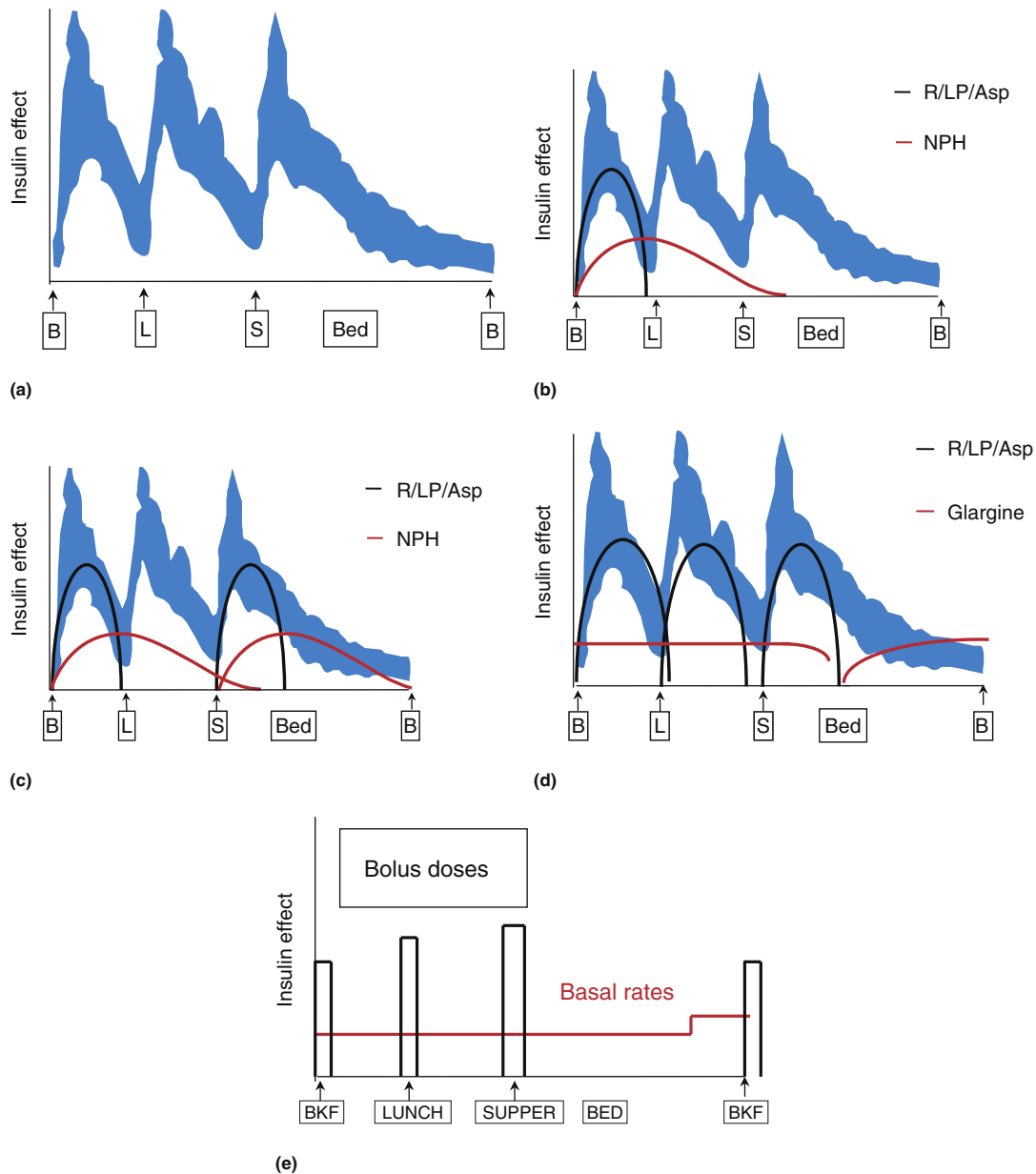


Figure 1 Insulin regimens and notes on the nutritional intake required. (a) The normal insulin response to three meals (breakfast (B), lunch (L), and supper (S)). Note that insulin increases sharply after ingestion of a carbohydrate-containing meal, declining to baseline within several hours. (b) When a combination of short-acting and intermediate-acting insulin is given only at breakfast, the normal response to breakfast is reproduced and the intermediate-acting insulin 'covers' lunch. It is important that the patient ingest a regular breakfast and lunch in order to avoid hypoglycemia from the insulin present at these times. (c) When a combination of short-acting and intermediate-acting insulin is given at breakfast and supper, there is better 'coverage' of the supper meal, but the intermediate-acting insulin peaks near bedtime and the middle of the night, so a bedtime snack may be necessary. (d) A more intensive regimen provides insulin as a 'basal' dose at bedtime, lasting the full 24 h, and short-acting insulin with every meal, for a total of four doses per day. This regimen usually requires patients to monitor their own blood glucose before each meal to adjust their short-acting insulin dose to both the amount of carbohydrate to be ingested and the blood glucose level at the time of ingestion. This regimen does provide more flexibility of meal timing. (e) Use of an external insulin pump infuses insulin at a precise basal rate, and the patient signals the pump to deliver bolus doses of insulin with each meal. As with D, regular monitoring is required as well as accurate understanding of the content of the meal to be ingested. Basal rate can be adjusted, for example, to avoid nighttime hypoglycemia, and there is flexibility of when meals are eaten.

insulin dose. Alternatively, if weight loss and caloric restriction are desired, then the prebreakfast insulin dose can be decreased.

Especially with intensive insulin therapy (three or four daily injections or an external insulin pump), there is

some flexibility in the timing of the meals but also a need for more accurate assessment of meal content. Some patients will learn their own ratio of grams of carbohydrate to insulin dose necessary to maintain blood glucose in a good range.

Eating disorders pose a serious problem to the management of type 1 diabetes. Presumably because people with diabetes are often diet conscious, the prevalence of eating disorders is surprisingly high among teenagers with diabetes. The problem is especially dangerous because young people may skip insulin injections in order to induce glucosuria, a sort of 'metabolic purging.' These conditions clearly require prompt professional help.

Growth and Development

The total daily energy intake of a person with type 1 diabetes should be calculated to maintain normal growth and development in a child and normal weight in an adult. Examples of these calculations are provided in **Table 3**. As most people with type 1 diabetes are not overweight, they do not need low-energy diets. In fact, underfeeding is a poor way to maintain blood glucose control. The energy needed to establish and maintain normal weight should be matched with the insulin needed to control glycemia. There is no need for a thin or normal-weight person with type 1 diabetes to be perpetually hungry.

Special Aspects of Dietary Management of Other Types of Diabetes

Other types of diabetes include those with relatively well-recognized etiologies, such as pancreatectomy-induced diabetes, diabetes due to pancreatitis, cystic fibrosis, iron infiltration of the pancreas (hemochromatosis), or rare syndromes of insulin resistance.

Pancreatitis may be secondary to severe hypertriglyceridemia (triglyceride content $> 1100 \text{ mmol l}^{-1}$ (1000 mg dl^{-1})). In this case, a very low-fat diet is often indicated. When there is widespread destruction of pancreatic cell mass, as with cystic fibrosis, pancreatectomy, or extensive cancer, the exocrine as well as endocrine functions are affected, leading to malabsorption and impaired glucagon secretion. Malabsorption causes steatorrhea and may require pancreatic enzyme replacement to avoid marked variability in carbohydrate as well as fat absorption. Lack of the hormone glucagon increases the risk of severe hypoglycemia after insulin administration because there is less counterregulatory ability to raise blood glucose levels after mild hypoglycemia.

Effects of Ingested Nutrients on Blood Glucose

Carbohydrate

Carbohydrate ingestion causes blood glucose to increase and is one of the major determinants of postprandial glucose levels. In people without diabetes, the normal increase in blood glucose is approximately $0.5\text{--}2.8 \text{ mmol l}^{-1}$ ($10\text{--}50 \text{ mg dl}^{-1}$) above baseline, returning to baseline within 1–3 h. The pancreatic hormonal response to dietary carbohydrate mediates the return to normal. Insulin is the central mediator of energy metabolism. The basics of insulin-dependent energy metabolism in the fed and the fasting states are depicted in **Figure 2**.

Although carbohydrate intake plays the major role in postprandial blood glucose, there are other factors to consider. Diet is not the only source of glucose in blood; hepatic gluconeogenesis maintains blood glucose in the absence of dietary intake. For example, when a person is ill and dietary intake is curtailed, it would be a mistake to stop insulin administration because hepatic glucose production may in fact be increased. Sick-day instruction is essential for people with diabetes so that they do not simply stop their treatment if they are not eating well although doses may need to be modified. Pharmacologic therapies (insulin or oral agents), of course, also affect blood glucose.

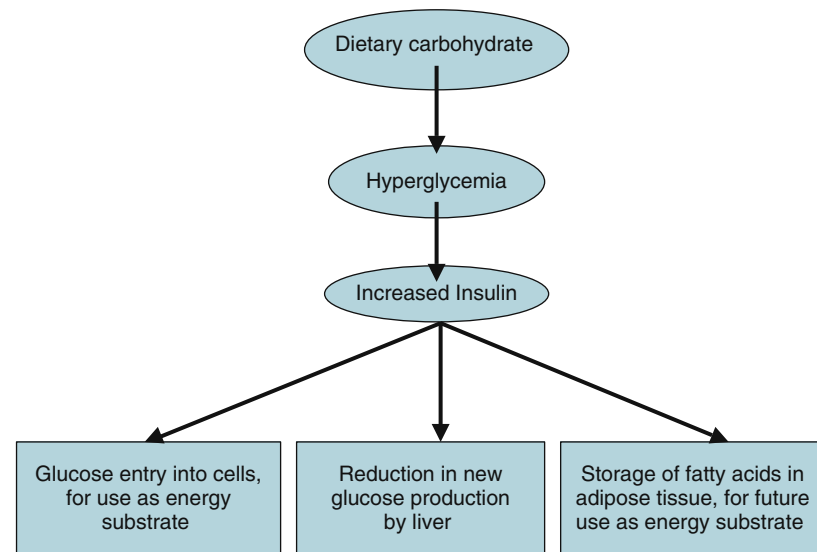
A long-standing debate has surrounded the optimal proportion of intake from carbohydrate, fat, and protein. People with diabetes, especially when insulin is administered, will discover that if they hold back carbohydrate their blood glucose does not increase as much. However, holding back carbohydrate unless the diet is hypocaloric, inevitably leads to a high-fat diet, and carbohydrate restriction leaves insulin with no substrate to act on. In our experience, this can cause blood glucose levels to be more unstable, susceptible to swings of hypoglycemia and hyperglycemia. The authors support the recommendation of most professional guidelines that carbohydrate should make up a substantial percentage (50–60%) of total nutrient intake, particularly in the form of complex carbohydrates (see below).

Two areas of controversy and of nutrition research deserve special attention: the glycemic response to oral sucrose (concentrated sweets) versus complex carbohydrates and the glycemic index (GI).

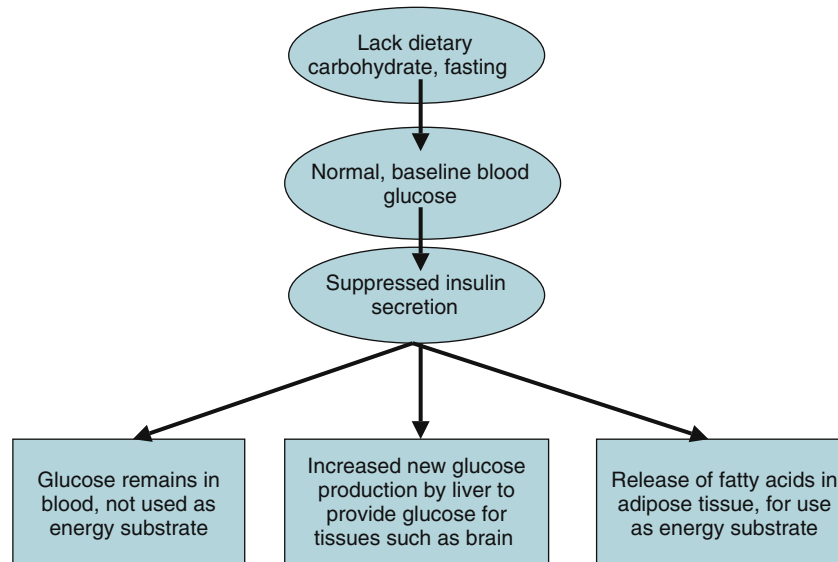
Sucrose Versus Complex Carbohydrate

Careful metabolic studies suggest that, gram for gram, sucrose does not increase blood glucose more than complex carbohydrates, either acutely or over a matter of weeks. In these studies, sucrose was isoenergetically substituted for other carbohydrates, mostly under carefully defined research ward conditions in which precise substitutions can be made. Because complex carbohydrates and sucrose are both digested to monosaccharides before they are absorbed, it is not unexpected that each should cause the same glycemic excursion if administered in the same number of grams. It does run counter, however, to the traditional advice that people with diabetes should avoid concentrated sweets.

A number of organizations have cited these research studies in support of a recommendation that allows ingestion of concentrated sweets. The caveat, in the words of the American Diabetes Association, is that "sucrose should be substituted for other carbohydrate sources in the food/meal plan." In the authors' view, there is a practical fallacy in this recommendation: People are unlikely to substitute sucrose for complex carbohydrates in equal amounts. Owing simply to taste, concentrated sweets are likely to be taken in far greater quantity than the more filling and less sweet starches. Thus, in reality, people who routinely eat concentrated sweets are likely to have greater and less predictable glycemic excursions than those who stick to complex carbohydrates. There is also the



(a)



(b)

Figure 2 Influence of insulin on basic energy metabolism. (a) With dietary carbohydrate intake, hyperglycemia induces insulin secretion that acts to enhance glucose entry into cells for utilization as metabolic fuel. Simultaneously, insulin decreases new glucose production in the liver, as dietary glucose is already available and excess stored in liver as glycogen, and stores the remaining excess caloric intake in adipose tissue as fat. (b) With lack of dietary carbohydrate, as in fasting, the reverse occurs: With lower blood glucose, insulin secretion is suppressed. This minimizes entry of glucose into cells but stimulates enough new glucose production from the liver to provide for obligate glucose-using tissues such as the brain. Meanwhile, low insulin concentration promotes fatty acid release from adipose tissue to serve as an alternate fuel for metabolism.

significant risk that excess concentrated sweet intake will cause weight gain (as well as dental caries). However, if a person with diabetes can include a fixed amount of concentrated sweet in his or her diet and can demonstrate that his or her diabetes is well controlled and the postmeal glycemia is not excessive, there is no reason to deny the person the sweet. The other consideration is that complex carbohydrates are superior sources of fiber, vitamins, and minerals, and therefore, should be the larger portion of carbohydrate intake.

Glycemic Index

The GI is defined as the area under the 2-h curve of blood glucose after the ingestion of a set amount of carbohydrate compared with ingestion of the same amount of carbohydrate from a reference food (white bread or glucose). The GI is expressed as a percentage of the standard food value:

$$GI = \frac{\text{Area under the curve of the food}}{\text{Area under the curve of standard food}} \times 100$$

The glycemic load (GL) is an additional measure in which the amount of carbohydrate in a typical portion is taken into account. **Table 6** provides examples of foods high and low in GI and GL. These indices have been calculated for more than 500 different carbohydrates, and values are readily available on the Internet. A number of factors in addition to the reported GI and GL actually affect the blood glucose response to meals, however, because mixed meals are ingested in everyday living. Among these are the fat and fiber content of the meal, type of cooking, the patient's absorptive rate, and micro-nutrient content.

In the authors' opinion, the concept of GI is valid in a research sense: Certain carbohydrates, gram for gram, do raise blood glucose levels more, or with different glycemic patterns, than others. However, they believe that basing nutrition plans on the GI and the GL of foods is usually too much of a burden for people with diabetes, who have to closely monitor the total amount of carbohydrates. It is more practical to encourage people to learn their own glycemic response to different foods from experience. They may learn, for example, that far more insulin is needed before eating pizza or a bagel; they may learn to avoid certain 'desserts.' A general awareness of what preferred foods, in what amounts, raise blood glucose may be more practical than memorizing GI or GL.

Protein

Since the classic experiments by Benedict in the 1910s, it has been known that protein ingestion causes hyperglycemia and glucosuria. The effect of protein ingestion on blood glucose, however, is far less pronounced than the effect of carbohydrate ingestion. A rule of thumb is that a gram of protein raises blood glucose approximately one-third as much as a gram of carbohydrate. In most diets, approximately 50–100 g protein is ingested per day, compared with approximately 200–300 g carbohydrate. Therefore, protein is a calorically less significant part of the diet and far less important in regulating blood

glucose. Although protein ingestion affects postprandial blood glucose significantly less than carbohydrate, high-protein diets are not recommended particularly in anyone with diminishing renal function. In people with type 2 diabetes, protein does not slow the postprandial absorption of carbohydrate. The same cannot be said about dietary fat.

Fat

Dietary fat has little, if any, immediate effect on blood glucose concentration because the constituent fatty acids do not produce new glucose and the glycerol moieties are insignificant in their contribution to blood glucose. However, there is considerable evidence that circulating free fatty acids promote gluconeogenesis and hyperglycemia. In a normal overnight fast this is good: Fatty acids help maintain normoglycemia. However, in uncontrolled diabetes, when fatty acids can be very high, they significantly worsen hyperglycemia. This has been referred to as 'fat toxicity.'

Fat ingestion slows gastric emptying. The delayed delivery of carbohydrate to the circulation can cause a late, slow postprandial rise in blood glucose, although people who do not self-monitor frequently are unlikely to be aware of this effect of dietary fat.

Non-nutritive Sweeteners

Sweeteners are important to the quality of life of people with diabetes. An essential distinction is to differentiate those with from those without significant energy content. **Tables 7 and 8** provide many of the available non-nutritive and nutritive sweeteners. The non-nutritive sweeteners have no or virtually no energy content, and they can be consumed without concern about their effect on blood glucose. They have been determined to be safe when consumed within the daily intake levels established by the Food and Drug Administration (FDA).

Many 'diet' sweeteners, such as sorbitol or fructose-based snacks, do cause at least some degree of hyperglycemia. Sugar

Table 6 Examples of foods high and low in glycemic index (GI) and glycemic load (GL)^a

	GI	Serving size (g)	GL
Low GI/low GL			
Apple, NS (USA)	40	120	6
Oranges (Sunkist, USA)	48	120	5
Healthy Choice hearty seven-grain bread (USA)	55 ± 6	30	8
Ice cream, premium, French Vanilla – 16% fat (Australia)	38 ± 3	50	3
Kidney beans (USA)	23	150	6
Pizza, Super Supreme, thin, and crispy – 13.2% fat, Pizza Hut (Australia)	30 ± 4	100	7
Low GI/high GL			
Barley (<i>Hordeum vulgare</i>) (India)	43 ± 6	150	26
High GI/low GL			
Watermelon, raw (Australia)	72 ± 13	120	4
White wheat flour bread	70	30	10
High GI/high GL			
Cornflakes (Kellogg's, USA)	92	30	24
Bagel, white, frozen (Lenders, Canada)	72	70	25
White rice, type NS, boiled 13 min (Italy)	102	150	31

^aHigh GI is considered >70 and low <55. High GL is considered >20 and low <10.

Source: Adapted with permission from Foster-Powell K, Holt SH, and Brand-Miller JC (2002) International table of glycemic index and glycemic load values. *American Journal of Clinical Nutrition* 76(1): 5–56.

alcohols (polyols) such as sorbitol, mannitol, and xylitol are classified as hydrogenated monosaccharides, hydrogenated disaccharides, and oligosaccharides. They do contain calories, but because they are only partially absorbed in the small intestine, they have a reduced energy value per gram. Excessive use of sugar alcohols has laxative effects and can cause diarrhea.

It is important for people with diabetes to understand clearly these distinctions because many calories can be ingested with foods labeled as 'diet' under the false assumption that they are without effect on blood glucose.

Trace Elements, Vitamins, and Minerals

There has been recurrent interest in whether such trace elements and minerals as chromium, potassium, magnesium, vanadium, and zinc affect blood glucose control in diabetes. It would obviously be attractive if simple oral supplements could facilitate normoglycemia. The evidence, though, is slim and unconvincing that supplementation of any of these trace elements has a beneficial effect except when there is a true deficiency. Such deficiency may occur in an undernourished setting, in the elderly with poor dietary intake, or in certain strict vegetarian diets.

The same conclusion can be reached regarding vitamin supplementation: it is indicated when vitamin deficiency is suspected or likely. For example, populations such as the elderly, those pregnant or lactating, strict vegetarians, or those on a calorie-restricted diet may require vitamin supplements. Folate supplementation is well documented to improve the outcome of pregnancy, with or without diabetes. However, there is no clear evidence that supplementation is helpful for those eating an adequate diet.

Antioxidants such as vitamins C, E, or A and α -lipoic acid are the object of intensive research. It is unclear whether or which of these actually prevent long-term complications of diabetes, but the literature should be monitored. Vitamins B₁, B₆, and B₁₂ are sometimes used to treat diabetic peripheral neuropathy, but without much supporting evidence of benefit. On the contrary, calcium supplementation is indicated, particularly in the elderly, if daily intake is <1.0–1.5 g.

In summary, evidence is weak that vitamin or trace element deficiencies occur due to diabetes. Supplementation in more normal circumstances has little or no role in the control of diabetes, and general nutritional guidelines for vitamins and trace elements should be followed. This may be particularly the case with vitamin D, because deficiencies have been reported in many populations due to decreased sun exposure, aging, and lactose intolerance.

Table 7 Non-nutritive sweeteners

Type	US brand names	kcal g ⁻¹	Description
Saccharin	Sweet and Low, Sweet Twin, Sweet 'N Low Brown and Necta Sweet	0	200–700 times sweeter than sucrose; noncarcinogenic and produces no glycemic response
Aspartame	Nutrasweet, Equal and Sugar Twin (blue box)	4	160–220 times sweeter than sucrose; noncarcinogenic and produces limited glycemic response
Acesulfame-K	Sunett, Sweet & Safe, and Sweet one	0	200 times sweeter than sucrose; noncarcinogenic and produces no glycemic response
Sucralose	Splenda	0	600 times sweeter than sucrose; noncarcinogenic and produces no glycemic response

Table 8 Polyols and novel sugar sweeteners

Type	kcal g ⁻¹	Description
Monosaccharide polyols or novel sugars		
Sorbitol	2.6	50–70% as sweet as sucrose; some people experience a laxative effect from a load ≥ 50 g
Mannitol	1.6	50–70% as sweet as sucrose; some people experience a laxative effect from a load ≥ 20 g
Xylitol	2.4	As sweet as sucrose
Erythritol	0.2	60–80% as sweet as sucrose; also acts as a flavor enhancer, formulation aid, humectant, stabilizer and thickener, sequestrant, and texturizer
Disaccharide polyols or novel sugars		
Isomalt	2	45–65% as sweet as sucrose; used as a bulking agent
Lactitol	2	30–40% as sweet as sucrose; used as a bulking agent
Maltitol	2.1	90% as sweet as sucrose; used as a bulking agent
Polysaccharide polyols		
HSH	3	25–50% as sweet as sucrose; other names include hydrogenated starch hydrolysates and maltitol syrup

Source: Adapted with permission from the Journal of the American Dietetic Association Position Paper: Use of Nutritive and Non-nutritive Sweeteners.

Complementary and Alternative Medicine: Herbs

Herbal-derived compounds and supplements are commonly used in many cultures for the treatment of diabetes. Although the benefits of some of these compounds have been observed, currently there is insufficient data to recommend any herbal remedies in the treatment of diabetes. In addition, although generally well tolerated at the doses reported, some compounds have significant herb–drug interactions which may prove harmful and require further investigation. For instance, one of the most widely used medicinal herbs, especially in Asia, is ginseng (*Panax ginseng*). The active compounds are thought to be ginsenosides which in some preclinical studies suggest improvements in insulin resistance. However, concomitant administration with warfarin appears to reduce warfarin's therapeutic effects. In addition, the purity and advertised amounts of the active ingredients of many dietary supplements have been questioned. Further research is needed to adequately establish the role of herbal medicines in diabetes management.

Major Non-nutrient Factors that Regulate Blood Glucose

No element of diabetes management exists in a vacuum, so it is essential to consider how dietary therapy interacts with other elements of management. Besides glucose-lowering therapies, these other major nonnutrient factors include medications (e.g., glucocorticoids), acute illnesses (i.e., infection), estrogens, physical activity, and stress. Some of these nonnutrient factors are discussed below.

Insulin

As two major types of diabetes (1 and 2) differ, so the use of insulin differs for each. As described earlier, in type 1 diabetes the insulin doses must be closely matched to the meals ingested. Too much insulin or too little ingested carbohydrate can cause serious hypoglycemia. Frequently, patients are on intensive insulin regimens, sometimes four doses per day, and sometimes using an insulin pump. People with well-controlled type 1 diabetes have usually learned to pay close attention to their carbohydrate intake, recognize portion sizes, or even count grams of carbohydrate. They often adjust insulin dose or carbohydrate intake, but this can be done effectively

only if they have a good quantitative understanding of both. Meals skipped or eaten later than usual can be a problem.

Intensive insulin regimens may involve the use of an insulin pump or insulin doses based on a 'sliding scale.' Ordinarily, sliding scales are developed for the patient based on the self-monitored blood glucose at the time of the meal. For higher blood glucose levels, more short-acting insulin is administered. In more intensive regimens, often with insulin pump use, the amount of insulin delivered is also adjusted depending on both blood glucose level at the time and the carbohydrate to be ingested. To be safe and effective, this self-adjusted fine tuning of insulin dose requires considerable knowledge of diet as well as insulin.

Examples of sliding scales are provided in Table 9. Some people, particularly those with intensive insulin regimens, learn to adjust their insulin dose according to both the blood glucose at the start of a meal and the estimated amount that a unit of insulin will reduce their blood glucose – some people may be more sensitive to insulin than others, thus, the correction factor and meal factor needs to be individualized. Examples are provided in Table 10. Also, although nutritionists do not usually prescribe changes in insulin dosage, it is useful to know the various types of insulin available (Table 11) and patterns of insulin action (Figure 1).

With type 2 diabetes, because there is usually some endogenous insulin secretion remaining from the pancreas and considerable insulin resistance, dietary intake may not have to be so precisely regulated according to insulin dose. There is a risk of hypoglycemia but it is usually less than that seen in type 1 diabetes. As discussed previously, the nutritional emphasis is usually on reducing the caloric intake, with careful control of fat as well as carbohydrate. Weight gain is unfortunately common with insulin initiation due to the anabolic effect of insulin as well as the reduced glycosuria mentioned above.

Oral and Noninsulin Injectable Antidiabetic Agents

Oral antidiabetic agents are not insulin; insulin is delivered only by injection or infusion. The variety of oral agents in use has escalated dramatically in recent years, so it is worth knowing how the various classes act and how they may interact with diet.

Sulfonylureas (e.g., glyburide, glimepiride, and glipizide) or meglitinides (e.g., repaglinide and nateglinide) commonly act by stimulation of pancreatic insulin secretion. They

Table 9 Examples of sliding scale insulin doses based on blood glucose level at the time of the meal^a

Meal	Blood glucose (mmol l ⁻¹)	< 3.33	3.33–6.7	6.8–8.3	8.4–11.1	11.2–13.9	14–16.7	> 16.7
	Blood glucose (mg dl ⁻¹)	< 60	60–120	121–150	151–200	201–250	250–300	> 300
Breakfast	Fast acting	0	3	5	6	7	8	9
Lunch	Fast acting	0	3	5	6	7	8	9
Supper	Fast acting	0	4	6	7	8	9	10
Bedtime	Long acting	14	14	15	15	18	18	18

Example: Prebreakfast blood glucose 10 mmol l⁻¹ (180 mg dl⁻¹): Take 6 units of fast-acting insulin.

^aEach patient will have individual insulin requirements and needs. Longer acting insulin (such as glargine) may be used at bedtime, and faster acting insulins (such as lispro or aspart) may be used premeal.

Table 10 Examples of mealtime insulin dose based on blood glucose level and amount of carbohydrate to be ingested^a*Short-acting or fast-acting insulin dose*

'Correction factor': 1 unit per 2.8 mmol l^{-1} (50 mg dl^{-1}) $> 5.5 \text{ mmol l}^{-1}$ (100 mg dl^{-1})

Plus

'Meal factor': 1 unit per 15 g carbohydrate

Example: Before ingesting a meal estimated to have 45 g of carbohydrate, the blood glucose is measured to be 150. Insulin dose would be 1 unit (for the blood glucose) + 3 units (for the carbohydrate) = 4 units.

^aBoth the correction factor and the meal factor will vary from patient to patient. Long-acting insulins are used in addition to these prandial doses.

Table 11 Types of available insulin by onset, peak, and duration of action

Category	Insulin type	Approximate onset	Approximate peak (h)	Approximate duration (h)
Fast acting	Aspart	5–15 min	1–2	3–5
	Lispro	5–15 min	1–2	3–5
	Gulisine	5–15 min	1–2	3–5
Rapid acting	Regular	30 min to 1 h	2–4	5–8
Intermediate acting	NPH	1–3 h	4–10	10–16
Long acting	Glargine	2–3 h	'Peakless'	20–24
	Levemir	1–3 h	6–8	6–24
Premixes				
50/50: 50% lispro-protamine suspension (similar to NPH), 50% lispro				
70/30: 70% NPH, 30% regular				
Mix 70/30: 70% aspart-protamine suspension (similar to NPH), 30% aspart				
75/25: 75% lispro-protamine suspension (similar to NPH), 25% lispro				

therefore can cause hypoglycemia if taken in excess or without normal food intake.

The other most popular oral agent is metformin, which primarily decreases hepatic glucose production and increases peripheral glucose utilization; therefore, it does not stimulate insulin secretion and should not cause hypoglycemia by itself. Metformin can cause bloating and diarrhea, but it can also be mildly weight reducing in conjunction with diet.

The drugs called thiazolidinediones (TZDs), pioglitazone now being the only one primarily available on the market, improve insulin sensitivity but do not by themselves cause hypoglycemia. TZDs can, however, cause fluid retention and weight gain, so they are sometimes counterproductive in someone trying to lose weight.

A less commonly used class of drugs called α -glucosidase inhibitors (e.g., acarbose and miglitol) inhibit digestion and absorption of carbohydrate. They do not cause hypoglycemia, but they may interfere with the ability to treat hypoglycemia with oral carbohydrate.

Amylin analogs (e.g., pramlintide) acts as the naturally occurring gastric protein amylin, which is cosecreted with insulin by pancreatic β cells, to induce satiety and slow gastric emptying. This injectable agent can be used with insulin but hypoglycemia needs to be carefully monitored.

Two new classes of drugs have been introduced in the past few years. The first class is the oral dipeptidyl peptidase IV (DPP-IV) inhibitors (e.g., sitagliptin and saxagliptin). The second related class is the injectable incretin mimetics (exenatide and liraglutide). These drugs act through a related mechanism and stimulate glucose-dependent insulin secretion and slow gastric emptying. Weight loss has been reported with the injectable incretin mimetics, likely due to the delay in

gastric emptying and early satiety whereas DPP-IV inhibitors are weight neutral.

Physical Activity

The effects of exercise on blood glucose levels are complex and sometimes unpredictable. Although moderate, extended aerobic exercise generally causes progressive lowering of blood glucose, intense exercise may transiently increase the blood glucose. The authors generally recommend modification of diet to accommodate exercise, rather than changing the dose of insulin or oral agents, because the duration and intensity of exercise may be unpredictable. Trained athletes or 'weekend warriors' often learn to take extra carbohydrate before a strenuous workout rather than anticipating exercise by reducing the morning insulin dose. However, reducing insulin dosage in anticipation of exercise does work best for many individuals. They also learn that the time of greatest hypoglycemic risk may be 6–12 h after exercise.

Stress

Stress in normal life is difficult to quantify or study, but the usual experience is that diabetic control deteriorates under stress. This makes sense, considering the hyperglycemic effects of 'fight or flight' hormones such as adrenaline (epinephrine) and cortisol. However, it is likely that the main problem for people under unusual stress is less hormonal than behavioral – simply neglecting their diet or eating as a stress reliever. Therapists may be most effective by reminding patients to maintain their normal diet even when experiencing emotional stress.

Estrogens

Women often find that their blood sugar control varies based on their menstrual cycle. The data for this is poor, but should be individualized. Women may need to vary their insulin requirement during the month. There is no data to support improved or worsening of diabetic control after menopause.

Dietary Prevention and Management of Comorbidities**Accelerated Atherosclerosis**

Essentially the same nutritional approaches to the prevention of atherosclerosis apply whether or not a person has diabetes. However, they are even more important for the patient with diabetes because hyperglycemia is a risk factor, and most people with diabetes die of atherosclerotic cardiovascular disease. Therefore, anyone with diabetes should follow a 'heart-healthy' diet that focuses on lowering low-density lipoprotein (LDL) cholesterol level, which is a major contributor to the progression of atherosclerosis. Total fat intake can be held to 20–35% of total calories, <7% saturated fat, intake of trans fat should be minimized, and <200 mg day⁻¹ of dietary cholesterol and the remainder divided between monounsaturated fat and polyunsaturated fats. The recommendation allows for increased intake of unsaturated fats in place of carbohydrates in people with diabetes. In addition to the antiatherosclerotic diet, there should be routine screening for other specific risk factors, notably hypertension and dyslipidemia. If found, these risk factors, which are even more dangerous in diabetes, should be vigorously treated.

Dyslipidemia

If the dyslipidemia is predominantly elevation of LDL cholesterol, then dietary manipulations are no different in diabetes from those used to treat hypercholesterolemia generally: Low intakes of saturated fat and cholesterol are indicated, with cholesterol-lowering medications (usually statins) given as needed. Target goals for LDL cholesterol are <2.6 mmol l⁻¹ (100 mg dl⁻¹) in most patients with diabetes, however, a goal of <70 mg dl⁻¹ is an option in very high-risk patients with diabetes and overt cardiovascular disease. The dietary recommendation is to limit saturated fat to <7% of total calories, intake of trans fat should be minimal and to limit dietary cholesterol to <200 mg day⁻¹. Fish consumption of two or more servings per week is also beneficial for providing n-3 polyunsaturated fatty acids and may also be a safe and effective way to lower triglycerides.

Hypertriglyceridemia, on the contrary, is the more common dyslipidemia in diabetes, and is especially dangerous when it is associated with low levels of high-density lipoprotein cholesterol. Insulin–glucose homeostasis is intrinsically and complexly related to triglyceride metabolism. Insulin stimulates both very low-density lipoprotein–triglyceride synthesis in the liver and its clearance via lipoprotein lipase in the periphery and better glycemic control can be associated with improved triglyceride levels.

In extreme cases, reduced chylomicron clearance causes 'diabetic lipemia,' characterized by chylomicronemia and

extreme hypertriglyceridemia. More moderate levels of hypertriglyceridemia, however, are considered to be due to overproduction of hepatic (endogenous) lipid under the influence of hyperinsulinism and peripheral insulin resistance.

Compounding the confusion is the phenomenon of 'carbohydrate induction of hypertriglyceridemia.' Most normal people switched isoenergetically from a low- to a high-carbohydrate diet (i.e., with less dietary fat) will actually increase their fasting triglyceride level. This carbohydrate induction of hypertriglyceridemia may be transient, lasting only a few weeks.

For the person with diabetes, treatment of hypertriglyceridemia begins with optimization of blood glucose control and a diet designed to achieve normal body weight. Weight reduction is often very effective, as is aggressive insulin treatment.

If hypertriglyceridemia persists, evidence favors the use of monounsaturated fats when dietary fat increases. Because hypertriglyceridemia is sometimes associated with excess alcohol and concentrated sugar, reducing the amounts of both consumed may be effective. For the unusual condition of fasting chylomicronemia, with triglyceride levels remaining more than approximately 11 mmol l⁻¹ (1000 mg dl⁻¹), a low-fat diet is necessary to avoid exacerbating the situation by adding dietary fat and precipitating pancreatitis from the chylomicronemia.

If pharmacologic treatment is necessary to treat hypertriglyceridemia, a fibric acid derivative, such as gemfibrozil or fenofibrate, or nicotinic acid should be used. ω -3 fatty acids from fish or fish oil supplements can also help lower triglycerides and are beneficial for cardiovascular outcomes in patients with known heart disease. Statins are usually the first drug of choice when LDL cholesterol is also elevated.

Hypertension

The current nutritional management of hypertension focuses on reducing dietary sodium intake and weight reduction, as well as the recently proven 'Dietary Approaches to Stop Hypertension' (DASH) diet. There has been long-standing evidence that in both normal and hypertensive people, a reduction in sodium intake lowers blood pressure. The DASH diet was shown to be effective in a large trial of dietary intervention. It is a fruit and vegetable diet, with a balanced consumption of foods emphasizing high fiber, grains, and low-fat dairy products. Potassium, magnesium, and fiber replace some snacks and sweets.

Modest amounts of weight loss and increased activity are also beneficial for the person with hypertension. Thus, overweight and obese individuals should be encouraged to lose weight as part of their medical therapy. In diabetes, ACE inhibitors or Angiotensin Receptor Blockers (ARBs) are usually the first line of medication used when diet and exercise are not effective in controlling blood pressure due to their additional renoprotective effect particularly in diabetes. Frequently, additional antihypertensives must be added.

Renal Disease

Nutritional therapy of established diabetic nephropathy continues to be studied. In both type 1 and type 2 diabetes,

persistent microalbuminuria is a strong predictor of gross proteinuria and developing nephropathy. Evidence suggests that microalbuminuria actually reverses in many cases, but gross proteinuria (more than approximately 300 mg per 24 h) in most cases eventually progresses to end-stage renal disease. Therefore, treatment focuses on reversing or at least retarding the progression of nephropathy. In recent years, the nutritional recommendation has been to lower dietary protein intake to 0.8–1.0 g kg⁻¹ of body weight per day for patients with microalbuminuria. For people with overt nephropathy, reducing dietary protein intake to 0.8 g kg⁻¹ of body weight per day may slow the progression of nephropathy. Protein restriction should not be attempted if serious protein loss from nephrotic-range proteinuria has reduced total serum albumin concentration, or a patient has end-stage renal disease and is on dialysis, in which case it is agreed that a low-protein diet is not indicated. In fact, a large study of the dietary treatment of kidney disease in nondiabetic subjects did not support the value of protein restriction in slowing progression of kidney damage.

The best documented therapies for proteinuria and reducing nephropathy are improved glycemic control and reduction of blood pressure using ACE inhibitors or ARBs. Additional dietary approaches include limited intake of refined and processed foods high in sugar and sodium, reducing dietary sodium, moderate alcohol intake, and achieving a healthy weight. Cessation of smoking is also of established benefit.

Conclusions

Medical nutrition therapy is essential to all people with diabetes, of whatever type or severity. A healthy diet should

contain important components, including foods containing carbohydrates from whole grains, fruits, vegetables, vitamins, and low-fat dairy products. Although blood glucose control and management of coexisting risk factors are overall goals, the implementation of dietary management is a highly complex and individualized process. Principles of medical nutrition therapy that generally apply to all diabetes, and specific features that apply to the various types of diabetes, have been discussed; but it must be emphasized that good nutritional management requires the close interaction of each individual patient with a knowledgeable expert in dietetics.

See also: Carbohydrates: Chemistry and Classification; Regulation of Metabolism. Diabetes Mellitus: Classification and Chemical Pathology; Etiology and Epidemiology. Glucose: Metabolism and Maintenance of Blood Glucose Level. Obesity: Complications. Sucrose: Dietary Sucrose and Disease. Weight Management: Approaches

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Etiology and Epidemiology

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Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by the disturbance in glucose metabolism leading to a state of hyperglycemia and is associated with microvascular and macrovascular complications in the long term. Diabetes is the leading cause of noncommunicable diseases worldwide and it is true to say that diabetes has reached epidemic proportions in certain parts of the world and in certain ethnic groups. This has widespread implications for health resources.

Diabetes mellitus is an etiologically and clinically heterogeneous group of disorders that share hyperglycemia in common. The two main types of diabetes, type 1 diabetes (T1D) and type 2 diabetes (T2D), are quite distinct from each other in their etiology and epidemiology. T2D is the most common form of diabetes worldwide accounting for 90% of cases globally and affecting approximately 4% of the world's adult population. T1D is an autoimmune disease that results in insulin deficiency. Both T1D and T2D are distinct from 'other causes of diabetes' as defined by the etiological classification of the World Health Organization (WHO); the authors will not comprehensively review the many types of diabetes but illustrate it with maturity onset diabetes of the young (MODY) and fibrocalculous pancreatic diabetes (FCPD). Gestational diabetes (GDM) is the fourth category defined by the WHO.

Type 1 Diabetes

Worldwide Prevalence

In 1997, there were 11.5 million people with T1D in the world; this figure is expected to rise to 23.7 million in the year 2010. These increasing figures will have most impact in Asia, where there are currently 4.5 million people with T1D, and this is expected to rise to 12 million by the year 2010. One of the best incidence studies has come from Europe as part of a European collaboration, where the highest incidence of T1D is found in Finland and the lowest rates in Romania (Table 1). The incidence of T1D follows a north–south gradient, with the notable exception of Sardinia. The figures from countries such as India are less precise, although one study in Chennai suggested an incidence equivalent to that found in Southern European countries. These different rates of T1D are likely to reflect both the genetic background of individual countries and the differences in exposure to environmental agents. In recent years, the incidence of T1D has been increasing in several different countries. These changes must reflect environmental influences.

Etiology

T1D is due to autoimmune destruction of insulin-secreting pancreatic β -cells of islets of Langerhans. T1D typically occurs

in young individuals with an age of onset of less than 40 years. The autoimmune reaction is likely to be triggered by an environmental agent *in utero* or in very early life (Figure 1). The earliest markers of β -cell destruction are the appearance of autoantibodies to glutamic acid decarboxylase (GAD), islet cells, and insulin. Autoantibodies have been detected 10–15 years before the onset of disease and, furthermore, have been known to disappear without T1D occurring in a few individuals. One to two years before the onset of the disease, evidence of β -cell impairment can be detected, initially evidenced by a reduction in the first phase of insulin response to intravenous glucose and in the later stages by an abnormal oral glucose tolerance. In contrast to the slow β -cell destruction, the onset of T1D is acute and is usually measured in weeks. At this stage in the etiological process, it is likely that 70% of β -cells have been destroyed and those remaining are inhibited by the action of cytokines.

There is a subgroup of patients who develop diabetes in adult life and do not require insulin during the first few years after diagnosis; they have an autoimmune component to their disease with positive GAD and islet cell antibodies. This condition is named as latent autoimmune diabetes (LADA). There are several common features between T1D and LADA, including T-cell insulinitis, islet antibody positivity, and high rates of HLA DR3 and DR4. The prevalence of LADA in newly diagnosed diabetics has been shown to range from 2.8% to 22.3% in different studies depending on the markers used and the characteristics of the patients. Although these patients present with T2D, they have been shown to progress to insulin dependency especially if the diabetes is diagnosed at a younger age and the patient is not overweight. Therefore, there may be a role for measuring GAD antibody in newly diagnosed patients with T2D to identify the LADA subgroup especially in

Table 1 Extremes of incidence of childhood type 1 diabetes mellitus in different ethnic groups

Higher	Incidence ^a	Lower	Incidence ^a
Sardinia	35–40	Venezuela	0–5
Finland	35–40	Peru	0–5
Sweden	25–30	China	0–5
Canada	20–25	Paraguay	0–5
Norway	20–25	Mauritius	0–5
UK	15–25	Chile	0–5
New Zealand	10–25	Japan	0–5
Portugal	5–20	Barbados	0–5

^aAge standardized incidence (per 100,000 per year) of type 1 diabetes in children <14 years of age.

Source: Data from Karvonen M, Viik-Kajander M, Moltchanova E, *et al.* (2000)

Incidence of childhood type 1 diabetes worldwide. *Diabetes Care* 23: 1516–1526, with permission from ADA.

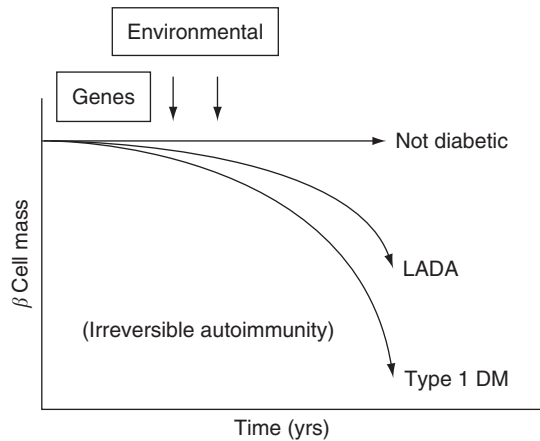


Figure 1 Etiology of type 1 diabetes mellitus. DM, diabetes mellitus; LADA, latent autoimmune diabetes.

the younger age groups. This group of patients is now classified as T1D by the new WHO classification.

Genetics

T1D is a multifactorial disease with both genetic and environmental components. The largest genetic contribution to T1D is determined by genes in the major histocompatibility complex (MHC) located on the short arm of chromosome 6 (IDDM1-HLA, 6p21). Initial associations between T1D and the MHC were described for the HLA class I antigens A1-B8 and B15. With the advent of HLA class II serology, closer associations were found with HLA-DR with an increased frequency of DR3 and DR4 and a decreased frequency of DR2 in T1D subjects. At the population level, the strongest genetic association with T1D is with HLA-DQ alleles. This is best defined by deoxyribonucleic acid (DNA) typing of HLA-DQ1, DQB1, and DRB1. However, due to the strong linkage disequilibrium between these loci it has been very difficult to study the effect of individual HLA-DQ or HLA-DR genes separately. For the individual, susceptibility is best defined by allelic combinations of MHC genes located to all three major regions (classes I, II, and III) called HLA haplotypes. Haplotypes occur because of strong linkage disequilibrium observed in the MHC whereby the combinations of alleles are seen more frequently than would be expected by their individual gene frequencies. An example of a haplotype would be A2, Cw1, B56, TNFa6, DRB1*401, DQA1*0301, or DQB1*0302. The haplotypes are likely to relate to functional groups of genes involved in the etiology of T1D. Thus, the critical residues of DR and DQ, accounting for the disease association with T1D, are located in the antigen-binding cleft of the HLA molecule and are likely to influence the binding of antigenic peptides for subsequent presentation to T helper cells. Similarly, polymorphisms of the HLA class I molecules are likely to relate to antigen presentation to cytotoxic T cells, and polymorphisms of tumor necrosis factor (TNF) have been associated with differing TNF responses to mitogenic stimulation.

The MHC accounts for approximately 40% of the genetic component to T1D. Evidence from genome scans and candidate

gene studies indicates the existence of a large number of putative non MHC genes contributing to the etiology of T1D, although all are of comparatively small effects compared to the MHC. The most reproducible T1D associations have been found with the insulin gene, cytotoxic T lymphocyte antigen 4 gene (*CTLA4*), and the vitamin D receptor. An association between the insulin genes (located on chromosome 11p15.5) is not sufficient (*INS*), and T1D was described in the 1980s and subsequently confirmed by linkage studies. The *INS* locus on chromosome 11p15.5 contains a major polymorphism 5' to the transcription site, which is a variable number of tandem repeats (VNTR) region. One functional hypothesis to explain the association between the insulin gene and T1D is that 'hypersecretors' of insulin determined by the disease-associated polymorphism might induce thymic tolerance to insulin, thus providing protection from the autoimmune reaction. A recent study showed an association of the T-cell regulatory gene *CTLA4* with susceptibility to autoimmune disease, including T1D. *CTLA4* (gene located on chromosome 2q33) plays an important role in the counter-regulation of CD28 T-cell antigen receptor activation of T-cells. In the mouse model of T1D, susceptibility was also associated with variation in *CTLA4* gene splicing with reduced production of a splice form encoding a molecule lacking the CD80/CD86 ligand-binding domain. There are associations reported between vitamin D receptor gene polymorphisms and T1D. The *VDR* gene is located on chromosome 12q and polymorphisms of the *VDR* gene may be related to T-cell-mediated autoimmune destruction of β -cells of pancreas. Vitamin D compounds suppress T-cell activation and significantly repress the development of insulinitis and diabetes in the nonobese diabetic (NOD) mouse, a mouse model of human T1D. Results from other candidate gene studies and a 'total genome' analysis have identified at least another 19 chromosomal regions that may be involved in pathogenesis of the disease. The finding that so many genes are involved in T1D raises the possibility that there are several disease processes that might lead to β -cell destruction. Furthermore, evidence is still emerging that the genetic susceptibility to T1D is graded both within and among populations.

Environmental Factors

Environmental factors play a significant part in the etiology of T1D and have been implicated in both initiation and progression of β -cell damage. The majority of evidence points to the effects of viruses and/or dietary factors as etiological agents.

Many viruses have been implicated in the pathogenesis of T1D; they may have a direct effect on β -cells by infection and cell lysis, or alternatively they may act as triggers to the autoimmune process. Among the viruses that have been implicated in humans are Coxsackie A, Coxsackie B, rubella, cytomegalovirus, mumps, and Epstein-Barr viruses. The enteroviruses (Coxsackie A, Coxsackie B, and Echovirus) are the most commonly associated viruses with diabetes and serve as a major trigger for T1D in the young possibly by induction of islet cell antibodies. The evidence for viral involvement in T1D came from several sources, including anecdotal case reports, epidemiological studies, seasonal incidence studies, and animal models. There is data to support the theory

that enterovirus infection either accompanies or precedes the development of T1D in young people in many instances.

Coxsackie B was first implicated in the early 1970s by Gamble, who found an increased titer of coxsackie B antibodies in newly diagnosed T1D patients. More recently, Coxsackie virus has been identified in very young-onset T1D (in patients under 5 years of age) using the polymerase chain reaction. Furthermore, when the Coxsackie virus was sequenced, although it had extensive homology to Coxsackie virus B4, there was some unique sequence variation indicating a T1D variant. There have also been many anecdotal reports of Coxsackie B virus causing T1D, presumably by a direct cytolytic effect on β -cells. A previously fit child died in diabetic ketoacidosis three days after a flu-like illness. At necropsy, there was an extensive lymphocytic infiltration into the β -cells of pancreas and Coxsackie B4 was found in the child's serum. This virus was extracted from the pancreas and when used to infect mice, led to diabetes.

There is a high incidence of T1D among patients with the congenital rubella syndrome. Clearly, this results from an *in utero* infection, but the diabetes that ensues is indistinguishable from the primary T1D: The disease presents in the second decade of life, the onset is preceded by islet cell antibodies, and the genetic predisposition is defined by the same HLA association as T1D. This is likely to be a good example of a virus triggering the immune process.

Dietary factors have also been implicated in the development of T1D. Among the dietary factors indirectly linked to either susceptibility or protection to T1D are cows' milk protein (including bovine serum albumin and β -lactoglobulin), β -cell-toxic drugs (alloxan, streptozotocin, and rodenticides), dietary toxins (in particular nitroso-containing compounds), and others such as coffee and sugar. There is an interesting interplay between vitamin D, vitamin D receptor (VDR), and association with T1D as discussed earlier. The contribution of vitamin D as a potent modulator of the immune system is well recognized. The main sources of vitamin D are ergocalciferol and cholecalciferol found in dietary sources and cholecalciferol produced in the skin by ultraviolet radiation of 7-dehydrocholesterol. Vitamin D deficiency in infancy and VDR polymorphisms may be risk factors for T1D. In NOD mice, long-term treatment with high doses of vitamin D₃ reduced the incidence of diabetes by changing the cytokine balance at the local pancreatic lesion.

The Future

The working out of the complete genetic basis of T1D will lead to a better understanding of the disease pathogenesis and, through studies of genetic and environmental interaction, the direct evidence of environmental factors. T1D may be the first multifactorial disease to benefit from primary prevention of both insulinitis (before immune process has been initiated) and T1D (once autoantibodies have been detected). In the Diabetes Prevention Trial, low-dose insulin was administered to persons with high risk of T1D as ascertained by family history, islet antibodies, and HLA typing. It was concluded that low-dose insulin does not delay or prevent the onset of T1D. In a European study (European Nicotinamide Diabetes

Intervention Trial, ENDIT), high-dose nicotinamide was used to protect the β -cells in high-risk individuals for T1D; unfortunately this trial also failed to show benefit on active treatment. However, 90% of T1D patients do not have a family history of T1D. The approach in this latter group might be to identify the genetically susceptible by the use of genetic markers and then test for autoantibodies. If the latter subjects are autoantibody positive then intervention may be considered in the future. The sensitivity and specificity that would be required for such sequential testing would depend on how safe the proposed intervention would be.

Type 2 Diabetes

Worldwide Prevalence

T2D is one of the most common noncommunicable diseases in the world with an estimated 147.2 million people suffering from this disorder; by 2010 this figure is expected to reach 212.9 million. Furthermore, it has been predicted that by the year 2010 more than half the people with T2D will be living in Asia. This trend is likely to be due to increasing urbanization and industrialization. According to WHO estimates, the figure is likely to double by the year 2025. The prevalence of T2D varies widely from the highest in Pima Indians (almost half of the population affected) to the lowest in Rural Africa (1%). As with T1D, the incidence of diabetes in different countries is likely to reflect the different genetic architecture as well as the differing environment. A good example is afforded by the population of Nauru. In full-blooded Nauruans greater than the age of 60 years the prevalence of T2D is 83%, whereas in those with genetic admixture as adduced by HLA typing the prevalence is 17%; this clearly reflects the genetic component. However, the rapid increase of T2D in the world in the past few decades, and the rise and a recent decrease in prevalence of T2D in the Nauruan community, can only be ascribed to environmental factors. This illustrates the multifactorial nature of T2D, with strong genetic and environmental contributions.

Etiology

T2D is a multifactorial disease with genetic and environmental factors playing a key role in its pathogenesis. Central to the etiology is a defect in insulin action, hepatic glucose output, and insulin secretion. Although insulin resistance is frequently the first detectable abnormality in the progression of T2D, insulin resistance by itself does not cause the disease, which is only manifested when there is a coexisting insulin secretory defect. T2D typically occurs in middle-aged and elderly people but there is an increasing trend of T2D occurring in young individuals. The main question yet to be answered is whether T2D is one disorder or a group of disorders with hyperglycemia as the end point in the disease pathogenesis. Insulin resistance is common to several other disorders, including ischemic heart disease, hypertension, dyslipidemia, central obesity, and coagulation defects; the clustering of these disorders is known as the metabolic syndrome or the insulin resistance syndrome. The interface of T2D with obesity is a complex one, highlighted by the discovery of leptin and

adiponectin. The cause of obesity and T2D in the ob mouse is a mutation of the *ob* gene. With administration of the ob gene protein (leptin) the ob mouse decreases its food consumption and increases exercise, leading to a dramatic weight loss; if given early enough it will also prevent diabetes. In contrast, common human obesity is associated with increased leptin levels, and which have been found to correlate with hyperinsulinemia. The newly discovered protein adiponectin signals adipose tissue mass; reduced levels are found in obese subjects and there is an important interplay between adiponectin, insulin resistance, T2D, and atherosclerosis. Ghrelin is a gut hormone that is a signal of satiety and therefore has a direct effect on obesity. In the obese subject with T2D, there may be interplay between leptin, adiponectin, ghrelin, and insulin, contributing to insulin resistance and the metabolic syndrome.

Genetics

T2D is a complex disease and the heterogeneity both at the phenotypic and pathophysiological level indicates that the genetic component is likely to be heterogeneous with no single locus accounting for the disease. There are many strands of evidence to support a strong genetic component to T2D; these include a near 100% concordance in identical twins, familial clustering, genetic admixture and migration studies, complex segregation analysis, and the detection of gene variants leading to diabetes including the identification of genes responsible for human monogenic diabetes (see the section on MODY below).

Many groups worldwide have completed the first stages of genome scans for genes that predispose an individual to T2D. Currently, a large international research effort is being directed to those diabetes-associated linkage peaks that are overlapping in several genome scans; specifically on chromosomes 1, 12, and 20. One genome scan has been taken to completion with the identification of the *calpain10* gene (located on chromosome 2q37) as a major susceptibility gene in Mexican-Americans. The majority of subsequent studies have confirmed an association between *calpain10* and T2D as well as insulin action, insulin secretion, endothelial function, and aspects of adipose metabolism. This putative diabetes susceptibility gene encodes a ubiquitously expressed member of the calpain-like cysteine protease family, calpain-10. Functional studies would suggest a role in insulin secretion, insulin action, and adipocyte metabolism. For instance, reduced levels of *calpain10* have been found in skeletal muscle associated with disease-associated polymorphisms, and inhibition of calpain affects insulin secretion and translocation of glucose transporter-4 in an adipocyte cell line.

A large number of candidate genes have been studied in T2D. Only a few have produced consistent results, that is, the genes for the insulin receptor substrate (*IRS*)-1, insulin, *KCNJ11*, and peroxisome proliferator-activated receptor gamma (*PPAR* γ), and are critically dependent on the power of individual studies. *IRS* 1 (*IRS-1*) is a protein involved in insulin signaling. After insulin binds to its insulin receptor, it stimulates autophosphorylation of its β -chain, which, in turn, leads to phosphorylation of several multisite *IRS* docking proteins including *IRS-1*. This then generates one of the signals

for insulin action. Several variants of *IRS-1* have been detected, one of which (due to the substitution of a glycine for arginine at position 972 (G972R) in the molecule) is associated with insulin resistance but only in the presence of obesity. This is a good example of a gene variant that is common in the population (at least 8% in the white population) and will only lead to disease in association with other contributing factors for diabetes. Recently, a metaanalysis has confirmed the role of the G972R variant in T2D with an odds ratio of 1.25.

KCNJ11 encodes *Kir6.2*, which is an essential subunit of the pancreatic β -cell potassium ATP (K_{ATP}) channel. Rare mutations of this locus lead to the monogenic syndrome of familial hyperinsulinemia, confirming the important role of *KCNJ11* in insulin secretion. Although there are a large number of rare variants of the *KCNJ11* gene, only one common variant (E23K) has been associated with T2D, although by no means consistently in all studies. It is likely that the studies mentioned above were underpowered as a metaanalysis, which additionally included a large new study, has demonstrated a significant odds ratio for T2D of 1.23. However, it should be borne in mind that the E23K variant does not change protein function and therefore it is unlikely to be the predisposing mutation.

The *PPAR* γ gene is mainly expressed in the adipose tissue and is the target of the thiazolidinedione class of drugs used to treat T2D by improving insulin action and secretion. In man, rare mutations of *PPAR* γ are associated with a monogenic syndrome of severe insulin resistance, T2D, and hypertension. In contrast, a common amino acid polymorphism (Pro12Ala) in *PPAR* γ has been shown to be associated with an increased risk of typical T2D, confirmed by a metaanalysis with an odds ratio for diabetes of 1.25 for the common proline allele. Furthermore, a gene nutrient interaction has been demonstrated with an important interaction of the Pro12Ala variant and the ratio of dietary polyunsaturated fat to saturated fat in the diet. *PPAR* γ is a nuclear receptor, which on activation stimulates the transcription of genes responsible for growth and differentiation of adipocytes. This clearly indicates a role for *PPAR* γ in fat cell biology and pathophysiology of obesity, diabetes, and insulin resistance.

One of the first candidate genes to be studied in T2D was the insulin gene with an association described with the class 3 allele. As mentioned in the section on the genetics of T1D, the insulin gene hypervariable region is a determinant of insulin secretion. Although consistent associations were found of the insulin gene and T1D, this was not the case for T2D. However, the earlier T2D studies were very much underpowered and the controls and patients not well matched. Using a family-based design, the association between T2D and the class 3 allele has been confirmed demonstrating paternal but not maternal transmission. This is in keeping with the fact that the insulin/insulin growth factor II gene locus is maternally imprinted. In addition to the importance of adequately powered studies, the paternal transmission is likely to be another explanation for variable results in a case-control study design, emphasizing the importance of family-based designs as an additional strategy in association studies. The insulin/insulin growth factor II gene locus is a determinant of fetal and postnatal growth, which is also an important factor in susceptibility to T2D.

Environmental Factors

Evidence of a strong environmental element to T2D has come from the studies of Barker and Hales. In a number of separate studies, a strong relationship of the development of glucose intolerance and other associated factors of the insulin resistance syndrome with low birth weight or thinness at birth has been demonstrated. Furthermore, these associations are not confined to those with growth retardation *in utero* but extend to the whole range of birth weights. As a consequence of these epidemiologic studies, the 'thrifty phenotype' hypothesis has been proposed, whereby nutritional deficiencies *in utero* lead to poor fetal and infant growth and the subsequent development of T2D in later life, especially when combined with obesity due to excess food intake and lack of physical activity. These changes are recognized to be due to insulin resistance, which is favorable for survival in the immediate postnatal period but plays a significant role in the progression to T2D and metabolic syndrome, and to a certain extent insulin secretion. Although there is much discussion regarding this hypothesis, it illustrates the importance of environmental factors in early life, which might prime the fetus for T2D in later life.

Dietary factors and physical inactivity undoubtedly affect the progression of abnormal glucose tolerance to diabetes in a genetically predisposed individual. The best way to lower the risk of diabetes is to lead a healthy life style by eating a healthy balanced diet, engaging in regular physical activity, and balancing the energy intake with energy expenditure. Indeed, recent evidence would suggest that the adoption of a healthy life style in high-risk subjects can decrease the risk of developing T2D by 60%. There is a close relationship between diabetes and obesity, especially when the latter has central distribution. Apart from obesity, several other nutritional factors affect glucose metabolism and the risk of T2D. Current evidence suggests an association between different types of fats and carbohydrates and insulin resistance and T2D. Diets rich in saturated fats are associated with insulin resistance; a multicentre study in a group of healthy individuals showed that a diet high in saturated fat decreased insulin sensitivity compared with a diet high in monounsaturated fat with the same total fat content. Prospective and cross-sectional studies suggest a role of specific types of fat rather than the total fat content in the development of T2D, where high intake of vegetable oils, oils consisting primarily of polyunsaturated fat, was associated with reduced risk of developing diabetes and a positive association between saturated fat and hyperglycemia or glucose intolerance. In a 20-year follow-up of the Finnish and Dutch cohorts of the Seven Countries Study, it was found that a high intake of fat (in particular saturated fatty acids) contributed to the risk of glucose intolerance and T2D. Dietary carbohydrates are classified into simple or complex carbohydrates depending on their chemical structure. The traditional view is that simple carbohydrates should be avoided and substituted with complex (starchy) carbohydrates to reduce postprandial glucose response, but this has been challenged by various studies that recognized that starchy foods such as baked potatoes and white bread produce even higher glycemic responses than simple sugars. Glycemic index (GI) was developed to quantify the different glycemic responses induced by different carbohydrate foods. A low GI

diet with a greater amount of fiber and minimally processed whole-grain products seems to improve glycemic and insulin responses and lowers the risk of T2D. This shows that dietary recommendations to prevent and manage diabetes should focus more on the quality of fat and carbohydrate than the quantity alone.

A number of environmental toxins have been shown to cause diabetes in humans, including nitrosated compounds, as well as streptozotocin, the rat poison Vacor, and foods such as smoked mutton; depending on the amount consumed they could lead to either T1D or T2D, presumably dependent on the amount of direct β -cell destruction. It has also been proposed that vitamin D might modulate the diabetic process. Vitamin D deficiency has been shown to reduce insulin secretion. In a UK study of Bangladeshi subjects living in east London who were particularly prone to vitamin D deficiency, vitamin D levels were found to be low in those most at risk of diabetes. Furthermore, there was a correlation between vitamin D levels and 30 min oral glucose tolerance test, blood glucose, insulin, and C-peptide levels.

The Future

Research into the identification of genes involved in T2D is beginning to lead to insights into the pathogenesis of this common condition. With knowledge of the precise biochemical variants involved in disease pathogenesis, we will be in a better position to classify the disease and design more rational therapeutic maneuvers to prevent and ameliorate this condition. Research also needs to be directed at the gene-environment interaction, as this will indicate the appropriate population strategies to combat the increasing incidence of this common noncommunicable disease. Recently, life style interventions (healthy diet and exercise) have demonstrated significant reduction in onset of diabetes in high-risk individuals for T2D. In the future, genetic profiling might be used to identify those likely to respond to such strategies including pharmacological treatment.

Other Types of Diabetes

MODY

MODY are a group of monogenic disorders inherited in an autosomal dominant pattern. MODY is characterized by early onset (usually before the age of 25 years) of T2D β -cell dysfunction and there being a family history (at least two generations) of early onset diabetes. The defect is in insulin secretion due to mutations in the glucokinase and β -cell transcription factor genes (Table 2). Hepatocyte nuclear factors (*HNF*) 1α , 1β , and 4α , insulin promoter factor (*IPF1*), and neurogenic differentiation-1 (*NEUROD1*) play an important role in the normal development and function of the β -cells of the pancreas. In the UK, mutation in *HNF1 α* is the commonest cause of MODY accounting for 63% of cases, followed by mutations in the glucokinase gene (20% of cases). The clinical presentation and progression of diabetes is different among patients with mutations of glucokinase, *HNF1 α* , and *HNF1 β* . Subjects with glucokinase mutations are frequently asymptomatic but can be identified when diagnosed with GDM or

Table 2 Maturity onset diabetes of the young

<i>MODY subgroup</i>	<i>Gene</i>	<i>Chromosome</i>	<i>MODY frequency</i>
MODY1	<i>HNF4α</i>	20q	Rare
MODY2	<i>GCK</i>	7p	10–65%
MODY3	<i>HNF1α</i>	12q	20–75%
MODY4	<i>IPF1</i>	13q	Rare
MODY5	<i>HNF1β</i>	17q	Rare
MODY6	<i>NEUROD1</i>	2q	Rare

with a milder form of diabetes, which is frequently treated with diet alone and is not associated with the complications of diabetes. In contrast, subjects with *HNF1 α* mutations are more like lean patients with T2D with susceptibility to micro-vascular complications and progressive loss of β -cell function exacerbated by increasing body mass index. In comparison to patients with T2D, subjects with *HNF1 α* mutations are very sensitive to sulfonylurea treatment as might be predicted from the genetic defect. Finally, patients with *HNF1 β* mutations in addition to T2D have renal cysts that may lead to renal failure and hence such patients are more frequently found in the renal clinic.

GDM

GDM is defined as glucose intolerance first recognized in pregnancy. This, therefore, excludes those women with either T1D or T2D diagnosed before conception. GDM is a relatively common occurrence in pregnancy affecting 1–14% in White European and North American populations and higher in certain ethnic groups such as South Asian and Afro-Caribbean populations.

GDM increases the risk to both mother and fetus, although the levels of maternal glycemia that leads to an adverse outcome are not well defined. Furthermore, there is controversy as to who should be screened in pregnancy, the best available diagnostic test that has high sensitivity and specificity, and the timing of the test during gestation. This is reflected in the lack of international agreement on diagnostic criteria ranging from the WHO criteria to a more pragmatic approach based on fasting and postprandial glucose levels. Those at particular risk for GDM are ethnic groups with a high prevalence of diabetes, women with a previous history of delivering large babies, a family history of diabetes, obesity, older women, and multiparous women.

Pregnancy is associated with an increase in insulin resistance and an increase in hormones with actions opposing insulin (i.e., cortisol, progesterone, growth hormone, and human placental lactogen). GDM develops at a time when the β -cell reserve cannot cope with the prevailing state of insulin resistance. Therefore, by definition after pregnancy when the insulin resistance reduces, then the subject becomes normoglycemic. However, GDM can be considered as a prediabetic condition with an increased future risk of developing T2D during their lifetime, which is approaching 20–50%. The treatment of GDM consists of maintaining strict glycemic control. If dietary intervention fails to achieve normoglycemia, the treatment of choice is insulin.

The maternal risk due to GDM include increased risks in pregnancy, accelerated fetal growth leading to macrosomia, and increased rates of caesarian section. The fetal risks include stillbirth, congenital malformations, shoulder dystocia, birth trauma, and the risk of neonatal hypoglycemia and calcium and bilirubin disturbances in the neonatal period. There is also an increased risk of the child subsequently becoming obese and developing diabetes in adult life as a result of *in utero* hyperglycemia.

FCPD

In tropical countries, there is a form of nonalcoholic chronic pancreatitis characterized by pancreatic exocrine and endocrine insufficiency and associated with pancreatic calcification. This disease, tropical calcific pancreatitis, affects young individuals who are malnourished and present with abdominal pain, extreme emaciation characteristic of protein-energy malnutrition, glucose intolerance, and at a later stage diabetes. The diabetic stage of the illness is referred to as FCPD. Several reports of FCPD have been reported from the tropical countries and many cases have been reported from the Indian subcontinent. The pathogenesis of the disease is still unclear and is attributed to various possible causes – malnutrition, cassava toxicity, oxidant stress due to micronutrient deficiency, and genetic and environmental factors. Recently, a study showed the N34S variant of the *SPINK1* trypsin inhibitor gene as a susceptibility gene for FCPD in the Indian subcontinent. Although by itself it is not significant to cause FCPD, it indicates the role of gene–environment interaction in the pathogenesis of diabetes.

Patients with FCPD are at risk of long-term diabetic complications and require insulin to control their hyperglycemia. Given the underlying problem of malnutrition, they benefit from high calorie intake, especially the protein content. There is a need for further investigation into the roles of nutritional, environmental, and genetic factors to establish the etio-pathogenesis of this illness.

See also: Diabetes Mellitus: Classification and Chemical Pathology; Dietary Management. **Glucose:** Metabolism and Maintenance of Blood Glucose Level. **Obesity:** Complications

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DIARRHEAL DISEASES

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Glossary

Dehydration A condition which arises from loss of fluids and electrolytes from the body characterized by thirst, loss of skin turgor, rapid pulse, sunken eyes, increased breathing, decreased urinary output, etc.

Diarrhea An increased frequency of bowel movement with increased fluidity of stool.

Hyponatremia and hypokalemia Low blood sodium and potassium, respectively.

Metabolic acidosis Production of excess acid diagnosed by low pH and bicarbonate in arterial blood. A range of

symptoms may result including rapid breathing, vomiting, abdominal pain, and weight loss.

Oral rehydration therapy Provision of an oral rehydration solution (ORS) to prevent dehydration. ORS, often distributed in sachets, usually contain sodium, glucose, chloride, potassium, and citrate in amounts that provide a total osmolality of 245 mOsm/L when diluted in water. Home preparations of water with added sugar and table salt are also effective.

Introduction

Diarrheal diseases are one of the leading causes of morbidity and mortality worldwide, especially in children in the low and middle income countries (LMIC). Diarrhea causes loss of body fluid, which may lead to severe dehydration, electrolyte imbalance, shock, and even death. Hyponatremia, hypokalemia, and metabolic acidosis are common electrolyte and acid–base abnormalities in children with diarrhea.

In the early eighties, an estimated one billion diarrheal episodes and approximately five million deaths occurred annually among children under 5 years old globally. Recent estimates show that diarrhea causes 1.3 million deaths annually in children younger than 5 years of age or one out of every five child deaths occurs due to diarrhea. The diarrheal attack rate is highest in the 6–11 month age group and the rates vary from 0.9 to 9.8 episodes per child per year. The highest diarrhea associated mortality is among infants less than 1 year of age. Although diarrheal mortality rates have fallen during the last decade, there is no evidence of reduction in the incidence of diarrhea.

Definition of Diarrhea

Diarrheal diseases are commonly defined as an increase in the frequency of bowel movement with increased fluidity of stool relative to usual patterns in an individual. There is a wide variation in the literature in the definition of a diarrhea episode, ranging from mothers' perceptions to operational definitions based on the frequency of loose stools in 24 h. The

operational definitions vary, but require at least three, four, or five watery or loose stools in 24 h.

Diarrhea can be classified in a number of ways: duration of an episode (acute-short duration – less than 14 days; persistent – ≥ 14 days), type of stool (watery, bloody, or dysentery), consistency of stool (loose, liquid, and watery), dehydration status (dehydrated, nondehydrated), pathophysiologic mechanism (invasive, secretory), or based on causative agents (e.g., *Escherichia coli*, cholera, rotavirus, shigella, etc.).

Pathophysiology of Diarrhea

During diarrhea there is an increased frequency of bowel movement and alteration in stool consistency. Normal fluid and solute movement across the intestinal membrane is altered resulting in increased loss of water and electrolytes (particularly sodium, potassium, chloride, and bicarbonate). Four mechanisms have been postulated to be responsible for these alterations: decreased fluid absorption, increased intestinal secretion, increased luminal osmolality, and altered intestinal motility. It has been suggested that a common mechanism exists in cases of bacterial, viral, and parasitic diarrhea where levels of cyclic nucleotide are enhanced. Loss of fluids and electrolytes may lead to dehydration, acidosis, hyponatremia, and hypokalemia. The degree of dehydration depends on the net amount of fluid lost. WHO classifies the grades of dehydration as none, some, or severe dehydration. The estimated fluid deficits in the different types of dehydration are: no dehydration (loss of $< 5\%$ of body weight),

some dehydration (loss of 5–10% of body weight), and severe dehydration (loss of > 10% of body weight).

Causes of Diarrhea

The causative agents of diarrhea are numerous and include bacteria, viruses, and parasites. However, the etiologic agents vary by geographical locations, seasons, and age groups. Most common bacteria are *E. coli*, *Shigella*, *Salmonella*, *Vibrio cholerae*, *Campylobacter jejuni*, *Clostridium difficile*, and *Yersinia*. Rotavirus and Adenovirus are the common viral agents causing diarrhea. The parasites that cause diarrhea are *Entamoeba histolytica*, *Giardia lamblia*, and *Cryptosporidium*.

Management

Rehydration is the cornerstone of the management of acute watery diarrhea. The development of inexpensive and simple oral rehydration therapy (ORT) has greatly simplified the treatment of diarrhea, regardless of the etiology of the diarrhea or age of the patient. Fluids and electrolytes that are normally available at home can be used to treat diarrhea. Approximately 90–95% of patients with acute diarrhea can be managed effectively with oral rehydration solution (ORS) alone. Zinc supplements are also recommended for acute diarrhea because zinc reduces the severity and duration of diarrheal episodes and lowers the risk of reinfection during the subsequent 2 to 3 months. Those who are unable to take ORS orally or with severe dehydration require intravenous fluids. However, ORS does not cure diarrhea symptoms or correct intestinal pathology. The principles of treatment of diarrhea consist of correction of dehydration and maintenance of hydration, elimination of etiologic agents (if possible and necessary), and maintenance of nutrition. Antimicrobials are not needed in most cases of diarrhea and useful only in selected cases, particularly in shigellosis, cholera, severe traveler's diarrhea, amebiasis, giardiasis, *C. difficile* enterocolitis, and *Cyclospora* infections.

Risk Factors

Several risk factors have been identified for diarrhea. Complex interactions between the host, etiological agents, environmental, and socioeconomic factors operate at different levels. Cell-mediated immune deficiency and malnutrition are independent risk factors for persistent diarrhea in children. In developing countries, early weaning, lack of exclusive breast feeding up to age 6 months, and introduction of contaminated complementary food at an inappropriately young age have been identified as important risk factors for diarrhea in infants. Age and nutritional status appear to be the most important host factors in determining the severity and the duration of diarrhea. Studies have shown that male children have more diarrhea compared to female children. Inappropriate prescribing of antibiotics encourages the risk of emergence of drug resistant microorganisms.

Intervention Studies

There have been significant reductions in mortality from diarrhea worldwide. Reductions in diarrhea mortality have been attributed to general improvements in living conditions, including better nutritional situations, access to medical care, increased vaccine coverage, increased coverage of potable water and sewage systems and the growing use of ORT. Intervention programs to improve sanitation have documented a 26% reduction in diarrhea prevalence and an 11% reduction in incidence of diarrhea in Brazil among preschool children. It has been suggested that combining improved water supply, sanitation and hygiene education projects might be able to reduce diarrheal morbidity rates by 30–50%. Secondary infection rates of shigellosis were reduced by 67% with promotion of hand washing. Simple techniques like hand washing before preparing food help to prevent diarrhea.

Prevention and Control

Proper management of diarrheal cases at health facilities and the home are important in preventing deaths from dehydration. Workers at the health service should be trained to use fluid to rehydrate severely dehydrated patients. Mothers and other family members should be able to recognize dehydration and conditions which require referral to a health worker or a health service. Approximately 90% of diarrhea cases recover within a short period without further intervention if appropriate fluid replacement at home is initiated without delay. With training, provision of intravenous fluids and ORS, the case fatality rate of diarrhea can be brought down to 1% even in a simple, makeshift community treatment center. Breast feeding, improved weaning practices, use of safe water, hand washing, use of latrines and proper disposal of stool and measles vaccination are important in prevention of diarrhea. In addition preventive measures include making food safe for consumption by thorough cooking of high risk food (especially sea food) and health education through mass media. The optimal period of exclusive breast feeding should be up to 6 months of age. Breast milk has both immune and nonimmune antimicrobial factors; also exclusive breast feeding eliminates intake of contaminated food and water. There is a strong inverse association between appropriate safe complementary feeding and mortality in children of age 6–11 months. Measures should be taken to improve nutritional status as improvement in nutritional status reduces infection and can help to break the vicious cycle of malnutrition and infection. Vaccinations against a range of diarrheal pathogens are helpful to control global diarrheal disease burden. Two new rotavirus vaccines – Rotarix by GlaxoSmithKline and Rotateq by Merck – have been developed. Both the vaccines were tested in industrialized, middle, and low income countries which demonstrated safety of the vaccine and efficacy in preventing deaths from rotavirus diarrhea. Dukoral, an oral vaccine that consists of killed *V. cholera* organisms along with the cholera B subunit stimulates both antibacterial and antitoxic immunity. Typhim Vi, a purified capsular polysaccharide parenteral vaccine and Vivotif Berna, an enteral live attenuated strain of *S. typhi* vaccine are available to prevent typhoid.

Probiotics are live microbes of healthy normal human gut microflora which improve intestinal microbial balance and mucosal-associated immune defenses.

See also: Breast Feeding. Dehydration. Electrolytes: Acid–Base Balance. Growth and Development: Physiological Aspects

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DIETARY FIBER

Contents

Physiological Effects and Health Outcomes Role in Nutritional Management of Disease

Physiological Effects and Health Outcomes

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Glossary

Nonstarch polysaccharides Polysaccharides (generally of plant origin) which resist small intestinal digestion and enter the large bowel.

Prebiotics Selectively fermented food ingredients which allow specific changes, both in the composition and activity in the gastrointestinal microflora that confer benefits upon host well-being and health.

Probiotics Live microorganisms which when administered in adequate amounts confer a health benefit on the host.

Resistant starch Starch which resists small intestinal digestion and enters the human large bowel.

Whole grain foods Foods made from processed grains which contain the major constituents (endosperm, germ and bran) in the proportions that are present in the whole cereal.

Introduction

National and international health authorities recommend levels of dietary consumption for the general population and also for specific groups during the various stages of life cycle. This advice anticipates greatly improved physiological function and lowered disease risk with greater intake. These expectations are based principally on observational studies in various populations linking dietary intakes to better long-term risk status for a range of noninfectious health problems. They are reinforced by *in vitro*, animal, and human experimentations that show varying degrees of benefit for diverse conditions and physiological processes. However, a real appreciation of the significant opportunities for fiber in public health requires an understanding of how dietary fiber components can influence physiological processes. This is where there is a continuing global shift away from a relatively simple view of fiber action, based largely on intestinal indigestibility, toward a much more complex situation where resistance of fiber components to human small intestinal digestion is coupled with bacterial fermentation in the large bowel.

The Evolution of the Dietary Fiber Concept

The health potential of dietary fiber has attracted attention for a very long time with a primary focus on gastrointestinal

function, especially laxation. However, it is fair to say that most investigative activity was largely unfocused, nonsystematic, and limited by inadequate analytical measures for the food constituents that comprise dietary fiber. Much of the current high level of community interest in fiber is due to the pioneering work of a number of British medical practitioners working in South and East Africa in the mid-twentieth century. They noted that the native (black) Africans ate a largely plant-based diet with unrefined (whole) grains as staples. This population was essentially free from the noninfectious diseases (constipation, appendicitis, diverticular disease, colorectal cancer (CRC), etc.) that affected (white) Europeans living in the same environment. Based largely on observation (rather than dietary records), it was noted that the latter ate highly refined foods and ascribed the differential health status to apparent differences in fiber intake. Concurrently, the importance of indigestible feed carbohydrates (fiber) in the production of animal (ruminant) nutrition was being established and the standard analytical methodologies were orientated toward their measurement in forage. Stock feed fibers are largely insoluble plant cell-wall material, which is overtly 'fibrous'. By extension, a similar view developed of fiber in human food was leading to its definition in terms of indigestible plant cell-wall material. However, it emerged rapidly that other plant noncell-wall materials (e.g., exudates) were equally indigestible, leading to a modified definition as "Plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man."

However, the key feature of fiber small intestinal indigestibility remained and this concept was applied to the whole gastrointestinal tract, providing a ready explanation for the fecal bulking properties of fiber which is central to its laxating effects. This has been called the 'roughage' model of fiber, as it accorded with the apparently greater fiber intakes by low-risk native populations who are characterized by high stool outputs and low risk of associated diseases. The perception of fiber as a simple bulking agent focused research on nonstarch polysaccharides (NSPs) as these are the major components of plant cell walls. It led also to the development of the concepts of soluble and insoluble fiber, with much attention being paid to the latter (which is now under some scrutiny). Here, 'solubility' refers to the aqueous solubility of a particular fiber. However, the solubility under such conditions is not necessarily the same as that which occurs in the gut.

The inadequacies of the 'roughage' model are known and, even for the key property of laxation, it has been known since 1986 that the bowel regularity of native (black) South African children was not explicable in terms of dietary fiber intake despite the fact that they ate unrefined cereal foods. The focus on 'roughage' has led to an important population-wide experiment in Australia. There is no valid retrospective measure of total dietary fiber (TDF) in foods using current analytical procedures, but the limited data suggest that intake in the early 1980s was ~14 g per person per day. Since then, estimates of population-wide fiber consumption indicate that intakes are ~27 g per person per day for males and females across much of the life cycle. This change would have been expected to translate to a lowering of CRC risk. Quite the contrary, Australian CRC death rates remain among the highest in the world and incidence seems to be increasing. This paradox of apparently high TDF consumption but high CRC rates is the exact opposite of that in Southern Africa – low CRC rates with low TDF intakes. This anomaly can be resolved through examining the culinary practices of the African natives, who cook their staple (ground whole-maize meal) in water and then consume it after cooling. The cooking gelatinizes the starch making it much more susceptible to small intestinal α -amylase, but the subsequent cooling leads to reassociation of the starch chains. This process is called retrogradation and leads to resistance to amylolysis both *in vitro* and *in vivo*. This starch fraction is called resistant starch (RS) – a contraction of the original name, enzyme-resistant starch. The diet of native Africans is high in RS with average daily intakes of ≥ 20 g per person per day. However, the industrialization of food production means that RS intakes in Australia (and probably similar countries) are low and may be no more than 4–6 g per person per day. This difference seems to be a key factor in the differential rates of CRC (and other causes of morbidity and mortality) between populations. It also points to a major difference between RS and NSPs, i.e., their susceptibility to large bowel bacterial fermentation and its products.

Large Bowel Bacterial Fermentation and the Health Effects of Fiber

The general perception is that fiber survives passage through the gastrointestinal tract intact, but this is substantially wrong.

The fecal recovery of ingested NSP is highly variable, ranging from 100% (e.g., for purified cellulose) to ~0% (e.g., for pectin). A landmark study in humans showed that there are two distinct mechanisms for the fecal bulking action of fiber. Cereal fiber (comprising largely insoluble NSPs) increased fecal bulk through the passage of undigested fiber (plus some bacteria), whereas cabbage increased bacterial mass (plus some residual fiber).

Although much greater emphasis is being placed on the role of the major fermentative end products in effecting the benefits of fiber, it is equally important not to ascribe all of the benefits to them. More probably, the effects are a composite of physical presence (which dominate in the small intestine) and fermentation (which occurs in the large bowel). This is reflected in the current definition in Australia (and which is close to the Codex Alimentarius definition):

"Dietary fiber means that fraction of the edible part of plants or their extracts or synthetic analogs that –

1. are resistant to digestion and absorption in the small intestine, usually with complete or partial fermentation in the large intestine; and
2. promote one of the following beneficial effects:
 - (i) laxation,
 - (ii) reduction in blood cholesterol, and
 - (iii) modulation of blood glucose; and
3. includes polysaccharides, oligosaccharides (DP > 2), and lignins."

Evidence for the Health Benefits of Dietary Fiber

Much of the research direction in dietary fiber and health and of its expected health benefits comes from large human prospective cohort studies. These studies provide valuable insights into the relationships between dietary intakes, lifestyle factors, and disease risk, but they are limited. One clear drawback is the time taken from study initiation to the emergence of clinical end points (including death and nonfatal events). The elapsed time can be years so that there may well be substantial changes in key study factors – of great relevance for foods where there is a strong possibility of compositional variation with time. The situation for fiber is made more complex by changing definitions and improved analytical methods. However, the greatest drawback is that these studies show association, not causation, and often give no clue as to the mechanism involved. This is crucial for fiber where it is understood now that it comprises a range of components with quite divergent modes of action. This explains the Australian paradox for CRC and the inconsistent reports on the effectiveness of fiber. Some studies have failed to show any relationship between consumption (either as absolute amounts or on an energy adjusted basis) and CRC risk. In contrast, the large European Prospective Investigation into Cancer and Nutrition (EPIC) showed a strong dose-dependent reduction in CRC risk with greater fiber consumption. Importantly, this protection remains (even though attenuated) after correction for other protective factors. However, the Australian paradox highlights the fact that increased fiber consumption, defined in terms of fiber as 'roughage', can attenuate CRC risk. This is a very important issue for public health outcomes where

consumers are being encouraged to consume fiber-rich food for a benefit which is deferred for many years. The same is true for cardiovascular disease (CVD) where greater fiber (defined as TDF) consumption has been correlated with lower risk. Indeed, a portfolio of data from population studies shows convincing (i.e., statistically significant) dose-dependent relationships between fiber and diminished risk of the number of conditions. These are major global socioeconomic problems which are established in industrialized countries and are emerging in developing countries with greater affluence. Data are also being accumulated showing risk reductions in rather unexpected areas, for example, chronic obstructive airways disease (COAD), also called chronic obstructive pulmonary disease (COPD). Cigarette smoking is a known risk factor for COAD/COPD and it is relatively easy to link inhaled smoke with pulmonary disease, but it is much more difficult to develop a protective mechanism for nondigestible food components. One possible mechanism lies in the interaction between the gut microbiome (which is influenced heavily by diet) and gut-associated lymphoid tissue (GALT), but this is yet to be investigated.

Physiological Actions of Dietary Fiber and Improved Health Outcomes

The current definition of fiber identifies polysaccharides (including synthetic analogs) and oligosaccharides (OSs, degree of polymerization 2–10) as the main components and also lists key physiological effects, including fermentation in the large bowel to a degree which varies by carbohydrate type. Other important effects include laxation, plasma cholesterol reduction, and attenuation of blood glucose response to a meal (i.e., glycemic index (GI) or load). Health outcomes are major drivers for greater consumption because consumers expect some benefit in return for dietary change. However, the breadth of the expected benefits (and observed physiological effects) precludes a simplistic catalog of fiber type, especially in relation to mechanisms of action. One solution is to consider current knowledge of the physiological actions of fiber relevant to important health issues and attempt to link them to individual fiber fractions.

Specific Actions of Fiber

Satiety and Food Intake

Excess adiposity (especially abdominal obesity) is a growing global issue. It is a risk factor for causes of early morbidity and mortality including CVD, CRC, and type 2 diabetes (T2D). Obviously, obesity represents an accumulation of excess energy over expenditure, meaning that there are two strategies for weight management – increased energy expenditure or diminished energy intake (or a combination of both). Population studies have shown consistently that obesity risk is lessened dose dependently by dietary fiber intake. This does not seem to be through energy dilution of food or energy expenditure, meaning that fiber might act through satiety, secondary to greater bulking in intestinal contents. There are consistent reports of lower energy intake through consumption of fiber-

enriched foods but these studies are generally short term and do not translate to sustained loss of body weight. There appear to be no reliable human data on the long-term effectiveness of TDF or individual components in lowering food intake.

Soluble NSPs and Blood Glucose Control

T2D is emerging as a major global problem, especially in Asia. It is characterized by sustained hyperglycemia through impaired insulin action and, eventually, deficient secretion. Dietary modification can assist in improving glucose control and also reducing the glucose challenge to the pancreas. Population studies have shown inverse relationships between whole grain and dietary fiber intakes and T2D risk. This seems to be via soluble NSPs and clinical studies have shown that ingestion of meals containing soluble NSPs reduces the excursions in blood glucose following a carbohydrate meal. This lowering of the GI is linked to NSP viscosity in solution, which slows the passage of digesta through the small intestine and also the diffusion of glucose from the lumen. It also limits the access of amylase to the starch granule, slowing glucose release. In addition to this acute effect (the ‘first meal’ effect), there is also a reduction in the glycemic response to subsequent meals. This ‘second meal’ effect is linked to the onset of large bowel fermentation and the production of short-chain fatty acids (SCFAs). Clearly, this effect depends on fermentability of the fiber polysaccharide (i.e., the extent of fermentation plus SCFA production) and not solubility. Finally, there is also an adaptive effect of fiber leading to improved glycemic control, which is also related to fermentation. Although the lowering of GI by soluble NSPs has been shown in human trials, it is not clear whether this translates to a long-term improvement in T2D. This uncertainty may reflect the fact that fiber can improve glucose control through staged mechanisms acting acutely and in the medium and long term. It is also possible that both soluble and insoluble NSP can lower starch digestibility relatively modestly, thereby leading to a cumulative long-term diminished demand for insulin.

Soluble NSPs and Plasma Cholesterol

Coronary heart disease (CHD) occurs as sudden myocardial infarction or through progressive occlusion of the coronary circulation through infiltration of lipoprotein cholesterol into the intima. If unchecked, tissue necrosis and death follows. CHD has a number of fixed risk factors (such as age and gender), whereas modifiable risk factors include smoking, physical activity, and raised plasma low-density lipoprotein (LDL) cholesterol. The latter is the main vehicle for transporting plasma cholesterol to the tissues with obvious connections to CHD. Lowering of LDL cholesterol is an established strategy for reducing CHD events.

Cereals high in soluble NSPs can lower plasma total and LDL cholesterol by 3–5%. This has been shown particularly with oats and (to a lesser extent) barley. Both cereals contain soluble NSP β -linked glucans, which are thought to be the active agents. Studies in humans and animals have shown reductions with isolated glucans supporting this hypothesis. However, it must be recognized that there are some contradictory data with glucan-enriched fractions being ineffective. The reasons for this uncertainty are very important and may

reflect isolation procedures and food production among other factors. Glucans (and other soluble NSP such as psyllium) are thought to modulate digestion through their viscosity in solution, slowing digesta flow, thus delaying fat absorption and the reabsorption of bile acids. Bile acids are surface-active steroids synthesized from cholesterol in the liver and are secreted into gut (in bile) where they assist in lipid digestion and absorption. These acids are conserved through the enterohepatic circulation, being reabsorbed from the terminal small intestine and returned to the liver. Interruption of this cycle leads to greater fecal loss with the deficit leading to increased hepatic cholesterol catabolism and a fall in plasma LDL concentrations. The attractive hypothesis that insoluble 'NSP (or lignin)'-bound bile acids lowers LDL has not been translated effectively in human or animal trials. Indeed, it has been known for some considerable time that the effect may be an artifact of the procedures used to isolate lignin.

Fiber, Regularity, and Diverticular Disease

Promotion of regularity is the best documented and substantiated effect of fiber as isolates or fiber-rich foods. This is largely a property of insoluble NSP and relates to the passage of greater stool bulk. The relative effectiveness of a preparation relates to its capacity to increase fecal bulk per unit of fiber consumed. Of the foods consumed commonly in western countries, wheat bran appears to be the particularly effective with relative increases of approximately >4 g of additional stool per gram of product consumed. Although bulking is important, there is evidence that this is not necessarily the sole factor. It is a consistent finding that the presence of specific NSPs in a wheat product enhances its effectiveness considerably. This is the pentosan (arabinoxylan) fraction and these NSPs appear to be of high fermentability, which then introduces the potential role of SCFAs in the promotion of laxation as these acids are known to modify colonic muscular activity. It also raises the issue of the relative contribution of undigested fiber and fiber fermentation products to large bowel health and lowered risk of disease. Attention has been drawn to the observation that the greater ease of laxation among African children consuming unrefined foods was not related to high fiber consumption.

Large Bowel Fermentation and the Health Effect of Fiber

This is a subject of intense (and growing) community interest and it seems that some of the major actions ascribed to fiber may actually be due to the end products of large bowel microbial fermentation.

The Large Bowel Microbiome

The commensal microbiome is acquired on passage of the sterile infant through the birth canal when the gut becomes inoculated. This inoculum includes species of importance to the adult as well as the infant, highlighting their role through the entire postpartum life cycle of the host.

Consideration of the range of commensal species and their diverse activities lies outside the scope of this article. However, their fermentative products are central to the role of fiber. Their principal metabolic substrates for the large bowel

microbiome are undigested dietary components (mainly NSPs and RS plus some protein) and nondegraded body secretions (e.g., mucins). In adults, the major end products are SCFAs with gases (CO₂, H₂, and CH₄) and an increased bacterial mass. The major acids are acetate, propionate, and butyrate, generally in the rank order of abundance acetate > propionate ≥ butyrate. The actual proportion depends on substrate supply. In breast-fed infants, the products are rather different to those in adults, reflecting the fact that milk is the sole nutrient source. One of the key differences is the absence of propionate and butyrate, the predominance of acetate, and the presence of other acids (e.g., lactate and formate) and other metabolites (e.g., ethanol) not found in adults. This seems to reflect the presence of OSs in milk.

SCFAs and Human Health

One of the earliest studies of SCFA distribution (measured as volatile acid) was carried out more than 60 years ago and showed their abundant presence in the rumen of herbivores and in the large bowel of obligate herbivores including rabbits. However, they were found also in the hind gut of omnivores (pigs) and, much later, in humans. The importance of SCFAs to the energy needs of herbivores is accepted, but their potential human health significance took much longer to emerge.

SCFAs and Health Outcomes: Irritable Bowel Syndrome, Inflammatory Bowel Disease, and Colorectal Cancer

The production of SCFAs in the large bowel can be subdivided into two – a general effect on the colonic environment and specific actions of individual SCFAs.

Total SCFAs

Collectively, the major SCFAs have nonspecific effects including direct acidification of the digesta. This is believed to suppress the growth of potentially pathogenic organisms and also limit the absorption of cytotoxic and genotoxic compounds (such as ammonia), which are absorbed to a significant degree only in the unprotonated form, i.e., at pH values ≥ 7.

Acetate, Propionate, and Butyrate

These are the major acids in adults. Although acetate is the predominant SCFA throughout the lifecycle, there is little evidence for a specific effect unless it is to contribute to the control of pathogens. Much of the data for effects of the other two acids are derived from animal studies, so the link to health effects is by extension. Propionate appears to have a role in controlling aspects of colonic muscular contraction. However, of the major acids, butyrate seems to be pivotal in the promotion of large bowel health and is the most effective SCFA in modulating colonic muscular activity via enteric neurons. Butyrate infusion leads to a lowering of visceral sensitivity in humans, which points to a role in irritable bowel syndrome (IBS). This is a very common (generally nonlife threatening) condition characterized by abdominal pain and disordered large bowel leading to constipation or diarrhea. Fiber (as NSPs) appears to be quite ineffective in IBS.

Butyrate is a major fuel for colonocytes and its metabolism helps to drive the uptake of electrolytes and water, which assists in increasing water salvage in diarrhea in children.

Butyrate may have further roles in large bowel integrity. Animal trials have shown that raising large bowel butyrate appears to enhance intestinal anastomotic strength and enhance mucin production. Both would help to protect against bacterial translocation, a key element in the development of inflammatory bowel diseases (IBDs). IBD is another example where 'fiber' (i.e., NSPs) has proved to be of questionable value. There are two distinct forms of IBD, ulcerative colitis (UC) and Crohn's disease (CD), and both appear to be an aberrant inflammatory response triggered by the intestinal microflora. CD can occur outside the large bowel but UC predominates in the distal colon, so there is clear potential for butyrate to be of value. Finally, a large number of animal studies have shown that increased SCFAs promote a normal colonocyte phenotype with butyrate appearing to be the most potent. CRC is manifest as a progressive series of changes in the colonocyte population leading to frank malignancy. Although there is no direct evidence for a protective role for butyrate in human CRC, the data appear to be highly supportive.

Fiber and SCFAs

One of the key issues in linking dietary fiber, SCFA, and disease risk is establishing a nexus between fiber type and total and individual SCFA production. **Table 1** shows the potential substrates for the colonic microbiota and it is clear that, relative to a low-risk population, this western-type profile is relatively high in NSPs and lower in RS. Although there are animal trials with fiber isolates and some foods, the data from human studies are limited and are confined generally to fecal measures.

Oligosaccharides

OSs have gained much attention through their role as prebiotics in foods that promote the survival of probiotics. Current probiotics are predominantly lactic acid bacteria and bifidobacteria, species which predominate in breast-fed infants. Their major products do not include propionate and butyrate, which raises their usefulness in adults into question. However, in infants it appears that OS consumption promotes health and lowers the risk of inflammatory conditions such as asthma.

Nonstarch Polysaccharides

The range of food NSPs in nature is so large as to be almost impossible to catalog. These polysaccharides have fermentabilities ranging from 0 to 100%, but very few have been studied

systematically in human trials. Again, fecal SCFAs have been the general means of assay and the very limited number of trials has been with either whole foods or NSP isolates.

Resistant Starch

Population data support the concept that RS is an important substrate for the large bowel microbiome and that its fermentation favors butyrate generation. This may contribute to the low risk of large bowel and other diseases in populations consuming traditional diets. However, the number of studies linking RS consumption to improved health status is quite limited, certainly for CRC and IBD, and there appear to be none for IBS. Further, RS exists for a variety of reasons (including process modification of digestible starch) which is also in need of exploration.

Conclusions

There is good evidence linking greater fiber consumption to improved health outcomes and, in some specific instances, sound mechanisms for a physiological effect. However, there is a rapid and substantial change occurring with growing understanding of the potential role of the large bowel microbiome and its fermentation products in the health effects of fiber. This, coupled with a reappraisal of the precise contribution of individual fiber components to the activities of the large bowel microbiota, means that both mechanisms of action and the full potential for health improvement remain to be determined.

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Relevant Websites

www.foodstandards.gov.au

Food Standards Australia and New Zealand.

Table 1 Potential substrates for the colonic microbiota of adults on a 'Western' diet

Substrate	Amount (g d ⁻¹)
Resistant starch	1–5
Nonstarch polysaccharides	8–18
Insoluble NSPs	6–14
OSs	2–5
Simple sugars (mainly fructose, sucrose, glucose, and maltose)	2–8
Proteins	2–10
	3–15

Note: Approximately 5–10 g lipid and 6–9 g day⁻¹ of endogenous secretions and an unknown quantity of sloughed intestinal cells are substrates for the colonic microbiota.

Role in Nutritional Management of Disease

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Glossary

Dietary fiber Nondigestible carbohydrates and lignins from plants.

Diverticular disease Condition consisting of a combination of diverticulosis and diverticulitis.

Diverticulitis Inflammation of the diverticulae, which occurs in 10–25% of people with diverticulosis.

Diverticulosis A common condition in which the lining of the colon bulges outwards through weak spots in the colon wall, forming small pouches or 'diverticulae.'

Functional fiber Nondigestible carbohydrates that have established beneficial effects on humans.

Glycemic index (GI) A measure of the effects of carbohydrates on blood glucose levels. A food with a higher

GI generally causes a greater increase in blood glucose, although the effect is modulated by many other factors such as other foods consumed simultaneously and the method of food preparation.

Irritable bowel disease Inflammatory disease of the colon and small intestine, which may include Crohn's disease and ulcerative colitis.

Irritable bowel syndrome A common disorder characterized by cramping, constipation or diarrhea (which may be alternating), bloating, and abdominal pain. The cause is unknown.

Total fiber The sum of dietary fiber and functional fiber.

Introduction

Dietary fiber was a phrase unknown to all but a handful of individuals until the early 1970s when a wide range of potential therapeutic applications of dietary fiber were suggested by Hugh Trowell, Denis Burkitt, and Alexander Walker. Twenty-five years later only a few people have not heard of the term, although they may not be able to define it. Fiber has been classified into the following types: dietary fiber that consists of nondigestible carbohydrates and lignins from plants; functional fiber that consists of isolated, nondigestible carbohydrates that have established beneficial effects on humans; and total fiber that is the sum of the preceding two categories. In some cases, the claims of benefit remain largely unsubstantiated but in for three diseases, hyperlipidemia, diabetes, and disordered bowel function, there is sufficient evidence to allow dietary advice to be given.

Hyperlipidemia

Some forms of dietary fiber lower blood lipids, notably total cholesterol and low-density lipoprotein (LDL) cholesterol, and possibly triglycerides. The earliest observations on fiber preparations and blood lipids date from the mid-1930s when there was a fairly extensive investigation of the effects of pectin (polygalacturonic acid). The next period of investigation dates from 1974 when extracted and purified dietary fiber preparations such as guar gum – a glucomannan – were tested in normal, diabetic, and hyperlipidemic subjects and were found to lower blood cholesterol when given in sufficient quantities. In very large doses, these materials increase fecal excretion of fat and sterol compounds and would be expected to reduce

the body bile salt pool. Subsequent work has shown that at lower doses preparations of soluble dietary fiber have a mild cholestyramine-like effect: they bind bile salts rendering them unavailable for reabsorption in the terminal ileum, thus interfering with the normal enterohepatic cycle of bile salts and depleting the bile salt pool. Total and LDL cholesterol fall as cholesterol is diverted for the resynthesis of lost bile salts. However, this is likely not the only mechanism involved because the increase in fecal bile acids is small and not all soluble fibers increase fecal bile acids.

Preparations of soluble and functional dietary fiber lower blood cholesterol, whereas most preparations of predominantly insoluble fiber, such as wheat bran, have little or no effect. The major food sources of soluble fiber are oats, beans, lentils, rye, and barley, and these foods have naturally become the subject of clinical investigations. The addition of oats to the diet in normolipidemic and hyperlipidemic subjects following either their normal diets or when pretreated with low-fat diets has been the subject of extensive research. In sufficient quantity, oats, oat products, and oat β -glucan (providing at least 3 g oat β -glucan per day) lower blood total cholesterol and LDL cholesterol (usually by 5–10%), whereas leaving triglycerides and high-density lipoprotein (HDL) cholesterol largely unchanged. After a sufficiently large number of good-quality studies had been done on oats, the Food and Drug Administration (FDA) allowed the first ever food-specific health claim: 'Soluble fiber from oatmeal, as part of a low saturated fat, low cholesterol diet, may reduce the risk of heart disease.' Products that are labeled with this claim must provide at least 5.5% soluble fiber (as β -glucan) per serving. When considering the above claim, the FDA reviewed 37 studies and found that a sufficient number provided convincing evidence of efficacy. An earlier meta-analysis

of some of those trials had shown that the efficacy of oats and oat products was influenced by the initial values of blood cholesterol in the subjects: Patients with high starting values (more than 6.7 mmol l^{-1} total cholesterol) showed the greatest reductions when treated with oats, whereas healthy young subjects with low-to-normal starting values showed little response. A meta-analysis of 20 trials with oat bran found that cholesterol falls by $0.1\text{--}2.5\% \text{ g}^{-1}$ of bran with a resulting 2.4% reduction in risk of coronary heart disease per gram, other soluble fiber-containing products have been shown to lower blood cholesterol. The European Food Safety Authority approved a health claim for β -glucan in 2011. Extensive studies on psyllium (*Plantago ovata*) presented both as a pharmaceutical preparation and as a food product (a ready to eat breakfast cereal) have proven cholesterol-lowering properties where the dose–effect relationship is such that a useful additional therapeutically meaningful lipid-lowering effect can be achieved by prescribing a daily portion of psyllium-fortified breakfast cereal. Products of this type are now widely marketed and the US FDA allows a food-specific health claim for psyllium. Psyllium is also widely used in stool softener products sold over-the-counter.

There is also a small literature on the effects of beans on blood lipids and the findings of a blood cholesterol-lowering effect as expected. Total cholesterol and LDL cholesterol are reduced 5–8% by consuming half a cup of cooked or canned beans per day, and 19–24% by consuming a whole cup.

Virtually all of the reports of the effects of soluble fiber products on blood lipids report lowering effects on total cholesterol and LDL cholesterol without any effect on HDL cholesterol, and usually a much smaller or no effect on triglycerides – this contrasts with the effects of some drugs that may cause slight rises of triglycerides and falls of HDL cholesterol. The relationship between lowering of blood cholesterol and lowering of risk of heart disease is now generally accepted and a proven lipid-lowering effect is taken to mean a beneficial effect on risk of coronary heart disease. This means that in clinical practice it is reasonable to include advice on use of foods high in soluble dietary fiber in a lipid-lowering diet, and perfectly proper to emphasize the benefits of oats and oat products. Generally, a high-soluble fiber diet is more acceptable when the soluble fiber is drawn from smaller quantities of a larger range of foods; thus such a diet might include beans, lentils, rye breads, and barley as well as generous use of oats. A range of foods containing mycoprotein and fungal mycelial cell walls (chitin) may also help to lower blood cholesterol. These products are consumed in the UK and elsewhere in Europe but at present are not sold in the USA.

The US–Canada Dietary Recommended Intake (DRI) for dietary fiber has been set at 14 g per 1000 kcal, based on the amount associated with reduced risk of coronary heart disease in population studies. This amounts to 25 g day^{-1} for younger women and 38 g day^{-1} for younger men, and slightly less (21 and 30 g for women and men, respectively) for adults over the age of 50 years. The recommendation is an adequate intake, meaning that it likely meets or exceeds the actual requirement. This recommendation is also likely to be sufficient for reducing risk of diabetes and constipation.

Diabetes

Diabetes mellitus is characterized by either an absolute or relative lack of insulin, which has short- and long-term consequences. People with diabetes may develop both microvascular complications (mainly affecting the eyes, kidneys, and nerves) and macrovascular complications (essentially accelerated development of atherosclerosis presenting mainly as heart attack and peripheral vascular disease). Medical management aims to replace the insulin or modulate its production or efficacy using oral (hypoglycemic) drugs, in a metabolic environment enhanced by good control of diet and body composition. Medical management also aims to achieve early detection of complications and other risk factors for cardiovascular disease by regular testing of blood and urine biochemical variables and blood pressure and by regular physical examination of the eyes, neurological, and cardiovascular systems.

Control of dietary energy intake (in relation to the varying demands for growth, maintenance, physical activity, etc.) remains the key feature of dietary control affecting metabolic fluxes, blood glucose levels, and body weight. Views on the appropriate proportional sources of energy from fat, carbohydrate, and protein have changed enormously over the past century from seriously energy-restricted high-fat diets (with percentage energy from fat as high as 70% raising some doubts about the level of compliance) through to very high-carbohydrate diets (sometimes 60–65% energy from carbohydrate) used in specialist centers in the USA. Today, for the majority of patients with diabetes in most countries, the target is to achieve 50–55% energy from carbohydrate sources. Before the 1970s, when the move toward high-carbohydrate diets began, the high-fat content of the diet along with less tight blood glucose (and urine glucose) control than is customary today was partly responsible for the high relative mortality from cardiovascular disease seen among diabetic patients. At that time, young male diabetics were up to nine times more likely to die from heart attack than matched nondiabetic individuals. Reduction of fat in the diet and achievement of an optimal distribution from saturated, monounsaturated, and polyunsaturated sources (<10%, 10–20%, and no more than 10%, respectively, for patients with diabetes in the UK) remain a major aspect of dietary management of diabetic people in order to reduce the risk of developing coronary heart disease.

Control of blood glucose is critical in order to achieve avoidance of prolonged periods of hyperglycemia, which is associated with glycation of proteins and the risk of development of microvascular complications, and avoidance of hypoglycemia with its attendant risks of coma. In day-to-day practice, the avoidance of hypoglycemia is very important to patients and any new method of achieving normalization of blood glucose profiles is an advance. Dietary fiber offered such an advance from the mid-1970s when some forms (notably isolated polysaccharides such as guar gum, a glucomannan, and pectin, polygalacturonic acid) were shown to reduce the area under the blood glucose and insulin curves after acute test meals. Subsequent long-term (6 week) clinical trials showed that diets high in foods containing soluble dietary fiber, such as beans, oats, and barley, were more effective in reducing the

area under the 24-h blood glucose profiles than diets containing more high-fiber foods based on wheat products.

Research in this area led Jenkins to describe (in 1981) the concept of the glycemic index (GI), which is a numerical expression of the ability of a food to raise blood glucose levels. In practice, it is measured by comparing the blood glucose response to a 50-g carbohydrate portion of food with the response to 50 g glucose (in some articles, the comparison is with a 50-g carbohydrate portion of bread). The dietary fiber (especially soluble fiber) content of a food slows down the rate of digestion and absorption of starch in foods giving flatter blood glucose responses and a lower GI; however, the structure of the starch (whether amylose or amylopectin) influences its rate of degradation and the extent to which the starch granules are hydrated by processing (including cooking) is also important. The physical structure of the food (particularly the extent to which plant cells are intact), the presence of fat, which may slow gastric emptying, and the presence of some antinutrient substances may all influence the GI. Low-GI diets have been shown in many clinical trials to improve important variables that are secondary indicators of blood glucose control and to reduce blood lipids. Low-GI diets have been shown in two large-scale epidemiological surveys published to be associated with a significant reduction in the risk of development of maturity onset (type 2) diabetes in middle-aged American men and women. Thus, there is some reason to believe that there should be greater emphasis on the GI of diabetic diets and the fiber content, as well as emphasis on GI for those at risk of developing diabetes, especially the older obese person. Expert committees in many developed countries of the world have set target values for dietary fiber intake for diabetic patients (e.g., the American Diabetes Association (ADA) recommends 20–35 g total dietary fiber daily and many, especially the Australian Diabetes Association and with the notable exception of the ADA, have recommended an increase in low-GI foods). Diabetes UK (the UK Diabetes Association) notes that there might be merit in taking account of GI in dietary management for those with diabetes. Some physicians and other health professionals believe that the GI of foods is too complex an issue for patients to grasp, but in essence, it simply requires a partial substitution of bread and potatoes with pasta products, an increased use of high-fiber breakfast cereals including oats, increased use of beans and lentils, and emphasis on the use of temperate fruits (e.g., apples and pears).

Obesity (body mass index ≥ 30 based on weight in kilograms divided by height in meters squared) is becoming more prevalent in developing countries and carries an increased risk of the development of diabetes mellitus; a high proportion of established type 2 diabetics are obese and overweight. It is plausible that dietary fiber would help people lose weight by a number of mechanisms, including reducing the efficiency of dietary energy absorption and by making people feel full for longer after meals thus having an overall effect on reducing food intake. There is an inverse association between body mass intake and dietary fiber intake in epidemiological studies. However, studies on the effects of dietary fiber on postprandial satiety where experimental meals are carefully designed to differ little except for fiber content have given variable results. However, there is a clear effect of fiber on

chewing (the number of chews necessary to eat the same energy equivalent of food) where high- and low-fiber types of commonly consumed foods are eaten and this may have an important satiating effect. Clinical trials of high-fiber weight loss regimens have given variable results. Double-blind placebo-controlled trials using pressed barley fiber and pectin tablets compared to a starch control have been undertaken in Scandinavia and have demonstrated statistically significantly greater weight losses in the fiber-treated groups up to 26 weeks of treatment. It seems reasonable to conclude that under some conditions, the right kind of high-fiber diet can facilitate weight loss, but may not always do so.

People with diabetes are more likely to have dyslipidemia than nondiabetic people. When control of diabetes is lost, patients may demonstrate gross hypertriglyceridemia due to increased production of very-low-density lipoprotein particles in the liver as a consequence of the increased flux of free fatty acids from the peripheral tissues. At the same time, total and LDL cholesterol may be raised. Improvement in diabetic control often achieves normalization of blood lipids, but where hyperlipidemia persists there may be a place for use of dietary fiber, especially soluble fiber, and oat β -glucan-containing foods as an adjunct to dietary and pharmacological therapy (see above).

Bowel Disorders

Denis Burkitt first suggested a role for dietary fiber in bowel disorders in 1971. In the intervening period, understanding of the normal physiology and pathophysiology of the colon have improved enormously. During the same period, methods of analysis have been refined and a distinction is drawn between dietary fiber (nondigestible plant carbohydrates and lignin in which the plant matrix is largely intact) and nonstarch polysaccharide (NSP) (determined by analysis of component sugars), and starch not digested in the small gut, which is now defined as being resistant and can be naturally present in a food or created by processing. Three types of resistant starch have been described. These advances in analysis have helped physiologists appreciate the contributions of various substrates to colonic fermentation and stool bulking.

The intake of dietary fiber (NSPs) is directly related to the amount of wet stool passed each day in large population groups. An average wet stool weight for the UK is approximately 105 g day⁻¹. Nearly half of the members of groups studied in the UK have stool weights of less than 100 g per day, below which complaints of constipation are common. Stool weight has been shown to be clearly inversely related to colon cancer incidence in population groups: a mean daily stool weight of 105 g corresponding to a relatively high population colon cancer incidence of approximately 22 per 100 000 per annum. An incidence rate of 11 per 100 000 per annum corresponds to a mean daily stool weight of approximately 175 g daily. This information was used as the numerical basis for calculating the UK's dietary reference value for NSP in the late 1980s. In UK, the population is urged to increase NSP intake by 50% to a population average of 18 g day⁻¹ in order to shift the distribution of wet stool weight upwards. The DRIs of fiber for the US and Canada are based on prevention of

coronary heart disease operating via reduction in LDL cholesterol levels, although they do accept that both dietary and functional fiber increase stool weight, and that in most studies, higher fiber intake was associated with reduced risk of constipation.

Constipation is generally considered to be infrequent opening of the bowels with straining to pass stools (less than three defecations per week and straining and/or the passing of hard stools in more than one in four defecations). Constipation is sometimes caused by another specific disease of either an endocrine nature (e.g., myxedema – reduced thyroid function) or physical obstructive nature (e.g., colon cancer). Where constipation has developed recently in a previously nonconstipated individual above the age of 40 years, colon cancer must be excluded as the cause of the change of bowel habit. In the absence of evidence that the constipation is secondary, it is probably due to dietary and life-style factors. The mucosa of the lower colon has a great capacity to desiccate its contents. If the call to stool does not occur or is ignored, residual material dries out and individual fecal pellets become smaller. There is experimental evidence to suggest that greater abdominal pressures are needed to expel pellets that are 1 cm in diameter than those that are 2 cm in diameter. Thus, factors that result in the call to stool being ignored, like not allowing sufficient time for defecation after a stimulus such as breakfast or the walk to the station or being unprepared to defecate anywhere except at home (a common characteristic consistent with mammalian behavior), are likely to cause constipation. Simple solutions include going to bed earlier and getting up earlier in the morning, and finding another acceptable location for defecation at the workplace. Increasing fiber in the diet, most easily achieved by making breakfast a high-fiber meal with either high-fiber breakfast cereals or high-fiber breads, will increase stool bulk, shorten transit time (the time for a marker to pass from the mouth and be passed in the stool), and alleviate symptoms in many cases. The importance of exercise in maintaining normal colon function is gradually being recognized – the importance of brisk walking should not be underestimated. However, some specific types of simple constipation have been identified, which do not necessarily respond to high-fiber diets. Grossly prolonged transit times reflecting seriously slow colonic motility has been seen particularly in young women and do not respond well to high-fiber diets, and some ‘outflow abnormalities,’ which sometimes have a basis in abnormal rectal conformation, may also not respond.

Diverticular disease of the colon, characterized by the development of protrusions of mucosa through the bowel wall, is common and usually asymptomatic. It has been shown to be less likely to develop in those following a high-fiber diet, and once acquired can be managed, in many cases, by ensuring an adequate amount of fiber in the diet. Experimentally, various fiber supplements and ‘bulking agents’ have been shown to reduce the abnormally high-peak intracolonic pressures that are characteristic of diverticular disease. Sometimes 10–20 g of coarse wheat bran as a supplement is all that is required, but some patients develop flatulence and distension at least initially. Other fiber supplements such as ispaghula husk (psyllium) may be as effective, without the initial adverse side effects. Sometimes, simple dietary changes to

achieve an adequate total daily intake of dietary fiber particularly from wheat-based foods are effective. Diverticulitis (inflammation of the diverticula) is a complication requiring medical management, which will usually include a short period of abstention from food. Many patients remain largely without symptoms once the right ‘fiber’ regimen has been determined.

The irritable bowel syndrome (IBS) is a ‘functional’ disorder of the bowel, which is said to affect up to 15% of the population and is characterized by some, but not necessarily all, of a range of symptoms, including alternating diarrhea and constipation, recurrent abdominal pain, and urgent or frequent defecation. An important part of management is the exclusion of other serious organic disease such as inflammatory bowel diseases (IBDs). In IBS, the gut is abnormally sensitive to distension, and symptoms may be related to or exacerbated by external emotional events. The role of high-fiber diets in IBS has been investigated and not surprisingly is only of benefit in some cases: in those patients in whom the predominant feature is constipation. In some patients, high-fiber diets may make their symptoms worse.

In IBD, high fiber diets have no special part to play. In the management of Crohn’s disease, enteral feeding (with formula low-residue, low-fiber preparations) is especially beneficial where there is acute extensive small bowel disease. In ulcerative colitis, specific dietary advice is usually unnecessary, although fiber supplements may be of benefit in patients whose disease is limited to proctitis (inflammation of the rectum).

The treatment of newly diagnosed colon cancer does not include diet therapy, but treatment of those at increased risk of developing colon cancer by dietary and other means will become increasingly common as more information about the effects of high fiber diets and supplements on colon function becomes available. The critical step in the adenoma–carcinoma sequence in the human large bowel is the enlargement of the small adenoma (which has a low risk of malignant transformation) to a large adenoma (which has a high risk of malignant transformation); dietary factors, including low amounts of fiber in the diet, enhance adenoma growth. Bile acids are strongly linked to adenoma growth and bile acid concentrations in the colon are influenced by dietary fat and dietary fiber. Other effects of fiber may also be protective: bulking the stool and accelerating material through the colon, and provision of substrate for fermentation particularly with production of butyrate, which may have antineoplastic properties. However, despite a great deal of epidemiological and experimental work, the potential role of dietary fiber in modulating the risk of colon cancer remains controversial. In setting the DRIs for the US and Canada, it was concluded that the evidence was conflicting and currently inadequate to use as a basis for setting a recommended fiber intake that would reduce risk of colon, breast, or any other cancer.

See also: Cereal Grains. Cholesterol: Factors Determining Blood Levels. Diabetes Mellitus: Classification and Chemical Pathology; Etiology and Epidemiology. Fiber: Physiological and Functional Effects. Glucose: Metabolism and Maintenance of Blood Glucose Level. Glycemic Index. Hyperlipidemia: Overview. Lipoproteins.

Nutritional Considerations for the Management of Hypertension

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Relevant Websites

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DIETARY GUIDELINES, INTERNATIONAL PERSPECTIVES

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Introduction

The use of food based dietary guidelines (FBDGs) has emerged as an important food and nutrition policy and education program since the late 1970s and is valuable for addressing issues of both nutrient adequacy and excess. Importantly, FBDG communicate nutritional principles in a manner that is relevant to the population. Most FBDG encourage energy balance; physical activity; a healthful variety of foods, including fruits and vegetables, whole-grain products, food sources of protein, calcium, and unsaturated fatty acids; and safe food handling. Cautionary messages focus on excess energy intake, saturated and *trans*-fatty acids, added sugars, salt, and alcohol. To develop relevant FBDG, each country must identify local public health issues and appropriate diet-related strategies for the population.

Historical Background

Throughout the ages, religious and philosophical writings have included dietary recommendations, and this is reflected in an oft-quoted line from Hippocrates: "Let thy food be thy medicine." In the late 1800s, modern science began to influence the nature of recommendations regarding foods and beverages. The original focus of guidelines developed from Pasteur's discoveries of the disease-causing organisms that could be present in foods such as milk; thus, recommendations emphasized sanitation in food handling. In the early 1900s, the discovery of vitamins and minerals led to the realization that foods contain factors that are essential for health. This 'vitamin theory of disease' led to research throughout the first half of the twentieth century to discover these factors and determine their essential functions in the treatment of deficiency diseases. The knowledge that food was important in the prevention of diseases that were major public health problems, such as scurvy, beri-beri, night blindness, and pellagra, led to early efforts to develop and promote dietary recommendations or guidelines, although the specific curative factors in foods had not been identified. Among the earliest examples, Egyptians were known to promote the use of liver to correct night blindness, the British Navy used lemons or limes to prevent scurvy, and alkali treatment of corn was associated with a lower incidence of pellagra in Mexico. The understanding of the linkage between certain foods and the prevention of disease resulted in the development of food guides or groups illustrating a pattern of food choices that was most likely to prevent deficiency diseases. As the chemical nature of the factors in food that prevented or cured

nutritional deficiencies became known, it was possible to determine the specific amount required in the diet to maintain health. These studies led to the development of recommended dietary allowances (RDAs), which are numeric recommendations of the nutrient intakes that will meet the needs of the majority of the population. RDA has also been used to evaluate the adequacy of diets in many populations.

By the second half of the twentieth century, it had become clear that in many developed countries the primary causes of disease were shifting from dietary deficiencies to those associated with dietary excess. As noted by the Surgeon General of the USA, by 1988, micronutrient deficiencies were no longer major public health problems in the USA; and diseases associated with excess intakes of energy, saturated fat, total fat, cholesterol, alcohol, and sodium, in conjunction with inadequate fiber intake, were the major causes of death in the USA. The economic transition experienced by many developing countries has led to a similar pattern; this is sometimes referred to as the double burden of disease. Although nutritional deficiencies continue to be prevalent in large segments of the population, an increasing proportion of the population is at risk of developing diet-related chronic diseases, such as obesity, cardiovascular disease, cancer, and diabetes. This emerging pattern of disease has resulted in the development of FBDG, which recommend dietary patterns that are adequate in nutrient content and encourage food choices to lower the risk of non-communicable diet-related diseases.

Types of Guidelines

This section outlines the evolution of three interrelated general types of nutrition recommendations that are developed and used in most areas of the world by national or regional government agencies: technical recommendations, which provide specific numeric criteria for nutrient intake; FBDG, which outline strategies to lower the risk of chronic disease; and food guides, which illustrate dietary patterns or food choices to encourage individuals to meet the recommended nutrient requirements and to follow the advice in dietary guidelines. The more technical quantitative guidelines are typically used by health professionals to develop educational materials and evaluate the adequacy of diets. Food guides and FBDG are important components of educational materials for healthy individuals. Although this section will focus on FBDG, it is important to understand how they are related to the other types of nutritional recommendations and also that recommendations categorized as FBDG ideally are related to and supportive of other types of nutritional recommendations that

are part of national or regional health policy. Frequently, nongovernmental groups develop food guides or FBDG to suit a specific purpose (such as weight loss, treatment of cardiovascular disease, or promotion of a food culture) or a specific population segment (such as older individuals); however, before accepting these recommendations, it is important to determine how they have been validated in terms of other criteria such as the dietary reference intakes. These non-

governmental recommendations do not have the same policy status as recommendations developed by government agencies or through government-sponsored scientific organizations and may be suitable only for a specific targeted function.

In 1992, a recommendation of the International Conference on Nutrition organized by the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) was that each country should develop nutritional

Table 1 Reorientating from nutrients and food components to foods

What are the important public health issues for the population? Do they have diet-related factors?
Health statistics will indicate the major causes of morbidity and mortality in a population. Diet-related diseases include nutritional deficiency diseases and noncommunicable diseases, such as obesity, type 2 diabetes, certain types of cancer, and cardiovascular disease. It is important to determine whether nutrition is the primary cause of the disease or secondary to some other more prevalent problem (e.g., smoking and infectious agents).
What are the target nutrients linked to the major public health issues? Are there related nutrients or other factors?
In many nutrition-related problems, several nutrients or food factors may interact to cause the nutritional problem. For example, the fat content of the diet affects absorption of fat-soluble vitamins, obesity can be related to either excess energy intake or inadequate expenditure, multiple factors contribute to adequate bone formation, folic acid can mask anemia due to vitamin B ₁₂ deficiency, and so on. Simply increasing the intake of a target nutrient and ignoring these other factors may not address the problem adequately.
What foods are high in nutrient(s) or consumed in sufficient quantity to be a significant source of the nutrient(s)?
Using both food-composition databases and food-consumption data, foods that are good sources of the nutrient and foods that are consumed in sufficient quantity to meet the target intake can be identified. Likewise, dietary patterns that lower the risk for the public health problem and are associated with adequate intake of the nutrient can be identified.
What is likely to be acceptable to the target audience?
For nutrition interventions to achieve success, recommendations must target food choices that can be integrated into the diet based on cost and acceptability of the foods.
How do diet strategies integrate with other food policies?
Economic, agricultural, and trade analysis is useful to determine which diet strategies are sustainable.

Table 2 Steps in the development of FBDGs

1. Develop support from key government agencies. The successful implementation of FBDG will depend on support from key ministries, such as health, agriculture, education, sports, and recreation. Building consensus among these agencies will result in consistent messages regarding diet, health, and lifestyle for the public. Examples of support include technical support for data analysis or a secretariat to maintain and coordinate activities.
2. Form a working group of experts. The working group should include diverse expertise in areas, such as public health, nutrition, food science, agriculture, and behavioral sciences.
3. Solicit public comment and input. The expert panel needs to gather and evaluate scientific information to determine the guidelines that are most relevant to the target population. This information can be obtained from the scientific literature. In addition, professional groups may have important information to submit to the panel for consideration. Solicitation of information is consistent with an open process; however, the panel is responsible for evaluating the relevance of the information submitted.
4. Review and identify key public health issues and evaluate the diet–health relationships of concern for the population; determine the critical health, food, and nutrition issues to be targeted in the FBDG; and define the purpose, target groups, and content of the FBDG. Even if data are limited, it is important for the working group to identify the key public health issues. This step may be especially important in countries in which both undernutrition and overnutrition are of concern. Identification of the public health issues allows the working group to address the questions in **Table 1**.
5. Develop and draft the main messages for the FBDG. The working group will need to decide whether the draft document will be targeted primarily at health professionals, and hence may be more technical, or will be targeted toward the general public. In developing the main messages, they may identify consumer-orientated materials, such as a food guide, that will be useful in communicating the FBDG to the public.
6. Assess the cultural and economic appropriateness and credibility of the messages as perceived by the target groups. Through focus groups or other types of consumer testing, the effectiveness of the FBDG can be assessed. This information can be used to revise the guidelines before developing the final draft.
7. Release and implement the FBDG. It is valuable to have government leaders from key ministries involved in the release and implementation of the FBDG so that there is a commitment to integrate the guidelines into departmental policies. In addition, the implementation can require development of educational materials for different target groups as well as public–private partnerships to aid in dissemination of the messages to the public.
8. Monitoring and revision. Monitoring can be used to assess the impact and implementation of the FBDG. In addition, monitoring data are useful for making appropriate revisions and updates to the guidelines on a periodic basis.

Table 3 Dietary-guideline messages from three countries

<i>USA</i>	<i>China</i>	<i>Thailand</i>
The <i>2010 Dietary Guidelines for Americans</i> has 23 recommendations in the following categories for the general population and six additional recommendations for special populations (http://www.health.gov/Dietary Guidelines)	Eat a variety of foods, with cereals as the staple	Eat a variety of foods from each of the five food groups, and maintain proper body weight
Balancing Calories to Maintain Weight	Consume plenty of vegetables, fruits, and tubers	Eat adequate amount of rice or alternative carbohydrate sources
Foods and Food Components to Reduce	Consume milk, beans, or dairy or bean products every day	Eat plenty of vegetables and fruits regularly
Foods and Nutrients to Increase	Consume appropriate amounts of fish, poultry, eggs, and lean meat; reduce fatty meat and animal fat in the diet	Eat fish, lean meats, eggs, legumes, and pulses regularly
Building Healthy Eating Patterns	Balance food intake with physical activity to maintain a healthy body weight Choose a light diet that is also low in salt	Drink milk in appropriate quality and quantity for one's age Eat a diet containing the appropriate amounts of fat
	If you drink alcoholic beverages, do so in limited amounts Avoid unsanitary and spoiled foods	Avoid sweet and salty foods Eat clean and safe food Avoid or reduce the consumption of alcoholic beverages

Table 4 Common themes for food-based dietary guidelines

<i>Foods or behaviors that are encouraged</i>	<i>Cautionary messages</i>
Energy balance	Saturated fatty acids and <i>trans</i> -fatty acids
Includes physical activity	Energy balance
Encouraging a healthful variety of foods	Total energy from fat
Fruits and vegetables	Consumption of foods high in added sugar
Use of whole grains	Use of salt and salty foods
Protein-based foods	Alcohol
Foods that are calcium sources	
Sources of unsaturated fatty acids	
Safe food handling	

Table 5 Food and Agriculture Organization initiative: Get the best from your food

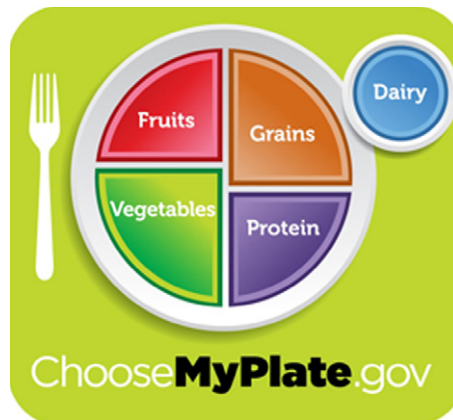
Enjoy a variety of food	Recognizing the importance of food in understanding nutrient requirements, nutrient and nonnutrient interactions, and diet–health relationships
Eat to meet your needs	Importance of energy balance and different needs across the life cycle
Protect the quality and safety of your food	Recognizing the importance of food and water sanitation, especially in developing countries
Keep active and stay fit	Importance of physical activity in maintaining well-being

recommendations that included FBDG. To encourage this activity, FAO and WHO convened a group of experts to recommend a process for developing FBDG; their findings are published in a WHO technical report.

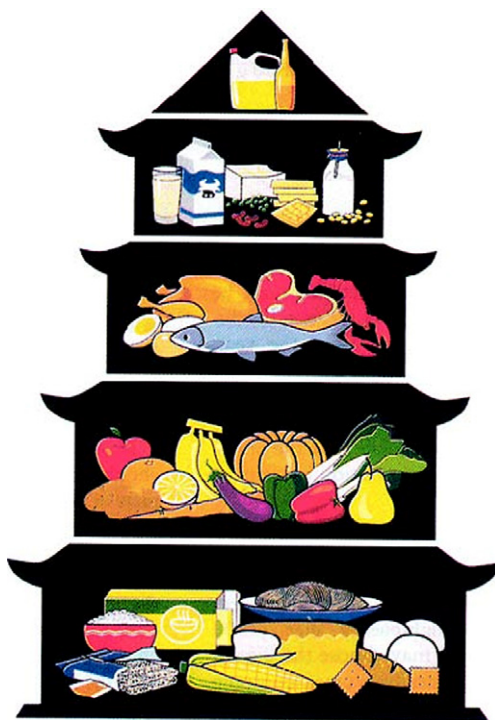
The Development of FBDG

FBDG express the principles of nutrition education in terms of the food and food choices available to the population rather

than in terms of specific nutrients or food components. Scientifically, these guidelines are based on the association between dietary patterns and the risk of diet-related diseases and incorporate recommendations that address major diet-related public health issues. In addition to communicating scientific knowledge about the association between food, dietary patterns, and health, development of FBDG provides an opportunity to strengthen consensus among various government and nongovernment organizations on important nutrition recommendations to be incorporated into educational programs. In



(a) <http://www.choosemyplate.gov>



(b) Fats and oils, 25 g
Milk and milk products, 100 g bean and bean products, 50 g
Meat and poultry, 50–100 g fish and shrimp, 50 g eggs, 25–50 g
Vegetables, 400–500 g fruits, 100–200 g
Cereals 300–500 g



(c)

Figure 1 The food guides that accompany the FBDG in (a) the USA, (b) China, and (c) Thailand. <http://www.choosemyplate.gov>

addition, by expressing scientific principles in terms of food, FBDG recognize the consumer awareness of food rather than nutrients and emphasize to consumers the importance of meeting nutrient needs with foods. Thus, both the content of the FBDG and the process of development are important.

Researchers often focus their studies on a specific nutrient or food component that may alter the risk of developing a disease. These studies are reviewed in the development of FBDG, but the information must be reorientated from a

nutrient-based focus to a food recommendation by addressing the questions in [Table 1](#). As indicated by these questions, the process is driven by the identification of diet-related public health issues and the development of food-based strategies that are relevant to the target population.

The process for developing FBDG is based on building consensus among various sectors and groups involved in public health. [Table 2](#) provides a general outline of the steps in the process, which can be adapted to the specific needs of a

country or region. The goal is to have a set of guiding principles for food-based recommendations that lay out the overall policy agreed by various agencies and groups.

The product of the working group is likely to be a document that outlines recommendations and includes background information on the rationale for the guidelines as well as guidance on implementing the recommendations. The guidelines from three countries are shown in **Table 3** as an example of the types of message developed during this process. In all cases, the messages are accompanied by a document containing background information. **Table 4** presents common themes emerging from the FBDG that have been developed in a variety of countries. Based on foods available and cultural practices, the types of fruits, vegetables, and whole grains and the specific types of food that are emphasized as sources of protein, calcium, or unsaturated fatty acids may vary.

All countries concerned about the increasing incidence of obesity have placed greater focus on energy balance, in terms of both food selection and physical activity. As a part of their effort to support the development of FBDG, the FAO launched a public information initiative for consumers entitled “get the best from your food.” This initiative promoted four simple principles (**Table 5**) that can be adapted for educational programs in a variety of settings.

Most countries that have developed FBDG have also developed a food guide to accompany the messages in the guidelines. The food guide is typically a simple graphic illustration of food choices and dietary patterns. The food guides that accompany the FBDG shown in **Table 4** are illustrated in **Figure 1**. Criteria for a food guide should include representation of foods common to the population, consistency with the FBDG, use of simple graphics that are meaningful to the target population, and developing a food pattern that meets the nutrient requirements of the population. Although a simple graphic is useful for visual communication, it should be clear that proper use of the food guide depends on understanding the more complete information in the FBDG.

Conclusion

The use of FBDG has emerged as an important food and nutrition policy and education program since the late 1970s and

is valuable for addressing issues of both nutrient adequacy and excess. Importantly, FBDG communicate nutritional principles in a manner that is relevant to the population. As a policy document, they should be revised periodically so that the information reflects current science on food and nutrition factors that promote health and prevent disease. Additionally, it is important for each country to develop their own set of FBDG so that the recommendations and presentation are relevant to the local population.

See also: Nutritional Surveillance: Developed Countries; Developing Countries

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DIETARY INTAKE MEASUREMENT

Methodology

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Glossary

Calibration A method of adjusting for systematic error. An example would be to use a more precise measure of dietary intake or a biomarker to adjust for measurement error with a less precise dietary method.

Measurement error Error that can occur when measuring information in studies. An example would be error that occurred when estimating dietary intake of an individual.

Qualitative analysis A technique for less structured or unstructured information and uses more open ended

information such as open ended survey responses or other non-numerical information such as photographs.

Quantitative analysis A technique of analysing numerical information or data and is used in statistical techniques that provide numerical outcomes.

Validation A method of identifying the type and scale of measurement error. An example would be to use a biological marker such as plasma vitamin C to compare with dietary intake of vitamin C.

Introduction

Dietary intake measurements are used to assess food, nutrient, or bioactive intake of individuals, groups, or populations. The purpose of collection of measurements varies from individual assessments in clinical situations (nutrition screening) or the adequacy of intake of population groups (nutrition surveillance) to use in research relating diet to health status, particularly in epidemiology. Measurements are also used to establish exposure to food-borne contaminants, in the evaluation of nutritional intervention programs, and to develop nutritional guidelines for governmental health policy.

This article describes the dietary intake measurements available, issues associated with data collection, conversion to nutrients and food types, measurement error when using dietary intake methods, validation and calibration of dietary methods, and future developments.

Dietary Intake Measurements

Table 1 describes the advantages and limitations of the main types of dietary methods, which are suitable for different purposes.

In all methods 'foods' refers to consumption of foods, beverages, and snacks both inside and outside of the home.

Of the individual methods weighed records, estimated food records, 24-hour recalls (24-HR), and dietary histories are more intensive. The quantity of food consumed may be weighed directly or estimated using household measures such as cups and spoons, photographs, standard units, or average portions (see **Table 2**). More recent methods in development utilize photographs of portion size recorded with mobile

phone or digital camera technology. For all methods the amount consumed can be measured or described either including or excluding wastage material usually discarded during food preparation, e.g., outer leaves and peel from vegetables or bones from cuts of meat.

Data should be derived from weighed intakes, government surveys, and research groups in populations similar to the one to be studied.

Some considerations when choosing a dietary method are shown in **Table 3**.

Methods for Measuring Food Consumption at the National Level

Food balance sheets

The Food and Agriculture Organization (FAO) publishes food balance sheets (FBSs) for approximately 200 countries. FBSs present a comprehensive picture of the pattern of a country's food supply during a specified reference period. Food balance sheets may also be termed national food accounts, food moving into consumption, food consumption statistics, food disappearance data, and consumption level estimates, reflecting differences in the method of calculation but providing similar information.

The supply available during a period is calculated from the total quantity of foodstuffs produced in a country, added to the total quantity imported and modified for any change in stocks that may have occurred. Calculation of quantities used for purposes other than human consumption (exports, livestock, used for seed, nonfood uses) and losses during storage and transportation are made. The per capita supply of each food item available for human consumption is calculated by

Table 1 Names and characteristics of dietary methods used for estimating food and nutrient intake

<i>Name of method</i>		<i>Advantages</i>	<i>Limitations</i>
<i>National level</i>			
Food balance sheets		Available for 200 countries; suitable for monitoring change	Per caput not individual intake; intake overestimated as nutrient losses during storage and preparation not accounted for; should not be used to provide estimates of nutritional adequacy of particular regions
<i>Household level</i>			
Food account method		Low respondent burden; relatively inexpensive	No estimates of change in larger stocks; measurements confined to food brought into the home (unless method modified to measure food consumed outside the home, which can be quite large); consumption of confectionery, alcoholic, and soft drinks excluded
Inventory method		Low respondent burden; relatively inexpensive	Consumption of confectionery, alcoholic, and soft drinks excluded
Household record		Suitable for populations with high proportion of homemade foods; useful if literacy levels are low; provides direct measure of food available for consumption	High input from field workers or interviewers
List recall methods		Relatively rapid and inexpensive; only one interview required; suitable for populations with higher proportion of purchased than home-produced food	Advance warning of interview may distort food consumption patterns; subject may fail to record items from memory; no record of foods eaten outside the home
<i>Individual level</i>			
Retrospective methods			
	24-Hour recall (24-HR) (single or multiple days)	If interviewed, respondent literacy not important; not reliant on long-term memory; providing not forewarned, individuals do not alter food consumption; interview length 20–45 min	Single 24-h should not be used for estimating intake of individuals but can be used for group assessments
	Diet history	Respondent literacy not required	
	Food frequency questionnaire (FFQ) (if includes portion estimates termed semiquantitative FFQ)	Useful for large numbers; relatively straightforward to complete; administration simpler and less costly than other individual methods; more rapid data processing	Report of past intake is influenced by current diet; trained interviewers required; average interview length 1–1.5 h; high processing costs
Current methods	Weighted food record (weighed inventory technique)	No requirement for memory retrieval as it records current intake; food intake weighed so estimates of quantity consumed not required	Needs to be developed for specific population group to ensure important food items are covered and requires updating to accommodate changes to supply of foods; less flexible for later analysis as food lists are fixed; responses governed by cognitive, numeric, and literacy abilities of respondents also by length and complexity of the food list
	Food record with estimated weights	No requirement for memory retrieval as it records current intake	Literate, cooperative respondents required as burden is high; possible that respondents change usual eating patterns to simplify the record; high processing costs
	Duplicate analysis	Greater accuracy	Literate, cooperative respondents required as burden is high; possible that respondents change usual eating patterns to simplify the record; necessary to find values for estimates of quantity of food consumed; high processing costs
Records using electronic equipment e.g., mobile phones, digital cameras		Visual records of foods. Avoids need for paper records. Data can be sent to investigators electronically.	Highly labor intensive; requires laboratory to do food composition analysis; limited applicability in population studies
			Currently involves labor intensive programmes to convert to usable data i.e., quantities and types of foods, although systems are in development to deal with this.
			Limited use in older people who experience difficulties with using newer technology.

Table 2 Types of portion used for methods using estimated portions

Portion types
Average or small, medium, large portions, weights – available from studies of weighed intake
Photographs (ideally there should be five or more representing the population range of intake)
Household measures (spoons, cups, mugs, liquid measures)
Standard units (1 apple, 1 banana)
Food models/replicas (three-dimensional models representing foods)

Table 3 Factors determining choice or suitability of method

Size and scale of the data collection
Screening, clinical, research, surveillance purposes?
Literacy or numeracy of the population
Age of the individual or population (the very young or very old may need assistance with completion)
Intended or potential use of the data (immediate short-term assessment versus prospective research)
Requirement for group or individual estimates for nutrient intake
Requirements from the data for nutrients, food groups, or bioactive nutrients
Detail and comprehensiveness of the information to be extracted for analysis (if information only required for particular nutrient or food type, shortened questionnaires may be administered)
Has repeatability of the method been assessed?
Have previous validation studies performed on the method by other researchers in similar population group to be studied?
Availability of resources for interviewers and including training
Availability of suitable coding program (record and recall methods require greater resources than frequency methods but frequency programs are more complex to develop)

dividing the total of available food by the number of the population actually consuming it and expressed in terms of quantity and nutrients. Estimates from FBSs include household wastage material, plate waste, and food fed to pets. Nutrient losses during storage, preparation, and cooking are not calculated and so figures for available food are greater than those reported by individual dietary surveys.

Food balance sheets can be used to formulate agricultural policies concerned with production, distribution, and consumption of foods and as a basis for monitoring changes and forecasting food consumption patterns, as well as to provide inter-country comparisons of available supplies.

Methods for Estimating Dietary Intake at the Household Level: Household Budget Surveys

Techniques for estimating intake at the household level include the food account method, the inventory method, the household record, and the list recall method. These methods measure all foods and beverages available for consumption by a household or family group during a specified time period of

between 1 and 4 weeks, although some lasts for 2–3 months. Wastage factors are sometimes applied. Household surveys provide data for per capita consumption of foods or nutrients, not intake for specific individuals. Data are calculated irrespective of the age and gender distribution in the household. These methods provide population data for annual mean food consumption and selection patterns, and are used for analyzing trends in intake. Household budget surveys are used more widely in Europe than elsewhere. As countries may not produce compatible data the Data Food Networking Project (DAFNE) has developed the methodology to allow the data from 11 European countries to be combined and compared.

Food Account Method

A record is made by a respondent of details of all quantities of food entering the household (purchased, home grown, or received over a period), usually over a period of 7 days. Changes in larder stocks are not estimated as on average some households will gain and some will use up stocks. Estimates of losses and wastage during preparation are made. This method is used for Living Costs and Food (LCF) module of the Integrated Household Survey (IHS) (until 2001 the LCF was called the National Food Survey and from 2001–2008 the UK Expenditure and Food Survey), and has included consumption of food, confectionery, soft drinks, and alcohol outside the home since 1992. As consumption outside the home now accounts for a substantial proportion of dietary intake in the UK the method was modified in 2001 to include the use of till receipts and individual 2-week diaries for each household member aged 7 years or older. This method can be used to measure seasonal variation in intake over 1 year.

Inventory Method

The inventory method is similar to the food account method and respondents record all foods coming into the household. A wastage factor is often applied and a larder inventory is included at the beginning and end of the survey period.

Household Record

Foods available for consumption (either raw or processed) are weighed or estimated. Foods for each meal are recorded separately to give a total for the household. Waste is measured directly or estimated. Interviewers visit the household early in the day to determine the quantity of food used to prepare the first meal and the number of individuals who consumed it. The midday meal may be weighed or recorded using estimated measures. A further interview is required later in the day. This method is appropriate for use in preindustrial societies where literacy is low and units for buying foods not standardized.

List Recall Methods

The respondent is asked by a trained interviewer to recall the amount and cost of food obtained for household use over a period, usually of 1 week. The method takes into account food use, purchases, and acquired food, but not waste. Quantities

consumed are weighed or estimated using household measures. The interview can take up to 2.5 h. Response rates are usually high. Information on the age and sex of people in the household and the number of meals eaten both in and outside the home, income, and other socioeconomic characteristics may be collected. It is helpful to notify the respondent in advance so that records of purchases can be kept before the interview. This method was used by the United States Department of Agriculture (USDA) National Food Consumption Survey between 1931 and 1988.

Individual Dietary Intake Methods

Many methods are available for estimating individual dietary intake measures and can be divided into two types: retrospective measures of intake such as 24-HR, dietary history or food frequency questionnaires (FFQs), or current measures of intake such as weighed or estimated food records. Qualitative information is available from all methods but quantitative estimates for nutrient consumption are possible only if data for weighed or estimated portion weights are available. Most methods may be either self-completed or completed by a surrogate. Surrogates may be required if study individuals are too young, old, or infirm but data will be less reliable than when reported directly.

24-HR and FFQs may be self-completed or interview administered either face-to-face or by telephone and can be mailed. Data collection costs can be reduced if questionnaires can be self-completed or mailed.

The number of days of report required for adequate measures of nutrients using 24-HR, weighed, or estimated records varies depending on the day to day variability of nutrient consumption. The number of days is partly dependent on the variation in nutrient concentration in foodstuffs. The concentration of macronutrients such as protein and carbohydrate in foods varies less than micronutrients such as vitamin C or iron. The number of days required to classify individuals into the correct third of the percentage distribution for usual intake, for 80% of individuals, has been calculated in British and Swedish populations. Up to 7 days of recall would be required for energy, protein, sugars, and calcium. Nutrients with greater variability and requiring between 4 and 14 days of records were alcohol, vitamin C, riboflavin, and iron. More recent analysis for the number of days required to estimate energy intake, using doubly labeled water estimates of energy over 14 days as the reference, suggest that 3 days of record are optimal with no improvement with 4 or more days of records. Also one of the 3 days of record should include a weekend day.

24-Hour Recalls

24-HRs determine intake during the preceding 24 h. Interviews can be recorded on paper or using interactive computerized software. Day-to-day variability in nutrient intake is large and a single day will not categorize individuals correctly within a distribution of intake. Therefore, single 24-HR are better used for group assessments than estimates for

individuals. However, multiple 24-HR can be used to overcome this problem. The sampling protocol for studies should include an equal proportion of all days of the week and coverage of all four seasons. Newer methods for recording of on-line recording of 24-HR recalls are under development.

Diet History

The diet history consists either of an interview administered 24-HR or establishing usual eating pattern over a 1-week period, followed by a frequency questionnaire to provide additional information. The dietary history provides a representative pattern of usual intake and is interview administered only.

Food Frequency Questionnaires

FFQs consist of a list of specific foods or food types associated with frequency of consumption. They are termed semi-quantitative if portions are included. Most questionnaires specify a frequency response in relation to an average or medium portion but some request records of specific portions. The period of record is usually the previous month or year. FFQs provide an indication of usual intake and can be used to obtain population estimates of frequency of consumption of food types.

FFQs need to be developed for specific population groups otherwise important foods may be missed. FFQs may become outdated if the supply of foodstuffs changes. FFQs consist of a fixed food list, which may be a disadvantage for prospective studies as hypotheses to be tested are limited by the list. Factors that affect the response to FFQs are the literacy and numeracy of respondents as some mathematical ability is necessary to calculate relative frequencies, the length and complexity of the food list, and the influence of current diet. Not all respondents will relate frequency to portion size accurately.

In the US examples of FFQs are the Block and Willett questionnaires. In Europe FFQs were developed for the European Prospective Investigations into Cancer and Nutrition (EPIC) study in the Netherlands, Germany, Greece, Italy, Denmark, France, and the UK.

Weighed Food Record Inventory and Estimated Food Record

For weighed food records (WRs) all food consumed over a period is weighed and recorded with details of food type and method of preparation, on preprinted forms or booklets, to obtain consumption over a period of days. Portable scales need to be supplied. WRs may include some estimated items eaten out of the home. Leftover food should be weighed and deducted. The recommended time period for records is 4–7 days or more, although the number of days depends on the nutrient of interest, study population, and objectives of the study. As some populations have different eating habits at weekends, weekend days should be included proportionately.

For estimated food records all foods consumed over a period are recorded with details of food type, method of preparation, and estimated portions over a period of days (see

Table 2). If recorded over 7 days, this may be called a '7-day diary'.

Both these methods have a high respondent burden and need cooperative, literate respondents. Respondents require training in the level of detail needed to describe foods. It is also possible that respondents may change usual eating patterns to simplify the process of the record. It is also beneficial to include a review of weighed records during the period of recording either after the first day or at the end.

Duplicate Sample Technique

Duplicate samples of all foods consumed are made and the nutrient content analyzed. This method is used for metabolic studies and though providing greater accuracy than other methods, its use is not feasible for most purposes.

Further Information

Although nomenclature for dietary methodology is reasonably consistent, care should be taken when reading the literature as methods with the same name may have been applied differently. The final decision over which method to choose will depend on the aims of the study, the population for study, the potential burden on respondents, and the resources available. Household surveys and food balance sheets provide data for per caput but not individual intake. In general, individual and the more intensive methods are associated with higher costs and respondent burden, whereas household methods are more economical and have a lower respondent burden.

Clinical Practice

Dietary methodology for clinical practice requires rapid assessments of nutritional intake in order to prescribe dietary change or to improve nutritional status. Traditionally, 24-HR of 'usual' intake or diet histories have been used for this purpose. Food frequency questionnaires and weighed or estimated food records are not generally used due to the more intensive burden on respondents and on the resources required for coding and processing the data.

There is considerable discussion over the optimum method to use for establishing individual dietary intake and studies designed to measure the validity of methods suggest that those that are more intensive and detailed lead to greater measurement precision, justifying the greater cost. Confirmation of these findings is required. Despite these potential benefits if resources are unavailable less intensive methods tend to be used.

Factors Affecting Individual Ability to Report Intake Accurately

Factors governing individual accuracy and quality of reports are respondent's literacy and numeracy skills; preconceived ideas on the purpose of the inquiry and, for list-based methods, the interpretation and meaning of food names. Individuals may make errors when measuring and recording food weights or estimating weights of foods consumed. There is

also respondent variation in the perception of the size of portions represented by photographs.

Interviewers

The aim of using interviewers with dietary methods is to obtain a complete, accurate, and detailed record of what respondents eat. Therefore, it is important for interviewers to be well trained and have an awareness of food composition and preparation techniques. Ideally, interviewers should be educated in nutrition (dietitians or nutritionists), although non-nutritionists can be trained to standardized techniques, and come from the same cultural or ethnic background as the study population. Interviewer protocols should be developed.

Computerized Interview Procedures

Computerized interview systems can aid interviewers by prompting for specific questions to elicit sufficient and specific detail and reduce the burden on interviewers. Examples are the Minnesota Nutrition Data System and the EPIC-SOFT systems, used in the US and a number of European countries. Although computerized interviews have advantages in improving accuracy and standardization, and in saving time and effort when recording and coding data, interviewers do have to be competent with computers and the resources required to develop systems are high.

Using Dietary Methods in Different Populations

Ethnic subpopulations may consume different food types than a main population and baseline surveys will be required to establish what types of foods and method of preparation are common. This information would be required before list-based methods such as the FFQ could be developed.

Recall of Remote Diet

Investigators may wish to recall diet in the remote past, perhaps of many years. However, interpretation of remotely recalled dietary data is complex as recalled diet is heavily influenced by current dietary habit. Some studies have found that the correlations between recalled past diet and current diet were higher than the correlations between actual past diet and recall of past diet. The onset of diseases such as cancer may affect the appetite and dietary intake of study participants and as recall of remote diet is strongly related to current diet, may affect recall of remote diet. As diet before the onset of disease is the measure of interest, it is preferable to collect dietary information prospectively, that is before disease onset. Case-control studies in which the diet of cases with disease is compared with controls may be affected by altered perception of recalled diet, particularly by cases.

Reproducibility of Dietary Methods

The reproducibility of a method may also be referred to as reliability, repeatability, or precision and is a measure of the extent to which the same results can be obtained when

repeated under the same conditions. Repeated measures provide an estimate of the within-person variability of intake. However, interpretation of the repeatability of measures is difficult as a lack of consistency may be due to genuine change over a time period or a lack of sensitivity or specificity of the method used to measure intake.

Use of Data and Conversion of Reported Intake to Nutrients and Food Types

Qualitative Analysis

Dietary method data can be used qualitatively, for instance during the process of reviewing nutritional intake for the purpose of dietary treatment as in clinical practice. Data on frequency of consumption may also be collected and analyzed by the FFQ method without conversion to nutrient intakes. However, even for qualitative analyses it is likely that paper-based dietary methods will require conversion to an electronic format. The majority of uses of dietary methods are targeted toward quantitative analyses.

Quantitative Analysis

The data collected by dietary methods are converted into food and nutrient consumption by calculating the amount of food eaten and linking this to a database with values for the nutrient composition of foods.

The databases of nutrient composition of foods are provided by the governments of many countries. They consist of nutrient composition data for the average composition of commonly consumed foodstuffs and are usually available as printed publications, computerized databases, or as part of software packages. Values in nutrient composition databases are expressed as either per 100 g of food or per common household measure. Nutrient databases vary in the coverage and comprehensiveness of the foods and nutrients. They are revised periodically to cover newer foods of different nutrient compositions or to modify or extend the nutrient coverage. Some issues concerning the choice of nutrient databases are shown in **Table 4**. It is important to read the information distributed with the printed or electronic versions of databases to determine the uses and limitations of the data.

Several steps are involved in calculating nutrient intake (also known as coding or processing). The first is to choose an item in the database, which corresponds most closely with the food consumed. If the food consumed is not in the database a suitable alternative can be chosen by considering food type, general characteristics, and likely nutrient profile. Once the food has been chosen the nutrient composition of the food quoted in the database is multiplied by the amount of food eaten, e.g., for 60 g food the nutrients would be multiplied by 0.6 (where nutrients are expressed per 100 g of food).

To calculate daily intake for an individual the contribution of each food is calculated and all the foods for a day summed. If more than one day's data have been collected it is usual to calculate the average of the number of days recorded. Data from FFQs are usually computed to consumption per day but can also be computed per week.

Table 4 Factors to consider when choosing a nutrient database to calculate nutrient intakes

Comprehensiveness of food item and beverage coverage?
Does the database contain entries for important foods consumed by the population to be studied?
How comprehensive is the coverage of nutrients?
Does the database contain data for mixed or multiple ingredient recipes or dishes?
What analytical techniques were used to derive nutrients in the database? (There can be differences in nutrients measured by different techniques.)
Are the data officially evaluated?
What compilation methods were used to construct the database?
Which conversion factors are used to calculate metabolizable energy content of foods for protein, fat, carbohydrate, and alcohol?
What proportion of missing values exists within the database? (Missing values are counted as zero in calculations and so result in systematic underestimates of intake.)
For international studies or comparisons how do the analytical methods for determining nutrient composition and compilation techniques affect the resulting data?

Although it is possible to compute intake by hand, using a calculator and a printed copy of a nutrient database, this is very labor intensive and in practice for most purposes has been superseded by computerization.

Data Processing and Computing Dietary Intake

The same care as that invested in data collection should be applied to data processing as errors of great magnitude may be introduced.

Estimated Food Quantities

To obtain quantitative information for nutrients or food groups, actual or estimated food weights are used. For methods using estimated food weights, values also need to be found for foods described such as standard units, average portions, or household measures. Sources of data are national publications, surveys of weighed dietary intakes, and food manufacturers. Data may also be included in nutrient calculation programs. Portion weights need to be population specific and, if unavailable, studies to establish values will be needed. Intensive methods used for large-scale surveys will require databases of more than 20 000 values for portion weights.

Data Entry and Nutrient Calculation Systems

A number of computerized data entry systems and nutrient calculation programs exist; factors that need to be considered when choosing a system are given in **Table 5**. The features required depend on the intended use of the data but as a minimum should include a list of foods, weights of portions, and a nutrient composition database. Ideally, systems should enable entry of data in sufficient detail to fulfill hypotheses for investigation and include measures to ensure consistent entry by staff such as defaults for inadequately reported foods,

Table 5 Factors to consider when choosing a computerized entry or interviewing program

Speed of the assessment
Requirements of the study for detailed or general data
Food composition database used
Food portion database used
Cost of the system
Facilities for organization of data
Ability to extract nutrients or food groups from the system
How up to date are the nutrient composition databases included in the system?
Commercial availability

portions, or mixed component foods. They should also include a method for entering newer foods with different nutrient composition from the existing nutrient database. This is particularly important, as the range of new foodstuffs and products with different nutritional characteristics is ever increasing.

Computerized systems and nutrient databases become outdated and for large-scale prospective studies it is desirable to develop systems with a flexible approach to updating by using database technology.

Data Processing Errors

Errors arising during the coding (data entry) and processing of individual dietary methods (24-HR, diet history, weighed and estimated records) need to be avoided. Misclassification can arise due to human error if incorrect foods are chosen during coding, for instance, if milk was consumed in the full-fat form but was coded for skimmed milk. This may also arise where a food has local or alternative food names, which may be unknown to the coder. It is important to have a qualified nutritionist available to develop a protocol for training staff, answering queries, and dealing with ambiguities. Coders should have knowledge of food composition and food preparation techniques. It is difficult to control entry of incorrectly matched foods but careful checking and staff training are crucial in preventing this. Other potential errors are entry of incorrect quantities or multiplication factors for portion weights and missed items, problems that can occur even with structured computer programs. So, systematic post-entry checks to identify extremes of portion weights or nutrient values and the verification and correction of data are necessary.

Issues Associated with Measurement of Dietary Intake

Measurement Error

There is potential for the occurrence of measurement error with the measurement of any exposure such as when using dietary methods to measure nutritional intake. Errors may arise as a result of flaws in the design of the measurement instrument or during data collection or processing. Measurement error may also occur as a result of individual

characteristics of participants in studies. Measurement error can be defined as the difference between the measured exposure (or measure of dietary intake) and the true exposure. All measurement of dietary exposures is subject to some degree of measurement error making it difficult to achieve measurements of true intake.

Efforts to reduce measurement error during data collection and processing should be introduced into the protocol of all studies, however, even if preventative measures are taken it is impossible to eliminate it altogether. It is difficult to identify the type and structure of measurement error associated with dietary intake. Measurement error may occur because of inaccurate reporting by respondents. It may also vary according to dietary method, for instance, food items within record methods may be intentionally or unintentionally omitted and with FFQs frequency of consumption may be inaccurately reported. Systematic bias, interviewer bias, recall bias, and social desirability bias have been identified but there are likely to be other sources of error. (Bias can be defined as the modification of a method of measurement by a factor, which influences the measurement in one or more directions.) Measurement error associated with dietary methods may consist of one or more types of error.

Measurement Error in Data Collection and Processing

Systematic Bias

Systematic bias is a systematic mis-measurement of data and can occur, for instance, if equipment such as weighing scales under- or overestimates values or if an interviewer consistently fails to use questions to probe for consumption of snacks and additional foods. If systematic bias can be identified solutions can be found, for instance, by calibrating equipment or training and monitoring interviewers.

Interviewer Bias

The behavior of an interviewer can influence the response of interviewees leading to interviewer bias. The degree of rapport between interviewer and respondent also influences results. Bias may occur if interviewers omit responses or record them incorrectly. Trained interviewers should ask open-ended questions in a neutral or nonleading manner, and not imply that a food or beverage should or should not have been consumed and avoid value judgments.

Social Desirability Bias

Social desirability bias can influence dietary measures as respondents strive to report what they think is required not what was actually consumed, for example, reporting less alcohol consumption than is the case or greater consumption of foods with perceived health benefits such as fish, fruit, or vegetables. This is likely to be the cause of mis-reporting, under-reporting, or low energy reporting, which occurs in certain respondents. It is possible to predict how much energy a respondent should report, as this is the amount required to maintain a stable weight. (Weight will be either gained or lost if more or less energy is consumed than required.) As energy intake should equate to energy expenditure, expenditure effectively measures intake. Techniques for measurement of energy

expenditure such as whole body calorimetry and doubly labeled water can be used. Using these techniques those individuals classified as low energy reporters are likely to be older, more overweight, and of lower educational and socioeconomic status than the rest of the population. Low energy reporters tend to have lower consumption of foods in the groups cookies, cakes, puddings, confectionery (candy), and sugary foods and, in some populations, lower consumption of spreads, cooking fats, and potato chips. Interviewers should be aware of low energy reporting, aim to be entirely nonjudgmental, and also request participants make complete records of food intake.

Impact of Measurement Error

As the proportion of error within a measurement increases, the accuracy of the measurement decreases and the results using the measurement will become less interpretable. Hence, greater measurement error reduces the likelihood that the truth has been measured with accuracy and increases the likelihood that analyses relating diet to disease status will tend toward null results. The effect of measurement error is to misclassify an individual within a range of intake.

Validation of Dietary Methods

Validation is used to quantify the measurement error that occurs when measuring dietary intake exposures. It requires two measures: a main measurement and a second measurement subject to less measurement error than the first. The errors of the two measurements should be independent. Validation is used to estimate the proportion of measurement error within the main method by modeling the differences between the main and the secondary measurement. It had been considered that dietary methods had errors independent of each other and that record methods such as 24-HR could be used, but it is now known that the errors are not independent as individuals report in the same way with different methods. Therefore, it is better to use biological variables measurable in blood or urine (also known as biomarkers) as the second measure for dietary validation. There are two types of biomarkers, recovery biomarkers, which allow quantitative estimates of intake over a specific time period or concentration markers, which measure relative concentration across a distribution. Examples of recovery biomarkers are urinary excretion over 24 h of nitrogen, potassium, and sodium. Examples of concentration biomarkers are measures in blood of vitamins such as vitamin C and carotenoids, minerals, and individual fatty acids. Examples of validation studies are those performed within EPIC-Europe and the Observing Protein and Energy Nutrition Study (OPEN) in the US. Work is ongoing to extend the number of biomarkers available and to define further and elicit the structure of measurement error.

Use of Calibration Methods to Adjust for Measurement Error

In contrast to validation, which attempts to identify the type and scale of measurement error, calibration is designed to adjust for systematic over- or under-estimation in dietary

intakes within populations. It may also be used at the individual level to attempt to correct for attenuation bias (or dilution) in relative risk due to errors in dietary measurements. Calibration of data has been proposed for large multicentre nutritional studies that have used different dietary methods to capture population-specific diets. Calibration studies require a highly standardized second dietary measure to be used in a representative subsample from each cohort to form a common reference measurement across populations. An example of this approach has been used by the European EPIC Study using a computerized, standardized 24-HR in 10 countries.

Future Developments

Future developments in methodology involve using computing, digital, and Internet technology, such as videos of food eaten and online programs for self-reported intake. Use of Dictaphones and combinations of weighing and other recording equipment are also possible. Development of statistical techniques to combine the optimal properties of different dietary methods is also ongoing and is a promising avenue for the future.

The number of foodstuffs available, particularly of manufactured foods and readymade meals, will continue to increase, presenting challenges for those attempting to estimate nutrient intake. Nutrient databases will continue to be expanded and updated to incorporate newer food items and nutrient measurements available using improved analytical techniques.

In some populations more than 40% of individuals have been shown to consume supplements and as very few comprehensive databases of vitamin and mineral supplements exist these need to be developed, as supplements can make a major contribution to nutrient intakes.

See also: Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Food Composition Data

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Relevant Websites

- <http://www.eurofir.net/>
EuroFIR Project website with information on European food composition databases.
- <http://www.fao.org>
INFOODS information for nutrient database compilers and suppliers.

DIETARY MODULATION OF INFLAMMATION

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Glossary

Damage-associated molecular patterns

(DAMPs) Endogenous molecules derived from tissue injury or stress that can be recognized by pattern recognition receptors (PRRs) to induce sterile inflammation.

***n*-3 PUFAs** *n*-3 Polyunsaturated fatty acids that are abundant in marine lipids.

Pathogen-associated molecular patterns

(PAMPs) Conserved molecules in pathogenic microbes recognized by PRRs.

Pattern recognition receptors (PRRs) Innate immune receptors that activate proinflammatory signaling pathways by recognizing PAMPs or endogenous molecules.

Sterile inflammation Inflammation induced by the activation of PRRs by endogenous molecules of nonmicrobial origin.

Toll-like receptors (TLRs) Mammalian homolog of *Drosophila* toll that can recognise PAMPs and DAMPs.

Introduction

Inflammation is a heightened innate immune response caused by infection or wound. It is a part of the essential immune responses for host defense against invading pathogens and wound healing, which are the key biological processes necessary for the survival of all multicellular organisms. In mammals, it is orchestrated by leukocytes (neutrophils, monocytes, and eosinophils) designed to eliminate pathogens and to heal tissue injury using multifactorial chemical signals (chemokines, cytokines, adhesion molecules, and lipid mediators). Pattern-recognition receptors (PRRs) mediate both infection-induced and sterile inflammation by recognizing pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs), respectively. The activity and/or the expression of PRRs can be modulated by dietary components and metabolic intermediates. Dietary saturated fatty acids (SFAs) and imbalance or disturbance in metabolic homeostasis can serve as an agonist signal to PRRs, whereas dietary factors such as *n*-3 polyunsaturated fatty acids (PUFAs) and certain anti-inflammatory phytochemicals can act as extrinsic resolution factors for PRR-derived inflammation. These results suggest that chronic inflammation and consequent risk of chronic disease can be dynamically modulated by what we eat.

What Mediates Inflammation?

PRRs, unlike antigen receptors involved in adaptive immune responses, are germline-encoded receptors that are highly conserved in both invertebrates and vertebrates. PRRs include toll-like receptors (TLRs), retinoic acid-inducible gene 1-like receptors, and nucleotide-binding oligomerization domain proteins (NODs)-like receptors (NLRs). PRRs induce innate

immune responses by recognizing invariant PAMPs leading to the activation of downstream signaling pathways and the expression of diverse arrays of proinflammatory marker gene products that are required for host defense against invading pathogens. Proinflammatory marker gene products, such as cytokines and chemokines, in turn activate their cognate receptors leading to amplification of proinflammatory signals. In addition, PRRs are activated by endogenous molecules derived from tissue injury and elicit sterile inflammation to initiate wound-healing processes. PRRs can also detect metabolic disturbances and bridge immune responses to metabolic homeostasis. Such functional diversity of PRRs may be achieved by their ability to recognize a wide variety of so-called 'DAMPs'. However, the broad specificity of PRRs in sensing agonists can make them vulnerable to dysregulation leading to chronic inflammation, which in turn can promote the development and progression of chronic diseases, including atherosclerosis, insulin resistance, Alzheimer's disease, and cancer (**Figure 1**). Among the PRRs, TLRs and NODs are known to be modulated by dietary components and metabolic intermediates.

Resolution of Inflammation by Negative Regulators of PRRs

The highest priority of our immune system is to combat infection and to heal wounds, which can sometimes override the safety of our own tissues, resulting in tissue damage. Therefore, timely resolution of heightened immune responses is critical to prevent such collateral tissue damage. Inflammation is usually self-limiting. It is normally resolved after its purpose is achieved; however, dysregulation of any of the converging factors can lead to chronic inflammation promoting pathogenic processes that lead to the development of major chronic diseases. There are many negative regulators of

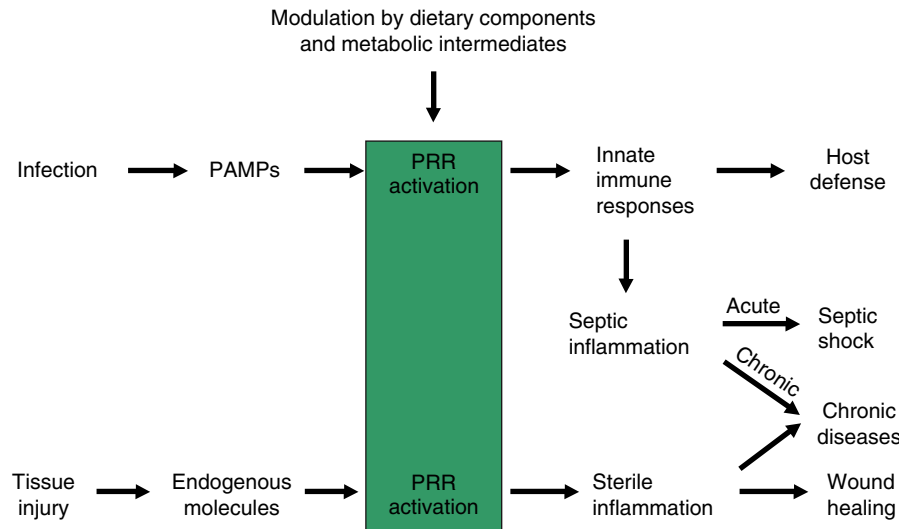


Figure 1 PRR-mediated inflammation and its modulation by dietary components and metabolic intermediates.

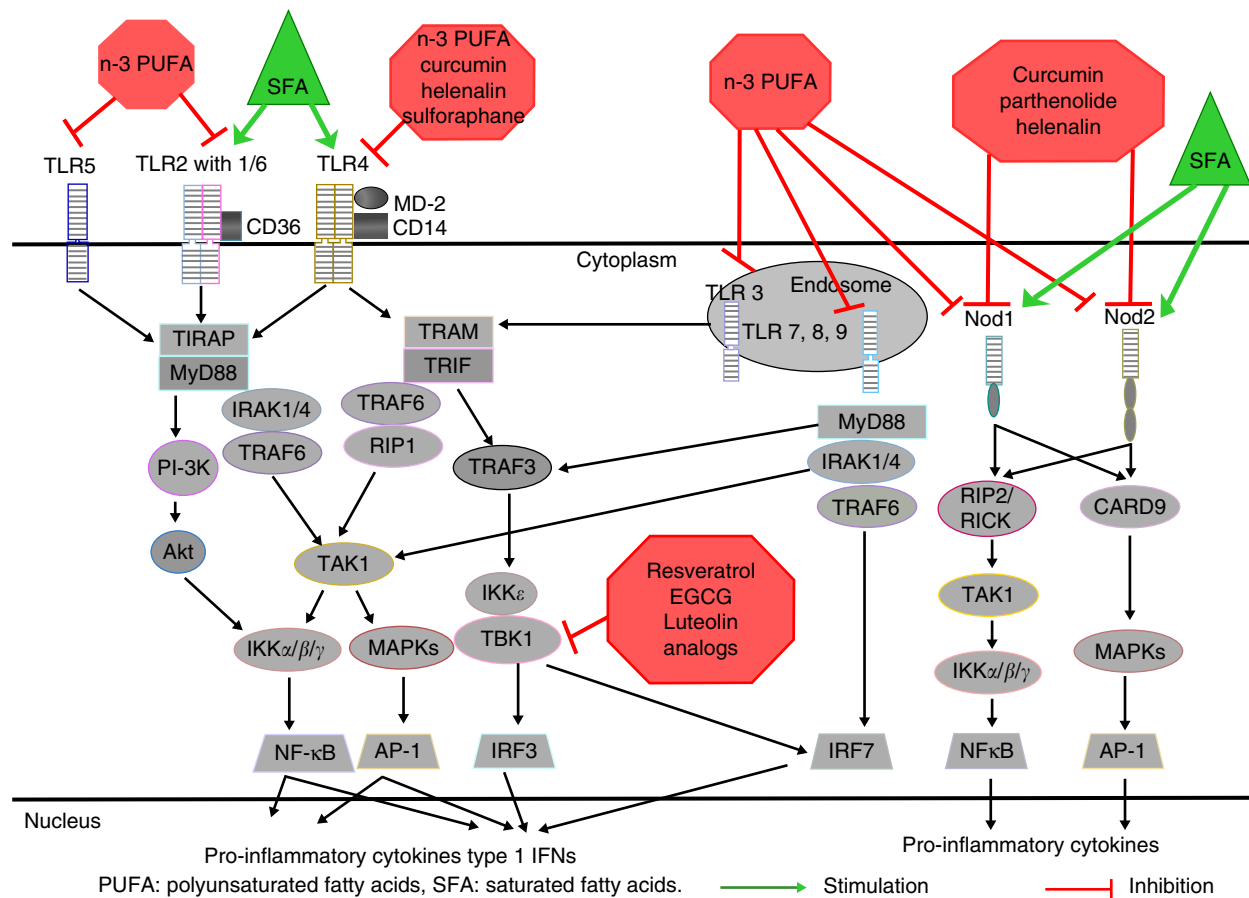


Figure 2 Differential modulation of PRR signaling pathways by fatty acids and polyphenols. Modified from Zhao L, Lee JY, and Hwang DH (2011) Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals. *Nutrition Reviews* 69(6): 310–320, with permission from Wiley.

PRRs so far identified. These negative regulators include soluble decoy TLRs that interfere with ligand binding or receptor dimerization, and intracellular negative regulator molecules that interfere with recruitment of downstream signaling

molecules, or stimulate degradation of signaling molecules. Many of these negative regulators are induced by ligands of PRRs suggesting that PRRs can activate both proinflammatory and anti-inflammatory signals in negative feedback and

temporal manners. Thus, negative regulators of PRRs represent intrinsic resolution factors of inflammation.

Modulation of PRR-Mediated Inflammation by Dietary Components and Metabolic Intermediates

Results from genetic, clinical, and biochemical studies suggest that PRR-mediated inflammation is an important determinant in increasing the risk of the development of chronic diseases. Dietary components that can suppress PRR-mediated inflammation may reduce the risk of chronic disease resulting from dysregulation of PRR activation, whereas those stimulating PRR-mediated inflammation may increase the risk of such chronic disease. Recent studies demonstrated that the activation of PRRs can be dynamically modulated by different fatty acids or metabolic intermediates. SFAs stimulate, but PUFAs, particularly *n*-3 PUFA docosahexaenoic acid, and certain plant polyphenols inhibit PRR-mediated proinflammatory signaling pathways (Figure 2). These results suggest that propensity to PRR activation can be affected by the delicate balance between SFAs and unsaturated fatty acids in cellular lipids, which is in turn affected by the types of dietary fat we consume. SFAs are stored primarily as triglycerides in adipose tissue. Thus, increased lipolysis in obesity, or lipolysis resulting from insulin resistance in adipose tissue, can lead to elevated plasma-free fatty acids that are rich in SFAs. This can enhance the propensity to activate PRRs in vascular endothelial cells and blood leukocytes, particularly monocytes, which can transigrate across the endothelium of peripheral tissues promoting a proinflammatory state (Figure 3). Many studies with animal models also showed that a high saturated fat diet activates PRR-derived proinflammatory

signaling pathways and induces insulin resistance, and that TLR4 or TLR2 deletion and mutant mice were protected from high fat diet-induced inflammation and insulin resistance.

It has been reported that the expression of certain TLRs is increased in tissues derived from patients with chronic inflammatory disease including atherosclerosis, rheumatoid arthritis, cancer, and myocardial ischemia/reperfusion injury. Increased expression of PRRs can enhance the sensitivity of cells to their endogenous agonists. The expression and activation of TLR4 can also be enhanced by high blood glucose, and by oxidized low-density lipoproteins. In addition, incomplete β -oxidation of long-chain fatty acids, partly due to a relatively low tricarboxylic acid cycle capacity, is associated with insulin resistance and leads to accumulation of C12–C14 acylcarnitines. Recent studies found that these acylcarnitines activate proinflammatory signaling pathways. These results suggest that disturbance or imbalance in metabolic homeostasis can enhance propensity to PRR activation.

Postprandial Inflammation: Blood Monocyte Activation

Blood monocytes are sentinel innate immune cells that are directly exposed to, and respond to, dietary components absorbed from the gut and nutrient metabolites. SFAs derived from a high-fat meal can activate PRRs (TLRs and NLRs) in monocytes leading to the production of proinflammatory cytokines, chemokines, and adhesion molecules, the hallmark of monocyte activation (postprandial inflammation). The postprandial inflammation can increase microvascular permeability, binding of monocytes to the endothelium, and endothelial cell apoptosis with release of endothelial cells from

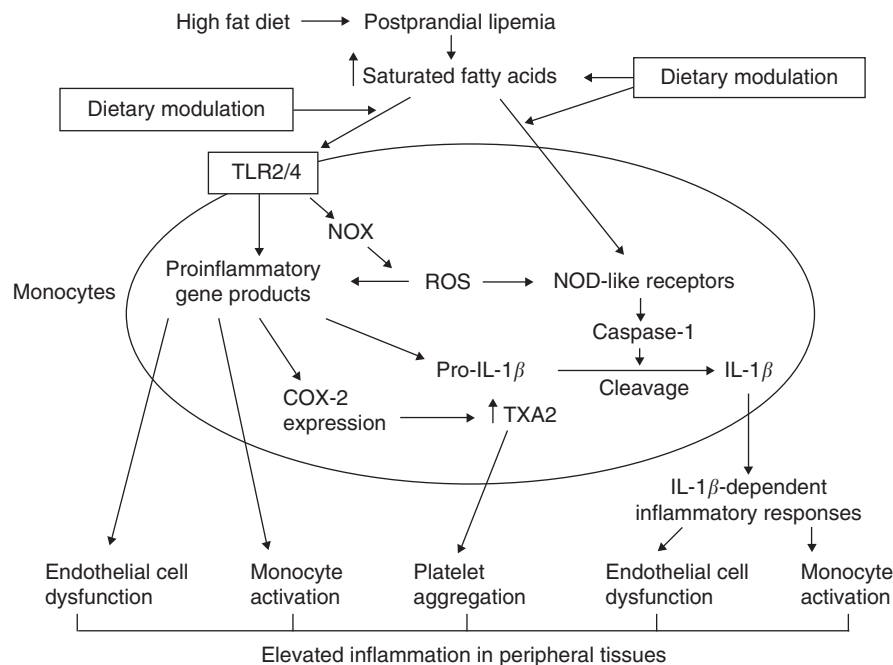


Figure 3 High-fat diet induces monocyte activation leading to elevated inflammation in peripheral tissues.

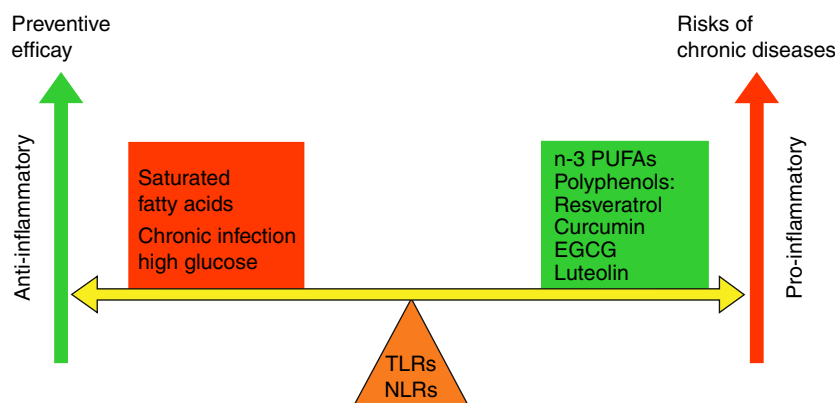


Figure 4 PRRs (TLRs and NLRs): Fulcrum for Ying and Yang of inflammation. Modified from Zhao L, Lee JY, and Hwang DH (2011) Inhibition of pattern recognition receptor-mediated inflammation by bioactive phytochemicals. *Nutrition Reviews* 69(6): 310–320, with permission from Wiley.

the basement membrane. Increased microvascular permeability allows a variety of proinflammatory molecules to enter peripheral tissues leading to increased local inflammation. Therefore, the activation of proinflammatory pathways by blood monocytes is the gateway to enhanced inflammation in various peripheral tissues and brain (Figure 3).

Monocyte Activation: PRR (TLR4 and NOD2)-Mediated Expression of Prointerleukin-1 β and Inflammasome-Mediated Secretion of Mature Interleukin-1 β

Interleukin-1 β (IL-1 β) is a major cytokine involved in monocyte activation and activation of proinflammatory signaling pathways in peripheral tissues and brain. IL-1 β expression and its secretion are tightly regulated. The expression of pro-IL-1 β is induced by activation of PRRs (TLR4 or NOD2). The expression of preformed IL-1 β (pro-IL-1 β) in monocytes by SFAs derived from high-fat meals is mediated by the activation of TLR4 or NOD2, the primary signal (Figure 3). Pro-IL-1 β stays inside cells but is secreted as a mature IL-1 β after proteolytic cleavage by caspase-1. Caspase-1 is normally present as inactive procaspase-1 but can be activated by the inflammasome. The inflammasome is a cytosolic proinflammatory signaling complex that can be activated by endogenous, microbial, and environmental stimuli, which has been shown to have major actions on cell injury and inflammation (Figure 3). The inflammasome is composed of NLR, procaspase-1, and an adapter molecule. The activation of the inflammasome stimulates cleavage of pro-IL-1 β by caspase-1, resulting in mature IL-1 β that is released from the cells. IL-1 β activates its receptor in a paracrine manner leading to the expression of proinflammatory gene products. Thus, the expression and secretion of IL-1 β that are mediated through PRRs (TLRs and NODs) and the inflammasome are the important hallmarks of monocyte activation. In monocytes, caspase-1 is present in a constitutively active form. Therefore, primary signals derived from the activation of PRRs are sufficient to induce inflammasome-mediated IL-1 β production. This fact suggests that dietary components that can activate TLRs or NODs can activate blood monocytes.

Inhibition of PRR Activation by Bioactive Phytochemicals

In addition to *n*-3 PUFAs, certain bioactive phytochemicals inhibit PRR-mediated proinflammatory signaling pathways. Curcumin, helenalin, cinnamaldehyde, and sulforaphane, containing α , β -unsaturated carbonyl, or isothiocyanate group, respectively, and that are known to interact with free SH groups in cysteine residues (Michael addition), inhibit TLR4 activation by interfering with receptor dimerization. Similarly, curcumin, as well as parthenolide and helenalin containing α , β -unsaturated carbonyl group, but not resveratrol or epigallocatechin (EGCG), also inhibits NOD2 activation by interfering with NOD2 receptor dimerization. These results suggest that other phytochemicals containing structural motifs that can modify free SH groups in cysteine residues can inhibit PRR receptor activation by interfering with receptor dimerization. In contrast, resveratrol, EGCG, and structural analogs of luteolin such as quercetin, chrysin, and eriodictyol that do not contain the structural motif conferring Michael addition to free sulfhydryl groups, specifically inhibit the TLR3 and TLR4 signaling pathway by targeting TBK1 and RIP1 in the TRIF complex. Together, these results indicate that PRRs and their downstream signaling components are important molecular targets for dietary strategies to reduce PRR-mediated chronic inflammation and consequent risks of chronic diseases (Figure 4).

See also: Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Phytochemicals: Health Effects

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DIETARY SURVEYS

Surveys of Food Intake in Groups and Individuals

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Glossary

24-Hour dietary recall Individual respondents are asked to recall all food consumed during a given 24-hour time period.

Food account method The person most responsible for acquisition and use of food in a household is asked to keep a daily record of all food that enters the household for a given period of time.

Food balance sheets Surveys that measure overall national food production, inflow, and outflow of food commodities.

Food frequency questionnaire Individual respondents report their usual frequency of consumption, and in some cases portion size, for a pre-specified list of foods.

Household diet record Report of preparation and use of foods at the household level with consideration of number of consumers.

Interviewer-administered list recall An interviewer asks the responsible household member to recall food purchases, production or gifts in the household during a specified period.

Research Questions and Data Needs

At the national level, information on food use is needed for economic and agricultural policy decision making. For policy makers to advise on food production, food imports, pricing of staple foods, and other factors that affect food availability, they require information on the production, inflow, and outflow of food commodities and products at the national level. Most countries use food balance sheets to measure these flows, and total available nutrients are estimated in relation to the size and composition of the population. These surveys measure overall national food production, imports and available food stocks, and subtract exports, food used for animals, and losses that occur during production, storage, and manufacturing. The FAO has compiled food balance sheets for many countries since 1949, allowing useful intercountry comparisons of food availability. However, the aggregate information obtained with food balance sheets does not consider food distribution within a country and does not quantify food intake or needs of subgroups of the population.

Most countries need more information on household-level food use to target food and nutrition policies toward groups in need. Household food surveys capture the amounts and types of food that enter a household, and per capita intake equivalents are calculated by dividing the total nutrients available in the household from the edible portion of entering foods by the number of household members, weighted by specific age and sex. This information allows identification of groups at risk of inadequate intake of energy or of

specific nutrients. For example, these surveys may highlight rural–urban differences, inland–coastal differences, differences by socioeconomic strata, and so on. Such surveys provide critical information within countries for the development and targeting of economic, agricultural, and nutritional policies specific to regions or other subgroups of the population. They do not, however, provide information on individual intakes within the household and are not useful for understanding age- and sex-specific intakes.

To describe food and nutrient intake by age, sex, and physiologic state, data are needed at the individual level. Surveys of individual dietary intake use methods that range from qualitative food checklists to multiple detailed records of food intake, with quantification of preparation methods and portion sizes. Individual-level data are used for a variety of purposes and the survey design will depend on the primary data needs. At the national level, a primary objective is to identify subgroups at risk of inadequate intake of energy or specific nutrients. The important advantage of individual-level data is that target age and sex groups may be identified in addition to groups identified by region or other household-level characteristics. A further objective is to determine the extent of under- or overnutrition in relation to energy or specific nutrients in subpopulations. This requires consideration of the distribution of intakes in specific age, sex, and physiologic status groups. A more ambitious objective of some national or targeted dietary surveys is to associate aspects of individual dietary intake with the existence of health conditions. This objective requires that usual intake data be valid and reliable at the individual level.

Issues in Survey Design

National data on food availability are generally collected with food balance sheets. Although not a survey in the formal sense, this is a collection of data from the food sector regarding wholesale distribution. After adjusting for expected wastage, these data are compared to nutrient values and then to the size and composition of the population to calculate per capita nutrient availability. Because this is a crude assessment, it does not account for all losses and, therefore, tends to overestimate availability.

For a household-level survey to be nationally representative, one must carefully consider the sampling design. This is generally done by multilevel selection of regions, then subregions, then households, in such a way that the resulting data may be generalized to the national level. When results are necessary at the regional level, coverage of all regions is necessary, although this will usually increase the cost of the survey. When the objective is more specific than national description, target areas may be selected, based on risk status or relevance to the question being addressed.

Similarly, for individual-level data to be representative of the greater population, complex sample design is employed. Decisions on sampling design will generally be a balance between equal opportunity for subject inclusion against logistic and cost considerations of full randomization. This design may be similar to that of the household-level survey, with the added step of randomly selecting individuals within households. Although surveys focused on the household level often interview all members of the household, this is not the most efficient way to get data representative of individuals in the population, due to the lack of independence of the observations. Members in the same family consume similar foods and therefore are more like each other than others in their community. Although this lack of independence can be adjusted in the analysis, it will require larger number of interviews to achieve representative stability of data estimates. The multistage approach of region, subregion, and community is less representative than a pure random sample, but this is corrected by consideration of the 'design effect,' which is calculated by comparing variation within versus between sampling units at each level. Although the design effect leads to the need for higher overall number of surveyed individuals, this is generally considerably less expensive than expanding coverage to all locations.

In addition to representation of the general population, many surveys also consider subgroups that will not be well represented unless specifically oversampled. Examples include pregnant women, ethnic subgroups, or low-income groups. Individuals who meet the specified characteristics are identified within the existing sampling design but are selected in larger numbers than would be representative of the entire population. This allows sufficient sample size to present valid estimates separately for these groups. When included in measures of the total population, the overrepresentation of these subgroups is adjusted using sampling weights.

Another design consideration is the timing of the survey. Intake may vary considerably by season, and it is therefore important that all seasons are represented. Although logistic and cost constraints often limit ideal design planning, it is

optimal if data from all seasons are collected in all survey locations. If different locations are covered at different times of the year, comparisons across regions may be compromised. Additionally, intakes may be misrepresented if certain days of the week are not included in the data collection plan (Table 1).

Selection of Dietary Assessment Measure

Household Level

Several alternative methods of dietary assessment are available. At the household level, one commonly used approach is the food account method. The person most responsible for the acquisition and use of food is asked to keep a daily record of all the food that enters the household for a specified period – often 1 week. This includes food purchases, food production, and food received as gifts. There are several limitations to this approach, including the assumption of constant food stores, which may not be the case.

In some locations, it is not feasible for many individuals to accurately record this information. In this case, an interviewer-administered list recall method is often used. The interviewer asks the responsible household member to recall food purchases, production, or gifts in the household during a specified period, following a list of major foods that are relevant for that location. Additional information on age and sex of household members and number of meals each consumes at home is collected to calculate adult equivalent per capita food availability for the household. Although edible portions of foods are generally considered in quantifying availability, most such surveys do not account for wastage or use by animals and, therefore, may overestimate household food use. However, if they do not account for food consumed away from the home, they may underestimate food intake. Although useful for economic and food commodity flow information, this type of survey is, therefore, limited with respect to nutritional intake assessment.

To understand dietary intake within households, more elaborate methods are needed. One approach is to use a household diet record, where the household respondent is asked not only to report inflows of food, but also to record actual use and preparation of foods in the household over a specified period of time. Food consumed outside the home may also be assessed for each household member, and the number of individuals, including guests, who are present at each meal is recorded. While demanding for the respondent, this approach provides a better estimation of the total food consumed by the household than the inventory methods described earlier. Estimation of waste is included in some but not all such surveys and is a limitation of most. Because of the heavy respondent burden, incomplete response is also a major problem, which threatens the validity and generalizability of the survey.

Individual Level

A variety of methods are available for use at the individual level, and their selection depends on the questions to be addressed

Table 1 Advantages and disadvantages of dietary survey methods

<i>Level</i>	<i>Survey type</i>	<i>Advantages</i>	<i>Disadvantages</i>
National	Food balance sheets	Inexpensive	Crude estimate; no consideration of wastage; does not allow disaggregation to sublevels
Household	Food account method	Inexpensive	Does not account for food consumed away from home, inventories, or wastage
	Interviewer-administered list recall	More detail obtained on foods than in the food account method	List may limit responses; waste usually not accounted for
	Household diet record	Usually covers 1 week with great detail; most accurate of household methods	High respondent burden; expensive
Individual	24-h dietary recall	Detailed information on food intake, good estimate of mean intakes by subgroup	Misclassifies individuals; single recall per person is not useful for correlative investigation
	Multiple recalls	Average of multiple days can give good quantitative estimate of usual intake; with two recalls, intra-/interindividual variance can be calculated and used to correct correlations and linear coefficients for random error	Expensive; variability ratios are useful but not sufficient to correctly classify individuals; has limited utility in nonlinear analyses
	Food frequency questionnaire (FFQ)	Inexpensive, measures usual intake	Semiquantitative; dependence on food list and recipe assumptions may lead to error in estimation of intake in subgroups
	Combined approach with two 24-h recalls and a qualitative FFQ or propensity questionnaire	Allows improved estimates of individual intakes by adjusting for the probability of consumption of food groups that may appear as zeros in two 24-h recalls	Expensive; heavy respondent burden; requires considerable statistical expertise

balanced with cost considerations. As noted earlier, major uses of individual intake data from dietary surveys include the description of mean intakes by subgroups, description of the proportions of the population with inadequate intake of specific foods or nutrients, and comparison of dietary intake with individual characteristics, including health status.

For the purpose of describing mean intake of groups and subgroups, the most efficient method is the use of a single 24-h dietary recall per selected individual. This is the methodology that was, until recently, used in the US National Health and Nutrition Examination Survey (NHANES), providing a good description of average intakes of nutrients by age, sex, and ethnic group. As an aggregate measure, this design has worked very well. However, there are limitations to these estimates, and validation against quantified energy expenditure measurements has shown that most people tend to underreport intakes with the 24-h dietary recall method. Although it has often been assumed that this underreporting is random, more recent investigations have shown that it may also be associated with individual characteristics such as obesity, restrained eating behavior, or social desirability bias in reporting. Underreporting will affect the mean intake of groups and, if nondifferential, may introduce bias in subgroup comparisons.

In addition to underreporting, a major limitation in the use of single 24-h recalls in a dietary survey is the misclassification of individuals that results from day-to-day variation in individual intake. An individual who is usually a

heavy consumer of energy and fat, for example, may on any single day eat uncharacteristically lightly or vice versa, leading to severe misclassification of individuals in the intake distribution. Although (underreporting aside) the mean intake may be reliable and valid, the tails of the intake distribution are extended, leading to overestimation of proportions either above or below a specified cutoff point, relative to what is seen when usual intake is assessed as the average of multiple days. The misclassification of individuals relative to their actual usual intake also severely limits the ability to correlate intake data with individual characteristics, including health status and biomarkers.

Because of the importance of using national or regional survey data to identify the extent of inadequate nutrient intake, there has been considerable discussion on how to assess diet efficiently, yet estimate prevalence of inadequacy. With repeated recalls on a representative subset of the population, the day-to-day variability may be quantified and used to adjust the distribution to better represent usual intake. Although they require specialized training to use, statistical methods have been developed to adjust distributions for this purpose. Using these techniques, we are able to pull in the tails and get a more realistic distribution of usual intake and thereby a more accurate estimate of the proportion of the population that falls below or above a specified cutoff point.

There have been differing approaches to deciding on the optimal cutoff points for use in determining inadequate

intakes. Because actual requirement differs across individuals, any cutoff point used may misclassify some. Historically, two-thirds of the recommended dietary allowance (RDA) were used, based on the concept that the RDA was designed to meet the requirements of most healthy individuals and, therefore, was higher than necessary for many. Current US nutrient intake recommendations also define the estimated average requirements (EARs) for most nutrients, and the proportion of individuals who fall below this EAR. Using an intake distribution adjusted for day-to-day variability is a good estimate of the proportion of the population with inadequate intake when the requirement distribution is normally distributed. In some cases, however, the nutrient requirement distribution is not normal. In that case, a more precise, but also more complex, way to estimate the relationship between intake and actual requirements is to use information on the probability that a specific nutrient is inadequate by comparing the distribution of requirement to the distribution of intake. However, the actual distributions of requirement are not known. An exception is iron, which is skewed, and low intakes are best estimated using the probability approach.

A third important objective of dietary survey data is to gain a better understanding of the correlates of nutrient intake – with individual characteristics on the one hand and indicators of health on the other. For many nutrients, the day-to-day variation in intake is considerable, and multiple days would be required to achieve stable estimates of intake at the individual level. Without this, the misclassification of individuals in the distribution leads to a weakening in the ability to see associations that may truly be there. An extreme example is vitamin A, which tends to be concentrated in a few foods. If one frequently consumes liver and carrots but happened not to have them on the day of the recall, that individual would be classified as having low vitamin A intake although his/her usual intake is quite large. Conversely, one who almost never eats these foods but had liver on the day of the recall would be incorrectly placed at the upper end of the vitamin A distribution. This misclassification weakens correlations or regression coefficients so that true associations between intake and health measures may not be observed or may be observed as less strong than they really are.

Because day-to-day variation is likely to be random, information from multiple recalls on a subset can be used to calculate the ratio of the day-to-day intake variation within individuals to the variation across individuals. To the extent that intra-/interindividual variance ratios are large, as in the case of vitamin A, the ability to see associations will be severely limited. Unfortunately, in most cases, this variance ratio is sufficiently large that a single day of intake will not allow valid correlational analyses. The collection and averaging of multiple days of intake will greatly improve this situation. For most nutrients, 3 or 4 days are acceptable. However, for some nutrients of interest, including vitamin A and vitamin B₁₂, the variability ratios are so high that an unrealistic number of days are needed for stable estimates. Because random error is predictable, equations have been developed to use the variance ratio to estimate the true correlation. The variance ratio can be obtained with two nonconsecutive days of intake. For this reason, many studies now incorporate two recalls in their design.

Although it can be used to adjust for attenuation of correlations and linear coefficients after analysis, the use of variance ratios does not allow the true estimation of usual intake at the individual level, and correction is much more complex and not always possible with categorical analyses frequently used in epidemiology. Therefore, the food frequency questionnaire (FFQ) remains the method of choice in most studies where diet–disease relationships are the primary objective. The FFQ asks respondents to report the frequency of consumption of a prespecified list of specific foods. Additional questions on portion size and preparation methods are added in differing ways to different questionnaires. The FFQ provides a lower-cost alternative to multiple recall days, but also has limitations. Because it relies on a food list, its validity is dependent on the representativeness of that list and of portion size and recipe assumptions. Most FFQs in wide use have been developed using data that represent the major sources of nutrient intakes in a national population. However, individuals with divergent eating patterns will not be well represented using this tool.

Recently, the US National Cancer Institute (NCI) has proposed a combined method to better assess usual intakes in population-based studies. The NCI method uses the data from two 24-h recalls to adjust distributions for day-to-day variability and adds information from an FFQ on the frequency of food consumption to estimate the probability of consumption when the foods are listed as zero intakes in the 24-h recalls. This method has not yet been widely used but promises to be an important advance in estimating usual intakes. For this reason, the most recent NHANES includes two 24-h recalls plus a qualitative FFQ. For many large studies, however, the cost and the respondent burden of including these three assessments limit the use of this approach.

With the emergence of human genome data, there is increasing demand for good estimates of usual intake for use in studies of gene–diet interactions. Because these usually require very large samples, it is rarely feasible to include three dietary assessments. Further, there is a trend toward combining data from multiple studies to obtain sufficient sample size and this is not easily done when studies use different dietary assessment methods. More efficient methods of valid dietary assessment, which can be collected consistently across studies, are needed.

Data Analysis and Limitations

Whatever dietary assessment measure is used, the utility of the data is dependent on the translation of reported food intake to nutrient intakes. This requires detailed and accurate nutrient databases. The US Department of Agriculture has the most extensive nutrient database in the world, allowing for good estimation of dietary intakes. Most other countries have not conducted this level of food composition analysis for their own locations. Therefore, most existing databases have obtained the majority of their values from the US nutrient database, adding information as possible from locally analyzed products. However, because the nutrient composition of many foods, including fruits, vegetables, and even animal products, can vary widely by growing conditions and specific

subvariety, most available nutrient databases remain inadequate. Many use extrapolated values from similar foods when chemical analysis has not been completed. Furthermore, it is common for many country-specific databases to include information only on macronutrients and a few selected vitamins and minerals. The continuous arrival of new manufactured products also complicates the upkeep and management of food composition databases. Considerable database work remains to expand the utility of worldwide dietary surveys.

Once the nutrient data are calculated, survey data are generally tabulated to present age, sex, and, sometimes, ethnic-specific mean intakes and standard deviations for individual nutrients. Further disaggregation by region, socioeconomic group, or other group characteristics can be very helpful in understanding the macrodistribution of nutrient intake and for targeting specific groups with nutrition intervention programs. If a complex survey design was used, or if systematic nonparticipation was observed, sampling weights must be applied to adjust the means and standard errors.

Using methods described earlier, estimates of the population with low intakes of specific nutrients are also calculated. Beyond these descriptive measures, comparison of nutrient intakes with individual characteristics and health measures generally requires multiple regression analysis with appropriate adjustment for potentially confounding variables. Again, when complex survey designs have been used, the inclusion of sample weights and appropriate adjustment of variances is needed. Specialized statistical software for use with survey data is available. In cases where a single recall has been used, substantial weakening, or attenuation, of associations is likely, but use of deattenuation methods described earlier can, at a minimum, provide information on the likely extent of this attenuation. When FFQ data are used, it is important to include some validation methods, preferably with comparison

to key biomarkers of nutritional status, but at least to multiple recalls on a subset. This is particularly true when a new questionnaire is being used, but is also important over time as the food supply and food habits change in the population. Available biomarkers are currently limited, but active investigation is under way to expand these to improve validity of measures of dietary exposure.

See also: Nutritional Surveillance: Developed Countries; Developing Countries

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DOWN'S SYNDROME

Nutritional Aspects

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Glossary

Bruxism Grinding of the teeth which can cause the teeth to wear and damage the tooth enamel through time.

Chromosome One of the thread-like structures in a cell nucleus that carry the genetic information.

Cystathionine β -synthase Enzyme that catalyses the first step of the transulfuration pathway from homocysteine to cystathionine.

Lipid peroxidation Refers to the oxidative degradation of lipids. It is the process whereby free radicals oxidize lipids in cell membranes resulting in cell damage.

Nonstarch polysaccharide (NSP) Complex carbohydrates other than starches found in foods. Contribute to dietary fiber in the diet. Insoluble NSP found in wheat, maize and rice and have a stool bulking effect.

Soluble NSP found in oats, barley, rye and beans, can help lower blood cholesterol.

Osteoporosis Condition resulting in loss of bone tissue resulting in bones becoming brittle and liable to fracture.

Purine A nitrogenous compound with a two-ring molecular structure. Examples are adenine and guanine which occur in DNA structure, and uric acid.

Superoxide dismutase An enzyme which catalyses the dismutation of superoxide into oxygen and hydrogen peroxide. It is an important antioxidant defense in nearly all cells exposed to oxygen.

Trisomy A condition where there is one extra chromosome present in each cell in addition to the normal pair.

Down's syndrome, named after John Langdon Down, is a most widely recognized chromosomal disorder found in humans and falls into a category of chromosomal disruptions known as trisomies, hence, the other term for the condition; Trisomy 21. People with Down's syndrome vary widely in their abilities, but the syndrome is the most common genetic cause of intellectual disability.

More than 90% of Down's syndrome individuals have a total of 47 chromosomes in cells instead of the usual 46. The remaining cases are mainly either translocations, where there is a rearrangement of fragments of chromosomes, or mosaics in whom there are both normal and trisomic cells, that is, mosaic trisomy 21. There is a relationship between the frequency of Down's syndrome births and age, with both very young mothers and older mothers having a higher incidence of affected infants. It has been suggested that nutrition may be implicated in the nondisjunction of the chromosomes. The additional chromosomal material in Down's syndrome usually comes from the mother, but it can, on occasion, come from the father. This observation may be indicative of hormonal changes in the older mother that reduce the likelihood of spontaneous abortion in an abnormal pregnancy.

The incidence of Down's syndrome is approximately 1 in 600–1000 live births. Prevalence is rising as life expectancy has improved over recent years with advancing medical knowledge and higher standards of care. In addition, women are having

children at the older age and the incidence of Down's syndrome is increasing with increasing maternal age, although there are significant differences between various racial and social groups.

Physical defects common in Down's syndrome include congenital anomalies of the gastrointestinal tract (e.g., duodenal atresia and intestinal aganglionosis), which occur in approximately 12% of infants with Down's syndrome. Most of these anomalies require the neonate to be operated on immediately to allow nutrition. Congenital heart disease occurs in approximately 40% of infants with Down's syndrome. Children with congenital heart disease may present with failure to thrive, but after surgical repair of heart defects these children usually improve. Immune dysfunction, increased susceptibility to leukemia, and premature ageing with Alzheimer-like changes in the brain are major features of the syndrome.

Thyroid dysfunction is more common in people with Down's syndrome with the incidence increasing with age. Hypothyroidism is most frequently reported but hyperthyroidism can also occur. Correction of the thyroid function is essential to allow normal learning processes to take place and to help weight control.

There are many biochemical anomalies associated with the syndrome, mainly quantitative rather than qualitative. It is presumed that the overexpression of genes on chromosome 21

Table 1 Nutritional complications of Down's syndrome

Physical	Problems with muscle tone, oral health and dentition, chewing and swallowing
Metabolic	Anomalies in carbohydrate protein and lipid metabolism Increased demands on antioxidant defence system and methylation pathways Increased incidence of diabetes, celiac disease, obesity and thyroid disorders and leukaemia Growth retardation
Behavioral	Food consumption and exercise choices

contributes to both the structural and functional pathology. Overdose effects of the genes already mapped to chromosome 21 are thought to alter pathways controlling the production of monocarbons, purines, pyrimidines, tubulins, and myelin.

The nutritional complications associated with Down's syndrome are summarized in **Table 1**.

Nutritional Status

It is debatable how relevant reference data from normal groups are for people with Down's syndrome.

Dietary Assessment

In children with Down's syndrome, conflicting reports have shown energy intake to be less than, similar to or greater than age-matched comparison groups, with a small percentage of children exceeding the recommended daily intake by more than 50%. However, as children with this syndrome tend to be shorter than age-matched children, energy intake comparisons need to be calculated per unit of body height.

Lower than recommended intakes of nonstarch polysaccharide coupled with higher than recommended consumption of protein and fats have also been reported. Researchers have reported low intakes of calcium, particularly in preschool and school-age children who refuse or limit milk consumption. Iron intakes have been reported to be low, particularly nonhem iron. Vitamins A and C intakes are limited in those who have a poor intake of fruit and vegetables. Intake of vitamin B has also been reported as low.

Laboratory Assessment

Carbohydrate metabolism

Fasting blood glucose levels are usually in the normal range, but the glucose tolerance curve has been reported to be flatter and often with a double humped curve, suggestive of delayed absorption. There is an increased incidence of both Type 1 (insulin dependent) and Type 2 diabetes in Down's syndrome. Studies have shown increased insulin resistance in obese and overweight females and adults with Down's syndrome but further research is required.

Protein Metabolism

Disturbances in protein metabolism are common in Down's syndrome. An increased level of immunoglobulin A and immunoglobulin G antibodies to food antigens has been

reported, and several studies have reported an increased prevalence of celiac disease. Abnormal levels of fasting plasma and urinary amino acids have been reported.

Lipid Metabolism

One study reported no significant differences between study and control groups, drawn from within the same families, in levels of total cholesterol, low-density lipoprotein, apolipoprotein B, and the apolipoprotein B to apolipoprotein A-I ratio. Triacylglycerol levels were significantly increased and serum high-density lipoprotein cholesterol to total cholesterol ratio significantly decreased in Down's syndrome. This suggests increased risk for coronary heart disease. The results of this and other studies reporting no difference between Down's syndrome and comparison groups in atherosclerosis, contrast with early reports that suggested a decreased incidence of coronary artery disease in Down's syndrome. It is not clear whether the differences reflect nutritional variables or population variable changes reflecting the increased survival rate in infancy.

There is evidence of increased lipid peroxidation in Down's syndrome.

Vitamins

Some studies have reported biochemical evidence of deficiency of thiamin, nicotinic acid, pyridoxine, cobalamin, folate, ascorbic acid, retinol, β -carotene, and α -tocopherol in patients with trisomy 21. Vitamin D metabolites have been reported to be in the normal range in a Spanish study that demonstrated wide seasonal variation linked to intensity of solar radiation.

Minerals

Low iron, calcium, manganese, and zinc blood concentrations have been reported in patients, and the iron to copper ratio has been reported to be decreased. Recent studies reported that intracellular zinc in blood mononuclear cells was approximately 47% lower than normal controls and it is possible that this could play a role in thyroid dysfunction, immunodeficiency, retarded growth, and faulty DNA repair. Low zinc status may contribute to the chemical disturbances that usually appear with ageing in individuals with Down's syndrome. Further research is required to determine whether zinc supplements are beneficial and if so at what dose. Supplementation with selenium aimed at increasing levels of the selenium-dependent enzyme glutathione peroxidase is reported to have led to a decrease in initially high blood mononuclear cell levels of copper, but did not affect iron or zinc.

Vitamin and mineral levels have been held to reflect not just nutrient intake, but also abnormal metabolism. Assessments of antioxidants and of oxidation by-products are useful indicators of nutritional status in people with Down's syndrome. The overexpression of the superoxide dismutase system, the purine synthesis pathway and cystathionine β -synthase are thought to create extra demands for antioxidants and for folate, but despite gene dosage effects the many biochemical anomalies that have been reported in people with Down's syndrome show a great deal of individual variation.

Anthropometric Assessment

Growth delay is one of the main characteristics of Down's syndrome but impaired growth velocity is particularly evident at certain stages of development.

The fetal growth has usually been reported to be relatively normal and the length of the neonate is often within normal limits, allowing for gestation. Some studies have reported the prenatal growth delay and a major Italian study comparing neonatal length, weight, head circumference, and weight/length squared reported all percentiles of growth variables lower in Down's syndrome infants except for weight/length squared percentiles.

At approximately 6 months of age, when growth starts to become regulated by growth hormone, growth velocity usually begins to show a marked reduction from normal levels. Although for the Down's syndrome child the period between birth and 2 years and the period between 6 years and 10 years of age are times of accelerated growth, the deviation from normal levels remains significant. Slow growth velocity is also a particular feature of adolescence, although there is a pubertal growth spurt. The deviation of adult stature from the means of reference groups is greater than the deviations in early infancy.

The short stature in Down's syndrome seems to be mainly the result of the impaired growth of the long bones of the leg, as sitting height measurements show that the growth of the vertebral column is closer to normal.

Why there is growth delay in Down's syndrome is not entirely clear, and several hypotheses have been advanced. Both human growth hormone therapy and zinc sulfate supplementation of the diet have been reported to accelerate the growth.

Children with Down's syndrome tend to be not only shorter, but also heavier than reference children. Charting the height and weight of a child with Down's syndrome using reference norms from the general population, will show the abnormality of the growth pattern. However, it is more useful clinically, to compare the height and weight of an individual against syndrome-specific norms, as that will show up any deviation from the growth patterns of children with Down's syndrome.

Italian percentile charts have been drawn up for neonates with Down's syndrome based on a large sample of consecutively born infants. The specific growth charts for children with Down's syndrome have been constructed based on anthropometric assessments of US children, Sicilian children (thought to be representative of southern European children)

and Dutch children (thought to be representative of northern European children). On average the Dutch children were taller than the US children and the US children were taller than the Sicilians. More recently growth charts have been developed for UK children and in the US work is currently being undertaken to update growth charts to encompass current research that shows children with Down's syndrome are growing better.

Nutritional Requirements

Children and adults with Down's syndrome need the same range of nutrients as the general population. Energy intake standards based on age groups are not appropriate for children with Down's syndrome. Energy intakes in both children and adults need to be tailored to height and weight and to the physical activity.

Nutritional Therapy

In the 1970s and 1980s hopes were raised that megadoses of vitamins and minerals would boost intelligence in children with Down's syndrome, but rigorous studies have shown these doses lead neither to higher intelligence nor to better health. In addition, there is anxiety about possible side effects, particularly of the fat-soluble vitamins.

As more has been learned about the genes on chromosome 21, interest shifted to targeted nutritional intervention aimed at correcting the metabolic anomalies that are common in Down's syndrome owing to genetic overexpression, with the emphasis on nutrients to maintain health and prevent disease. Targeted nutritional supplementation with vitamins, minerals, amino acids, digestive enzymes, and essential fatty acids remains controversial. Clinicians have reported differences between children treated and not treated in health, growth, and cognitive and speech functions but few well-designed studies have been performed.

Dietary Management

Dietary Guidelines

Dietary recommendations are as for the general population until research proves otherwise. There are, as yet, no specific dietary guidelines for the woman pregnant with a Down's syndrome child. Periconceptual folic acid supplements of 400 μ g daily in addition to folate-rich foods may be beneficial in decreasing risk of Down's syndrome, as well as neural tube effects. There are also indications that antioxidant and essential fatty acid intake may be particularly important, but at present dietary advice is the same as for other pregnant women.

The situation is similar for infant feeding. Brain lipids in the human infant are known to change with changing intakes of fatty acids. The needs of a newborn with Down's syndrome for the long-chain polyunsaturated fatty acids docosahexenoic acid and arachidonic acid have not yet been determined. Because breast milk contains the preformed dietary very long-chain fatty acids that seem to be essential for the

development of the brain and the retina, it seems prudent to encourage breast-feeding.

The antioxidant defense system has a particularly important role in Down's syndrome and parents and caregivers can be advised on providing a diet rich in antioxidants. Dietary intakes need to be considered for the sulfur amino acids (which are needed for glutathione synthesis), of fat-soluble vitamins A, C, and E, water-soluble vitamins B₆, B₁₂, and folic acid, and of the minerals selenium and zinc. In latitudes where no vitamin D is synthesized in the winter months, it is particularly important to ensure exposure to sunlight during summer months to maintain adequate stores of the vitamin throughout the year as recent studies indicate an increase in the incidence of osteoporosis in Down's syndrome. Those who are housebound or have poor mobility may benefit from a vitamin D supplement.

Feeding Behavior

Feeding skills tends to be delayed in the young child with Down's syndrome, but the sequence of the emergence of the skills is the same as with other children if appropriate learning opportunities are provided.

Infants with Down's syndrome have a smaller oral cavity, which makes it easier for liquids to spill from the sides of the mouth. If a child is hypotonic, the tongue is likely to flatten out when the child sucks instead of forming a groove round the nipple, so the child will have a weak suck, may gag, and milk will leak from the mouth. Feeding will be exhausting, and particularly where the child has a cardiac defect, the child may have difficulty taking in enough milk to meet energy requirements. Tube feeding may be necessary until the child develops better tongue control. Feeding will be easier if the infant is wide awake and extra support for the infant during feeding, in particular supporting the infant's chin to help steady the jaw, can all help encourage intake. Because of the benefits of breast-feeding, it is essential that nursing mothers are given help and advice when their infants have initial difficulties. Breathing during feeding may be helped if the mouth and nose are cleared of mucus with a syringe before feeding.

As with other children, it is important to introduce textured food when the child is developmentally ready, and information should be provided for parents and caregivers regarding appropriate expectations and helpful feeding techniques as well as dietary advice. In children with Down's syndrome, poor neuromotor control of the tongue may result in the continued use of pureed food. There may be slow initiation of the swallow response, possibly because of hypotonic pharyngeal muscles, and oral sensitivity problems may also make the transition to textured foods difficult. Persistent feeding problems merit multidisciplinary assessment and therapy. Impaired swallow can result in food being aspirated and contribute to respiratory problems. The presence of the tongue protrusion reflex past the age of 12–18 months can result in delayed progression to solid food and can contribute to malocclusion of teeth. Also, dental abnormalities can exacerbate difficulties with chewing and can contribute to poor nutrition, because children who have problems chewing may be offered soft often high-energy food and be given little

opportunity to accept meats, fresh fruits and vegetables, which are lower in energy.

Fresh fruit and vegetables provide the nonstarch polysaccharide that can help prevent the constipation common in Down's syndrome. Fruit juices and water between meals also help with constipation. Because the hypotonia in Down's syndrome also contributes to sluggish bowel habits, this is another reason for children and adults to be encouraged to take part in physical activity. If constipation does not respond to dietary management, there should be a medical assessment to exclude gastrointestinal and thyroid problems.

Dental Problems

Dental anomalies in Down's syndrome include changes in the tooth structure, reduced total number of teeth and delayed or abnormal eruption. Together with the physical abnormalities of the facial appearance and oral cavity, these can all impact on feeding. Dental disease is common in Down's syndrome as teeth are more at risk of wear through bruxism and decay due to fragile enamel. In addition, gum disease (gingivitis), and oral infections due to mouth breathing can lead to teeth becoming loose and falling out. A healthy balanced diet, including plenty of fruit and vegetables, low in sugar-containing fluids and fizzy drinks (including 'diet' varieties), and avoiding frequent snacks will help preserve teeth.

Obesity

Obesity is common in Down's syndrome having been reported from different cultures and ethnic backgrounds. From Australian and North American studies it has been reported that by 2–3 years of age more than 30% of children with trisomy 21 are overweight and that by 9 years of age the average child with Down's syndrome is obese.

High rates of overweight and obesity have been reported in adults with Down's syndrome, both living in the community and at home, and more commonly in females than males. Overweight and obesity are particularly associated with living in the family home compared to supervised community units or hospital, but they are not significantly associated with the degree of learning disability.

Because excessive weight gain in childhood often leads to adult obesity, it is important to encourage healthy choices in childhood. Why children with Down's syndrome have a tendency to become fat is not clear, but probably several factors influence the weight gain. Retardation of growth resulting in short stature may be of prime importance. Obesity in people with Down's syndrome has also been linked with several physiologic features of the syndrome (Table 2).

Prepubescent children with Down's syndrome have a decreased resting metabolic rate compared to control children matched for the body mass index. Children of approximately the same body composition, whether or not they have Down's syndrome, expend similar levels of energy in the physical activity. Since obesity is negatively correlated with the motor performance, it is likely to lead to a reduction in sporting and physical recreation activities, and thus obesity has social as

Table 2 Factors predisposing to obesity in Down's syndrome

	<i>Increased</i>	<i>Decreased</i>
Poor eating behavior	↑	
Calorie intake	↑	
Resting metabolic rate		↓
Muscle tone		↓
Exercise		↓
Thyroid function		↓
Substrate fat oxidation		?↓
Leptin levels	?↑	

well as health implications, in children with Down's syndrome as in other children. However, children and adolescents with Down's syndrome have been shown to have difficulty with sustained physical exercise in both laboratory and recreational situations, and this has been attributed to physiological impairments, notably cardiovascular, as well as to lack of motivation.

Children, adolescents and adults with Down's syndrome have a deficit in isokinetic strength, and by the age of 14 years adolescents with testosterone levels in the normal range fail to show the pubertal muscle strength increase. Progressive resistance exercise programs can help to build muscle strength, and regular aerobic exercise will improve exercise tolerance. Often individuals can attain high standards in competitive gymnastics and swimming. The overexpression of collagen genes on chromosome 21 affects both muscle and connective tissue, and it has been claimed that targeted nutritional treatment leads to rapid improvement in both muscle strength and joint stability.

In a cross-sectional study of men and women with Down's syndrome, the body mass index declined with increasing age. Further research is needed to clarify whether individuals lose weight as they age, or whether there is a shorter life expectancy for individuals with higher body mass indices.

Celiac Disease

There is an increased incidence of celiac disease in Down's syndrome compared with the general population. Symptoms such as gut dysfunction (both diarrhea and constipation), abdominal bloating, dyspepsia, mouth ulceration, mood change, arthritis, general fatigue, and mild anemia may be indicators of celiac disease, although many of these are common in Down's syndrome without celiac disease. Blood tests such as antiendomysial antibodies (AEA) and tissue transglutaminase antibody status can give an indication of celiac disease but are not conclusive. Definitive diagnosis remains the identification of villous atrophy on small bowel biopsy. Quality of life can be greatly improved on a gluten-free diet so implementation following diagnosis and good compliance should be encouraged.

Ageing

The rapid ageing that characterizes Down's syndrome is in line with the accumulating evidence that many degenerative

diseases are associated with deleterious activated oxygen species reactions. Activated oxygen species can damage genetic material and inactivate membrane-bound enzymes as well as cause lipid peroxidation in cell membranes. Of particular relevance to Down's syndrome is the evidence relating to cancer, inflammatory joint disease, diabetes, degenerative vascular disorders, degenerative eye disease and senile dementia – all reported to have increased prevalence in Down's syndrome.

The gene for copper/zinc-superoxide dismutase is on chromosome 21, and copper/zinc-superoxide dismutase levels are elevated by 50% in a range of cells of people with Down's syndrome, including erythrocytes, blood platelets, leucocytes, and fibroblasts. The increase has also been reported in fetal cerebral cortical cells. Although copper/zinc-superoxide dismutase usually functions as an antioxidant it seems likely that in Down's syndrome the raised levels lead to oxidative stress. When the increased production of hydrogen peroxide through catalysis of superoxide free radicals is not matched by a sufficient increase in glutathione peroxidase to metabolize the additional hydrogen peroxide to water and oxygen, there is thought to be an increase in highly reactive hydroxyl radicals leading to increased lipid peroxidation.

Fibroblasts derived from people with Down's syndrome show elevated lipid peroxidation, and levels of thiobarbituric reaction products, which indicate extent of lipid peroxidation, have been reported to be raised in erythrocytes from Down's syndrome subjects compared to control subjects.

A reported increase in the activity of the hexose monophosphate pathway in Down's syndrome is thought to be a compensatory mechanism to deal with increased hydrogen peroxide, allowing greater production of the reduced form of nicotinamide-adenine-dinucleotide phosphate, thus improving the ability of cells to reduce oxidized glutathione. However, it has been suggested that this shift of glucose utilization from energy production to reducing power may compromise cellular cation pumps.

Among the genes so far identified on chromosome 21 is that for β -amyloid precursor protein. Amyloidosis is evident in the brain tissue of both patients with Alzheimer's disease and those with Down's syndrome. Studies are investigating the implications of the anomalies in the expression of the β -amyloid precursor protein and also of the effect on cobalamin/folate metabolism of the gene for the enzyme cystathionine β -synthase, also on chromosome 21. The overexpression of both these genes is believed to contribute substantially to the development of dementia. Although all people with Down's syndrome have evidence of brain pathology similar to Alzheimer's disease by their early thirties, not all show Alzheimer-like behavior changes as they age.

It may be that an increase in dietary antioxidants could delay the onset of Alzheimer's type symptoms but more research is required. However, standard dietary recommendations for healthier lifestyles (i.e., eating more fruit and vegetables and including more oily fish in the diet) may have the added potential benefits of increasing antioxidant intake. Unfortunately these are often the foods least favored by individuals with Down's syndrome.

Low vitamin E levels have been found to be associated with dementia, not only in the elderly but also in those with

Down's syndrome. Vitamin E may have a potential therapeutic role in Alzheimer's like neurological changes by protecting the integrity of the muscarinic receptors. In addition, vitamin E is a strong antioxidant. Likewise, vitamin B₁₂ deficiency has been reported in cases of Down's syndrome and Alzheimer's disease; however, replacement therapy does not change the evolution of the underlying disease. Continuing research into the etiology of the Down's syndrome phenotype is expected to lead to advances in the treatment of both Down's syndrome and Alzheimer's disease.

Care in the Community

Most people with Down's syndrome live in the community; some live with parents or caregivers, but adults often live independently or semi-independently. Many people with Down's syndrome can learn about healthy eating and manage their own diets. A dietitian's role in a community learning disability support team is likely to encompass not only individual assessment, but also teaching and educating people with Down's syndrome as well as parents, caregivers and other professionals.

See also: Antioxidants. Dental Disease: Etiology and Epidemiology. Fatty Acids: Metabolism. Growth and Development: Physiological Aspects. Obesity: Complications; Definition, Etiology, and Assessment; Prevention; Treatment. **Weight Management:** Approaches; Weight Cycling/Weight Change

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National Down's Syndrome Society.

DRUG–NUTRIENT INTERACTIONS

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Glossary

Cytochrome P-450 The cytochrome P450 superfamily (officially abbreviated as CYP) is a large and diverse group of enzymes. The function of most CYP enzymes is to catalyze the oxidation of organic substances.

Drug A substance recognized in an official pharmacopoeia, used for the diagnosis, cure, mitigation, treatment, or prevention of disease.

Herbs An herb is a plant that is valued for flavor, scent, medicinal, or other qualities. Herbs are used in cooking, as medicines, and for spiritual purposes.

Pharmacokinetics Includes the study of the mechanisms of absorption and distribution of an administered drug, the rate at which a drug action begins and the duration of the effect, the chemical changes of the substance in the body and the effects and routes of excretion of the metabolites of the drug.

Introduction

Understanding the interactions between dietary constituents and pharmacological compounds is essential for monitoring drug therapy correctly. Assessment of potential nutritional impact on medications is important to determine, resulting in increased toxicity or therapeutic failure. Bioavailability of drugs caused by food-induced changes is the greatest cause of clinically relevant drug–nutrient interactions. Most therapeutic agents exhibit some form of interaction that ultimately affects the nutritional status of the host, by altering absorption or utilization of nutrients. These changes are frequently not readily identified or may be obscured by the underlying disease. A need for strategies to identify and prevent the development of drug–nutrient interaction is important to reduce adverse events.

The interactions between therapeutic agents and nutrients are part of the large number of interactions occurring between nutritional and nonnutritional constituents of the human diet. These constituents include all substances added to the food chain – incidentally or deliberately – during harvesting, processing, packaging, distribution, and preparation of foods. Examples of these agents are pesticides, food additives, antibiotics, hormones, and environmental toxins.

Drug–nutrient interactions operate in two directions: drugs can have a significant impact on nutrient absorption and utilization. The nutritional status of the host also affects the drug's ability to be absorbed and transported and to exert an effect on the target tissues.

Drug–nutrient interactions can be broadly classified into two categories: direct physicochemical interaction and physiological or functional interaction. They can also be divided into pharmacokinetic and pharmacodynamic interactions. The most common interactions are pharmacokinetic. An example of pharmacokinetic interactions is food's effect on absorption, distribution, metabolism, or elimination of a drug. Interactions where food affects the drug action at a

receptor level are less common but would be classified as a pharmacodynamic interaction.

Drug–nutrient interactions can also be classified according to their site of occurrence: within the food matrix, in the gastrointestinal (GI) tract, or during transport, metabolism, and excretion. The mechanisms and sites of drug–nutrient interactions are listed in [Table 1](#).

Physicochemical interactions usually involve some form of molecular interaction between the drug and a nutrient, and occur primarily during digestion and absorption. The usual consequence of this interaction is a reduction in the bioavailability of the drug and/or the nutrient. These pharmacokinetic effects on bioavailability are an important parameter of drug–nutrient interactions. The characteristics of a drug's physical and chemical structure play an important role in its potential for interactions with nutrients. Individual drugs must be tested to determine the exact interaction when given with food. A well-known example of this is the binding of metals by the antibiotic ciprofloxacin. This would be an example of a chemical reaction between the drug and nutrient, which decreases the concentration of ciprofloxacin.

Functional interactions in the GI tract are particularly significant. Alterations in GI function are likely to affect the digestion and absorption of both the drug and nutrients. Absorption of a drug can be the most important factor in the drug–nutrient interactions. The most common GI functional effects are as follows.

Changes in GI motility: A reduction in transit time may lead to decreased absorption. There are a large number of drugs that affect gut motility, whether this is their primary therapeutic effect or not. Conversely, food composition also affects motility. Dietary fiber not only increases motility but also may trap other nutrients and drugs and reduce their bioavailability. Fiber will bind to digoxin and lovastatin and decrease their bioavailability. High-fat content meals can affect the bioavailability of lipophilic drugs. Albendazole and

Table 1 Mechanisms and sites of drug–nutrient interactions

<i>Site</i>	<i>Mechanism</i>	<i>Effect</i>
Food matrix	Binding and chelation	Decreases bioavailability
Gastrointestinal tract	Changes in gastrointestinal motility, binding and chelation, bile-acid concentration, and gastric pH	Increase in transit time reduces absorption, decreases bioavailability, and reduces absorption of fat-soluble nutrients
Circulation	Albumin concentration	Affects absorption of iron, vitamin B12, and other substances
	Competitors for albumin binding	Decreases transport of bound substances; displaces albumin-bound nutrients (fatty acids, tryptophan, etc.)
Target tissues	Antagonistic effects	May increase requirements for antagonized nutrients
	Enzyme activities	Reduced concentration of enzyme product
Excretion	Renal function	Increased excretion may lower nutrient levels, increasing requirements
	Sequestration	As above

isotretinoin are examples of drugs that have increased bioavailability with a high-fat meal.

Changes in Gastric-acid Output

Reduced production of chloride with a subsequent increase in gastric pH retards gastric emptying and may alter the balance between ionized and nonionized forms of therapeutic agents. Prolonged use of acid suppression drugs such as proton pump inhibitors can lead to B₁₂ malabsorption.

Reduction in the Concentration of Bile Acids

A reduction in the concentration of bile acids will affect the absorption of most fat-soluble compounds. Lower bile-acid concentration may result from increased binding and excretion or from decreased production. For example, the antibiotic neomycin binds to bile acids and increases their fecal excretion, thus reducing their luminal concentration. This will lead to a decrease in the absorption of fat-soluble vitamins. Drugs can be used therapeutically to reduce bile-acid turnover in patients with certain liver diseases, to lower cholesterol levels by reducing their re-absorption.

Alterations in the GI Micro Flora

Alterations in the GI micro flora may affect the availability of nutrients produced by the normal gut flora, such as vitamin B₁₂. The process of B₁₂ absorption starts by R-protein binding in the saliva; this R-protein is cleaved in gastric acids of the stomach. The gastric parietal cell secretes intrinsic factor, which binds B₁₂. The final absorption is facilitated by receptors in the terminal ileum, and since many drugs are susceptible to bacterial metabolism, changes in the gut flora may also affect drug bioavailability. In certain cases, drug cleavage by intestinal microorganisms is an expected and necessary step for adequate drug action. For example, the anti-inflammatory agent 5-aminosalicylic acid is given as its precursor sulfasalazine, which is converted into the active compound by colonic bacteria. An altered colonic flora will affect the production of the active compound. Drugs can also affect nutrient

absorption by directly inhibiting protein synthesis in the enterocyte. Since most transport systems require active protein synthesis and turnover, such inhibition results in a decreased rate of nutrient absorption. Furthermore, certain drugs undergo initial metabolism in the enterocyte, before reaching the bloodstream. Alterations in protein synthesis in the enterocyte, or an impaired turnover of the intestinal epithelia, will also affect this process.

Interactions Affecting Transport, Metabolism, and Excretion

Functional Synergism or Antagonism

The biological actions, of nutrients and drugs can be synergistic or antagonistic, occur at different times after exposure and affect a variety of target tissues. Some of the most common mechanisms are as follows.

Alterations in Drug Transport

Drugs circulate in the bloodstream as free compounds or bound to other constituents, usually proteins. Drugs vary greatly in their propensity to bind to circulating proteins, covering virtually the entire spectrum from 0 to 100%. For a given drug, the bound fraction tends to be relatively constant under normal physiological conditions. Changes in pH, electrolyte balance, and the presence of competing molecules can change the percentage of drug bound. The major transport protein in plasma is albumin. Albumin concentration and the presence of other compounds with an affinity for albumin binding will affect the amount of drug that will ultimately be transported by this protein. Phenytoin is a very good example of how protein binding and how serum protein concentrations affect the free phenytoin.

Increase in nutrient catabolism. Certain drugs stimulate detoxifying systems, such as the cytochrome P-450 pathway. Activation of this system may result in increased catabolism of certain nutrients. In other cases, drugs directly affect nutrient catabolism, as in the case of anticonvulsant drugs, which stimulate vitamin D catabolism in the liver.

Table 2 Major drug–nutrient interactions of clinical relevance

<i>Drug</i>	<i>Drug Class</i>	<i>Food/Nutrient</i>	<i>Effect/Mechanism</i>
ACE Inhibitors captopril, enalapril, lisinopril	Antihypertensive	Potassium	High doses increase urinary potassium losses
Aspirin	Analgesic	Food	Reduced absorption
		Folic acid	Decreased rate of absorption
		Amino-acids	Increased excretion of folate
			Decreased intestinal absorption of amino-acids, increased urinary excretion of tryptophan
		Iron	Chronic high dose 3–4 g day ⁻¹ , iron deficiency possible
		Alcohol	Gastric irritation, leading to possible gastric bleed
		Curry powder, liquorice, teas, raisins, and paprika	Potential salicylate accumulation
		Ascorbic acid and fresh fruits	Increased urinary excretion; decreased concentration in serum and platelets
Albendazole	Antihelmintic	Food	Increase absorption with fatty food
Astemizole	Antihistamine	Grapefruit juice	May result in cardiotoxicity
Atenolol	Antihypertensive	Food	Decreased bioavailability
Atovaquone	Antibiotic	Food	Delayed absorption
			Bioavailability increased, especially in high-fat foods
Atropine	Anticholinergic	Iron	Delayed absorption
Azithromycin	Antibiotic	Food	Decreased rate and delayed absorption
Barbiturates	Anticonvulsants	Alcohol	Enhanced CNS depression
		Calcium, vitamin D	Increased vitamin D requirements, owing to increased metabolism
		Cyanocobalamin	Increased bone resorption
		Folic acid	Decreased serum levels, leading to megaloblastic anemia
		Serum lipids	Decreased CSF folate and erythrocyte concentration; may increase cholesterol, HDL triacylglycerols
Benzodiazepines	Anticonvulsants	Food	Enhanced CNS depression
Clonazepam		Calcium	Increased vitamin D requirements secondary to increased metabolism
Clorazepate dipotassium		Vitamin D	Increased bone resorption
Diazepam			
Lopazepam		Cyanocobalamin	Decreased serum levels, leading to megaloblastic anemia
Oxazepam		Folic acid	Decreased CSF folate and erythrocyte concentration
		Serum lipids	May increase cholesterol, HDL triacylglycerols
Buprenorphone	Analgesic	Alcohol	Enhanced CNS depression
HCL/tartrate	Narcotic		
	Agonist–antagonist		
Calcium carbonate	Antacid	Iron	Decreased Iron absorption
		Fats	May cause steatorrhea
Carbamazepine	Anticonvulsants	Sodium	SIADH
		Food	Enhanced absorption, increased bile production
Cefadroxil		Food	No effect (may take with food)
Cefpodoxime proxetil		Food	Bioavailability increased with food
Cefuroxim axetil		Food	Bioavailability increased with food
Cefixime		Food	Decreased rate of absorption
Cefachlor		Food	Decreased rate of absorption
Cephalexin		Food	Absorption reduced for suspension, delayed for capsule
Cephadrine		Food	Rate of absorption delayed
Cetirizine	Antihistamine	Food	Delays time to serum peak; no effect on overall absorption
Chlorambucil	Antineoplastic	Food	Reduced absorption

(Continued)

Table 2 Continued

<i>Drug</i>	<i>Drug Class</i>	<i>Food/Nutrient</i>	<i>Effect/Mechanism</i>
Chloramphenicol	Antibiotic	Iron	Increased serum level iron; increased total iron-binding capacity
		Folic acid	Antagonist to physiological action; increased requirements of folic acid
		Vitamin B ₁₂	Increased requirements of vitamin B ₁₂ can cause peripheral neuropathy
Chlorothiazide	Diuretic	Food	Increased drug absorption owing to delayed gastric emptying
Chloroquine	Antimalarial	Food	Increased bioavailability
Chlorpromazine	Antiemetic	Food	Decreased absorption owing to delayed gastric emptying
Chlorpropamide	Antidiabetic	Glucose	Decreased blood glucose concentration
		Sodium	Hyponatremia, SIADH
		Alcohol	Flushing, headache, nausea, vomiting, tachycardia
Cimetidine	Histamine 2 antagonist	Food	Delays absorption
Ciprofloxacin	Antibiotic (quinolone)	Caffeine	Decreased rate of absorption
		Food	Decreased elimination of caffeine
		Calcium	Calcium can bind quinolones
		Mineral supplement	Absorption of divalent and trivalent cations decreased by binding to quinolones
Clarithromycin	Antibiotic (macrolide)	Food	Decreased onset of absorption; no change in total amount absorbed
Cloxacillin	Antibiotic (penicillin)	Food	Decreased rate of absorption
Codeine	Narcotic agonist, analgesic	Alcohol	Enhanced CNS effect
		Glucose	Can cause hyperglycemia
Corticosteroids	Steroids	Calcium, phosphorus, vitamin D	Decreased absorption of calcium and phosphorus; increased urinary excretion; chronic high dose can cause osteomalacia
Prednisone			
Prednisolone			
Dexamethazone			
Methylprednisolone			
Hydrocortisone			
		Nitrogen	Increased urinary nitrogen losses
		Zinc	Increased urinary excretion and decreased serum levels
		Glucose	Impairs glucose tolerance; increases plasma levels
		Triacylglycerols, cholesterol	Increased serum levels
Co-trimoxazole	Antibiotic	Potassium	Decreased excretion hyperkalemia
		Sodium	Increased excretion hyponatremia
		Folic acid	Potential for folate deficiency
Cyclosporine	Immunosuppressant	Milk, fat, pineapple juice, grapefruit	Increased absorption
Demeclocycline	Antibiotic	Food, calcium, iron	Decreased absorption of dairy products and divalent and trivalent cations
Dicumarol	Anticoagulant	Food	Increased absorption with high-fat meals and delayed gastric emptying
Didanosine Tab	Antiviral	Food	Decreased rate and extent of absorption
Oral suspension		Fruit juice or acid liquid	Didanosine unstable in acid
Digoxin	Cardiac	Food	Delayed absorption; adsorbent to high-fiber high-pectin foods
Divalproex	Anticonvulsant	Food	Decreased rate of absorption; extent of absorption not affected
Doxycycline	Antibiotic	Food	Decreased absorption of food and milk
Erythromycin stearate	Antibiotic (macroide)	Food	Increased absorption by delayed gastric emptying
Ethionamide	Antituberculosis	Pyridoxins	Reports of peripheral neuritis and paraesthesia
Etodolac	NSAID	Food (milk)	Decreased total bioavailability of tolmetin; decreased absorption of ibuprofen
		Sodium	Hyponatremia (indomethacin/ketorolac)
		Potassium	Hyperkalemia (indomethacin/ketorolac)
		Food	Increased rate of absorption

(Continued)

Table 2 Continued

<i>Drug</i>	<i>Drug Class</i>	<i>Food/Nutrient</i>	<i>Effect/Mechanism</i>
Felbamate	Anticonvulsant	Glucose Magnesium Phosphorus Potassium Sodium	Hypoglycemia Hypomagnesemia Hypophosphatemia Hypokalemia Hyponatremia
Fenoprofen Fenoprofen calcium	NSAID	Food (milk) Sodium Potassium Food (Calcium)	Delayed absorption Hyponatremia (indomethacin/ketorolac) Hyperkalemia (indomethacin/ketorolac) Absorption not affected peak level delayed and diminished
Fluconazole	Antifungal	Potassium	Hypokalemia
Flucytosine	Antifungal	Food	Decreased rate of absorption; no change in extent of absorption
Foscarnet	Antiviral	Calcium	Hypocalcemia; drug chelates; divalent metal ions
		Magnesium Phosphorus Potassium	Hypomagnesemia Hypophosphatemia and hyperphosphatemia Hypokalemia
Furazolidone	Anti-infective	Tyramine-rich foods (avocados, canned figs, aged cheese, cola beverages, coffee, chocolate, wines, soy sauce, fermented meats, yeast preparation, yoghurts) Alcohol	Prolonged large doses result in increased risk for hypertensive crisis Rushing, headache, nausea, vomiting, sweating, tachycardia
Furosemide	Diuretic	Food	Delayed absorption
Ganciclovir	Antiviral	Food	Increased area under curve plasma concentration
Glipizide	Antidiabetic	Food Alcohol	Delayed absorption Flushing, headache, nausea, vomiting, sweating, tachycardia
		Sodium	Hyponatremia, SIADH
Griseofluvin	Antifungal	Alcohol	Can increase alcohol effect, flushing, tachycardia
		High-fat food	Increased drug absorption rate
Hydralazine	Diuretic	Food	Increased absorption
Hydrochlorothiazide	Diuretic	Food	Increased absorption by delayed gastric emptying
HMG-CoA	Antihyperlipidemic	Food (grapefruit)	Increase drug serum concentration; increase area under curve concentration
Reductase inhibitors Simvastatin Lovastatin Atrovastatin Ibuprofen	NSAID	Food (milk)	Decreased total bioavailability of tolmetin; decreased absorption of ibuprofen Hyponatremia (indomethacin/ketorolac) Hyperkalemia (indomethacin/ketorolac)
		Sodium Potassium	Hyponatremia (indomethacin/ketorolac) Hyperkalemia (indomethacin/ketorolac)
Indinavir	Antiviral	Food Food	Increased rate of absorption Decreased absorption of high-calorie, high-fat and protein-rich foods
		Grapefruit juice	Decreased area under curve concentration
Indomethacin	NSAID	Food (milk)	Decreased total bioavailability of tolmetin; decreased absorption of ibuprofen
		Sodium Potassium	Hyponatremia (indomethacin/ketorolac) Hyperkalemia (indomethacin/ketorolac)
		Food	Increased rate of absorption
Iron	Mineral	Ascorbic acid Amino-acids Calcium phosphate	Increased absorption Increased absorption Decreased absorption

(Continued)

Table 2 Continued

<i>Drug</i>	<i>Drug Class</i>	<i>Food/Nutrient</i>	<i>Effect/Mechanism</i>
Isoniazid	Antituberculosis	Tea/Coffee	Decreased absorption due to iron tannate formation
		Zinc	Inhibits absorption
		Vitamin A	Vitamin A deficiency inhibits iron utilization and accelerates the development of anemia
		Food	Decreased intestinal absorption
		Pyridoxine	Decreased metabolism, antagonism
Itraconazole	Antifungal	Food and histamine, tuna, liver, aubergine, parmesan cheese, tomato, spinach, tyramine containing foods	Headache, redness, itching of eyes and face, chills, diarrhea, palpitation; potential hypertensive crisis due to monoamine oxidase inhibitor activity
		Food	Increased absorption, increased triacylglycerols
Ketoconazole	Antifungal	Potassium	Hypokalemia
		Alcohol	Flushing, headache, nausea, vomiting, sweating, tachycardia
Lansoprazole	H/K proton-pump inhibitor	Food	Delays absorption
Labetalol	Antihypertensive	Food	Increased absorption
Lamivudine (3TC)	Antiviral	Food	Decreased rate of absorption
Levodopa	Anti-Parkinson's	Food	Decreased absorption; with high-protein meals amino-acids compete for absorption
Lithium	Antimanic	Low-sodium diet	Increased lithium concentrations
		High-sodium diet	Increased lithium clearance
		Food	Increased absorption
Linezolid	Antibiotic	Tyramine-rich foods	May result in blood-pressure changes
Loracarbef	Antibiotic	Food	Decreased rate of absorption
Mebendazole	Anthelmintic	Food	Increased absorption
Melphalan	Antineoplastic	Food	Reduced absorption
Mercaptopurine	Antineoplastic	Food	Reduced absorption
Methenamine mandelate	Urinary anti-infective	Milk products, citrus fruits	Excessive amounts inhibit drug conversion
Methotrexate	Antineoplastic	Food	Increased absorption
Methyldopa	Antihypertensive	Vitamin B ₁₂ , folate	In high doses methyldopa can increase vitamin B ₁₂ and folate losses
Metoprolol	Antihypertensive	Food	High-protein meals compete for absorption
		Food	Increased absorption
		Alcohol	Flushing, headache, nausea, vomiting, sweating, tachycardia
Metronidazole	Antibiotic	Food	Decreased peak serum concentration but total amount of drug absorbed is not affected
		Food, calcium	Decreased absorption
		Food	Decreased absorption; decreased serum levels due to altered gastric pH
Minocycline	Antibiotic	Potassium	High doses can cause hypokalemia owing to increased urinary losses
		Food	Increased absorption by delayed gastric emptying
Nafcillin	Antibiotic	Food	Decreased peak serum concentration but total amount of drug absorbed is not affected
		Food, calcium	Decreased absorption
Nitrofurantoin	Antibiotic	Potassium	High doses can cause hypokalemia owing to increased urinary losses
		Food	Increased absorption by delayed gastric emptying
Norfloxacin	Antibiotic (quinolone)	Food, dairy products	Decreased rate of absorption
		Multivitamin and mineral supplements	Decreased absorption due to formation of divalent and trivalent cation complexes with quinolones
Nifedepine	Antihypertensive calcium-channel blocker	Grapefruit juice	Increased serum level of nifedepine flavonoids inhibits cytochrome P-450
		Food	Decreased bioavailability, formulation dependent
Ondansetron	Antiemetic	Food	Increased extent of absorption
Omeprazole	H/K proton-pump inhibitor	Potassium	Hypokalemia
		Food	Delays absorption
Oral contraceptives		Ascorbic acid	Decreased ascorbic-acid concentration in plasma, platelets, leucocytes

(Continued)

Table 2 Continued

<i>Drug</i>	<i>Drug Class</i>	<i>Food/Nutrient</i>	<i>Effect/Mechanism</i>
Oxacillin	Antibiotic	Vitamin C, folic acid Vitamin B ₁₂ Amino-acids, vitamin A, vitamin E, copper	Decrease in serum levels Impairs tryptophan metabolism Increase in serum levels
		Food	Decreased absorption and decreased serum concentration
Paromomycin	Amoebicide	Fats	Oxacillin can cause steatorrhea
		Food	Increased absorption by delayed gastric emptying
Penicillamine	Antidote (chelating agent)	Vitamins A, D, E, K	Malabsorption of fat-soluble vitamins owing to hypocholesterolemia
		Food	Decreased absorption
Penicillin G and VK	Antibiotic	Iron, zinc	Decreased absorption 30%–70% of increased zinc absorption; decreased penicillamine absorption
		Food	Decreased absorption by delayed gastric emptying
Pentamidine	Antibiotic	Glucose	Hyperglycemia
		Calcium, magnesium Potassium	Hypomagnesemia, hypocalcemia Hyperkalemia due to nephrotoxicity
Phenobarbital	Anticonvulsant (see Barbiturates)	Food	Decreased absorption due to protein binding
		Protein	Low-protein diet increases duration of action of phenobarbital
		Vitamin D, calcium	Decreased serum vitamin D by cytochrome P-450
		Vitamin B ₁₂ , folic acid	Decreased absorption and serum levels of folates; inhibits vitamin B ₁₂ transport
Phenytoin	Anticonvulsant (hydantoins)	Copper	Increased serum levels
		Fresh fruits and vitamin C	Increased urinary excretion
		Vitamin D, calcium	Decreased serum vitamin D by cytochrome P-450 hypocalcemia
		Enteral feeds	Decreased absorption
Pimozide	Antinerveoleptic	Food	Increased absorption by delayed gastric emptying
		Food (grapefruit)	Increased risk of cardiotoxicity
Praziquantel	Anthelmintic	Food	Decreased rate and extent of absorption
Primidone	Anticonvulsant	Fresh fruits and vitamin C	Increased urinary excretion of primidone
		Protein	Low-protein diet increases duration of action of primidone
Propranolol	Antihypertensive	High-protein foods	Increased absorption
Pyrimethamine	Antimalarial	Folic acid	Decreased serum folate concentrations
Quinidine	Antiarrhythmic	Food	Delayed absorption due to protein binding
Riboflavin	Vitamin	Food	Increased absorption by delayed gastric emptying
Rifampin	Antibiotic	Food	Decreased absorption
Ritonavir	Antiviral	Vitamins	Can cause vitamin deficiency
		Potassium	Hyperkalemia and hypokalemia
		Cholesterol	Hypercholesterolemia
		Triacylglycerols	Hypertriacylglycerolemia
Oral solution	Analgesics	Food	Delayed absorption
		Food	Increased extent of absorption
		Iron	Long-term chronic use decreases serum iron
		Vitamin C	Decreases concentration in serum and platelets
Salicylates	Analgesics	Amino-acids	Decreases their intestinal absorption and increases urinary secretion
Magnesium salicylate			
Choline salicylate			
Sodium salicylate			
Saquinavir mesylate	Antiviral	Food	Increased absorption of high-calorie, high-fat foods
		Calcium	Hypercalcemia
		Glucose	Hyperglycemia and hypoglycemia

(Continued)

Table 2 Continued

<i>Drug</i>	<i>Drug Class</i>	<i>Food/Nutrient</i>	<i>Effect/Mechanism</i>
Spironolactone	Diuretic	Phosphorus Potassium Food	Changes in serum phosphorus Hyperkalaemia and hypokalemia Increased absorption by delayed gastric emptying
Sulfonamides Sulfisoxazole	Antibiotic	Food Folic acid	Delayed with no effect on extent of absorption Decreased intestinal synthesis, absorption, and serum levels
Sulfamethoxazole Tetracycline	Antibiotic	Food Minerals	Decreased absorption Inhibits absorption of iron, calcium, zinc
Theophylline	Bronchodilator	Charbroiled beef High-fat meals	Increased metabolism of theophylline Increased absorption dependent on formulation
Tolazamide	Antidiabetic	Sodium	Hyponatremia and SIADH
Tolbutamide	Antidiabetic	Ethanol	Prolonged hypoglycemia, disulfam reaction
Trimethoprim	Antibiotic	Folic acid	Decreased serum folate levels
Valproic acid Divalproex Sodium valproate Sodium oral solution Warfarin	Anticonvulsant	Milk, food, carbonated drinks	Delayed absorption but no effect on extent of absorption
	Anticoagulant	Alcohol, vitamin K	Inhibits warfarin metabolism; beef liver, pork liver, green tea, leafy green vegetables high in vitamin K inhibits anticoagulant effect
Zalcitabine	Antiviral	Vitamin E	Can increase warfarin response
Zafirlukast	Selective leukotriene antagonist	Food	Decreases rate and extent of absorption
Zidovudine	Antiviral	Food	Delayed absorption
			Decreased rate of absorption

SIADH, Syndrome of inappropriate antidiuretic hormone excretion; CNS, central nervous system; CSF, cerebrospinal fluid; NSAID, nonsteroidal anti-inflammatory drug.

Changes in Drug Metabolism

Certain nutrients (such as those found in grapefruit) can inhibit the activity of cytochrome P-450. Cytochrome P-450 3A is one of the isoforms affected in a clinically significant way. The mucosal cells of the small intestine are affected to a greater degree than the hepatic cytochrome P-450-3A. Certain HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase inhibitors (simvastatin, and atorvastatin) as well as immunosuppressants (tacrolimus).

Biological Antagonism

Biological antagonism occurs when drug and nutrient have opposite biological actions, as is the case, with vitamin K and salicylates in the coagulation process. Small amounts of vitamin K have also been shown to stabilize warfarin effects, whereas large amounts of vitamin K can reverse warfarin's effect.

Increased Nutrient Losses

Many drugs directly or indirectly enhance the urinary excretion of nutrients. Examples are the increased urinary losses of electrolytes caused by aminoglycoside antibiotics and amphotericin B antifungal, and the increase in urinary ascorbic acid excretion induced by barbiturates.

Host-related Functional Interactions

Nutrients and nutritional status can also affect drug action and disposition. Perhaps the most significant host-related factor affecting drug disposition is protein synthesis. Altered protein synthesis usually results from insufficient dietary protein intake or severe diseases. This has a pharmacokinetic affect on absorption, transport, metabolism, and excretion, as these are all protein-dependent processes. The role of plasma albumin in drug transport is discussed above and is certainly affected by impaired albumin synthesis and/or sequestration in the extravascular space, as seen in protein-energy malnutrition. It should be noted, however, that malnutrition affects many aspects of drug metabolism, not all in the same direction. For example, drug delivery may be reduced by impaired albumin concentration, but the drug concentration in the bloodstream may be increased as a result of impaired clearance, which is also affected by malnutrition. The plasma amino-acid profile may affect the efficacy of drug entry into the central nervous system. At the blood–brain barrier, certain drugs are transported into the brain by the same transport system that carries the large neutral amino-acids; thus they must compete with them for use of the carrier binding sites. Diet composition, by affecting the postprandial amino-acid profile, may significantly affect the clinical efficacy of drugs such as L-dopa, used in the treatment of Parkinson's disease.

Table 3 Herbal–drug interactions

Herbal	Drugs	Effect/mechanism
Echinacea	Methotrexate, aminodarone, ketoconazole, steroids (anabolic)	Increased hepatotoxicity
Feverfew	NSAIDs	Decreased herbal effect
	Anticoagulants	Additive platelet inhibition
Garlic	Aspirin, anticoagulants	Reduced clotting time
Ginkgo biloba	Aspirin, anticoagulants, NSAIDs, tricyclic antidepressants, anticonvulsants	Decreased seizure threshold; increased risk of bleeding
Ginseng	Monoamine oxidase inhibitors	Headache, tremors, mania
	Corticosteroids	Increased steroid toxicity
	Warfarin	Decreased INR
	Digoxin	Increased digoxin levels
Kava kava	Benzodiazepines	Increased CNS depression
Ephedra	Antidepressants, CNS stimulants	Increased herbal effect
St John's wort	Antidepressants, CNS stimulants	Additive effects
	Piroxicam, tetracycline	Increased photosensitivity
	Theophylline	Decreased theophylline levels
Saw palmetto	Estrogen	Increased effect of herbal
Valerian	CNS depressants	Additive CNS depression

CNS, central nervous system; INR, International Normalization Ratio; NSAIDs' nonsteroidal anti-inflammatory drugs.

Body composition is also a relevant determinant of drug disposition and action. Although most drug dosages are calculated by total body weight, most drugs act only in the fat-free body mass. Thus, at a given body weight, individuals with more body fat will tend to receive a higher effective dose than those with less body fat. The amount of body fat is also important for drugs that are stored in adipose tissue. This is why aminoglycosides are best dosed on ideal body weight in obese patients.

Major Drug–Nutrient Interactions of Clinical Relevance

Table 2 provides information on the major drug–nutrient interactions of clinical relevance. The list reflects well-known interactions of drugs that have been on the market for some time. The US Food and Drug Administration (FDA) maintains an online database of recently reported interactions and interactions of new drugs. The database can be accessed at <http://www.fda.gov>.

Herb–Drug Interactions

Herbal botanicals have been used in many cultures throughout the world for hundreds of years. These products are usually seen as natural; they should not be synonymous with safe. Possible interactions can involve hepatic metabolism via cytochrome P-450 and changes in intestinal absorption, distribution, and renal excretion. Herbal interactions with certain drugs are listed in **Table 3**.

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EARLY ORIGINS OF DISEASE

Contents

Fetal

Non-Fetal

Fetal

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Glossary

DNA methylation Chemical modification of DNA in which methyl group is added usually to cytosine.

Epigenetics The study of inherited changes in phenotype (appearance) or gene expression that occur without any changes to the underlying DNA sequence.

Histones Proteins that package and order DNA.

Impaired glucose tolerance Prediabetic state of increased levels of glucose in the blood.

Insulin resistance Condition in which insulin is less effective in lowering blood sugar levels.

Metabolic syndrome A combination of medical disorders including high blood pressure, high triglyceride

levels, high cholesterol, obesity, and insulin resistance, that increase the risk of developing heart disease, type 2 diabetes, and stroke.

Mitochondrial dysfunction Inability of the mitochondria to convert the energy derived from food into the adenosine triphosphate (ATP), which powers most cellular functions.

Oxidative stress Imbalance between the production of reactive oxygen species within cells and the ability of the antioxidant machinery to detoxify the reactive intermediates, or to repair the cellular damage.

Introduction

Chronic diseases such as cardiovascular disease, type 2 diabetes, and cancer account for 60% of all deaths worldwide. In recent years it emerged that these diseases, thought of predominantly as diseases of adulthood, are increasingly prevalent amongst children. Although a nutritionally unbalanced diet and sedentary lifestyle play a contributing role, a growing body of evidence suggests that the ability of an individual to respond to metabolic challenges encountered throughout its

lifetime may be determined during its fetal life. Although fetal growth and development follows the route encoded within an individual's genome, the environment in which a fetus grows can influence this process. The intrauterine environment provides a forecast of conditions for the fetus after birth. The fetus can respond and adapt to a variety of stimuli or insults. However, the adaptations include irreversible changes in the structure and function of the body, that are detrimental to the long-term health of an individual, especially if postnatal conditions differ to the environment experienced *in utero*.

Epidemiological Data

It has been over 75 years since it was suggested, that the decline in overall death rates in Sweden and the UK between 1751 and 1930 was attributed to improvements in childhood nutrition and living conditions. Focus on the very early environment was prompted when David Barker and colleagues reported strong correlations between the prevalence of ischemic heart disease and rates of mortality amongst newborns suggesting that increased risk of disease was linked to environmental factors affecting fetal development. The association between birth weight (a proxy for a compromised fetal growth) and development of type 2 diabetes was shown for the first time in a study of men born in Hertfordshire, UK, who were 64 years old at the time of study. The prevalence of impaired glucose tolerance amongst these men steadily increased as the birth weight decreased and men with lower birth weights were six times more likely to have type 2 diabetes than those born heavier. Subsequently, it was shown that for every 1 kg increase in birth weight there is 25% decrease in type 2 diabetes risk. In the original cohorts this was linear across the entire birth weight spectrum. However, in contemporary populations as rates of maternal obesity increase and the prevalence of women with gestational diabetes rises, more infants are born large for gestational age (>4000 g). These newborns have increased adiposity and are at a higher risk of developing obesity and features of the metabolic syndrome including impaired glucose tolerance. Therefore, infants with birth weight at both ends of the body weight spectra are more susceptible to the development of chronic diseases in later life giving rise to U-shaped relationships. Suboptimal *in utero* conditions have now been linked to a broad spectrum of diseases (Table 1).

Developmental Origins of Health and Disease Hypothesis

On the basis of the epidemiological data, in 1992 Nick Hales and David Barker proposed the thrifty phenotype hypothesis to explain the relationship between fetal growth and development of diseases in later life. They postulated that in response to undernutrition, a growing fetus adopts strategies to ensure its immediate postnatal survival in conditions of continued poor nutrition. These strategies include redistribution of blood flow to preserve brain growth at the expense of other tissues such as the liver, kidney, endocrine pancreas, and skeletal muscle. In addition, alterations in hormone production (e.g., decrease in fetal insulin and insulin-like growth factor 1 (IGF-1) concentrations) and tissue sensitivity to hormones, as well as programming of whole body metabolism to promote storage of nutrients when available also occur. If the individual is born into conditions of poor nutrition these adaptations are beneficial for his survival. However, if the individual is born into an environment of plenty or adequate nutrition, the adaptations become detrimental to long-term health. Since the proposal of the thrifty phenotype hypothesis, it has become apparent that critical windows of development extend into postnatal life and that the nutrient overload during fetal life is also detrimental. Therefore, the concept that

Table 1 Diseases associated with suboptimal intrauterine environment in humans

<i>Metabolic disorders</i>
Impaired glucose tolerance
Insulin resistance
Obesity
Dyslipidemia
Type 2 diabetes
Nonalcoholic fatty liver disease
<i>Cardiovascular disorders</i>
Hypertension
Coronary Heart disease
Stroke
Atherosclerosis
Coagulation disorders
Pre-eclampsia
<i>Reproductive system disorders</i>
Polycystic ovary syndrome
Early adrenarche/menarche
Early menopause
<i>Endocrine disorders</i>
Hypercortisolism
Hypothyroidism
<i>Respiratory disorders</i>
Chronic obstructive lung disease
Asthma
<i>Nervous system disorders</i>
Neurological disorders
Schizophrenia
Dementia
<i>Skeletal system disorder</i>
Osteoporosis
<i>Renal disorders</i>
Chronic renal failure
<i>Cancer</i>
Breast cancer

experiences of early life influence an individual's long-term health is now referred to as the 'developmental origins of health and disease hypothesis (DOHaD)' (Figure 1).

Evidence from Human Studies

Studies of twins have provided a very strong evidence in support of the link between the intrauterine environment and the risk of developing chronic disease. A study of Danish twin men discordant for type 2 diabetes revealed that in both monozygotic (identical) and dizygotic (nonidentical) twin pairs, the twin born with lower birth weight developed diabetes. As monozygotic twins are genetically identical, the differences in birth weight must reflect differences in the fetal environment. Similar observations were also made in younger Italian twin men (mean age 32 years). The link between nongenetic intrauterine factors and blood pressure has been proven to exist in a huge study involving over 20 000 Swedish twin pairs. Decreased birth weight was associated in this study with increased risk of hypertension independent of risk factors for hypertension in adulthood, including body mass index (BMI).

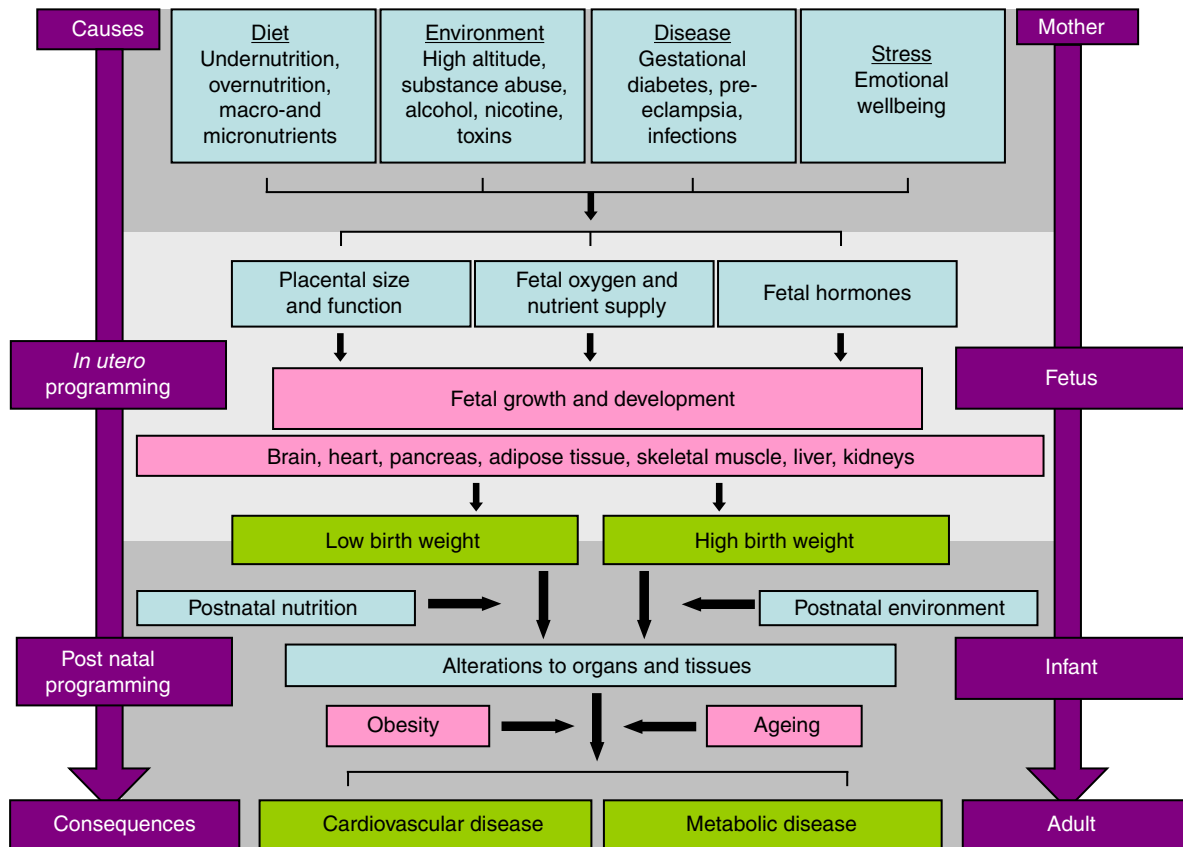


Figure 1 Intrauterine programming of adult disease.

In humans it is difficult to access the direct impact of maternal nutrition on the health of the offspring. Studies of individuals who underwent famine whilst *in utero* are therefore invaluable in accessing the effect of undernutrition on long-term health. The Dutch famine occurred for 5 months at the end of World War II (from late November 1944 to early May 1945) and before the famine the population of the affected area was reasonably well-nourished. When comparison was carried out between the individuals born during the year before the famine and those *in utero* during the famine, the latter had impaired glucose tolerance as shown by raised plasma glucose concentrations following an oral glucose tolerance test. The timing of the exposure to famine has also proven to be very important. Obesity rates at 19 years of age were increased amongst individuals exposed to famine in the early gestations, whereas they were decreased amongst those exposed in late gestation. Exposure to famine in early gestation has also been associated with greater risk of developing coronary heart disease at the age of 50 years.

In addition to fetal undernutrition, fetal overnutrition can also influence individual's long-term health. Some of the strongest evidences in support of the role of nutritional excess during pregnancy and subsequent development of diseases such as obesity and type 2 diabetes in adulthood have come from the study of the Pima Indians of Arizona, a population with very high incidence of type 2 diabetes, believed to be due to genetic inheritance. A six-fold increase in the prevalence of type 2 diabetes has been shown in this population amongst

children born to diabetic mothers in comparison to children of nondiabetic women. This increase was still present after adjustments for paternal diabetes, age of onset of diabetes in parents and obesity in the offspring. Moreover, compared to siblings born before the mother developed diabetes, children born after her diagnosis were at higher risk of developing diabetes and obesity by early adulthood. The effect was observed in addition to genetic transmission and hence must be related to the diabetic milieu experienced *in utero*. Similar findings have also been obtained from studies in lower-risk populations and in the developing world.

Animal Models

In order to gain insight into the mechanisms underlying the association between the early environment and adult health, a number of animal models have been established. These include studies in large animals such as nonhuman primates, sheep, horses, or pigs. However, the majority of research has been conducted in smaller animals such as mice and rats to take advantage of their shorter gestation and lifespan.

Maternal Undernutrition Models

Maternal Calorie Restriction

In this model of prenatal undernutrition caloric intake of mothers is restricted and the outcome is dependent on the

timing, length, and magnitude of the caloric restriction. Reduction in maternal nutritional intake to 50% of *ad libitum* during both pregnancy and lactation leads to the birth of low birth weight offspring, who remain smaller throughout adulthood. If such reduction is limited to gestation the offspring are smaller than controls at birth but become heavier than control offspring by weaning. By the age of 9 months they have a greater percentage of body fat, increased plasma leptin and triglyceride levels, hyperglycemia, hyperinsulinaemia, and impaired glucose tolerance. A 50% decrease in maternal food intake during the first two weeks of pregnancy does not have adverse effect on insulin secretion and action in adult male offspring. However, if 50% maternal nutrition is given during the last week of pregnancy the offspring are born with low birth weights and have decreased pancreatic β -cell mass and insulin content. Even if these animals are fed a standard laboratory diet postnatally, pancreatic deficiencies persist into adulthood. Much more severe maternal caloric restriction of 30% of *ad libitum* food intake results in the birth of growth-restricted offspring, who develop hyperphagia and adult-onset obesity with hyperinsulinaemia, hyperleptinaemia, and hypertension.

Maternal Protein Restriction

This is one of the most extensively studied rodent animal models of developmental programming. The regime involves feeding pregnant dams a low (5–8%) protein (LP) diet during pregnancy and lactation in comparison to a 20% protein diet given to the control group. This dietary manipulation does not lead to changes in conception rates or litter size. Offspring of LP rat dams have a 15% reduction in birth weight and if offspring are suckled by their mothers, permanent growth restriction occurs despite weaning the animals onto a control diet fed *ad libitum*. After the initial increase in insulin sensitivity in LP offspring in young adult life (6 weeks–3 months of age), these animals undergo an age-dependent loss of glucose tolerance in a sex-dependent manner. Male offspring of LP dams have impaired glucose tolerance by 15 months of age and frank diabetes by 17 months. Female LP offspring develop hyperglycemia and have impaired glucose tolerance at the older age of 21 months. The insulin resistance is associated with decreased protein expression of key insulin signaling proteins including insulin receptor substrate1 (IRS1), protein kinase zeta (PKC ζ), and glucose transporter GLUT4 in skeletal muscle; and the phosphatidylinositol 3-kinase (PI3K) catalytic subunit p110 β in adipose tissue. The profile of insulin signaling molecule deficiencies identified in the LP model is strikingly similar to the pattern observed in tissues from low birth weight young men. As the changes observed in humans occurred prior to the development of insulin resistance or type 2 diabetes, they are unlikely to be a secondary consequence of hyperglycemia or hyperinsulinaemia and hence may help predict diabetes risk. Maternal protein restriction has also been linked to development of hypertension in the offspring due to the alterations in the activity of the renin–angiotensin system and to increased early mammary tumor risk amongst female LP offspring.

Maternal Overnutrition Models

Maternal Obesity

Maternal obesity has been associated with the development of insulin resistance and type 2 diabetes. Adult offspring of obese mouse dams are hyperphagic, have increased body weight, and raised fat-to-lean mass ratio. With age they develop beta cell failure. As with the maternal low protein model, they have alterations in key hepatic insulin signaling molecules. Diminished protein expression of IRS1 in the liver coupled with increased phosphorylation of IRS serine residues may therefore contribute to the development of type 2 diabetes. Not surprisingly, these offspring develop insulin resistance by 3 months of age and impaired glucose tolerance was observed in males at 6 months of age. Insulin resistance also contributes to the pathogenesis of nonalcoholic fatty liver disease, signs of which have been observed in these animals. Additionally, hypertension and impaired endothelial cell function were reported in the offspring of obese dams implying that maternal obesity can predispose offspring to the development of cardiovascular disease.

Maternal High-Fat Diet

Offspring exposed to maternal high-fat diet while *in utero* became obese in adulthood and demonstrated abnormal cholesterol and lipid metabolism, hyperleptinaemia, hyperinsulinaemia, and insulin resistance. They have reduced muscle mass and persistent accumulation of lipid in the liver, which predisposes them to the development of nonalcoholic fatty liver disease (NAFLD). In addition, these animals have increased risk of developing hypertension and cardiovascular disease. The phenotypic characteristics of metabolic syndrome are present in most models of maternal overnutrition regardless of type of diet the offspring is exposed to in postnatal life.

Surgical Model – Uteroplacental Insufficiency

Uteroplacental insufficiency is one of the most common causes of intra-uterine growth restriction (IUGR) in the western world and can be the result of maternal smoking, pre-eclampsia or abnormalities in the development of placenta. Unilateral and bilateral uterine artery ligation has been performed to induce asymmetric IUGR. Following this intervention fetuses shown to be were hypoxic, hypoglycaemic, and had reduced insulin and IGF1 levels, a profile also found in human growth restricted fetuses. Uterine artery ligation leads to the development of mild insulin resistance, defects in insulin synthesis, and secretion in early life. With time as beta cells fail to compensate, diabetes ensues. This phenotype is propagated to the next generation when intrauterine artery-ligated females develop gestational diabetes during pregnancy.

Pharmacological Models

Maternal Glucocorticoid Exposure

Glucocorticoids are administered to pregnant women predominantly to advance fetal maturation. In both humans and animals, fetal overexposure to glucocorticoids leads to the birth of IUGR offspring. In early gestation, exposure to glucocorticoids is minimal as the expression of placental

11 β -hydroxysteroid dehydrogenase 2 (11 β -HSD2) – an enzyme that catalyzes the conversion of active glucocorticoids into their inactive forms – is relatively high. However, as the adrenal glands are activated in the late gestation, the synthesis of glucocorticoids increases to enable maturation of fetal tissues. Overexposure to glucocorticoids in late gestation results in the birth of IUGR infants. This scenario can be mimicked by administration of synthetic compounds, for example, dexamethasone or inhibitors of 11 β -HSD2. Treatment with dexamethasone in rats leads to decreased insulin content in fetal beta cells, due to the down-regulation of the transcription factor PDX1 – a critical factor involved in the development, differentiation, and function of pancreatic beta cells. In the nonhuman primates, the number of beta cells has also been shown to be decreased in the offspring following maternal dexamethasone treatment. Dexamethasone has proapoptotic effect on beta cells, which further explains its negative impact on endogenous pancreas. Maternal overexposure to glucocorticoids has been associated not only with the development of glucose intolerance and insulin resistance, but also hypertension in the offspring.

Gestational Diabetes

Maternal administration of streptozotocin (STZ) is the most common pharmacological model for studying the effects of gestational diabetes on health of the offspring in rodents and sheep. STZ is a chemical that destroys pancreatic beta cells and acts in a dose-dependent manner. High doses cause severe maternal hyperglycemia and birth of IUGR offspring with hyperglycemia. The maternal hyperglycemia results in hyperstimulation of fetal pancreatic islets leading to their degranulation and consequently beta cell exhaustion. This leads to fetal hypoinsulinaemia, which coupled with decreased insulin receptors on target cells, leads to diminished fetal glucose uptake. Low doses of STZ induce mild gestational diabetes and macrosomic offspring that have enhanced development of their endocrine pancreas with hyperplasia and hypertrophy of islets. These offspring also have an increased beta cell mass, increased pancreatic insulin content, and enhanced insulin secretion. However, in adulthood these animals develop a deficit in insulin secretion and impaired glucose tolerance.

Cellular and Molecular Mechanisms

The phenotypic outcomes of nutritional, surgical, and pharmacological insults designed to challenge the fetal environment are very similar, indicating the existence of common pathways and mechanisms linking early life experiences to long-term health. These are therefore a major focus of the research in the field to define the mechanisms involved.

Epigenetic Mechanisms

Epigenetics refers to covalent modifications of DNA and histones that alter gene expression without affecting the DNA nucleotide sequence. Epigenetic alterations include DNA methylation and post-translational histone modifications such as methylation, acetylation, phosphorylation, ubiquitination,

and sumoylation. In recent years, microRNAs have also emerged as a potential epigenetic mechanism. These small, on average 22 nucleotides long noncoding RNAs have been traditionally associated with post-transcriptional gene regulation; however, recently they have been shown to also play a role in DNA methylation, thereby enabling them to further regulate transcription of their targets. The main role of epigenetic modifications is to heritably promote transcriptional silencing of specific gene regions so that varying gene expression levels can be achieved from identical DNA.

A number of studies to date have shown that maternal nutrition during pregnancy can lead to permanent changes in the epigenome of offspring. One of the first studies showing the link between maternal nutrition and methylation status in the offspring was conducted in mice that carried the epigenetically sensitive Agouti viable yellow (*Avy*) allele. Offspring of *Avy* dams fed a diet supplemented with methyl donors (folate, vitamin B12, choline, or betaine) were leaner and had a different coat color (pseudo-Agouti) in comparison to obese and yellow offspring of normally fed dams. The difference in phenotype was caused by hypermethylation of a retrotransposon element downstream of the *Avy* allele, which led to the silencing of the *Avy* gene. Depending on the pattern of methylation, a wide variation in coat color ranging from yellow (unmethylated) to pseudo-Agouti (methylated) could be achieved. Maternal methyl donor supplementation has also been shown to increase in DNA methylation, of another epigenetically sensitive allele *Axin*(*Fu*) resulting in a 50% reduction in the incidence of tail kinking in the offspring. Hypomethylation has also been reported in sheep that were exposed to a diet deficient in methyl donors during the periconceptional period. These sheep developed insulin resistance and hypertension in adult life. Studies in the offspring of protein-restricted dams showed increase hepatic DNA methylation of the insulin-like growth factor 2 (*IGF2*) gene and parallel reduction in gene expression. The methylation status of gene promoters in the offspring can also be affected by maternal protein-restriction. Both glucocorticoid receptor (*GR*) and peroxisome proliferator-activated receptor- α (*PPAR α*) gene promoters were found to be hypomethylated in the livers of protein-restricted offspring. Parallel changes in the expression of corresponding genes were also observed. Maternal diet has also been shown to influence epigenetic status in humans. Altered methylation of the *IGF2* gene was observed in the white blood cells of individuals who were exposed *in utero* to the Dutch Hunger Winter.

Alterations in histone modifications are also emerging as an important mechanism of developmental programming. Maternal nutrition has been shown to affect histone acetylation in the offspring. When the diet of *Avy* dams was supplemented with the phytoestrogen genistein (nonmethyl donor) the color of the offspring's coat was shifted toward pseudo-Agouti due to the increased DNA methylation of the *Avy* retrotransposon. It was proposed that genistein altered histone modifications consequently affecting the chromatic structure, DNA methylation, and gene expression. Recently it has been shown that hypomethylation in yellow *Avy* mice corresponds with enrichment of some activating histone acetylation modifications (H3 and H4 diacetylation), whereas hypermethylation corresponded with the repressive histone

H4K20 tri-methylation modification. Therefore, it appears that histone modifications act in concert with DNA methylation to affect interindividual variation of epigenetically sensitive genes. Intrauterine artery ligation has been shown to affect both DNA methylation and histone acetylation of the PDX 1 promoter. Additionally, losses in acetylation and an increase in dimethylation of histone, H3 at the GLUT4 locus persisting into adulthood, have been shown in the offspring of calorie-restricted dams. Recently, an association has been reported between periconceptual undernutrition in sheep and marked epigenetic changes in two hypothalamic genes: *GR* and an appetite-regulating neuropeptide, *proopiomelanocortin* (*POMC*). These epigenetic modifications could predispose the offspring to altered regulation of food intake, energy expenditure, and glucose homeostasis in later life.

Mitochondrial Dysfunction and Oxidative Stress

Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP) and are the main source of highly destructive reactive oxygen species (ROS). Evidence of oxidative stress in the fetus has been found following uteroplacental insufficiency. Pups born following this manipulation had increased pancreatic oxidative stress and impaired mitochondria function, which progressively worsens with age. This was associated with a decline in ATP production and accumulation of mitochondrial DNA damage. Pancreatic beta cells are especially vulnerable to ROS due to their high oxidative energy requirement and very low expression of antioxidant enzymes. Oxidative stress affects not just mitochondrial DNA but also genomic DNA. ROS induced attrition of telomeres located at the ends of chromosomes has been associated with development of chronic disorders. Impaired antioxidant defense mechanisms and increased ROS production have also been observed when offspring were exposed to intrauterine nutritional excess. ROS production through xanthine oxidase (XO) activation was increased in cord plasma and placenta of neonates born to mothers with gestational diabetes. It has been proposed that exposure to oxidative stress can directly mediate DNA methylation and chromatin remodeling; however, further research is required to understand the underlying mechanisms.

Excessive Intrauterine Exposure to Lipids

Obese and diabetic mothers often give birth to large for gestational age (>4000 g) babies. However, as only 25% of the differences in birth weight can be attributed to maternal hyperglycemia, the majority of large infants are born to normoglycemic mothers. This would suggest that other factors besides glucose may be involved. Indeed maternal pre-pregnancy BMI, maternal fasting triglyceride, and free fatty acid levels have all been implemented in mediating excessive fetal growth. In rodents, fetal exposure to excessive lipid levels leads to lipid accumulation in the adult offspring's liver, predisposing the offspring to nonalcoholic fatty liver disease. Deposition of lipids in liver and muscle can cause mitochondrial dysfunction. Increased fetal lipids may also promote formation of adipocytes over other cell types such as myocytes

in early organogenesis. Circulating saturated fatty acids can activate kinases that cause an increase in IRS1 serine phosphorylation, an event that is associated with inhibition of insulin signaling and one of the hallmarks of insulin resistance. Excessive fetal lipid concentrations may also affect hypothalamic regulators of appetite and satiety. For example, consumption of a high-fat diet during pregnancy in the non-human primate may compromise the development of the melanocortin system in the fetal hypothalamus. Finally, increased pancreatic beta cell mass and excess insulin secretion, which can lead to islet cell failure and contribute to the development of diabetes, can be found in the models of maternal obesity during pregnancy.

Conclusions

There is no doubt that environmental challenges experienced by a growing fetus can determine risk of developing chronic diseases such as type 2 diabetes, obesity, hypertension, and other features of the metabolic syndrome in the individual. In light of the increasing prevalence of these disorders across the globe it is of critical importance to understand the mechanisms underlying the developmental origins of health and disease. Further research into the interactions between nutrition and chromatin dynamics and the role that particular nutrients play in fetal metabolic programming will be vital in allowing targeted interventions to be developed. This may ultimately lead to the establishment of prevention strategies.

See also: Breast Feeding. Diabetes Mellitus: Etiology and Epidemiology. Early Origins of Disease: Non-Fetal. Energy Balance. Glucose: Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level. Low Birth Weight and Preterm Infants: Causes, Prevalence and Prevention. Nutrient–Gene Interactions: Molecular Aspects. Obesity: Childhood Obesity; Complications. Pregnancy: Placental Regulation of Nutrient Delivery to the Fetus. Protein Deficiency

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Non-Fetal

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Introduction

A substantial body of evidence supports the hypothesis that adult chronic diseases have origins in early life. This is frequently labeled as the Developmental Origins of Health and Adult Disease or DOHAD. The basic premise of research in this field is that nutritional insufficiency during sensitive developmental periods results in structural changes or programming of metabolic functions. In the short term, such changes may enhance survival and spare brain growth at the expense of other organs. In the long run, the cost of such adaptive responses may be an increased risk of chronic disease. The main focus of research has been on fetal origins of adult disease, but there remains substantial potential for nutritional programming of later disease risk during infancy and childhood. The young infant has high energy and nutrient needs to support rapid growth and development. Birth weight typically doubles in the first 4–6 months of life, and length increases by approximately 30% between birth and 6 months. Many organ systems continue to mature after birth, notably the immunologic, gastrointestinal, and renal systems. This combination of rapid growth and continued development make the infant highly susceptible to the effects of environmental exposures and suboptimal nutrition, which might affect the development of disease risk. Differentiating postnatal from fetal origins is challenging, however, owing to the inevitable link between pre- and postnatal growth.

Instances of purely postnatal effects relate primarily to infant feeding or exposure to pathogens or toxins. The potential effects of infant feeding relate to nutritional adequacy, and to exposure or lack of exposure to specific substances in human milk or human milk substitutes. Effects of feeding may occur independently of the infant's nutritional status at birth. This topic is discussed further in a separate section below.

There is also a continuum of fetal and postnatal effects. Intrauterine growth-restricted infants may experience optimal or even excess postnatal nutrition, or they may continue to be exposed to nutritional insufficiency. Their responses to postnatal challenges may be conditioned by their fetal nutritional history, such that there is an interaction or synergism of fetal and postnatal effects.

Prenatal nutritional insufficiency may be thought to result in 'downsizing.' It may produce smaller organs, for example, kidneys with a reduced nephron number, a pancreas with fewer islet cells, or a low skeletal muscle mass. Nutritional insufficiency may also alter metabolic or hormonal regulation, for example, hormone secretion or sensitivity of the hypothalamic–pituitary axis. In either case, the effects may be permanent, or subject to compensatory responses once nutritional or other insults are removed. For example, a permanently reduced

nephron number is a hypothesized mechanism through which fetal growth restriction affects later blood pressure. Similarly, a reduced skeletal muscle mass may persist and affect insulin sensitivity in later life associated with a reduced number of insulin receptors. In such cases, the physiological capacity to respond to risk factors encountered later in life (e.g., diets high in sodium or excess calories relative to energy needs) may be compromised.

Alternatively, catch-up or compensatory postnatal growth may occur. Many infants who were underweight for length at birth typically undergo a period of rapid postnatal compensatory growth in weight, whereas those who are relatively short at birth have larger length increments (see [Figure 1](#) for an example from a Philippines infant cohort). A central finding in many studies is that chronic disease risk is most likely to be elevated in individuals who were growth restricted *in utero* and thus small at birth, but relatively large at the time health outcomes were measured, leading to the conclusion that excess postnatal growth contributes to disease risk. The extent to which rapid postnatal growth itself is a risk factor for the development of chronic disease has been the subject of extensive recent research. The relationship of early growth patterns to later disease risk is discussed in detail in a subsequent section.

Long-Term Effects of Infant Feeding

Much of the literature on the long-term effects of infant feeding is based on comparison of outcomes associated with human milk versus infant formula feeding. Postulated effects relate primarily to the different composition of human milk versus formula and different energy and nutrient intake by infants. The literature does not provide a clear and consistent picture of the long-term effects of feeding. When effects are found, they tend to be modest. Before discussing the results of

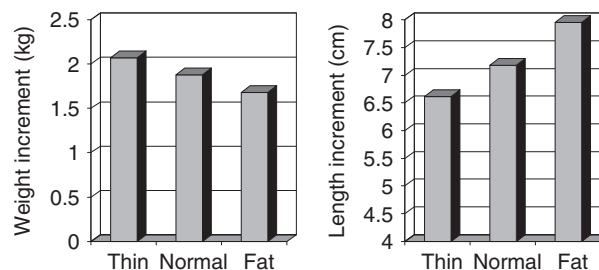


Figure 1 Early growth of Filipino infants is associated with relative weight at birth. Mean growth increments from birth to 2 months of age in children who were relatively thin (BMI <10th sample percentile) or fat (BMI >90th sample percentile).

these studies, it is important to raise several key methodological issues relevant to the interpretation of the literature.

First, breast-feeding is a complex behavior chosen by mothers. Women who choose to breast-feed are likely to differ in systematic ways from those who do not. The choice to breast-feed and the duration of breast-feeding may be related to other short- and long-term health behaviors that affect the ultimate health outcomes of interest. To isolate the effect of infant feeding, it must be assumed that other concurrent and subsequent exposures are not systematically related to feeding history, or such exposures must be taken into account in multivariate analysis. Unfortunately, most studies have insufficient data to adequately control statistically for these other behaviors, particularly because they are often unmeasured or poorly measured.

Second, many studies use historical cohorts in which feeding method is recalled by the mother or based on limited records. Although the decision to initiate breast-feeding is likely to be accurately recalled, information about breast-feeding duration and timing of introduction of other foods may be subject to recall bias.

Third, the composition of proprietary infant formulas has changed since their introduction in the 1920s. For example, sodium levels and fat sources have changed, and new ingredients such as n-3 fatty acids and nucleotides have been added recently. Therefore, results from older versus younger cohorts may differ either because true age-specific effects have emerged or because they were exposed to infant formula of different composition. Furthermore, the effects of breast and formula feeding on infant health are likely to differ depending on the environmental context.

The ideal study design for determining the long-term effects of infant feeding would require randomization to feeding regimens, and frequent follow-up of subjects up to the time when a disease risk factor or outcome is measured. Such designs are rarely ethical or feasible. An exception is a series of studies in the UK conducted by Alan Lucas and colleagues, which assessed long-term outcomes among preterm infants randomized to receive banked human milk or formula, and full-term infants whose mothers chose not to breast-feed randomized to different types of formula. Although many of the studies have focused on neurodevelopment, some are now looking at other health outcomes.

Selected Outcomes Related to Infant Feeding

The following are examples of some chronic disease-related outcomes studied in relation to infant feeding. The selected outcomes are intended to be illustrative of a range of effects rather than a comprehensive treatment of all outcomes related to infant feeding.

Serum Lipids

Based on a systematic review of literature relating infant feeding to blood lipids in infants, adolescents, and adults, total cholesterol was found to be consistently higher in breast-fed infants compared to bottle-fed infants. No consistent differences related to feeding history were found in children and adolescents; among adults, a majority of studies reported

lower mean total cholesterol in those who had been breast-fed. The proposed but unproven mechanism for the protective effect of breast-feeding in adults is downregulation of endogenous cholesterol synthesis.

Blood Pressure

Differences in the sodium and fat content and composition of breast milk versus formula are thought to be the relevant determinants of long-term effects of infant feeding on blood pressure. In a recent systematic review, data were compiled to compare exclusive breast-feeding to formula feeding, with adjustment for current age, sex, height, and body mass index (BMI). The analysis was based on 26 studies of systolic blood pressure and 24 studies of diastolic blood pressure. On average, subjects who were breast-fed had a modestly lower systolic blood pressure than those who had been formula fed, with an average effect of -1.10 mmHg, and no marked differences by age. However, the analysis suggested publication bias because the effect was significantly larger in small studies than large studies. The studies showed no effects of feeding on diastolic blood pressure.

Taking advantage of a 1980 randomized trial to study the effect of a low or normal sodium diet in Dutch infants, a follow-up study at age 15 years found systolic blood pressure to be 3.6 mmHg lower and diastolic to be 2.2 mmHg lower in the low-sodium group. These results suggest that sodium intake in infancy may affect blood pressure later in life.

Further evidence of the effects of diet composition comes from a long-term follow-up of the Barry Caerphilly Growth study cohort. In this study, mothers and their offspring were randomly assigned to receive a milk supplement or usual care. In young adulthood (age 23–27 years), blood pressure was positively associated with dried formula milk supplement consumed in infancy. The effect was attenuated but remained significant after controlling for current BMI, suggesting an effect of diet composition independent of growth.

Reproductive Function

The relatively high levels of isoflavones in soy-based infant formula have raised concerns about potential effects on endocrine and reproductive function later in life. A recent retrospective cohort study of young adults who as infants had participated in controlled feeding studies during infancy found no differences associated with soy feeding across a large number of outcomes potentially susceptible to estrogenic or antiestrogenic activity of phytoestrogens, including timing of maturation, sexual development, or fertility in adolescents or adults. Another literature review reported no meaningful differences in child growth related to feeding of soy formula. However, data are limited and further randomized controlled trials are needed to provide definitive evidence.

Growth and Body Composition

Mode of feeding may indirectly affect later disease risk through its effects on energy intake or aspects of metabolic regulation that affect growth and body composition. Numerous studies demonstrate different growth patterns in breast- and formula-fed infants that are hypothesized to reflect differences in nutrient intakes. In fact, evidence of systematic differences in breast- and formula-fed infants led the World Health

Organization to undertake the production of growth charts for breast-fed infants. In one careful study of body composition, total energy intakes and weight velocity from 3 to 6 months of age were higher in formula-fed compared to breast-fed infants. Estimates of fat and fat-free mass also indicate higher adiposity in formula-fed infants, however, none of these differences persisted into the second year of life. Similarly, in a study of nearly 5600 children who participated in the Third National Health and Nutrition Examination Survey, those who had been exclusively breast-fed for 4 months weighed less at 8–11 months than did infants who were fed in other ways, but few other meaningful differences in growth status through age 5 years were associated with early infant feeding.

Longer-term effects of infant feeding have been assessed in studies that examined whether breast-feeding protects against later overweight or obesity. A recent review found inconsistent results, with some large cohort studies showing a moderate protective effect, and others showing no effect. The studies were also inconsistent in showing a dose response. An illustrative large study in 3–5-year-old children found that after adjusting for potential confounders, risk of having a BMI >85th percentile was reduced among exclusively breast-fed children compared with those never breast-fed, but there was no reduced risk of having a BMI >95th percentile.

The findings are typically based on retrospective studies, in which breast-feeding data derive from maternal recall. This makes it difficult, if not impossible, to control for confounding, because a mother's decisions about breast-feeding may relate to subsequent child feeding and other factors associated with overweight. Thus, it is not clear based on the available data whether the effects of infant feeding are causal or whether breast-feeding serves as a marker for other health behaviors that may affect child and adolescent growth. Recent studies among siblings, which allow control for maternal characteristics, show no protective effects of breast-feeding on obesity in adolescents and young adults.

Exposure to Antigens and Development of Autoimmune Disease

The infant's diet is the main source of exposure to antigens suspected to be related to the development of autoimmune diseases. A likely protective effect of exclusive breast-feeding relates to lack of exposure to food allergens, though some other protective mechanisms related to specific substances in breast milk have been postulated. Exposure to bovine proteins by milk feeding, and to allergenic plant proteins such as those found in wheat is suspected to increase risk of developing diseases such as type 1 diabetes and celiac disease in genetically susceptible individuals.

Type 1 diabetes is one of the most prevalent chronic diseases with childhood onset. It is characterized by autoimmunity to pancreatic islet cells and is associated with a specific human leukocyte antigen (HLA) genotype. Not all individuals with the genotype develop the disease, suggesting an important role for gene–environment interactions. Hypothesized early exposures include infant feeding and enterovirus infections. Early introduction of cows' milk has received a great deal of attention as a potential risk factor. Numerous case–control studies associate increased risk with cows' milk, but a nearly equal number of studies show no

effects. These retrospective studies have been criticized as suffering from recall bias and inappropriate control groups, for example, controls without the susceptible genotype. Recent prospective studies of at-risk infants in Australia and Germany found no association of type 1 diabetes with feeding of cows' milk. However, pilot study data from an international primary prevention trial suggests that eliminating cows' milk proteins in at-risk infants reduces risk of developing islet cell autoantibodies. This study also supports a role for early enteroviral infections in the etiology of type 1 diabetes in genetically susceptible individuals. In fact, the research team has suggested that the effect of cows' milk may depend on viral exposures.

Recent studies suggest a role for other food antigens. A study of at-risk German children found that feeding of gluten-containing foods before 3 months of age was associated with risk of having pancreatic islet cell autoantibodies. Another study in the US also found an increased risk of islet cell autoimmunity among at-risk children given cereal before 3 months or after 7 months of age. Furthermore, they found that risks associated with cereal introduction were reduced by breast-feeding.

Other aspects of diet may have immunomodulatory effects. Vitamin D and the n-3 fatty acids EPA and DHA are suggested to be protective against immune-modulated diseases. For example, in a case-control study, Norwegian children given cod liver oil, a rich source of EPA and DHA, in the first year of life had significantly reduced risk of type 1 diabetes.

Type 2 Diabetes

Few studies have assessed the relationship of infant feeding to later development of Type 2 diabetes. Early feeding may affect patterns of insulin secretion in the newborn period, and thereby program subsequent development of metabolic control. Two studies in native American populations, one in Canada and one among Pima Indians, report a protective effect of breast-feeding on later development of Type 2 diabetes. In the Pima study, exclusive breast-feeding in the first 2 months of life was associated with a lower rate of Type 2 diabetes in children and adults. In the Canadian study, breast-feeding for more than 12 months was associated with decreased risk of Type 2 diabetes. Other studies have examined early risk factors related to subsequent development of Type 2 diabetes. For example, in a study of preterm infants randomized to human milk or formula of different composition, 32–33 split proinsulin, a marker of insulin resistance, was elevated in adolescents who had received a nutrient-enriched diet compared to those with a lower nutrient diet.

In sum, infant feeding, through nutritional adequacy, direct exposure to antigens, and protective substances provided in human milk, has the potential to alter response to subsequent exposures and to directly influence the beginning of disease processes.

Postnatal Growth and Later Risk of Disease

Small body size in childhood may reflect nutritional insufficiency that may program adult disease in ways similar to that observed in the fetal period. Independent of birth weight, low

weight at 1 year of age has been associated with increased risk of cardiovascular disease in adult men. Similarly, poor childhood growth manifested as short stature has been linked with insulin resistance.

More attention has recently been paid to the effects of rapid childhood growth in height and weight. The observation in much of the fetal programming literature that effects of birth size emerge or are strengthened when current body size (typically represented as BMI) is taken into account suggests an important role for postnatal growth in the origins of adult disease. Individuals who are born small, but who end up relatively large (taller or heavier than their peers) have clearly experienced more rapid growth at some point between birth and when health outcomes and current size are assessed. Whether rapid growth is an independent risk factor or whether it confers increased risk only in individuals with a history of intrauterine growth restriction is a question requiring further research. Moreover, even when strong associations of growth rate and chronic disease risk are found, it is unclear whether the association is causal or whether growth serves as a marker for other underlying causal processes.

Postnatal growth is clearly related to prenatal growth. Some metabolic changes associated with prenatal nutritional sufficiency may affect postnatal physiology and behavior that, in turn, affect growth. In addition, there is intriguing evidence from animal studies that prenatal nutritional restriction alters appetite and induces hyperphagia, and also reduces physical activity in adult animals (see Figure 2). If true in humans, this would be an important pathway by which disease risk is affected. Suggestive evidence comes from human infants whose cord blood leptin levels at birth were inversely related to weight gain in the first 4 months of life, independent of birth weight. Leptin may relate to subsequent growth by affecting appetite and energy intake.

Depending on the outcome under study, there are differences in whether linear growth or growth in weight, particularly weight relative to height, matters. Most often, more rapid weight gain is the risk factor, owing to the fact that excess adiposity is an important risk factor for many chronic diseases of adulthood. Another key issue concerns the timing of effects. There is controversy about whether early infancy compensatory growth following intrauterine growth restriction confers risk, or whether it is only later growth that matters.

Where many potential adverse outcomes might be affected by postnatal growth, the following sections focus on adiposity, blood pressure, coronary heart disease, insulin resistance, diabetes, and cancer.

Adiposity and Obesity

Early undernutrition followed by later overnutrition as well as early overfeeding independent of prior growth restriction are thought to increase risk of later obesity. Rapid postnatal weight gain occurs in a significant proportion of infants who are born small for gestational age. Prospective studies in the US, South African, and British cohorts show that rapid growth in early infancy increases later risk of overweight. Longitudinal data from the US National Perinatal Collaborative study show

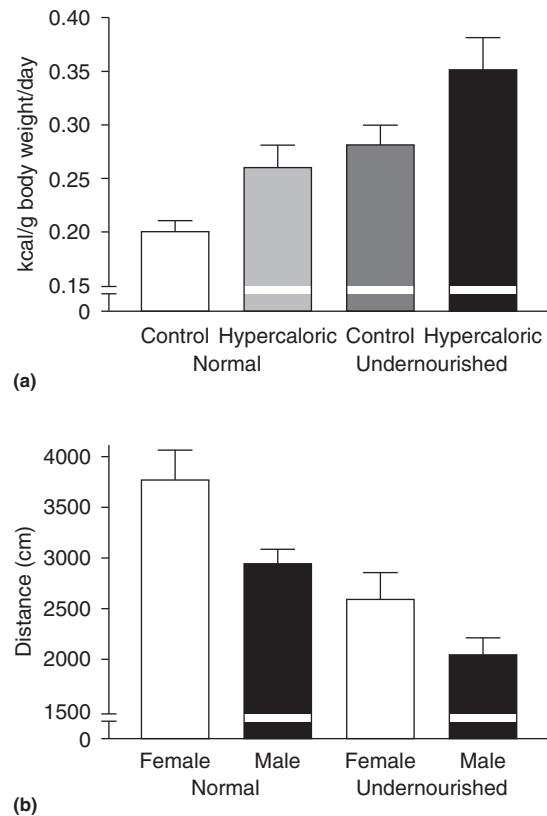


Figure 2 Locomotor behavior and food intake in Wistar rats as a consequence of a normal or adverse fetal environment ($n=6-8/\text{group}$). (A) Food intake (kcal per gram body weight per day over a 5-day period) in females at day 145; $P<.005$ for effect of fetal programming, $P<.05$ for postnatal hypercaloric diet. (B) Locomotor activity at 14 months in males and females; $P<.005$ for effect of fetal programming and gender. Data analyzed by factorial ANOVA, and data are shown as means \pm SE. Reproduced from Vickers MH, Breier BH, McCarthy D, and Gluckman PD (2003) Sedentary behavior during postnatal life is determined by the prenatal environment and exacerbated by postnatal hypercaloric nutrition. *American Journal of Physiology. Regulatory Integrative and Comparative Physiology* 285(1): R271-R273, with permission from APS.

that, independent of birth weight, one-third of obesity at age 20 is attributable to rapid weight gain in the first 4 months of life. In a Bristol, UK cohort, nearly one-third of children had an increased weight standard deviation (SD) score of more than 0.67 units from birth to age 2 years, and these children remained fatter, having more central fat distribution at age 5 years compared to children with lower early growth rates. Similarly, data from the South Africa Birth to Ten cohort showed that children with rapid weight gain in infancy were significantly lighter at birth and significantly taller, heavier, and fatter throughout childhood.

Early postnatal growth rates may program insulin-like growth factors, IGF-I and IGF-II. Figure 3 illustrates this point with data on 5-year-old children from Bristol, UK, in whom IGF levels were strongly related to current body size, but also that, independent of current size, children who had experienced catch up growth (change in Z-score >0.67 SD) from

birth to age 2 had higher IGF levels. Childhood IGF levels are important as determinants of later linear growth and timing of puberty, and are associated with later risk of hormone-dependent cancers.

Cancer

A large body of literature relates adult height to cancer risk, with the largest volume of evidence on breast, prostate, and colorectal cancers. In each case, risk of disease is increased with taller stature. A role for accelerated childhood growth is inferred, because taller individuals have experienced more linear growth. Possible mechanisms fall into two categories: childhood growth as a marker for other exposures that influence risk (fetal exposures, infections, timing of puberty, and energy intake) or growth as a mediator of risk (effects of growth promoting hormones such as IGF-I and IGF-II).

Few studies have directly addressed the effects of childhood growth, owing to lack of longitudinal data. Based on data from the UK Boyd Orr cohort, a one SD difference in height was associated with a 42% higher risk of overall cancer mortality in later life among males, but no effects were found in females. In another UK birth cohort, risk for breast cancer was elevated among women who were large at birth and tall at age 7. Based on data from the US Nurse's Health Study, rapid adolescent growth was associated with an increased risk of both pre- and postmenopausal breast cancer.

Blood Pressure and Coronary Heart Disease

Blood pressure is the one of the most well-studied outcomes in the context of fetal programming, with fairly consistent findings of a modest inverse relationship of birth weight to adult systolic blood pressure that increases with age. Substantial evidence demonstrates a synergistic relationship of fetal growth restriction with rapid postnatal growth. **Figure 4** presents the classic picture for systolic blood pressure: the highest pressure is found among adolescent males who were relatively thin at birth, but relatively heavy as adolescents. Current BMI is typically the strongest anthropometric predictor of blood pressure, but at the same BMI, those with a history of fetal growth

restriction have higher mean systolic blood pressure and increased risk of having high blood pressure.

Owing to the existence of good longitudinal growth data in Scandinavia, child growth trajectories can be traced for individuals with and without hypertension or other adverse outcomes such as coronary heart disease. As shown in **Figure 5**, though initially smaller, adults with hypertension diverged in their BMI trajectory and were relatively heavier after age 7 compared to those without hypertension.

There remains controversy about the age at which higher growth rates pose risk of later disease. Some studies show elevated blood pressure in association with rapid weight gain in infancy, whereas other studies show no effect, or a protective effect (infants with larger weight increments have lower blood pressure as adults). The degree to which rapid infant growth represents risk may depend on whether it occurs in the context of recovery from fetal growth restriction and results in normalization of body weight versus excess growth leading to infant obesity.

There is more consistent evidence of increased risk associated with rapid weight gain in later childhood. In a Philippines cohort, larger weight increments from age 8 to 15 years increased risk of high blood pressure in boys who were relatively thin at birth. However, higher childhood weight gain

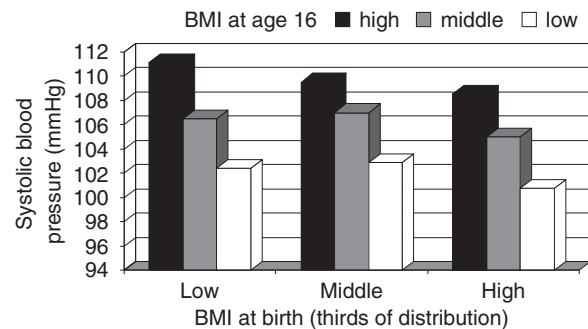


Figure 4 Synergistic effect of BMI at birth and age 16 on systolic blood pressure of Cebu (Philippines) boys: ■ high; ■ middle; □ low BMI. Data from the Cebu Longitudinal Health and Nutrition Survey.

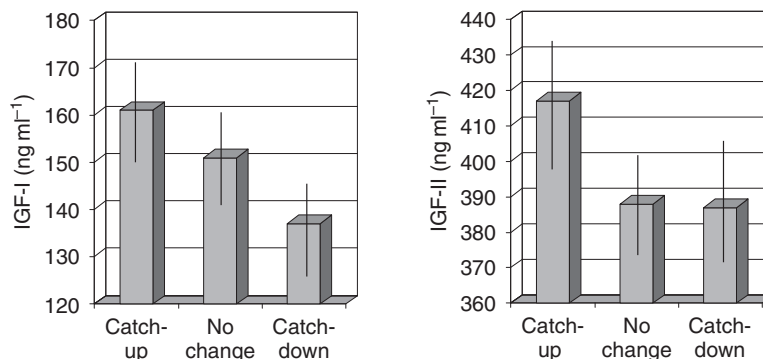


Figure 3 Hormone levels at age 5 years by change in weight Z-score from birth to 2 years of children in the ALSPAC cohort: means and 95% confidence intervals of IGF-I and IGF-II, adjusted for fat mass and fat-free mass. Data drawn from Ong K, Kratzsch J, Kiess W, Dunger D, and ALSPAC Study Team (2002) Circulating IGF-I levels in childhood are related to both current body composition and early postnatal growth rate. *Journal of Clinical Endocrinology and Metabolism* 87(3): 1041–1044.

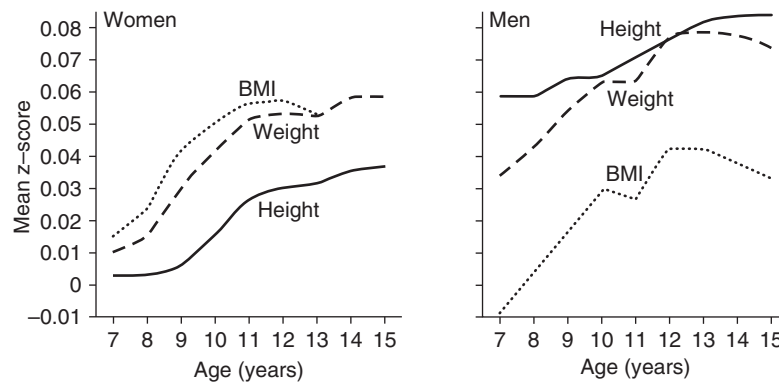


Figure 5 Z-scores for height, weight, and BMI from 7 to 15 years in 975 boys and 983 girls who later developed hypertension. Mean values for all 7086 subjects in cohort are zero. Reproduced from Eriksson J, Forsen T, Tuomilehto J, Osmond C, and Barker D (2000) Fetal and childhood growth and hypertension in adult life. *Hypertension* 36(5): 790–794, with permission from LWW.

in the absence of fetal growth restriction was not a risk factor in this population.

Fetal undernutrition may result in a reduced number of nephrons. Such deficits may not increase disease risk in individuals who remain small, but excess growth may challenge the ability of the kidneys to effectively regulate blood pressure. Catch-up linear growth has not been consistently implicated as a risk factor for later elevated blood pressure. In fact, continued poor linear growth, particularly in association with more rapid weight gain, increases risk of later elevated blood pressure.

Insulin Resistance and Diabetes

Most evidence relates to type 2 diabetes, but one large, population-based case-control study of type 1 diabetes in European populations found that height and weight were higher in cases starting at 1 month after birth, with maximum differences in cases and controls between 1 and 2 years of age. In the case of type 2 diabetes, both continued growth faltering in infancy and more rapid growth are associated with increased risk. Postnatal faltering in length is associated with impaired insulin metabolism.

As was the case for blood pressure, highest risk is associated with the combination of small size at birth and rapid postnatal growth gain. In a well-studied cohort in Finland, men and women who developed type 2 diabetes had lower birth weight, length, and ponderal index, and accelerated growth in weight and height from age 7–15 years. Precursors of diabetes such as insulin resistance have been studied. For example, in a follow-up study of British children who were born preterm, fasting split proinsulin and glucose concentration 30 min after a glucose load were highest in children with the greatest increase in weight centile between birth and time of measurement, regardless of early size.

Extensive studies of early origins of type 2 diabetes have been conducted in India, where rates are rising very rapidly. Indian babies who are small at birth have a deficit in skeletal muscle, but not body fat compared to normal size infants. These infants tend to grow into adults that retain a lower skeletal muscle mass, but have increased abdominal obesity.

This body composition is strongly related to increased risk of type 2 diabetes. Prospective studies of Indian children show an interaction between birth weight and subsequent growth. For example, children who were born small but were relatively large at age 4 had higher plasma glucose and insulin concentrations 30 min after an oral glucose load, and greater insulin resistance at age 8.

Higher growth rates in previously growth-restricted individuals may pose excessive demands on systems initially adapted to function in the face of limited resources, leading to increased risk of diseases, particularly those associated with the metabolic syndrome. Rapid growth in weight during infancy and childhood, and in particular, rapid growth following prenatal growth restriction, increases risk of developing obesity, especially abdominal obesity. Factors that contribute to early onset of obesity are therefore important to control, because obesity tracks from early life to adulthood, and is a well-recognized risk factor for diseases such as type 2 diabetes, hypertension, and coronary heart disease.

In sum, the continued vulnerability and responsiveness of the developing infant and child suggest the importance of a life course perspective on the development of diseases that are typically thought of as ‘adult onset.’

See also: Breast Feeding. Cancer: Epidemiology and Associations Between Diet and Cancer. Coronary Heart Disease: Lipid Theory. Diabetes Mellitus: Etiology and Epidemiology. Hyperlipidemia: Prevention and Management; Overview. Hypertension: Dietary Factors. Nutritional Requirements of Infants

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EATING DISORDERS

Contents

Anorexia Nervosa

Binge Eating

Bulimia Nervosa

Anorexia Nervosa

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Classification of Eating Disorders

Anorexia Nervosa

Anorexia nervosa is usually seen in younger women who restrict their food intake and increase exercise, causing a voluntary, stubborn malnutrition.

Bulimia

People who cannot control their hunger over a long period of time tend to have secret bingeing episodes. This is followed by an overwhelming feeling of guilt and depression, which frequently leads to self-induced vomiting. For this reason, the terms 'bulimia' (which only means binge eating) and 'self-induced vomiting' are sometimes used interchangeably.

Anorexoid Syndromes

These abnormalities are seen in individuals who can no longer control their weight by dieting and exercising and have to resort to abnormal subterfuges, such as:

- self-induced vomiting;
- ipecac abuse;
- laxative abuse;
- diuretic abuse;
- anorexic agents abuse;
- self-induced glycosuria in patients with insulin-dependent diabetes mellitus;
- thyroid hormone abuse; and
- excessive, compulsive exercising.

Professional Hyperthinness

This is a borderline condition, not necessarily pathological, in which individuals, usually with narcissistic tendencies, overvalue personal appearance and thinness as a way of obtaining professional success. It is commonly seen among models,

figure skaters, ballerinas, artists, gymnasts, etc. They do not use the 'subterfuges' of the anorexoid patients; they are not socially isolated; their weight loss is not extreme; they have normal psychosexual activity; and they do not see themselves as overweight, unlike people with anorexia nervosa. For them, thinness is a means of obtaining success, not the final goal as in anorexia nervosa (**Figure 1**).

Anorexia Nervosa

Anorexia nervosa is a serious disease with psychiatric, endocrine, and nutritional connotations. It is more frequent in young women, in industrialized countries, in upper socio-economical groups in recent years. Genetic factors predisposing to anorexia nervosa became apparent in studies that showed increased prevalence of concordance of the disease among monozygotic twins (66%) compared to dizygotic twins (0%). They also explain in part the family aggregation of cases in the same families. Sociocultural factors are also important as indicated by the recent increased incidence of the disease, and the increased distribution in certain parts of the world.

Anorexia nervosa has three main components.

Psychological Disturbances

Psychological disturbances are most likely to be the initial event; they result in a complex obsession characterized by the following features:

1. An intrusive body image delusion makes the patients see themselves as being overweight when they are actually severely undernourished. This leads to a pathological fear of fatness (dysmorphophobia), a chronic voluntary starvation, and resistance to any external pressures to gain weight. Anorexic patients hide and dispose of food in the most ingenious ways to avoid eating.

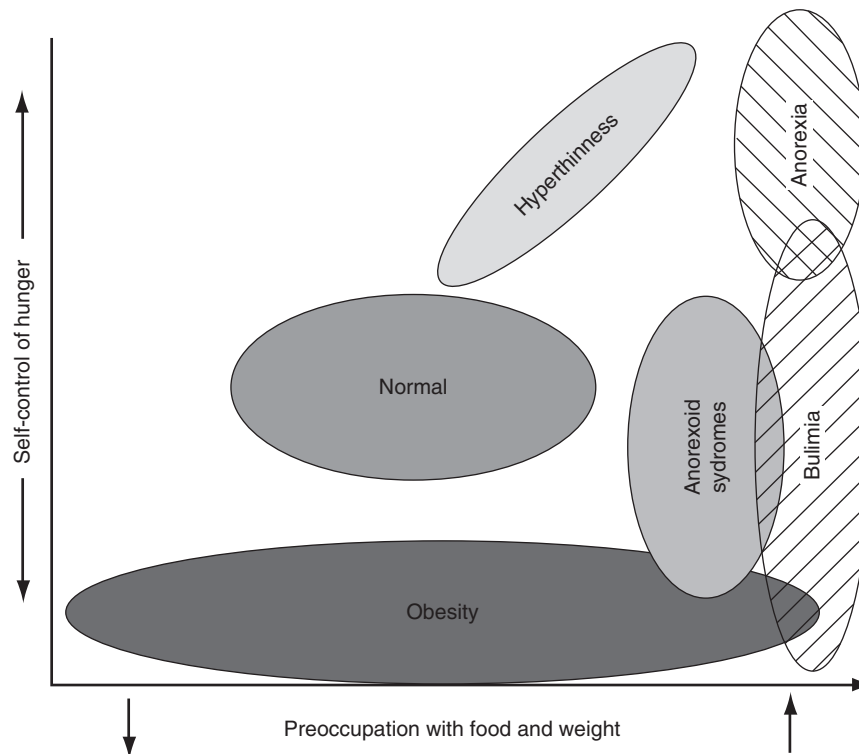


Figure 1 Classification of eating disorders based on the interaction between the preoccupation with food and body weight, and the self-control of hunger. © 1999 Academic Press.

2. An overwhelming sense of personal ineffectiveness makes anorexic patients believe that they cannot control the world around them. They continuously fear that they are going to lose their inner control. They therefore tightly control their world inside and slowly separate themselves from their social surroundings, with growing feelings of alienation and loneliness. There is no psychosexual development or interest, and no dating, unlike patients with bulimia.
3. Depression occurs which may be primary or secondary, obvious or atypical, and may or may not be amenable to treatment with psychotherapy and/or antidepressant medications.
4. Increased physical activity coexists with an apparent lack of hunger and fatigue, and is inappropriate for the degree of malnutrition and depression.

Malnutrition

Anorexia nervosa is a self-imposed starvation. The term 'anorexia' is a misnomer, because (at least initially) these patients are hungry. Anorexia nervosa is different from other forms of malnutrition because it is voluntary, resistant to nutritional treatments, accompanied by increased physical activity, without an initial organic cause (such as malignancy or surgery), and without associated infections. Because anorexia nervosa is a state of pure malnutrition without associated increased energy expenditure due to fever, immune response or tissue reconstruction, these patients have a lowered metabolic rate and do not tend to develop opportunistic infections.

The exact mechanism by which these patients are able to control their hunger is unknown. Disturbed brain neurotransmitters activity may be implicated primarily or secondarily.

Endocrine and Metabolic Changes

Amenorrhoea, decreased metabolic rate, hypothermia, hypotension, bradycardia, lanugo hair, carotinoderma, leucopenia, osteoporosis, etc. are mostly secondary to the severe malnutrition, and are reversible with weight gain.

Other Clinical Characteristics

Anorexia nervosa is more frequent among daughters of white, affluent, achievement-oriented families, in developed societies; it is extremely uncommon in areas of the world with poor nutrition. It tends to occur during the last years of high school or at the time of departure to the university.

The patients tend to be well-behaved, perfectionists, with good academic performance. Mothers of anorexic patients have a higher incidence of obsessive-compulsive personalities, and preoccupation with diets and weight loss. Anorexic patients have done everything their mothers or families have trained them to do and, when faced with the increasing demands (and choices) of adult life, they exaggerate the only control left in their lives: hunger. There is an unconscious wish to revert to childhood, or to a prepubertal state, by means of undernourishing.

The onset of anorexia nervosa is usually subacute, over a period of weeks, not uncommonly after an episode of weight gain, or after somebody has made a comment about the patient being overweight. Initially it appears as an innocent attempt to lose weight, but soon thereafter it starts showing its rebellious and progressive nature. Anorexia nervosa appears in small epidemics in cities and countries, probably owing to social pressures and to imitation behaviors.

In contrast to their poor dietary intake, these patients have a paradoxical enhanced interest in nutrition and cooking. They collect recipes, read nutrition textbooks, plan a career in nutrition or cooking, or find a job in a restaurant (usually waitressing). Anorexic patients enjoy cooking and feeding the rest of the family. They know the precise energy content of all usual food and use their knowledge to select low-energy items.

When forced to eat, anorexic patients will dispose of or hide food. They use their above-average intelligence to overcome all efforts to make them gain weight. They can be very resourceful in tampering with scales (adding weights to shoes or clothing, drinking large amounts of water just before weighing, etc.), and they have the most imaginative excuses as to why they are not gaining weight. They are extremely manipulative and master the art of confusing the different members of the treating team and family in their favor and against each other.

As they lose their natural insulation (subcutaneous tissue), it is difficult for anorexic patients to maintain their body temperature. They wear layer on layer of clothing, which also helps them to hide their malnutrition. Lack of body fat is sensed by the hypothalamus as a sufficiency of stored energy, and therefore the cycling and amplitude of gonadotrophins decrease. This leads to hypothalamic amenorrhoea, although approximately 30% of patients stop having menses before there is a significant weight loss. Depression may be another cause of hypothalamic amenorrhoea in these patients. Some of them will remain amenorrhoeic for several months after regaining normal weight.

In primary or classical anorexia nervosa the patients lose weight by dieting (restrictive) and exercising. These patients tend to be younger, more naive, introverted and obsessive, and they do not resort to subterfuges to lose weight. Their serum electrolytes, checked at frequent intervals, should be completely normal. Some patients find it impossible to control their hunger and start having binge episodes followed by forced vomiting (bulimia plus anorexia – ‘bulimarexia’). Others may start abusing laxatives or diuretics as they grow older.

There are patients in whom anorexia is secondary to an underlying, more serious psychiatric disorder such as depression, schizophrenia, hysteria or borderline personality disorder. In these cases, the course is longer and depends on the primary condition, as does its treatment. Men with anorexia nervosa are very uncommon, and in men the condition tends to be associated more often with these psychiatric problems and with homosexuality.

Physical Examination

The profound weight loss and cadaveric appearance contrast with the patient's increased physical activity. While hospitalized, if allowed, these patients try to perform some of the

nursing chores or even to counsel other patients. Many patients exercise secretly in their rooms, and jog or go for long walks when not supervised.

Pubic and axillary hair is preserved, and there is an increase in light, thin hair ('peach fuzz') on the face and neck, back, arms and thighs (lanugo hair). Patients have low body temperature and poor tolerance to cold exposure because of their malnutrition-induced lowered metabolic rate and the loss of the insulation of the diminished subcutaneous tissue. Layers of clothing tend to hide their cachectic appearance. Bradycardia and hypotension are common and secondary to decreased sympathetic drive due to malnutrition.

The skin is dry and has a peculiar bluish erythema over the knuckles and knees. Orange-yellow discoloration of the skin (carotinoderma), seen in palms and soles, is frequently found. It is caused, at least in part, by an increased intake of vegetables, because it may also be seen among vegetarians.

Symptoms

Symptoms are amazingly few. Most usually, these patients are forced to see a physician by their families. Spontaneous complaints may be amenorrhoea, constipation, abdominal pain or distension after eating, 'fluid retention,' and inability to lose weight, for which they may ask to be placed on special diets!

Laboratory Investigations

The following findings are typical:

- Mild normochromic, normocytic anemia.
- Leucopenia with relative lymphocytosis.
- Low sedimentation rates due to low fibrinogen levels.
- Serum albumin and transferrin levels are normal, except in severe cases.
- Serum carotene and cholesterol levels are normal or slightly elevated, which helps to rule out malabsorption.
- Low normal blood glucose levels are found, with low levels of glycohemoglobin.
- Electrolyte abnormalities, particularly low serum potassium values, occur only where there is self-induced vomiting or abuse of diuretics or laxatives.
- Low serum levels of luteinizing hormone, follicle stimulating hormone and oestradiol.
- Increased growth hormone levels with decreased levels of insulin-like growth factor I (IGF-I) (somatomedin C) in the serum.
- Plasma renin activity and aldosterone levels may be very high in patients who abuse laxatives or diuretics (pseudo-Bartter's syndrome).
- Electrocardiography shows sinus bradycardia, flat or inverted T-waves, and prolonged QT_c.
- Decreased bone density is due to decreased oestrogen and progesterone secretion, decreased calcium and vitamin D intake, protein malnutrition, and increased marrow fat content of the bones. The conversion of hematopoietic to fatty marrow is related to the severity of the malnutrition and may be demonstrated with magnetic resonance imaging.

Endocrine Changes

Insulin

Low serum insulin levels occur; with increased glucagon concentration. There is a tendency to asymptomatic low blood glucose levels and a low glycohemoglobin concentration. Fasting ketosis may be seen. The number and affinity of insulin receptors in target cells is increased, and abnormal glucose tolerance occurs due to prolonged fasting.

Adipose Tissue Hormones

The adipose tissue secretes different hormones called adipocytokines. Their secretion seems to vary in relation with the amount of adipose tissue accumulated although the exact mechanism is not known. During profound weight loss, like in anorexia nervosa, there is a marked decrease in the adipose tissue mass with the typical changes in adipocytokines secretion that occurs under these circumstances. One of the most studied adipocytokine changes is the decreased leptin secretion. Increased fat mass storage is accompanied by an increased leptin secretion; decreased fat mass stores decrease leptin secretion. Low serum levels of leptin reaching the hypothalamus increase the activity of the 'hunger center,' in part by increasing the local activity of neuropeptide Y. Individuals with anorexia nervosa have very low levels of leptin in blood and cerebrospinal fluid, in relation to their decreased adipose tissue. This should cause an increase in hypothalamic neuropeptide Y content and hunger, but this compensatory mechanism to maintain a normal body weight does not seem to be effective in anorexic patients. Another important effect of the serum levels of leptin on the hypothalamus is the modulation of the gonadal axis. Low levels of leptin are associated with decreased activity of the gonadal axis and explain the relationship between starvation and hypogonadism. After nutritional rescue and weight regain, the levels of leptin in the serum achieve normal levels.

Gonadal Axis

The female hypothalamus needs to 'sense' the presence of approximately 14–18 kg of body fat in order to allow fertility and menstrual cycles. With lesser amounts of fat, there is a progressive regression to the prepubertal state (low, nonspeaking serum gonadotrophin levels). The signal from the fat stores to the gonadal hypothalamus seems to be the levels of serum leptin. The very low levels of serum leptin, secondary to the decreased fat mass, seem to determine a decrease in luteinizing hormone releasing hormone (LHRH) secretion. The hypothalamic, hypogonadal state of anorexia nervosa is due to the combined effects of malnutrition and the psychological disturbances on the hypothalamus. Secretion of LHRH and gonadotrophins improves as weight is regained, and leptin levels increase; but in up to one-third of these patients menses do not return immediately after nutritional rescue and weight restoration are accomplished. The decreased oestrogen secretion from the ovaries brings about a significant loss of bone mass at a critical time, which will subsequently aggravate postmenopausal osteoporosis.

In males, malnutrition seems to have a less important influence on the gonadal axis. Severe weight loss of long duration decreases serum testosterone and gonadotrophin levels, but to a lesser degree than in women.

Thyroid

Thyroid stimulating hormone levels are normal, but there is a delayed response to thyrotrophin releasing hormone. Serum thyroxine, both total and free, are normal. The level of serum T_3 is low owing to decreased peripheral conversion (euthyroid sick syndrome), and there is concomitant increase in reverse T_3 .

The basal metabolic rate is decreased by 20–30%, and not fully corrected with T_3 replacement because it is also due to decreased sympathetic activity.

Sympathetic Nervous System

There is decreased peripheral sympathetic activity, with normal adrenomedullary function. This is due to decreased ingestion of energy, and it explains the tendency to bradycardia, postural hypotension, and low basal metabolic rate.

Adrenal Cortex

Serum cortisol levels are slightly raised, without diurnal variation, and may not suppress with dexamethasone overnight. Urinary 17-hydroxy and 17-keto steroids are decreased by 30–50%, but urinary free cortisol may be increased. Corticotrophin releasing factor (CRF) stimulation causes a subnormal corticotrophin rise, but a normal or supernormal serum cortisol response. Levels of CRF in the cerebrospinal fluid are elevated. These changes in the hypothalamic–pituitary–adrenal axis are very similar to those seen in untreated depression.

Growth Hormone

Basal and pulsatile secretion of growth hormone is increased, with a peripheral resistance to its effects. Serum growth hormone levels are elevated in 60% of patients, particularly in the most severe cases. This is due to decreased feedback from lowered serum concentrations of IGF-I, and to increased serum levels of ghrelin. Growth hormone levels do not rise normally after L-dopa or insulin hypoglycemia, but there may be an unexpected rise of growth hormone blood levels after stimulation with thyrotrophin releasing hormone.

Ghrelin is a recently discovered polypeptide secreted by the stomach that increases in circulation with weight loss. Ghrelin is an activating ligand for the GH secretagogue receptor in the hypothalamus. With starvation and weight loss, the increased serum levels of ghrelin increase the release of GH and hunger. Individuals with anorexia nervosa have high serum levels of ghrelin, which return to normal with normalization of body weight.

Vasopressin

There is decreased capacity to concentrate urine, due at least in part to sluggish vasopressin secretion in response to osmotic stimuli. Levels of vasopressin in the cerebrospinal fluid are increased.

Inflammatory Cytokines

Inflammatory cytokines have important endocrino-metabolic effects in persons with infections and neoplasias, one of them being anorexia and weight loss. In individuals with anorexia nervosa, the serum levels of interleukin 1β , interleukin-6, tumor necrosis factor α , and their soluble receptors are lower than normal, which may be due to decreased adipose production of these cytokines due to the decreased fat mass.

Hypothalamic Control of Hunger in Anorexia Nervosa

In normal individuals, fasting and weight loss increase hunger by multiple mechanisms (decreased serum levels of leptin, insulin, and blood glucose, and increased levels of ghrelin). At the level of the hypothalamus, there is an increase in the potent orexigenic neuropeptide Y and other changes in neurotransmitters secondary to the fasting state. Some of these neurotransmitters' changes may be the cause or a mechanism of anorexia nervosa, and for that reason they have received considerable attention in the last several years. One of the problems in understanding this data is that appetite control is a very complex hypothalamic function that involves many local and systemic neuropeptides, amines, and hormones.

Abnormal serotonin activity has been found in the brain of women with anorexia nervosa. More recently, an area in the chromosome 1 (p36.3–34.3) that contains genes for the serotonin 1D receptor and for the opioid delta receptor was associated with patients with anorexia nervosa by linkage analysis. One polymorphism in the agouti related protein (Ala67Thr) has also been found associated with anorexia nervosa. Melanocortin system stimulants in the hypothalamus, like agouti related protein, are also involved in appetite and energy regulation. However, these genetic abnormalities may amount only to a biological tendency, and do not explain the relatively short term of the illness during a life term nor the changes in prevalence in the past decades.

Bone Density

Decreased oestrogen and progesterone secretion, low serum levels of IGF-1, increased levels of serum cortisol, malnutrition with protein, calcium and vitamin deficiencies, and fatty degeneration of the bone marrow lead to decreased bone density. Increased exercising does not counteract this osteopenic tendency, which affects mostly young women during the years of skeletal growth. The osteopenia of anorexia nervosa is mostly asymptomatic, but some of these patients may present with stress fractures (diagnosed only with bone scans) related to their increased exercising. Many of these patients do not achieve their peak bone density even after their nutritional recovery and restoration of menses, and are left with a propensity to fracture bones for the rest of their lives. Treatment with oestrogens, calcium, and vitamin D are mildly effective. IGF-1 and DHEA-S have been used with partial success only. Rapid restoration of nutrition seems to be the best management of the anorexic osteopenia.

Differential Diagnosis

In the majority of cases, the severe and voluntary malnutrition accompanied by the typical delusion of being fat and resistance to gain weight make the diagnosis very clear. Malnutrition due to organic causes in adolescents usually has an obvious reason and the patients want to improve their nutrition. Hypothalamic tumors rarely may present with severe loss of appetite.

The differential diagnosis should include the anorexoid syndromes. In pure anorexia nervosa the weight loss is due only to restrictive eating habits and exercise. Some anorexic patients may start bingeing and inducing vomiting, in which case their condition is called 'bulimarexia.'

In some cases, anorexia nervosa is secondary to a serious, underlying psychiatric illness, with the weight loss being only an added problem. A particular diagnostic and therapeutic dilemma may occur with young women with personality disorder or chronic schizophrenia and anorexia nervosa.

Treatment

The multifaceted pathogenesis of anorexia nervosa requires an experienced team of psychiatrists, nutritionists, endocrinologists, internists or pediatricians, and nurses. Each patient should be considered individually because there are as many variations as there are patients. It is important to maintain communication between the different members of the team in order to present a unified front to the patient. Invariably, the patient will try to find and exploit the most minimal differences of opinion between the members of the team. Ideally, all the important decisions should be made by one central team leader. Nurses, aides, and other paramedical personnel should be instructed about how to deal with the patient's behavior and charming search for allies.

There is no specific treatment and the methods reported are, at best, controversial. The etiology of this disorder remains unknown, and etiological factors are probably different in each patient. It is important therefore to tailor the therapeutic approach to each patient.

Many cases of established and severe anorexia nervosa require prolonged hospitalization for psychological and nutritional rescue. Separation from parents and home environment is only part of what is to be gained from hospitalization. Administrators and health insurance companies must understand this need.

Hospitalization is indicated when there is:

- severe and rapid weight loss;
- serious metabolic or cardiovascular problems (hypokalemia less than 2.5 mmol l^{-1}) despite oral replacement, blood urea nitrogen more than 10.6 mmol l^{-1} of urea (30 mg dl^{-1}) in the presence of normal renal function, pulse less than 45 min^{-1} , systolic blood pressure less than 70 mmHg , or a body temperature less than 36°C ;
- severe depression and suicide risk;
- psychosis; and
- family crisis.

Psychiatric Treatment

From the outset the entire family should be interviewed to gain insight into the patient's previous behavior, to understand the family dynamics and enlist their help in therapy. Clear simple contracts with the patient are a form of behavior modification that is simple to carry out. Initially most daily activities and visits are curtailed and the patient is watched, particularly around mealtimes. As the patient improves, restrictions are lessened and privileges increased. Short-term goals are set from the beginning. Weight gains of 250 g daily or 1.3–1.8 kg a week are acceptable limits. Patients who accomplish these goals are rewarded by increasing levels of activity and autonomy within the hospital, as a positive reinforcement.

The general attitude of the team should be one of understanding, concern, and firmness. One should try to build a trusting relationship in which the patients feel understood, but without giving them a chance to deceive. The nature and course of the illness should be clearly explained to the patient and the family. This includes the serious complications of malnutrition and the fatal outcome of severe cases. Emphasize that the goal of treatment is not to make the patient fat, but to make the patient feel better and to improve self-confidence and eating habits. Weight is only a by-product of the improvement, and 'muscle mass and protein recovery,' not fat, is what has to be gained.

This firm understanding should engage the patient in a treatment alliance with the team. Remember that many of these patients are very polite and 'out to please you' at least superficially, and many times their initial acceptance hides deeper feelings of isolation and resentment. Psychotherapy is of help in some patients, usually accompanied by behavior modification and family therapy.

Despite the common use of antidepressants, several double-blind trials have been inconclusive or only slightly favorable. Patients with clear manifestations of depression and the more severe cases seem to benefit more from these medications. Tricyclic antidepressants tend to increase appetite and are more suited for patients with pure anorexia nervosa. Selective serotonin reuptake inhibitors may help decrease bingeing in patients with associated bulimia. Olanzapine, an atypical antipsychotic medication associated with weight gain, has been shown useful in some patients with anorexia nervosa in uncontrolled studies.

Nutritional Treatment

The psychiatric treatment is beneficial only as long as the patient's nutrition is improved. The nutritional rescue breaks down the vicious circle of the psychological consequences of starvation and makes the patient more receptive to psychotherapy. The team should be prepared to deal with the most ingenious ways to deceive. The patient should be told that because of the tendency to deceive frequently found in her illness, close supervision will be necessary at least in the beginning of the treatment. Patients should be weighed fasting in the morning, in nightgown without shoes and with the same scale, daily, or at regular intervals by a nurse.

Initially, oral intake should be monitored carefully with a nurse sitting through the eating period, and for 30 min

thereafter to prevent postprandial vomiting. The tray should be checked for any food not consumed. In this way, a careful energy count is obtained daily. If the energy intake is inadequate or if the patient is not gaining weight, the diet should be supplemented with low-residue, high-energy canned formulae dispensed by the nurse during medication rounds. These diet supplements should be consumed in front of the nurse. Many patients with anorexia nervosa have subclinical vitamin deficiencies and they should receive a multivitamin tablet every day.

It is not infrequent for these patients to complain of gastric distress after sudden increases in food intake; smaller and more frequent feedings and/or administration of metoclopramide or cisapride before meals may be of help. Tube feeding is poorly tolerated by most of these patients; it has connotations of a gastrointestinal 'rape.'

If severe malnutrition is present (low serum albumin and transferrin levels, anergic skin testing), parenteral hyperalimentation should be instituted from the beginning. It is recommended to start with small amounts of hyperalimentation fluid to avoid excessive sodium and water retention (refeeding oedema), which is very distressing to the patients. The rate of hyperalimentation solution administration should be modified according to the improvement in oral intake and weight. Staff should be continuously aware of the possibility of tampering with the central lines by the patient, with the potential for air embolization, infection, and bleeding. In many patients it is important to curtail all physical activity initially, to the point of confining them to absolute bed rest with only bathroom privileges. As the patient improves, the activities are progressively increased.

Oestrogen replacement is indicated to prevent the progressive decrease in bone density, but it is poorly tolerated and accepted by these patients. Ideally a birth control pill with good oestrogen content should be administered.

Prognosis

The outcome of patients with anorexia nervosa is variable; a worse outcome is associated with older age of onset, severity and duration of the illness, male sex and severe associated psychiatric disturbances. In general, 40–60% of patients achieve full nutritional and psychological recovery after 6–12 months. Approximately 20–40% attain a borderline normal weight and existence for the rest of their lives, but with the appearance of significant stress they may revert to their previous anorexic behavior. There is a mortality rate of 5–30% in the most severe cases, due to suicide, electrolyte imbalance, and starvation-induced myocardial damage causing intractable arrhythmias; it is rarely due to infection. Long term follow-up of these patients has recently shown an increased later mortality due to alcoholism.

See also: Adolescents: Nutritional Problems of Adolescents. Eating Disorders: Bulimia Nervosa. Obesity: Definition, Etiology, and Assessment. Starvation and Fasting: Biochemical Aspects

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Binge Eating

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Glossary

Abstinence from binge eating The absence of binge eating behavior for a specified period of time (usually at least one month).

Binge eating The ingestion of a large amount of food within a discrete period of time accompanied by a sense of loss of control over when, what, or the quantity of food that is eaten.

Compensatory behavior Inappropriate behavior designed to counteract the effects of binge eating or prevent weight gain. Examples of compensatory behaviors include self-induced vomiting; misuse of laxatives, enemas, diuretics, or diet pills; excessive exercise; and fasting.

Eating disorder A persistent pattern of aberrant eating or inappropriate dieting behaviors accompanied by

maladaptive thoughts and beliefs about eating, shape, or weight. Eating disorders are associated with significant medical morbidity, as well as psychological distress and dysfunction.

Loss of control The subjective feeling that one cannot stop eating or control when, what, or the amount of food that is eaten.

Obesity Refers to an excess of body fat. At present, there is no clear division between normal and abnormal levels of fat; however, body mass index (BMI), a ratio of weight to height calculated by weight in kilograms divided by the square of height in meters, is widely utilized to define obesity operationally given its robust associations with adiposity (i.e., BMI > 30: obese) and medical comorbidity.

Introduction

In 1959, Stunkard noted three patterns of eating behavior in obese patients: night eating, binge eating, and eating without satiation. However, it was not until the 1980s that binge eating began to receive attention as a distinct clinical syndrome. Spitzer and colleagues proposed diagnostic criteria for Binge Eating Disorder (BED) and subsequently evaluated them in two field trials. These initial investigations led to the inclusion of BED in the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition (DSM-IV) as an example of Eating Disorder Not Otherwise Specified (EDNOS) and as a proposed diagnostic category requiring further study.

BED is characterized by persistent and recurrent episodes of binge eating without the regular use of inappropriate compensatory behaviors seen in Bulimia Nervosa (BN) and the binge eating/purging subtype of Anorexia Nervosa (AN). Over the past decade, research on the phenomenology, epidemiology, and treatment of BED has burgeoned, and there now is convincing evidence for the validity of BED as a diagnostic category distinct from AN and BN. Consequently, the Eating Disorders Work Group for DSM-5 has tentatively proposed that BED be included in the next edition of the DSM. In this article, we address several issues pertaining to the BED diagnosis including assessment, prevalence, risk factors, and comorbid conditions. Evidence-supported treatments also are reviewed, including guidelines for choice of treatment approach (Figure 1).

Assessment of Binge Eating

A binge episode is defined as the consumption of a large amount of food within a discrete period of time, accompanied

by a sense of loss of control over eating. Researchers and clinicians have agreed that loss of control involves the subjective feeling that one cannot stop eating, or control what or how much is being eaten. Indeed, many observers have concluded that loss of control, rather than the amount of food ingested, is the hallmark of binge eating. There has been much less agreement about the size and duration of binge eating episodes. Specifically, there is no consensus as to what constitutes a large amount of food (other than agreement that the amount of food eaten is more than others typically would eat in a similar situation), and the duration of binge eating episodes can vary widely, sometimes continuing throughout an entire day. Because early data indicated that some individuals with BED have difficulty delineating binges into discrete episodes, DSM-IV research criteria for BED are based on binge 'days' rather than 'episodes.' However, recent work has documented that binge episodes can be assessed reliably in individuals with BED, and thus the Eating Disorders Work Group for DSM-5 has recommended that the BED diagnosis be based on binge episodes to make it consistent with BN. See Table 1 for the full DSM criteria for BED.

Several methods can be used to assess BED, including clinical interviews, self-reports such as questionnaires and food diaries, and observation of eating behavior in the laboratory. Currently, a clinical interview by a trained professional is the preferred assessment method, as it provides the opportunity to standardize definitions of key concepts such as a 'large amount of food' and 'loss of control.' Although questionnaires are relatively easy to administer, there is high potential for misinterpreting these terms. Interview-based assessments tend to yield ratings of binge eating that are lower, but more precise, than questionnaire-based surveys.

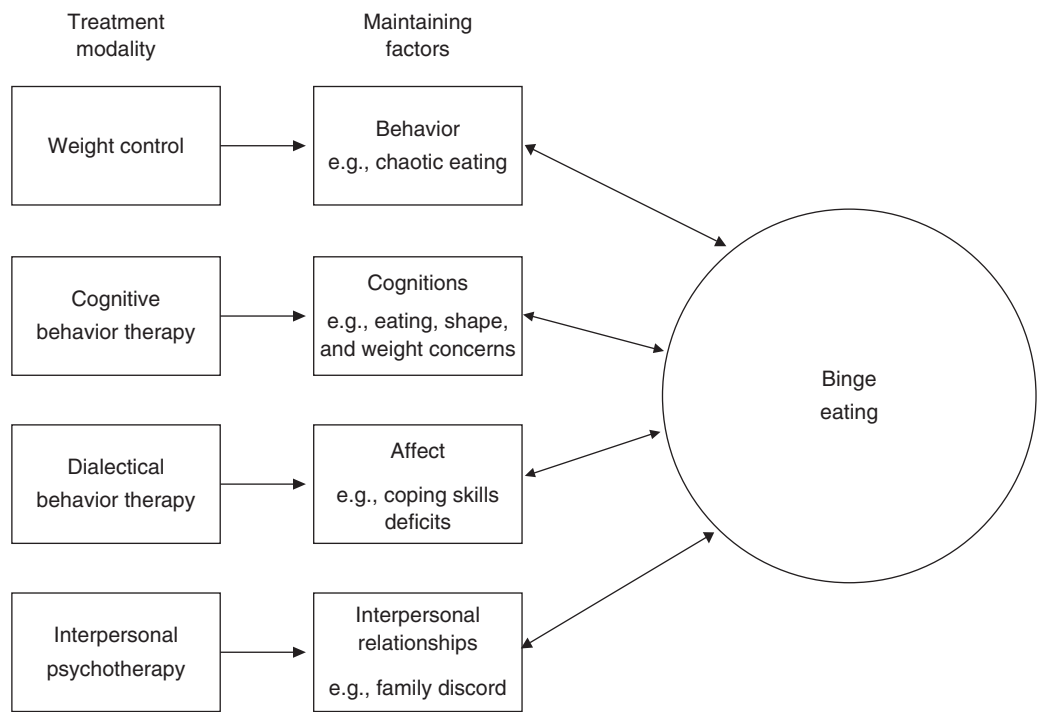


Figure 1 Interventions and treatment targets for BED.

Table 1 DSM-IV-TR (2000) Research Criteria for Binge Eating Disorder

Criterion
1. Recurrent episodes of binge eating. An episode of binge eating is characterized by both of the following: Eating, in a discrete period of time (e.g., within any 2-hour period), an amount of food that is definitely larger than most people would eat in a similar period of time under similar circumstances. A sense of lack of control over eating during the episode (e.g., a feeling that one cannot stop eating or control what or how much one is eating).
2. The binge eating episodes are associated with at least three (or more) of the following: Eating much more rapidly than normal. Eating until feeling uncomfortably full. Eating large amounts of food when not feeling physically hungry. Eating alone because of being embarrassed by how much one is eating. Feeling disgusted with oneself, depressed, or very guilty after overeating.
3. Marked distress regarding binge eating is present.
4. The binge eating occurs, on average, at least 2 days a week for 6 months. ^a
5. The binge eating is not associated with the regular use of inappropriate compensatory behaviors (e.g., purging, fasting, excessive exercise) and does not occur exclusively during the course of Anorexia Nervosa or Bulimia Nervosa.

^aNote: The Eating Disorders Work Group for DSM-5 has tentatively recommended that this criterion be changed to 'The binge eating occurs, on average, at least once a week for three months' to be consistent with proposed diagnostic criteria for Bulimia Nervosa.

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Food diaries involve having individuals keep a daily record of the specifics of eating episodes, including how much food was consumed, whether or not there was loss of control over eating, any use of inappropriate compensatory behavior, and the associated context. Food diaries can provide detailed assessment information without introducing the bias of retrospective self-report; however, self-monitoring also has been shown to affect eating behavior and is frequently employed in clinical treatment. Findings from studies that have utilized food

diaries indicate that BED patients report higher calorie intakes than nonbinge eaters on both 'binge days' and 'nonbinge days'. Observation of binge eating in the laboratory is a specialized technique that is limited to use in research settings, providing the opportunity to document actual eating behavior and measure consumption. Laboratory studies with relatively small samples have shown that, compared to equally overweight patients who do not binge eat, BED patients ingest more calories both during 'binges' and at 'regular' meals. This difference

in eating behavior in binge compared to nonbinge eaters supports the validity of BED as a distinct diagnostic category.

Prevalence and Risk Factors

Epidemiologic research has documented lifetime prevalence estimates for BED of approximately 2–3%, which is approximately twice as common as AN or BN. There is also evidence that the demographic profile of individuals with BED is more diverse than for other eating disorders, affecting relatively more men and minority groups. Finally, binge eating is more prevalent among obese individuals in both clinical and community samples. It is estimated that up to one-third of individuals presenting for treatment in university-based weight control clinics or bariatric surgery clinics report significant binge eating.

The exact causes of BED remain unknown, but research has identified several potential biological and environmental risk factors. For example, studies using family history and twin data have documented that BED is a heritable illness that aggregates in families independently of obesity. There also is preliminary evidence that individuals with BED show greater neural activation to food-related stimuli when compared to individuals without binge eating, but it is not clear to what degree this is a function of overweight status. Studies focusing on specific genetic polymorphisms (e.g., MC4R, cDNA 385C), hormones (e.g., cortisol), and peptides (e.g., ghrelin) generally have failed to produce consistent evidence for an association with BED. Finally, studies using case-control methodology in which individuals with BED are compared to age- and sex-matched individuals without BED have suggested several potential risk factors for binge eating; examples include childhood obesity, family overeating, low parental contact and high parental demands, and negative comments about shape, weight, and eating. One study using signal detection analysis to identify potential risk factors for BED and BN also reported that an elevated level of perceived stress before age 14 preceded the onset of binge eating in a significant number of individuals.

To improve our understanding of how multiple factors interact to determine the onset and maintenance of binge eating, prospective risk factor studies including males and females of different racial groups are needed. As suggested above, biological (e.g., obesity), psychological (e.g., perceived stress), and social (e.g., repeated exposure to negative comments about shape, weight, or eating) factors have been implicated in the pathogenesis of binge eating. However, no study has examined these factors prospectively to evaluate whether they predict future development of BED.

Comorbidity

Binge eating is strongly associated with obesity and psychiatric disorder. Although it is well documented that obesity is linked to adverse medical and psychosocial outcomes, research has indicated that BED is associated with poor health independent of the effects of obesity or comorbid psychopathology. Indeed, severity of binge eating is positively associated with degree of

overweight, and there are important differences between overweight individuals with and without BED. BED patients report earlier onset of obesity, along with a history of more severe obesity, dieting, and weight fluctuations. Moreover, when compared to equally overweight individuals without binge eating problems, BED patients report considerably less 'restraint' or control over eating, lower self-esteem, more fear of weight gain, more preoccupation with food, and higher body dissatisfaction. Finally, a recent report found that, compared to age-, sex-, and BMI-matched individuals without binge eating, obese individuals with BED were at increased risk for developing components of the metabolic syndrome (e.g., dyslipidemia, hypertension, type 2 diabetes) over a 5-year prospective follow-up.

With respect to psychiatric symptomatology, there is evidence that individuals with BED have significantly higher lifetime rates of major depressive disorder, anxiety disorders, substance use disorders, and personality disorders compared to equally overweight individuals without binge eating problems. Preliminary data also suggest that individuals with BED have higher rates of bipolar disorder and attention-deficit/hyperactivity disorder. Studies focusing on eating disorder symptomatology have documented that concerns about weight, shape, and eating are elevated in individuals with BED relative to obese and nonobese controls including other disordered eating groups. Considering these findings as a whole, a recent comprehensive review commissioned by the Eating Disorders Work Group for DSM-5 concluded that individuals with BED display significant psychiatric comorbidity that is comparable to other eating disorders and cannot be explained by the presence of obesity.

Treatment of Binge Eating

Among those who seek treatment, BED tends to be a chronic and fluctuating disorder. The clinical picture in BED often involves onset in late adolescence or the early 20s, with numerous periods of relative control over eating and weight loss alternating with periods characterized by binge eating and weight gain. Individuals with BED often seek obesity treatment rather than treatment of disordered eating *per se*.

A variety of psychosocial and pharmacological interventions can help individuals gain control over binge eating. There also is some evidence that behavioral weight control is associated with weight loss and short-term improvements in BED symptoms, although recent data suggest that eating disorder-focused treatments may be required to achieve longer-lasting remission from binge eating. Finally, it is important to note that a substantial number of patients are not abstinent from binge eating after treatment, suggesting the need for clinical trials of novel therapeutic approaches as well as combinations and sequencing of treatments.

Psychosocial Treatments

Treatments for BED have been adapted from those that have been shown to be effective in reducing binge eating among individuals with BN. The majority of the research

on psychosocial treatments has supported two structured, focused, short-term psychotherapies, Cognitive Behavior Therapy (CBT) and Interpersonal Psychotherapy (IPT), both of which have been shown to be more effective than no treatment in decreasing the frequency of binge episodes and improving the psychopathology associated with binge eating. In addition, the use of Dialectical Behavior Therapy (DBT) shows promise as an alternative treatment for BED. Finally, there is growing evidence that therapist-guided self-help interventions based on CBT principles have utility in the treatment of binge eating.

Cognitive Behavior Therapy

CBT has been the most extensively studied treatment for individuals with binge eating. CBT for BED is based on the assumption that binge eating is maintained in the context of ongoing dietary restraint, weight concerns, negative emotions, and low self-esteem. Treatment focuses first on normalizing eating, and then on the identification and restructuring of maladaptive thoughts and beliefs, particularly those related to eating, shape, and weight.

CBT for BED has been adapted to reflect important differences between individuals with BN and BED. Specifically, cognitions relating to having a large body size are directly targeted in treatment. Overweight individuals with BED may be helped to accept a larger than average body size and to change unrealistic expectations for weight loss. For the majority of BED patients, a five or ten kilogram weight loss does not correspond with their desired weight, even though a modest weight loss may relate to improvements in binge eating and overall health. It is therefore important to help patients adopt realistic goals for the body weight and shape they are likely to achieve.

Another adaptation of CBT for BED relates to differences in the role of dieting between individuals with BED and those with BN. Although the treatment of BN stresses the role of dietary restraint in precipitating binge episodes, and treatment focuses on decreasing dietary restraint, patients with BED do not necessarily binge eat in response to restraint or hunger. Indeed, the preponderance of evidence suggests that increasing dietary restraint may help to ameliorate binge eating in obese individuals. Thus, CBT for BED does not stress decreased dietary restraint; rather, treatment encourages the development of a moderate, structured, healthy eating pattern.

Interpersonal Psychotherapy

Klerman and Weissman's IPT also has received empirical support in the treatment of individuals with BED. IPT for binge eating is based on the idea that dysfunctional eating behavior is maintained in the context of interpersonal difficulties. Treatment focuses on identifying and addressing specific, problematic interpersonal patterns in an effort to ameliorate binge eating. Treatment can focus on: (1) role disputes, such as marital or family discord; (2) role transitions, such as the adjustment to motherhood or a new job; (3) grief, such as the loss of a spouse or loved one; or, (4) interpersonal deficits, such as loneliness and social isolation. IPT for BED does not directly target eating behaviors or attitudes about eating, shape, and weight. Although the ways in which CBT

and IPT conceptualize and treat binge eating differ, both appear to be effective in reducing the frequency of binge eating.

Dialectical Behavior Therapy

Developed by Linehan for the treatment of individuals with borderline personality disorder, DBT has shown promise in the treatment of BED. DBT is a comprehensive treatment program based on cognitive and behavioral principles and complemented by the use of mindfulness strategies derived primarily from Zen Buddhism. In addition to weekly individual outpatient treatment, traditional DBT prescribes a weekly group meeting in which the goal is to increase participants' behavioral skills. A group-only version of DBT for individuals with BED has been shown to decrease binge eating and maladaptive attitudes about eating, shape, and weight. However, despite its emphasis on emotion regulation, DBT has not shown superiority relative to a waiting-list control in reducing negative affect or enhancing adaptive affect regulation skills among individuals with BED. Thus, additional research is needed to determine the mechanisms by which DBT effects change in BED symptoms, and to evaluate the efficacy of DBT relative to CBT and IPT.

Guided Self-help

There is growing evidence for the efficacy of guided self-help programs, in which a therapist or other professional provides support to individuals in completing a manualized self-help intervention, in the treatment of patients with binge eating. Guided self-help programs have some advantages over more specialized psychotherapies for binge eating in that they can be implemented by therapists with little experience in the treatment of eating disorders and may offer a cost-effective approach to treating binge eating in community settings. For example, Striegel-Moore, Wilson, and colleagues demonstrated that an eight-session guided self-help program based on Fairburn's 'Overcoming Binge Eating' resulted in significantly greater rates of abstinence from binge eating and reductions in the cognitive correlates of disordered eating relative to treatment as usual when provided by nonspecialist clinicians in a community setting (i.e., a health maintenance organization in the USA). Another recent report found that guided self-help based on CBT principles was more effective than behavioral weight loss in producing remission from binge eating at two-year follow-up. Although these findings require replication, they suggest that CBT-based guided self-help may be a useful alternative to more specialized psychotherapies in the treatment of individuals with binge eating.

Behavioral Weight Control

Because the majority of individuals with BED are overweight and want to lose weight, and because obesity is associated with significant medical and psychosocial consequences, weight loss is a potentially important outcome in the treatment of BED. Numerous studies have documented that calorie restriction does not exacerbate binge eating in BED patients. Moreover, several reports have indicated that participation in a behavioral weight control program that focuses on calorie restriction, provides education about sound nutritional

principles, and promotes physical activity is associated with decreases in binge eating and improvements in mood among individuals with BED. There is also evidence that behavioral weight control interventions produce greater weight loss among individuals with BED than do specialized psychotherapies for binge eating (e.g., CBT, IPT), which generally have been shown to have little effect on weight status. Nevertheless, recent data have suggested that behavioral weight control programs may be less effective than specialized psychotherapies and CBT-based guided self-help in producing lasting remission from binge eating. Taken together, these findings suggest that the optimal approach to treating obese BED patients may be a combination of behavioral weight management to address weight loss and psychosocial interventions that specifically target disordered eating symptoms.

Pharmacotherapy

Pharmacologic approaches to the treatment of BED that have empirical support include antidepressant, anticonvulsant, and weight loss medications. Although there is little evidence that antidepressant medications increase the efficacy of psychotherapy for BED, preliminary data suggest that augmenting psychosocial treatments with anticonvulsant or weight loss medications may lead to greater reductions in body weight and higher rates of remission from binge eating among obese patients with BED.

Antidepressant Medications

Because of their efficacy in ameliorating binge eating and purging behaviors in individuals with BN, antidepressants have been used widely in the treatment of BED. Early research comparing tricyclic antidepressants, such as desipramine and imipramine, to placebo showed greater reductions in binge eating among obese binge eaters treated with active medication than with a placebo. More recently, several selective serotonin reuptake inhibitors (e.g., fluoxetine, fluvoxamine, sertraline, citalopram) have been shown to be associated with moderate reductions in binge eating in BED patients. Moreover, the effects of antidepressant treatment on binge eating are independent of any effects on mood.

Anticonvulsant Medication

Initial studies have provided support for the utility of an anticonvulsant medication, topiramate, relative to placebo in reducing the frequency of binge episodes and improving the cognitive correlates of disordered eating among individuals with BED. There also is preliminary evidence that augmenting group CBT for BED with topiramate is associated with greater reductions in body weight and higher rates of remission from binge eating compared to group CBT with placebo. However, the long-term effects of topiramate on binge eating and weight loss remain unknown.

Weight Loss Medications

Two anorectic agents used in the treatment of obesity, sibutramine and orlistat, have been investigated in BED patients with promising results. For example, a large, double-blind, randomized controlled trial documented the superiority of

sibutramine relative to placebo in reducing the frequency of binge days and episodes, decreasing weight and body mass index, and improving the psychological correlates of disordered eating. However, sibutramine has recently been withdrawn from the US market. Other research has shown that adding orlistat to CBT-based guided self-help is associated with higher rates of remission from binge eating at post-treatment and greater weight loss at both post-treatment and 3-month follow-up relative to CBT-based guided self-help plus placebo. These findings suggest that augmenting psychosocial treatments for disordered eating with weight loss medications may be an effective approach to treating obese BED patients.

Selection of Treatment for Specific Patients

No single treatment approach is effective for all patients. Although preliminary data have suggested that IPT may be more effective than CBT-based guided self-help and behavioral weight control for BED patients with severe eating disorder psychopathology and low self-esteem, additional research is needed to guide the selection of treatment for individual patients with BED. Until such information becomes available, clinicians and patients must decide on a course of treatment based on a careful assessment and thorough consideration of the pros and cons of available options.

Eating Disorder and Obesity History

A history of early onset of binge eating, binge eating in the absence of obesity, or obesity in combination with numerous bouts of weight loss and regain over time (i.e., 'yo-yo' dieting), suggest a course of eating disorders treatment. Such patients can be reassured that significant improvements in the aberrant eating and eating disorder psychopathology associated with BED can be obtained without weight loss.

On the other hand, clinical experience suggests that patients who report adult onset of binge eating and obesity, and do not have a history of marked weight fluctuations, may be likelier to benefit from a behavioral weight control approach. Behavioral weight control also may be indicated for patients who remain overweight after a trial of eating disorders treatment. Although behavioral weight control appears to be beneficial on average, it is important for each individual to evaluate the likelihood that he or she will be able to sustain lifelong changes in eating and exercise.

Psychiatric Status

Given the high psychiatric comorbidity in BED, a thorough evaluation is important for all patients who seek treatment. Although mild to moderate depression or anxiety is likely to improve during treatment of binge eating, the presence of marked or severe current illness suggests primary treatment of the mood or anxiety disorder. In addition, the presence of personality disorders characterized by emotional, dramatic, or impulsive behavior appears to be related to severity of binge eating, but does not appear to predict treatment outcome.

Available Resources

Clinicians trained in the use of psychosocial treatments for eating disorders are likely to be found in most metropolitan

areas, but may not be available in smaller cities or rural areas. Insurance companies vary in coverage for treatment of eating disorders, and some insurance plans may pay for obesity treatment only if there is a clear medical indication (e.g., hypertension or other cardiovascular risk). Thus, treatment decisions may need to take into account pragmatic factors such as clinician availability and training, or patient insurance plan coverage. Guided self-help programs based on CBT principles may be a promising alternative for patients who do not have access to specialized psychotherapies. There is growing interest in the use of computerized technologies (e.g., CD-ROM and internet-based interventions) as a means of disseminating therapist-guided self-help for BED, and preliminary data have been encouraging. However, comorbid psychopathology and high frequency binge eating may require more intensive clinical intervention.

Summary

BED is a chronic and fluctuating disorder that is common among obese individuals who seek treatment, and is associated with elevated rates of psychopathology. Although the exact causes of binge eating remain unknown, available data support a model of etiology in which biological, psychological, and social factors are implicated. Once established, binge eating is maintained by a complex interplay of eating behaviors, cognitions, affect, and interpersonal factors. Nevertheless, available research indicates that most people who binge-eat can be helped with specialized psychotherapy for eating disorders, therapist-guided self-help programs, or behavioral weight control. Pharmacotherapy also appears to reduce binge eating, and treatment with certain medications (e.g., topiramate, orlistat) may result in larger and more sustained weight losses when combined with psychosocial interventions than psychosocial intervention alone. A careful assessment, review of the benefits and disadvantages of the different therapies, and consideration of the availability of trained clinicians should guide the choice of treatment for an individual with BED. More research is necessary to fully understand this problematic eating pattern and to improve strategies for management and treatment.

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Bulimia Nervosa

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Introduction

Episodes of ravenous overeating, referred to as compulsive eating or binge eating, have been recognized clinically since the 1950s. However, the disorder of bulimia nervosa was not formally described until 1979. The description and understanding of the psychopathology of bulimia nervosa has continued to be refined. This is reflected in discussion over the way that bulimia nervosa and the other eating disorders will be described in future revisions to standard diagnostic manuals (e.g., DSM-V).

This article will focus on the features used to make a diagnosis of bulimia nervosa, the psychopathology and developmental course of the disorder, and the groups at risk. Specific attention will be paid to the nutritional consequences of bulimia nervosa and the ways in which dietary management is used in its treatment. Finally, long-term prognosis will be considered.

Diagnostic Criteria

The behavior at the center of the disorder, binge eating, has been progressively redefined. A priority has been to separate binge eating from mere indulgence and everyday overeating. Accordingly, three features of a binge episode have been identified: consumption of unusually large amounts of food (excessive) over a short period of time (brief) and a subjective sense of lack of control over eating (compulsive, unrestrained). The size of binges varies but is often between 1000 and 2000 kcal. It is of note that a distinction has arisen in the literature between subjective and objective binge episodes. Subjective binge eating involves the same loss of control as with objective binge eating. The difference is, small to moderate amount of food consumed. For many who binge, the amount of food is not the primary criterion for defining a binge; both can be associated with high distress.

Diagnostic schedules (such as DSM-IV-TR and ICD-10) agree on three features that must be present in someone with bulimia nervosa. The first is the presence of binge episodes, of a required frequency, for example, at least twice a week for 3 months. Second, the person must use compensatory behavior to control body shape or weight. The most common is self-induced vomiting, but these strategies also include use of laxatives or diuretics, excessive exercise, and extreme dieting or fasting. Third, the person must show over concern with body weight and shape. Importantly, the person should not be of low body weight, in which case a diagnosis of anorexia nervosa would be made.

The tightening of these formal diagnostic criteria has had the consequence of reducing misdiagnosis and prevalence, but has increased the numbers of those with atypical eating disorders. Failing to exhibit one or more of the key diagnostic

features, such as an insufficient frequency of binge eating, comes under the classification of 'eating disorders not otherwise specified' (EDNOS). Binge eating disorder (BED) is one of these exceptions. The key difference between BED and bulimia nervosa is the absence of the extreme compensatory behaviors that follow the binge. Those with BED are less likely to be restricting their eating but more likely to be overweight and to be older (most presenting between age 30 and 50).

Psychopathology

The description of body image disturbance that is central to both anorexia and bulimia nervosa has itself undergone revision. A distinction has been argued for dissatisfaction with body shape and overvalued ideas about weight and shape. Although body shape dissatisfactions are commonly found in these patients, it is their overvalued ideas about weight and shape that are the necessary diagnostic feature. In other words, concern should go beyond simply feeling fat to a point where a person's life is dominated by their feelings about body weight and shape.

If these overvalued ideas are accepted as the core psychopathology of bulimia nervosa, then the chaotic eating that typifies the condition can be seen as a behavioral consequence. Binges are often interspersed between periods of intense dieting, even fasting, themselves strategies to lose weight. Purging always follows a binge and is a way of expelling the food ingested or compensating for the food energy intake. Binges are secretive, planned, often expensive, and emotionally self-destructive. Paired with purging, they are cyclical and self-perpetuating, although their frequency may wax and wane. In addition, this behavior may have a long history before treatment is considered and clinical attention is sought.

Bulimic episodes may be triggered by a variety of factors, including anxiety, boredom, tension, or breaking the self-imposed dietary rules necessary to maintain rigid control over eating. Only rarely is hunger identified as precipitating a binge, even though the person may not have eaten for 24 h or more.

Sustained depressive and anxiety symptoms are common and are part of a range of psychological and social problems characteristic of bulimia nervosa. Impulsivity is also characteristic, with sexual promiscuity, self-harm, drug use, and stealing frequently noted. One suggestion is that impulsivity is linked to poor distress tolerance which is associated with an inability to regulate emotions effectively.

Etiology

As with anorexia nervosa, the picture of development is complex and multi-factorial, there is no single cause of

bulimia nervosa. Rather, a variety of psychological, biological, and social factors are involved in the emergence of the disorder. Although etiology is diverse, it has much in common with the forces held responsible for anorexia nervosa, and is clarified by looking at the groups of people most at risk. Overall, the balance of etiological factors is in favor of psychological and social causes, given that bulimia nervosa is a relatively new condition and has arisen at a time of profound social and cultural change, with little concurrent change in human biology.

The process of the development of eating disorders can be usefully divided into three stages. These conceptually separate the factors that predispose an individual to the disorder, precipitating events that lead to onset, and factors that perpetuate or maintain the disorder once initiated. Any framework drawn up for bulimia nervosa would be very similar to that for anorexia nervosa, as the etiologies of the two disorders appear to have a lot in common. Indeed, up to a third of patients with bulimia nervosa have a premorbid history of anorexia nervosa.

Although the etiological picture is very similar to that for anorexia nervosa, there are a few clues to differences. Genetic studies suggest that the disorder is less heritable than anorexia nervosa, although heritability estimates of 46–71% have been calculated for the key behaviors, binge eating, and self-induced vomiting. Evidence from case control and cohort studies suggests two groupings of factors that contribute independently to the risk of developing bulimia nervosa. First, there is increased exposure to dieting and related risk factors, including parental and childhood obesity, and critical family comments about weight, shape, or eating. Second, a greater number of general risk factors for psychiatric disorder has been observed. These include parental psychiatric disorders such as depression, alcohol, and substance abuse during childhood, low parental contact but high parental expectations, neglect, and abuse. Sexual abuse has been reported in 20–25% of patients with bulimia nervosa, a higher level than that found in restricting anorexia nervosa. Although the rate is increased compared to that of matched controls, it is no higher than the rate among young women with other psychiatric disorders. However, women with eating disorders in the context of sexual abuse appear to have higher rates of comorbid psychiatric conditions than other women with eating disorders.

The dominant perspective on bulimia nervosa is a cognitive behavioral one (Figure 1). Four points are emphasized in explaining this to patients. First, although dieting is a response to binge eating, it also maintains binge eating by both biological and psychological mechanisms. Second, compensatory purging encourages bingeing through a belief in its effectiveness at removing food for digestion. In other words, the barriers against overeating are removed because the food will not be absorbed. This is sometimes described as the reason why an individual initiated a binge–purge cycle of behavior. Third, extreme concern about body shape and weight promotes intense and rigid dieting, and maintains the eating problem. Fourth, extreme concern about shape and weight itself is commonly associated with negative self-evaluation manifested by cognitive thinking errors; perfectionist traits, and poor regulation of distress leading to negative effect. This cycle is

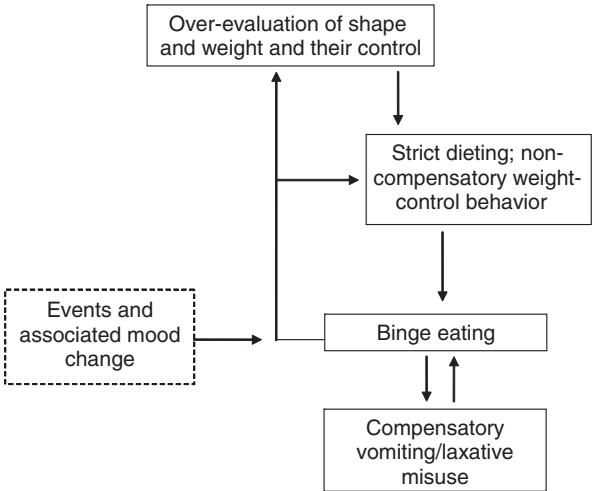


Figure 1 The cognitive behavioral view of bulimia nervosa. Reproduced from Fairburn CG (2008) *Cognitive Behaviour Therapy and Eating Disorders*. New York: Guilford Press.

self-perpetuating and habitual, and although there may be some immediate gratification (either physical or emotional) it is likely to lead to guilt and regret, which triggers further negative evaluation ensuring the cycle is maintained.

Groups at Risk

Like anorexia nervosa, bulimia nervosa is more common in women than in men at a female–male ratio for approximately 10:1. Exact prevalence is notoriously difficult to establish for eating disorders, and for bulimia nervosa in particular. Problems in this regard include the recency of the disorder, changing diagnostic criteria, and the secrecy and nonlife-threatening nature of the disorder preventing its routine appearance in clinical settings. Studies of American college students in the 1980s revealed up to 20% with bulimic symptoms. However, when such epidemiological studies investigate community samples and use interviews to follow up questionnaire surveys, the average point prevalence among young women using strict diagnostic criteria is approximately 1000 per 100 000, or 1.0%. In specific groups such as university students there may be more than twice this level of the disorder. Bulimia nervosa is rare in girls under 14 and the majority of cases are recognized between the ages of 18 and 25. Cases do present clinically in women in their late 20s and 30s, although they may have a long history of disordered eating.

The invisibility of bulimia nervosa is demonstrated by estimates of 1-year-period prevalence rates. These are calculated by adding together figures for point prevalence and annual incidence. The 1-year-period prevalence rates for bulimia nervosa per 100,000 young women have been reported as:

In the community	1500
In primary care	170
In specialist mental health care	87

These data indicate that only 11% of the community cases of bulimia nervosa are detected and of these, only half are

received by specialist services. Since the first clinical description in 1979, there has been a dramatic upsurge in the number of bulimia nervosa cases seen, and much greater than that for anorexia nervosa. Although improved detection may account for some of this increase, there is broad agreement that this represents a real increase in the number of women with bulimia nervosa.

Since women are most at risk, it is reasonable to ask why. Women are far more likely to diet than men and dieting is a behavior that places individuals at risk of developing bulimia nervosa. But the motivations for dieting are relevant to risk. Women diet more than men for several reasons. These are bound together as a socio-cultural perspective on bulimia, an approach that has become a powerful model for explaining who develops bulimia, and why. At the heart of this perspective are three issues: the importance of a thin body shape for women, the centrality of appearance in women's gender role, and the importance of appearance for societal success. The arguments and evidence to support this analysis are compelling.

Information on prevalence indicates that the average age of presentation with bulimia nervosa is older than that for anorexia nervosa. This may reflect the observation that bulimia nervosa can follow a period of anorexia nervosa or at least low weight. Developmental challenges and age-dependent life events are also seen as important. The developmental task of achieving a sense of identity during mid- and late-adolescence may be disrupted by relationship problems, peer or family difficulties, or events such as leaving home to go to college. The resultant erosion of self-esteem and perceived control can lead to problems with eating manifest as intensified dieting, or periods of overeating and weight gain. The disrupted pattern of eating that follows may be the early stage of the disorder.

Nutritional Findings

A key feature of bulimia nervosa is the extreme dietary restraint that is exhibited in between episodes of binge eating. Such behavior has been described as all or nothing, so that on a good day they may describe consuming a very low energy diet, whereas a bad day will consist of several episodes of uncontrolled eating. This will be accompanied by the purging behaviors previously described.

To sustain binge eating episodes, the person with bulimia nervosa may spend hundreds of pounds on food, selecting foods normally avoided during periods of dietary restraint, which are easy to eat and subsequently remove from the body. To them, it is this overeating that is seen as the basic problem, not the dietary restraint that precedes it. Yet, it is this dietary restraint that drives the disorder. When not binge eating, it is common for patients to avoid eating for long periods, with 80% reporting consumption of one meal a day or less. By restricting their intake, they will consume reduced energy foods, with a strong tendency to avoid fat.

It is often assumed that people with bulimia nervosa have good nutritional knowledge. Indeed, to the untrained eye, a diet history for a 'good day', consisting of foods, such as wholemeal bread, lots of vegetables and fruits, and skimmed

milk, can be interpreted as conforming to healthy eating guidelines. However, this is not the case. Such restrictive behavior may fail to achieve even half the recommended energy intake and consequently may be deficient in micronutrients. The anxiety experienced through consuming diet-breaking, 'unsafe' foods leads to the individual adopting extremely restricted diets between binges. Such intakes have been found to be lower in fat and higher in protein than the intakes of controls. People with bulimia nervosa also report feeling greater anxiety and guilt after eating foods they believed to be fattening.

Purging behaviors begin as a compensatory mechanism to offset episodes of binge eating. Consequently, it is a widely held belief that they are effective methods of weight control. However, the damage done to the body by these methods far exceeds any benefits in terms of weight. Any weight loss experienced is usually related to disruption of fluid balance rather than a loss of fat tissue. Furthermore, if self-induced vomiting is adopted, binges are likely to become more frequent and severe. If vomiting is prevented, the bulimic will consume significantly less food, thus maintaining the cycle previously described. Research has shown that vomiting fails to rid the body of all the food ingested. It has been estimated that only half the contents of the stomach are removed through vomiting, although this is variable and difficult to determine. Similarly, laxatives work on the system after food has been digested. One classic experiment looked at the amount of food energy lost through laxative abuse and found that, despite copious diarrhea, the amount of energy lost from the body was less than that found in the average chocolate bar.

What both laxative abuse and vomiting have in common is the depletion of fluid, leading to dehydration and electrolyte disturbances, particularly hypokalaemia (low potassium). In some cases, hypoglycaemia may develop as a response to fasting or binge eating and vomiting. In extreme cases, death may occur through cardiac arrest or gastrointestinal complications, such as esophageal or gastric rupture. Vomiting also leads to erosion of dental enamel, resulting in periodontal disease and an increased incidence of dental caries. Other effects of bulimia nervosa include menstrual irregularities, swelling of the salivary glands secondary to vomiting, and reflex constipation, which occurs as a consequence of laxative abuse and dehydration. Laxative abuse has also been found to cause steatorrhea and protein-losing enteropathy in some cases.

People with bulimia nervosa may have lower energy requirements. Using indirect calorimetry, it has been found that patients have a measured resting energy expenditure below that predicted by standard formulae, such as the Harris-Benedict equation. They also report consuming fewer kilocalories per kilogram body weight than control subjects. One explanation for this finding is that bingeing and purging may alter energy efficiency. These findings have implications for nutritional management, particularly in relation to the prescription of energy intakes. Therefore, diet plans need to be individualized with a dietitian to prevent binges and maintain a weight within the normal range. The dietitian will take into consideration the individual's appetite, weight- and binge-purge history, and their life style and will adjust the expected calories accordingly.

Dietary Management

Dietitians and registered nutritionists are increasingly involved in the treatment of bulimia nervosa. Their input is best utilized within a multidisciplinary team, ideally with some form of psychological intervention available. The National Institute for Health and Clinical Excellence (NICE) guidance is that dietitians or nutritionists should not be the only professional involved. Any professional working with eating disorders should be clear about what they can address and be aware of when it is appropriate to enlist other forms of help. Thus, nutritional intervention should aim to separate food from underlying issues, leaving these to be addressed by professionals more experienced in psychological techniques. Research suggests that nutritional intervention, alongside other psychological therapies, most notably cognitive behavior therapy (CBT), is an important part of treatment. In addition, training in psychological approaches such as CBT techniques is advised for dietitians and nutritionists involved in bulimia nervosa management.

The aim of dietary management of bulimia nervosa is to break the binge-purge cycle previously described. The individual should be informed about the problems of maintaining this cycle through dieting, and should be encouraged to stop dieting in an extreme way. They should also be educated about the damaging effects of vomiting and other purging behaviors. In some cases, this is enough to stop such behaviors. In others, this message should consistently be given to encourage them to work towards stopping these behaviors. An important part of breaking this cycle is to get the individual to monitor their intake through completing a food diary. In the example shown (Table 1), it can be seen how restricting intake earlier in the day can make the person more vulnerable to overeating later in the day. A food diary is a powerful cognitive tool that enables the individual to understand their eating behavior better.

Education is essential to ensure that the person understands why they are being asked to abandon what are some of the only coping mechanisms they have. They feel anxious that by giving up the pattern of dieting, binge eating, and purging they will gain excessive amounts of weight. These fears are very real and failure to address them with sensitivity can sabotage any attempt to control the disorder. This is particularly important when an individual has a history of overweight in the past. A detailed weight history should be carried out to include current, highest, lowest, and ideal weights and it should be stressed that recovery cannot be accomplished if the person is trying to maintain a weight below normal. Thus, those with a premorbid history of obesity may have to accept that they will need to reach a weight that is higher than they would like it to be. Weight stabilization should be an initial emphasis, particularly for those experiencing weight fluctuations. Initially, weight is likely to fluctuate through rehydration and repletion of glycogen stores. This effect should be explained to the individual to reduce unnecessary anxiety. They should also be discouraged from weighing themselves. If they must get weighed, this should be no more than once a week.

An important goal for nutritional management is to establish the individual on a regular pattern of eating. This is generally achieved by supporting them to monitor with increased awareness daily and weekly patterns of regular fluids and foods consumed. It is also worth getting them to compile a list of foods normally avoided or associated only with binges and to encourage the person to include them within their meal pattern, when they feel able to do so. Often, normal cues for hunger and satiety are disrupted through repeated cycles of binge and restrictive eating. So once the individual is mindful of these patterns, and can recognize their binge triggers (e.g., physical stimuli, distorted cognitions, negative affect), they can then be encouraged to plan a graded and gradual approach to eating a regular meal pattern. In turn, this should help the

Table 1 Example of a food diary

<i>Time</i>	<i>Food/drink eaten and amount</i>	<i>Binge/vomit/laxatives</i>	<i>Comment/feelings</i>
Breakfast	Nothing	–	Not hungry
Mid-morning	Cup of black coffee × 2	–	Need something to fill my stomach. Really busy at work so no time to eat
Lunch	2 Crispbreads, dry small tub of diet cottage cheese, 1 tomato, can of diet pop	–	Very hungry, feel as if I could eat more but must not
Mid-afternoon	Chocolate éclair	Vomited	Someone's birthday in the office so could not refuse. Feel really guilty and had to be sick
Evening meal	2 Dishes of blackcurrant cheesecake, a choc ice, 4 bowls of ice cream, 6 snack size chocolate bars, 5 cheese biscuits with butter and cheese, 5 slices of toast with butter and peanut butter, 2 packets of chocolate biscuits, 2 bowls of cereal, 1 packet of crisps and 1 chocolate and mint biscuit, 6 glasses of water	Binge!! Vomited and took 10 laxatives	Could not decide what to have for tea, so started on cheesecake. Could not stop this binge at any cost. I feel terrible
During evening			
Supper	–	–	Feel so terrible and bloated. Will have to cut back tomorrow

individual to identify hunger and fullness again. They should be gradually encouraged to eat regular meals and snacks and to maintain this pattern of eating even after a binge.

Each meal or snack should be based around carbohydrate, with moderate amounts of protein foods, vegetables, and fruits. They should be encouraged to include nondiet foods and to include foods containing fat. Whilst individuals are choosing from all food groups they should be educated on appropriate portion sizes to ensure a balanced meal plan (see the sample meal plan in **Table 2**). The amount of food needed to meet energy needs is greater than that needed to consume sufficient nutrients. Thus, consumption of some energy dense, less nutritious food should be encouraged. A minimum intake of 6.0 MJ (1500 Kcal) is usually an appropriate level to begin with, increasing to an intake corresponding to the estimated average requirement for women as recovery proceeds.

If the person is used to keeping their stomach empty, even a normal amount of food may seem excessive and may trigger the urge to vomit. They should be informed that stomach distension is a normal consequence of eating and reassured that they will get used to the feeling in time. Similarly, if someone has been abusing laxatives, they may suffer from constipation and should be encouraged to have soluble fiber along with plenty of fluids. Whilst reducing intake of insoluble fiber, symptoms of bloating and cramping are likely to emerge and may well continue after recovery.

Although it is important to give positive encouragement and feedback when working with individuals with bulimia nervosa, it should also be explained that relapse is a normal occurrence and should not be viewed so negatively that the individual feels a complete failure. Education on relapse prevention should thus be an important component of any treatment program. Furthermore, weight maintenance requires an individualized diet plan in accordance with lifestyle, weight history, and appetite.

Long-Term Prognosis

The evidence based on outcome is now sufficient to draw informed conclusions. Reviews conclude that 45% of patients show full recovery, 27% improve considerably, whereas 23% have a chronic protracted course. Around 1 in 10 still have the disorder 10 years after diagnosis. Cross-over to another eating disorder is observed in approximately 20% of cases. Studies report very low rates of spontaneous remission. Psychiatric comorbidity and symptom severity are indicators of poor prognosis.

In terms of treatment, several psychotherapies have shown their effectiveness in improving the symptoms of bulimia nervosa. Most evidence is available on cognitive behavioral therapy (CBT), and the outcome is generally impressive and replicable. The treatment, usually provided by clinical psychologists or psychiatrists, aims to modify both the disturbed eating habits and the extreme concerns about shape and weight (the core psychopathology). Consequently, it combines psychological and dietetic approaches to patient management. Given a treatment program of approximately 20 sessions over 5 months, between a half and two-thirds of patients make a full and lasting recovery. There is also

Table 2 Sample meal plan^a

Breakfast	Glass of fruit juice Bowl of cereal with milk, or 2 Slices of multigrain toast, spread with butter/margarine and marmalade/jam if desired
Mid-morning	Crackers and cheese or scone
Snack meal	Sandwiches made with 2 multigrain slices of bread, spread with butter/margarine and filled with lean meat, egg, or cheese, or Beans on toast, etc. Piece of fruit
Mid-afternoon	Yogurt or fruit
Main meal	Average helping of meat, chicken, fish, or vegetarian alternative Potatoes, boiled rice, or pasta equivalent to 2 exchanges Vegetables or salad
Sweet	Custard pot or mini chocolate bar
Supper	Yogurt or fruit

^aThis plan deliberately does not include specific portion sizes. However, some individuals may need the reassurance of a more detailed plan. The aim is to provide a minimum of 6.0 MJ (1500 kcal). However, the individual recovering from bulimia is likely to require approximately 1800 kcal per day.

promising evidence from new CBT associated mindfulness and acceptance based therapies. These enable individuals to increase their awareness of thoughts, feelings, and behaviors and to disentangle the automatic nature of these as well as any associated dysfunctional reactivity.

There is still uncertainty regarding prognostic indicators of treatment success. Patients with a less severe form of the disorder appear to do better in treatment. Self-help programs administered on their own or with modest support and encouragement from a nonspecialist therapist (guided self-help) may be of particular assistance to those in whom the disorder is less fully established. Conversely, those with childhood obesity, low self-esteem, or personality disturbance, appear to do worse. Importantly, there is no evidence that bulimia nervosa evolves over time into other psychiatric disorders, or of any persistent impairment in social functioning.

See also: Adolescents: Requirements for Growth and Optimal Health. Eating Disorders: Anorexia Nervosa; Binge Eating. Weight Management: Approaches

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Relevant Website

<http://www.b-eat.co.uk/Home>
UK Eating Disorders Association.

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Eggs have been a staple food in the human diet for thousands of years. From hunter-gatherers collecting eggs from the nests of wild birds to the domestication of fowl for a more reliable access to a supply of eggs to today's genetically selected birds and modern production facilities, eggs have long been recognized as a source of high-quality protein and other important nutrients. Over the years, eggs have become an essential ingredient in many cuisines due to their many functional properties such as water holding, emulsifying, and foaming.

An egg is a self-contained and self-sufficient embryonic-development chamber. At adequate temperature, the developing embryo utilizes the extensive range of essential nutrients in the egg for its growth and development. The necessary proteins, lipids, carbohydrates, vitamins, minerals, and functional nutrients are all sufficient for the transition from a fertilized cell to a newborn chick. The nutrient needs for an avian species are similar to human needs to make eggs an ideal source of nutrients for us. (The one essential human nutrient that eggs do not contain is ascorbic acid (vitamin C) because non-passerine birds have an active gulonolactone oxidase and synthesize ascorbic acid as needed.) This article summarizes the varied nutrient contributions eggs make to the human diet.

Egg Types

Although the majority of eggs consumed today are chicken eggs, a variety of eggs from different species of birds are

commercially available in different parts of the world, from the petite quail egg to the very large ostrich egg. The data presented in **Table 1** compare the caloric, protein, lipid, and cholesterol content per 100 g of eggs from various species. Eggs from commercial chickens differ from wild breeds in that they have a lower cholesterol and lipid content. This difference is thought to be the result of many years of genetic selection of breeds with increased feed to egg conversion ratios as well as faster rates of lay.

The commercial hen utilized in today's egg production has been selected for optimal feed conversion and egg production along with overall health, disease resistance, livability, and temperament. The majority of egg production is carried out using a battery cage system, which offers a high degree of control over environment, feed, water, hygiene, biosecurity, and egg collection. This system also facilitates mechanization. Other production systems include barn and free range (including organic production methods), which offer more freedom to birds, but often lead to higher disease and mortality rates in birds, and potentially increased susceptibility to bacterial contamination of the eggs.

Shifting dietary patterns in the population have resulted in compensatory changes in the egg industry. A major change has been the increased use of eggs going to egg products for the pre-prepared packaged food industry. In the USA more than 30% of the total egg production is used to make egg products, and egg product usage has been the most rapidly growing part of the industry accounting for the majority of the increased per

Table 1 Macronutrient composition of various raw eggs (per 100 g)

Nutrient	Species (average egg weight)				
	Quail (9 g)	Chicken (50 g)	Duck (70 g)	Turkey (79 g)	Goose (144 g)
Water (g)	74.35	76.15	70.83	72.50	70.43
Energy					
kJ	663	599	776	716	775
kcal	158	143	185	171	185
Protein (g)	13.05	12.56	12.81	13.68	13.87
Lipid (g)	11.09	9.51	13.77	11.88	13.27
SFA (g)	3.56	3.13	3.68	3.63	3.60
MUFA (g)	4.32	3.66	6.53	4.57	5.75
PUFA (g)	1.32	1.91	1.22	1.66	1.67
Cholesterol (mg)	844	372	884	933	852

Source: <http://www.nal.usda.gov/fnic/foodcomp>

capita egg consumption over the past decade. Another area of growth has been the special egg market. As consumers become more health conscience there has been an emphasis on functional components of foods that contribute to health and well-being. Eggs with enhanced nutrient benefits, especially with increased content of omega-3 fatty acids, are available worldwide. Eggs enriched with vitamin E, vitamin D, vitamin B₁₂, riboflavin, folate, selenium, and lutein are also available in limited markets. These nutrient enhancements are all achieved by modification of the hens' feed.

Egg Macronutrient and Micronutrient Content and Distribution

The levels of many nutrients in an egg are influenced by the age, and breed or strain of the hen as well as the season of the year and composition of the feed provided to the hen. Although most variations in nutrients are relatively minor, the fatty acid composition of egg lipids can be significantly altered by changes in the hen's diet. As noted above, the exact quantities of many vitamins and minerals in an egg are determined, in part, by the nutrients provided in the hen's diet.

Hen eggs contain 75.8% water, 12.6% protein, 9.9% lipid, and 1.7% vitamins, minerals, and a small amount of carbohydrates (Table 2). Eggs are classified in the protein food group and egg protein is one of the highest quality proteins available. Virtually all lipids found in eggs are contained in the yolk along with most of the vitamins and minerals. Of the small amount of carbohydrate (less than 1% by weight), half is found in the form of glycoprotein and the remainder as free glucose.

Egg Protein

Egg proteins, which are distributed in both yolk and white (albumen), are nutritionally complete proteins containing all of the essential amino acids. Egg protein has a 'chemical score' (essential amino acid level in a protein food divided by the level found in an 'ideal' protein food) of 100, a 'biological value' (a measure of how efficiently dietary protein is turned into body tissue) of 94, and the highest 'protein efficiency

ratio' (PER: ratio of grams of weight gain to grams of protein ingested in young rats) of any dietary protein.

The major proteins found in egg yolk include low-density lipoprotein (LDL), which constitutes 65%, high-density lipoprotein (HDL), phosvitin, and livetin. These proteins exist in a homogeneously emulsified fluid. Egg white is made up of some 40 different kinds of proteins. Ovalbumin is the major protein (54%) along with ovotransferrin (12%) and ovomucoid (11%). Other proteins of interest include flavoprotein, which binds riboflavin; avidin, which can bind and inactivate biotin; and lysozyme, which has lytic action against bacteria.

As shown in Table 3, egg protein contains substantial amounts of essential and nonessential amino acids. The first column shows the amount, in grams, of each of the amino acids in one large egg. The second column indicates the amount of each amino acid per 100 g of whole egg. The third column shows the dietary reference intake (DRI) for all of the essential amino acids (EAAs) per 50 g of total dietary protein, and the last column indicates the percentage of the DRI for each essential amino acid provided by one large egg. Although a large egg provides only some 3% of the energy in a 2000-kcal (8394 kJ) diet, it provides 11% of the protein needs. The EAAs in an egg contribute between 12% and 31% of the DRI for the various EAAs.

Egg Lipids

A large egg yolk contains 4.5 g of lipid consisting of triacylglycerides (65%), phospholipids (31%), and cholesterol (4%). Of the total phospholipids, phosphatidylcholine (lecithin) is the largest fraction which accounts for 26%. Phosphatidylethanolamine contributes another 4%. The fatty acid composition of egg yolk lipids is dependent on the fatty acid profile of the diet. The reported fatty acid profile of commercial eggs indicates that a large egg contains 1.55 g saturated fatty acids, 1.91 g monounsaturated fat, and 0.68 g polyunsaturated fatty acids. (Total fatty acids (4.14 g) does not equal total lipid (4.5 g) due to the glycerol moiety of triacylglycerides and phospholipids and the phosphorylated moieties of the phospholipids.) It has been reported that eggs contain less than 0.05 g of trans-fatty acids. Eggs yolks also contain cholesterol (211 mg per large egg) and the xanthophylls lutein and zeaxanthin. The lipid profile of a large egg is presented in Table 4.

Egg Vitamins

Eggs contain all essential vitamins except vitamin C, because the developing chick does not have a dietary requirement for this vitamin. As shown in Table 5, the yolk contains the majority of the water-soluble vitamins and 100% of the fat-soluble vitamins. Riboflavin and niacin are concentrated in the albumen. The riboflavin in the egg albumen is bound to flavoprotein in a 1:1 molar ratio. Eggs are one of the few natural sources of vitamins D and B₁₂. Egg vitamin E levels can be increased by seven- to 10-fold through dietary changes. Other than choline (see the section on Egg Choline), no single vitamin is found in very high quantity relative to its DRI value,

Table 2 Macronutrient distribution in raw chicken egg (per 50 g large egg)

	Whole egg	Egg albumin	Egg yolk
Weight (%)	100	66	34
Water (g)	38.1	28.9	8.9
Energy			
kJ	300	71	229
kcal	72	17	55
Protein (g)	6.28	3.60	2.70
Lipid (g)	4.75	0.06	4.51
Sugars (g)	0.36	0.24	0.61

Source: <http://www.nal.usda.gov/fnic/foodcomp>

Table 3 Amino acid content of a large egg

Amino acids	Grams per large egg	Grams per 100 g whole egg	DRI (g) EAA per 50 g protein d ⁻¹	Percentage EAA DRI per large egg
Alanine	0.37	0.74		
Arginine	0.41	0.82		
Aspartic acid	0.66	1.33		
Cystine ^a	0.14	0.27	1.25	12
Glutamic acid	0.84	1.67		
Glycine	0.22	0.43		
Histidine ^a	0.15	0.31	0.9	18
Isoleucine ^a	0.34	0.67	1.25	29
Leucine ^a	0.54	1.09	2.75	21
Lysine ^a	0.46	0.91	2.55	18
Methionine ^a	0.19	0.38	1.25	17
Phenylalanine ^a	0.34	0.68	2.35	15
Proline	0.26	0.51		
Serine	0.49	0.97		
Threonine ^a	0.28	0.56	1.35	24
Tryptophan ^a	0.08	0.17	0.35	31
Tyrosine ^a	0.25	0.50	2.35	12
Valine ^a	0.43	0.86	1.6	27

^aEAA are not synthesized by the body and must be consumed in foods; therefore, only EAA have a DRI value.

Source: <http://www.nal.usda.gov/fnic/foodcomp>

Table 4 Egg yolk lipid profile per large egg

Lipids	Amount
Fatty acids, total saturated	1.62 g
8:0–14:0	0.02 g
16:0	1.17 g
18:0	0.41 g
20:0–24:0	0.01 g
Fatty acids, total monounsaturated	1.99 g
16:1	0.16 g
18:1	1.82 g
20:1	0.02 g
Fatty acids, total polyunsaturated	0.72 g
18:2	0.60 g
18:3	0.02 g
20:4	0.07 g
20:5–22:6 n-3	0.02 g
Cholesterol	184 mg
Carotene, beta	15 µg
Carotene, alpha	6 µg
Cryptoxanthin, beta	6 µg
Lutein plus zeaxanthin	186 µg

Source: <http://www.nal.usda.gov/fnic/foodcomp>

however, it is the wide spectrum of vitamins present that make eggs nutritionally rich.

Egg Minerals

Eggs contain small amounts of all minerals essential for life. Of particular importance is iron found in egg yolks. Research evaluating the plasma iron and transferrin saturation in 6- to 12-month-old infants indicated that those who ate egg yolks had a better iron status than those who did not eat egg yolks.

Table 5 Vitamin content per large egg

Vitamin	Whole	Albumen	Yolk
Thiamin	0.02 mg	<0.01	0.03 mg
Riboflavin	0.23 mg	0.15	0.09 mg
Niacin	0.04 mg	0.04	<0.01 mg
Pantothenic acid	0.77 mg	0.06	0.51 mg
Vitamin B ₆	0.09 mg	<0.01	0.06 mg
Folate, total	24 µg	0	25 µg
Vitamin B ₁₂	0.45 µg	0.03	0.33 µg
Vitamin A	270 IU	0	245 IU
Choline	125. mg	0	116 mg
Retinol	80 µg	0	63 µg
Vitamin E	0.5 mg	0	0.44 mg
Vitamin D	41 IU	0	37 IU
Vitamin K	0.1 µg	0	0.1 µg

Source: <http://www.nal.usda.gov/fnic/foodcomp>

The study indicated that egg yolks can be a source of iron in a weaning diet for breast-fed and formula-fed infants without increasing blood antibodies to egg yolk proteins.

In addition to iron, eggs also contain calcium, phosphorus, sodium, potassium, magnesium, zinc, copper, and manganese (Table 6). Egg yolks also contain iodine (25 µg per large egg), which can be increased two- to three-fold by inclusion of an iodine source in the feed. Egg selenium content can also be increased seven- to nine-fold by dietary manipulations.

Egg Choline

Choline was established as an essential nutrient in 1999 with Recommended Daily Intake (RDI) of 550 mg d⁻¹ for men and 450 mg d⁻¹ for women. The RDI for choline increases during

Table 6 Mineral content per large egg

Mineral	Whole	Albumen	Yolk
Calcium, Ca (mg)	28	2	22
Iron, Fe (mg)	0.88	0.03	0.46
Magnesium, Mg (mg)	6	4	1
Phosphorus, P (mg)	99	5	66
Potassium, K (mg)	69	54	19
Sodium, Na (mg)	71	55	8
Zinc, Zn (mg)	0.65	0.01	0.39
Copper, Cu (mg)	0.04	0.01	0.01
Manganese, Mn (mg)	0.02	<0.01	0.01
Selenium, Se (μg)	15.3	6.6	9.5

Source: <http://www.nal.usda.gov/fnic/foodcomp>

pregnancy and lactation due to the high rate of choline transfer from the mother to the fetus and into breast milk. Animal studies indicate that choline plays an essential role in brain development, especially development of the memory centers, of the fetus and newborn. Recent studies have indicated that higher intakes of choline are associated with reduced risk of neural tube defects, reductions in plasma markers of inflammation, and decreased breast cancer risk. Studies have also shown that in the USA less than 10% of adult men and women attain the recommended AI for choline, including only 1 in 10 pregnant females. Egg yolk lecithin (phosphatidylcholine) is an excellent source of dietary choline, providing 125 mg of choline per large egg, and adding an egg a day to the diet can significantly increase the number of adults with adequate intakes.

Egg Carotenes

Egg yolk contains two important xanthophylls (carotenes that contain an alcohol group) which have important health benefits – lutein and zeaxanthin. It is estimated that a large egg has 0.33 mg of lutein plus zeaxanthin; however, the content of these xanthophylls is totally dependent on the type of feed provided to the hens. Egg yolk lutein levels can be increased five- to 10-fold through modification of the feed with marigold extract or purified lutein. An indicator of the lutein plus zeaxanthin content is the color of the yolk, the darker yellow-orange the yolk, the higher is the xanthophil content. Studies have shown that egg yolk xanthophylls have a higher bioavailability as compared with those from plant sources, probably due to the lipid matrix of the egg yolk facilitating greater absorption. This increased bioavailability results in significant increases in plasma levels of lutein and zeaxanthin as well as increased macular pigment densities with egg feeding.

Egg Cholesterol

Eggs are one of the richest sources of dietary cholesterol providing 215 mg per large egg. In the 1960s and 1970s, the simplistic view that dietary cholesterol equals blood cholesterol resulted in the belief that eggs were a major contributor

to hypercholesterolemia and the associated risk of cardiovascular disease. Although there remains some controversy regarding the role of dietary cholesterol in determining blood cholesterol levels, the majority of studies have shown that (1) saturated fat, not dietary cholesterol, is the major dietary determinant of plasma cholesterol levels, and eggs contain 1.5 g of saturated fat and (2) neither dietary cholesterol nor egg consumption are significantly related to cardiovascular disease incidence. Across cultures, those countries with the highest egg consumption actually have the lowest rates of cardiovascular disease mortality; and within-population studies have not shown a correlation between egg intake and either plasma cholesterol levels or heart disease incidence. A 1999 study of more than 117 000 men and women followed for 8–14 years showed that the risk for coronary heart disease was the same whether the study subjects consumed less than one egg a week or more than one egg a day. Numerous other epidemiological studies have failed to find a significant relationship between egg intake and cardiovascular disease risk.

Clinical studies show that dietary cholesterol does have a small influence on plasma cholesterol levels. Adding one egg per day to the diet would, on average, increase plasma total cholesterol levels approximately to 5 mg dl⁻¹. It is important to note, however, that the increase occurs in both the atherogenic LDL cholesterol fraction (4 mg dl⁻¹) and the anti-atherogenic HDL cholesterol fraction (1 mg dl⁻¹) resulting in virtually no change in the LDL:HDL ratio, a major determinant of cardiovascular disease risk. The plasma lipoprotein cholesterol response to egg feeding, especially any changes in the LDL:HDL ratio, varies depending on the individual and the baseline plasma lipoprotein cholesterol profile. As shown in Table 7, adding one egg a day to the diets of three hypothetical patients with different plasma lipid profiles results in very different effects on the LDL:HDL ratio. For the individual at low risk, there is a greater effect than for a person at high risk, yet in all cases the effect is quantitatively minor and would have little impact on their heart disease risk profile. Overall, results from clinical studies indicate that egg feeding has little if any effect on cardiovascular disease risk. This is consistent with the results from a number of epidemiological studies.

A common consumer misperception is that eggs from some breeds of birds have low or no cholesterol. For example, eggs from Araucana chickens, a South American breed that lays a blue-green egg, have been promoted as a low-cholesterol egg when in fact the cholesterol content of these eggs is 25% higher than that of commercial eggs. The amount of cholesterol in an egg is set by the developmental needs of the embryo and has proven to be very difficult to substantially change without resorting to hypocholesterolemic drug usage.

Undue concerns regarding egg cholesterol content resulted in a steady decline in egg consumption during the 1970s, 1980s, and early 1990s, and restriction of this important and affordable source of high-quality protein and other nutrients could have had negative effects on the nutritional well-being of many nutritionally 'at-risk' populations. Per capita egg consumption has been increasing over the past decade in North America, Central America, and Asia; has remained relatively steady in South America and Africa; and has been

Table 7 Changes in plasma lipoprotein cholesterol levels with addition of one large egg per day to the diet

	Cholesterol (mg dl ⁻¹)		LDL:HDL ratio (% change)
	LDL	HDL	
Baseline	125	50	2.50
+ 1 egg/day	129	51	2.53 (+ 1.2)
Baseline	150	50	3.00
+ 1 egg/day	154	51	3.02 (+ 0.6)
Baseline	175	50	3.50
+ 1 egg/day	179	51	3.51 (+ 0.3)

falling in Europe and Oceania. Overall, per capita world egg consumption has been slowly increasing over the past decade in part due to the change in attitude regarding dietary cholesterol health concerns.

Allergenic Aspects of Egg Proteins

Eggs are one of the most common causes of food allergies in infants and young children. Although the majority of egg allergies are caused by egg white protein, proteins in both the egg white and yolk are associated with allergies. The egg white contains 50% ovalbumin, which is the major allergen. Other egg white allergenic proteins are ovomucoid, ovotransferrin, and lysozyme. Most egg allergies in young children are outgrown by age 5 years following an elimination diet.

Owing to the allergenicity of egg proteins, it is advised not to feed egg yolks to infants younger than 6 months of age and to wait until children are 12 months of age to feed them egg whites. When feeding egg yolks to children between the ages of 6 and 12 months, the eggs should be prepared so that the egg white can be completely removed such as in hardcooked eggs.

Specialty Eggs

There is an increasing interest worldwide in production and marketing of specialty eggs with enhanced nutrient benefits. The nutrient composition of an egg can be significantly modified by the composition of the feed. Commercially available nutrient-enhanced eggs contain increased amounts of omega-3 fatty acids, vitamin E, selenium, and lutein. Other enhancements include increased content of vitamin D and the B vitamins as well as incorporation of conjugated linoleic acid (CLA).

Omega-3 fatty acids: The fatty acid content of eggs is easily and significantly affected by the fatty acid profile of the hen's feed. The omega-3 fatty acid content can be increased in the egg by feeding the hens a source of omega-3 fatty acids. In some countries, fish meal is used as a source of omega-3 fatty acids but this can result in eggs with a fishy odor and taste. Marine algae is another source of omega-3 fatty acid resulting in higher concentrations of eicosapentanoic acid

(EPA) and docosahexanoic acid (DHA) in egg yolks. Flax-seed oil is also used as a source of omega-3 fatty acids and results in increased levels of α -linolenic acid in egg yolks. The relative proportions of DHA to α -linolenic acid can be controlled by feeding a mixture of flax seed and marine algae. It is possible to attain levels as high as 200 mg of omega-3 fatty acids per large egg.

Although omega-3 fatty acid levels in eggs are well below levels found in fish, such as salmon or tuna, eggs can still be an important source of omega-3 fatty acids to the diet. For people who cannot eat fish, eggs with higher levels of omega-3 fatty acids can be an important way to include these beneficial fatty acids in the diet.

Other nutrients in specialty eggs: By altering the content in the feed, other nutrients in eggs can be enhanced such as lutein, vitamin E, and selenium. Vitamin E is usually added to the feed to serve as an antioxidant when the polyunsaturated fatty acids are increased. Vitamin E levels in eggs have been increased as much as 25-fold. The increased vitamin E in these eggs can provide an additional natural source of this important fat-soluble vitamin. Lutein (a xanthophyll) can also be increased in eggs by increasing the amount in feed, usually as marigold extract. Lutein is deposited in the egg yolk at levels as high as 2 mg per large egg and the human body readily absorbs lutein from the egg phospholipid matrix. Nutritional needs for selenium vary widely due to differences in the selenium content of regional soils. Egg selenium levels can be increased five- to eight-fold with addition of an organo-selenium source to the feed.

Egg Food Safety

Eggs pose a unique food safety problem because eggs can be contaminated internally with the pathogenic bacteria *Salmonella enterica* serovar Enteritidis (SE). If SE infects the reproductive tract of laying hens, it can be deposited in the egg during formation. In addition to internal egg contamination by SE, egg shells can be contaminated with a number of microorganisms. Caution is required when selecting eggs for consumption. Only clean eggs should be consumed. Vaccination of hens against *S. enterica*, temperature control, proper handling, and cooking are all important control measures to reduce the incidence of SE illness.

When SE internally contaminates an egg, it is thought that the SE is deposited at the yolk membrane in the egg white. The integrity of the vitelline membrane is very important to prevent SE from entering the yolk where it could grow very rapidly due to the nutrient-rich environment. The egg white has natural antimicrobial compounds, such as lysozyme, that help prevent SE from growing in the egg white.

In naturally contaminated eggs, scientists have documented that approximately 10–100 cells of SE may be deposited in an egg. The bacterial cell count will remain low unless the egg is exposed to temperatures that would allow rapid growth of SE or the vitelline membrane breaks down. Even when flocks are infected with SE, only a small percentage of the eggs produced will contain SE. Properly cooking the eggs to a temperature of 63 °C for 3 min, 65 °C for 1 min, or 70 °C for 1 s will destroy SE if present in an egg.

Role of Eggs in the Diet

The nutritional contributions of eggs to a diet are determined by the per capita consumption profile of a given country. In countries like Japan, with the highest per capita egg consumption, eggs play an important role as a source of nutrients whereas in countries like India, with very low per capita consumption values, its role is minor. Worldwide there are many different misperceptions and myths regarding eggs that influence consumption patterns (Table 8).

Eggs are a nutrient-dense source of many essential amino acids, vitamins, and minerals and, as shown in Figure 1, eggs contribute a number of nutrients to the American diet in amounts proportionally greater than their caloric contributions. While providing only 1.3% of the calories, they provide nine different nutrients ranging in amounts from 2 to 6% of the DRI. Such nutrient-dense foods can play an important role in diets of seniors who have decreased caloric

intakes as well as weight-reduction/weight-maintenance diets. Studies have shown that egg intake has a significant effect on satiety beyond what would be predicted from its protein and fat content. Egg intake slows the rate of gastric emptying resulting in a flatter blood glucose response as well as a lower insulin response. The effects on gastric emptying appear to be related to effects of egg yolk (not white) intake on secretion of cholecystokinin and gastric inhibitory peptide.

Summary

For nutritionally vulnerable populations including the poor, the very young, the very old, pregnant women, and those suffering from chronic diseases, eggs are an affordable, nutrient-dense source of high-quality protein important for maintaining health and facilitating recovery. Pregnancy is an

Table 8 Common myths and misperceptions about eggs

Myth	Fact
Brown eggs are healthier than white eggs. Fertile eggs have less or no cholesterol. Free-range eggs have more nutritional value than commercial eggs.	There are no substantive nutritional differences between white eggs, brown eggs, fertile eggs, and free-range eggs. Nutritional content is determined by the hen's diet.
Eggs contain the hormones they give the hen to force her to lay eggs when there isn't a rooster around.	Hens are not given hormones to produce eggs in the absence of a rooster. Hens lay eggs with or without a rooster. There are no harmful hormones in eggs.
Eggs contain the antibiotics they give hens to increase the number of eggs they'll lay.	Antibiotics have no effect on egg production and there is no value in using them unless needed for therapeutic reasons.
Eggs in the store are a mixture of fertile and non-fertile eggs. That stringy stuff is the embryo.	Commercial eggs are not fertile (can be included in a lactoovo- or ovo-vegetarian diet. That stringy stuff (chalaza) is an egg protein that anchors the yolk in the center of the egg.
Eating eggs can cause liver problems.	No study has ever shown that eggs cause liver problems.
Eggs with blood/meat spots are fertilized or are bad.	The tiny meat/blood spot is caused by the rupture of a blood vessel during egg formation. It has no adverse effect on the egg, and can either be removed or eaten.
If an egg floats in water it's bad.	As an egg ages the air sack expands and an egg will stand on end in water. This is not a sign that the egg is bad.

Source: www.aeb.org/eggencyclopedia/main_frame_page.html

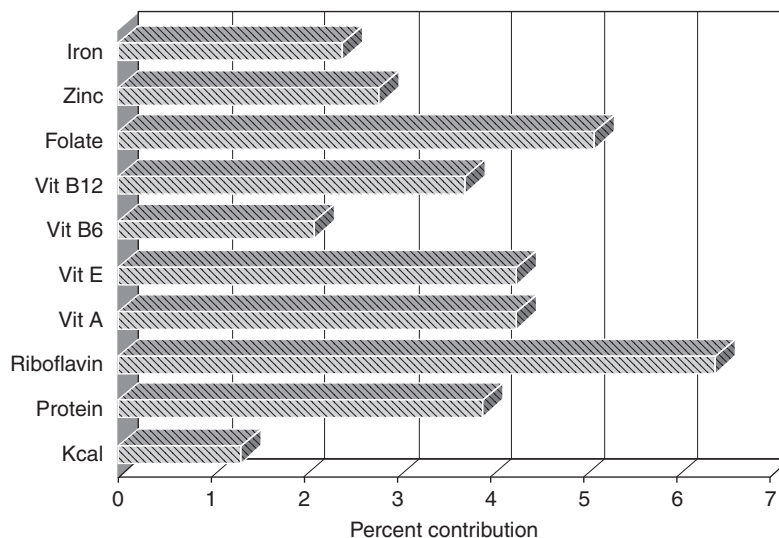


Figure 1 Nutrient contributions of eggs to the American diet.

especially important time to optimize intake of high-quality protein as well as other essential nutrients to reduce the risk of low birth weight and the associated development of chronic diseases and other health problems during the future adult years of the infant. Eggs also serve as an important dietary source of choline during pregnancy and lactation to provide choline to the fetus and newborn for brain development. In addition, eggs provide a satiety effect which, in view of the global problem of obesity, can be a valuable addition to weight-loss and weight-maintenance programs. For these various populations, from infant to aged, there are a multitude of health reasons to include nutrient-dense eggs as part of the diet, and for many of these groups it can be economically feasible.

The high-quality protein, many nutritional components, low caloric content, affordability, blandness, ease of digestibility, and satiety response are all characteristics that make eggs ideal for inclusion in the diet across the lifespan, from very young to very old, and under all conditions, health or convalescence. As noted in the Dietary Guidelines 2010, “Independent of other dietary factors, evidence suggests that one egg (i.e., egg yolk) per day does not result in increased blood cholesterol levels, nor does it increase the risk of cardiovascular disease in healthy people.”

See also: Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. **Phytochemicals:** Classification and Occurrence. **Pregnancy:** Nutrient Requirements. **Protein:** Quality and Sources; Requirements and Role in Diet. **Protein Digestion and Bioavailability**

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Relevant Websites

www.aeb.org

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www.enc-online.org

Egg Nutrition Center and related links to egg industry and health agency websites.

ELECTROLYTES

Acid–Base Balance

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Glossary

Acidemia The presence of a low blood pH.

Acidosis The clinical term for a state of excess acid in the blood, further defined by its etiology, either metabolic or respiratory in origin.

Alkalemia The presence of an elevated blood pH.

Alkalosis The clinical term for a state of excess alkali in the blood, further defined by its etiology, either metabolic or respiratory in origin.

Buffer Solution of a salt of a weak acid, which is able to bind hydrogen ions.

Renal tubular acidosis A group of conditions where metabolic acidosis results from diminished tubular secretion of hydrogen ions by the kidney.

Maintenance of cellular and extracellular pH (hydrogen ion concentration) is essential to life, in view of the exquisite pH dependence of processes such as enzyme function. Hydrogen ions (H^+) are generated by cellular metabolism and, to a lesser extent by the ingestion of acids in the diet. Acid–base homeostasis regulates pH between 7.36 and 7.44 (corresponding to a $[H^+]$ of 36–44 nmol l^{-1}) in extracellular fluids, such as blood, whereas intracellular pH is more acidic (pH 6.3–7.4) depending on individual organs and circumstances. The pH of subcellular organelles may be yet more acidic, reflecting their physiological function (e.g., lysosomes). Blood and extracellular fluid pH are tightly regulated by the presence of buffer systems, which effect change as a consequence of acid load. These buffer systems, both extracellular and intracellular, include hemoglobin, other proteins, phosphate, and bicarbonate – the latter being most important. However, the acid load must ultimately be eliminated by subsequent excretion of volatile acids by the lungs and fixed acids by the kidney.

Definitions, Acids, Bases, and Buffers

pH

The term pH is an expression of hydrogen ion (H^+) concentration (such that pH and H^+ are inversely related)(eqn [1]).

$$pH = -\log_{10}[H^+] \quad [1]$$

Acids and Bases

Acids are substances that dissociate to donate H^+ (eqn [2]); the stronger the acid, the more readily it dissociates. The

dissociation constant (pK_a) is the pH at which 50% of the acid is dissociated. At pH values greater than pK_a more H^+ will dissociate; the lower the pK_a , the stronger the acid. A base is a substance that accepts hydrogen ions. In the following text, ‘fixed acid’ is used to describe formed acid, and ‘volatile acid’ is used to describe the potential acid load imposed by carbon dioxide (CO_2). Where ‘A’ represents an acid, the following applies:

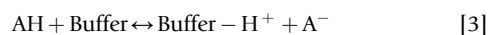


The importance of this relationship in physiological terms is that because the pK_a of most organic acids is much lower than the pH of extracellular fluids, most organic acids exist in a dissociated state – as acid anion salts – the free H^+ being buffered. In urine, where the minimum achievable pH is approximately 5, most strong acids (with a pK_a below this value) will be in a dissociated state, necessitating the excretion of H^+ together with urinary buffers.

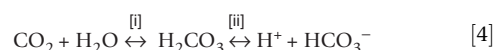
Acidosis is the term used to describe conditions where pH is low and those where pH would be low were it not appropriately buffered; similarly alkalosis is the term used for a high pH and for a potentially elevated pH that has been appropriately buffered. Acidemia and alkalemia reflect low or elevated blood pH. It is common to describe an acidosis/alkalosis as respiratory or metabolic depending on causation.

Buffers

Buffering is the ability of weak acids, present in excess, to accept H^+ donated from strong acids, thus limiting changes in free H^+ concentrations and pH changes (eqn [3]):



The principal buffer system in blood (and other extracellular fluids) is based on bicarbonate (HCO_3^-), accounting for approximately 70% of the buffering capacity of blood. In blood, CO_2 (the major product of oxidative metabolism) reacts with water, in the presence of the enzyme carbonic anhydrase (CA), to form carbonic acid (H_2CO_3). This compound is relatively unstable and tends to dissociate (eqn [4]). The rate of formation of carbonic acid is dependent on the concentration of carbon dioxide and the rate constant of reaction [i]; the dissociation of carbonic acid to generate H^+ and HCO_3^- is governed by the rate constant of reaction [ii]. In practice, these two reactions can be combined, and the relationship between pH ($[\text{H}^+]$), carbon dioxide, and bicarbonate is described by a single equation – the Henderson–Hasselbalch eqn [5]:



$$\text{pH} = 6.1 + \log_{10}([\text{HCO}_3^-]/K.S.\text{PCO}_2) \quad [5]$$

pH reflects $-\log [\text{H}^+]$; 6.1 is the value of $-\log (1/K)$, K being the equilibrium constant describing the overall eqn [4]; PCO_2 is the partial pressure of carbon dioxide; S is the solubility constant for carbon dioxide. $K.S.$ is constant and equal to 0.225 (when PCO_2 is measured in kPa , 0.03 when PCO_2 is measured in mmHg). Table 1 shows the normal range for these parameters in humans.

From eqn [5] the principles of acid–base balance can be appreciated. Acidification may occur in two ways: either by production of CO_2 or by the consumption of bicarbonate (as part of buffering of fixed acid). The excretion of CO_2 is controlled by the lungs, and excretion of fixed acid takes place in the kidney.

The Henderson–Hasselbalch equation allows basic understanding of acid–base physiology, in health and disease but has limitations. In the presence of either metabolic or respiratory derangement of acid–base homeostasis it does not allow assessment of the severity of the metabolic derangement, analogous to the respiratory component. It also does not assess the influence of other acids other than carbonic acid. For this reason some authors propose analysis of acid–base physiology using a more complex method based on the principals of physical chemistry. This method proposes that all changes in pH of plasma can be explained in terms of relative concentrations of CO_2 , relative electrolyte, and weak acid. This concept allows more rigorous interrogation of acid–base disorders and may permit greater insight into their pathophysiology and management.

Table 1 Normal ranges

Variable	Normal range
pH	7.36–7.44
Hydrogen ion (H^+)	37–44 nmol l^{-1}
Partial pressure CO_2 (PCO_2)	34–46 mm Hg 4.5–6.1 kPa
Bicarbonate HCO_3^-	24–30 mmol l^{-1}

Maintenance of the pH of Blood and Extracellular Fluids

Acid and Alkali Load

The sources of acids (and alkalis) are the diet and metabolism. The major potential source of acid is CO_2 ('volatile acid') (eqn [4]), generated by oxidative metabolism; a total of 12–20 moles of CO_2 are produced daily. Other metabolic products include lactic acid, other organic acids, and urea, the synthesis of which produces H^+ . Because of its role in the metabolism of lactic acid and in the synthesis of urea, the liver plays a major role in acid–base homeostasis that is often not appreciated.

The lungs excrete volatile acid, CO_2 , whereas the breakdown of sulfur- and phosphorus-containing compounds are 'fixed' acids. For example, cysteine or methionine metabolism leads to production of sulfuric and phosphoric acid (H_2SO_4 , H_3PO_4), and metabolism of other amino acids (lysine, arginine, and histidine) to hydrochloric acid (HCl). In contrast, organic acids (e.g., lactate, fatty acids) may be completely metabolized to CO_2 and H_2O and thus excreted by the lungs. In addition, absorption of dietary phosphate and fecal loss of bicarbonate represent an additional acid load. In total, the net acid load of fixed acid is approximately $1 \text{ mmol kg}^{-1} \text{ day}^{-1}$ and may be increased by a high protein intake or reduced by a strict vegetarian diet.

There is surprisingly little information on the direct contributions of individual foods to the acid burden. However, this source of dietary acid is of increasing importance in view of weight reduction diets (e.g., the Atkins diet). The major acids contained in food are citric acid (in fruit), acetic acid (as a preservative, pickles, vinegar), lactic acid (yogurt, fermented foods), malic acid (fruit), oxalic acid (vegetables, that contain smaller amounts of citric and malic acids), and tartaric acid (wine). Oxalic acid precipitates in the gut to form calcium salts, excreted in the stool, and little is absorbed. The others are absorbed but quickly metabolized and present an acid burden in the form of carbon dioxide. The largest source of fixed acid comes from the metabolism of amino acids (particularly those from animal proteins). The significance of this source of acid is readily demonstrated in patients taking a high-protein diet – particularly one rich in animal protein – who have increased urinary acid excretion. Based on studies on the relationship between diet, renal excretion of acid, and urine pH it is theoretically feasible to quantify urinary acid excretion for individual foods. However due to daily variation in diet (and therefore absence of a metabolic steady state) and inherent variation in the composition of foodstuffs, it has not been possible to estimate accurately the effects of diet on renal acid–base metabolism in circumstances reflective of normal dietary intake.

Alkalis are often prescribed to compensate metabolic acidosis and have often been used to neutralize gastric acidity. Milk and milk products are also alkaline but seldom cause any disturbance, unless consumed in great excess. Excessive consumption of milk or alkali is now rarely seen.

Regulation

Blood and extracellular fluid pH is regulated at three levels: (1) buffering within the blood and tissues; (2) excretion of

Table 2 Buffering and acid–base regulation

Mechanism	Site	Role (time)
Protein (e.g., Hb)	Cell	Rapid binding of H^+ (s)
Bicarbonate buffer	ECF	Buffering of H^+ (s)
Ventilation	Lungs	Excretion of CO_2 , respiratory compensation (h)
Fixed acid excretion	Kidney	Excretion of H^+ , reabsorption and regeneration of bicarbonate, renal compensation (h–days)

volatile acids by the lungs; (3) excretion of fixed acids by the kidney. Although buffering is immediate, respiratory compensation occurs over minutes to hours and renal excretion can take from hours to days (Table 2).

Blood/Extracellular Fluid

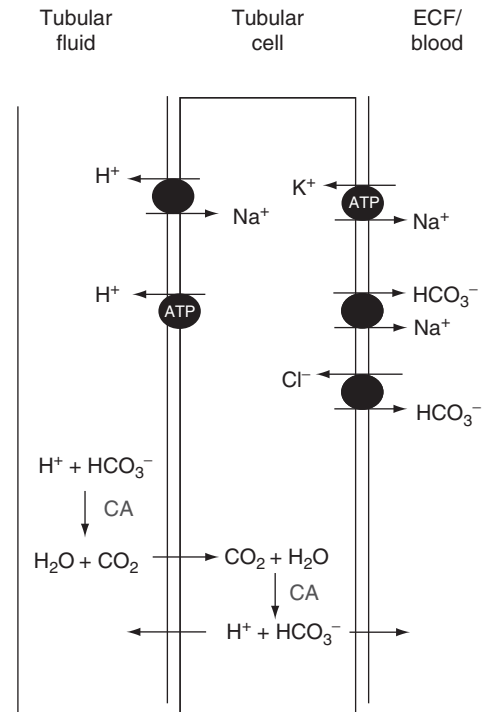
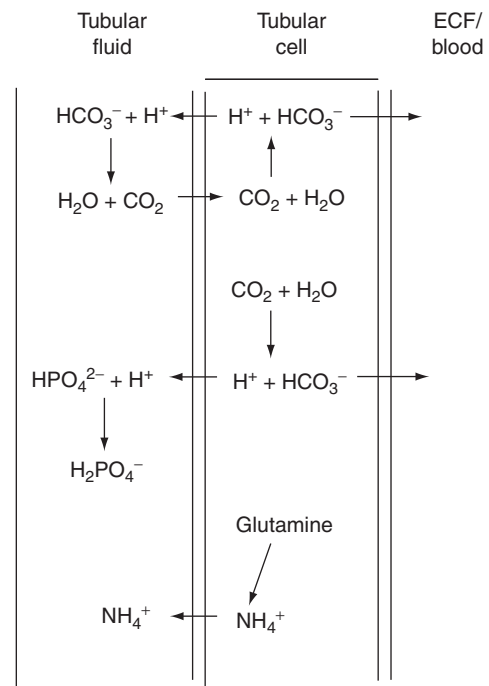
Immediate buffering of an acid load, for example by release of lactic acid and CO_2 by anaerobic and aerobic metabolism in exercising muscle, occurs in blood and other extracellular fluids. Together they contain approximately 350 mmol of bicarbonate buffer. 60–70% of the buffering capacity of blood is accounted for by the bicarbonate buffer system; 20–30% is dependent on direct binding to hemoglobin and to other proteins, including plasma proteins. Blood is in equilibrium with extracellular fluid H^+ . H^+ ions move across cell membranes depending on concentration and charge, thus H^+ ions may move into cells in exchange for K^+ (and to a lesser extent Na^+ ions) when extracellular H^+ is increased. Hence acidosis is often accompanied by increased serum K^+ , and alkalosis by low K^+ . Large amounts of H^+ may be ‘buffered’ by direct binding to proteins within cells and tissues, particularly bone where H^+ are also buffered by calcium salts, such as apatite.

Lungs

The lungs excrete volatile acid (CO_2) by changes in the rate and volume of respiration. This is regulated by respiratory centers in the brainstem, that respond to changes in the pH of cerebrospinal fluid (that is in equilibrium with extracellular fluids elsewhere in the body), and signals from chemoreceptors in the carotid and aortic bodies that are responsive to changes in pH and PCO_2 of arterial blood (increased PCO_2 or reduced pH cause an increase in respiration). Thus, acidosis leads to an increase in respiratory rate and ventilatory volume (the pattern in severe acidosis being described as Kussmaul breathing) and alkalosis to the opposite effect.

Kidneys

The kidneys have two major roles in acid–base homeostasis: the recovery of filtered bicarbonate and generation of new bicarbonate; and excretion of fixed acid (Figures 1 and 2; eqn [5]). Blood is filtered in the glomeruli and the glomerular filtrate is subsequently modified in the renal tubules so that the final urine volume is less than 1% of the glomerular filtrate volume. Plasma bicarbonate concentration is approximately

**Figure 1** Recovery of filtered bicarbonate in the proximal convoluted tubule.**Figure 2** Excretion of acid in the collecting duct.

25 mmol l^{-1} and glomerular filtration rate (GFR) 100 ml min^{-1} , thus 3600 mmol of bicarbonate must be reabsorbed daily. Bicarbonate reabsorption mainly occurs in the proximal convoluted tubule (PCT). 85% of filtered bicarbonate is reabsorbed here, 10% in the thick ascending limb of the Loop of

Henle, the remainder being titrated to regulate total acid excretion, in the collecting duct (Figures 1 and 2). As shown, different mechanisms are involved at each tubular site. The enzyme carbonic anhydrase, on the luminal brush border of tubular cells, catalyzes the combination of filtered bicarbonate with H^+ , secreted by the apical H^+ -ATPase and Na^+/H^+ exchangers on tubular cells, to generate CO_2 . CO_2 then diffuses into the tubular cells down its concentration gradient. Within the tubular cell, carbonic anhydrase catalyzes the reverse reaction, which generates H^+ and HCO_3^- . Hydrogen ions are then recycled to the tubular lumen and bicarbonate is secreted into the extracellular fluid by basolateral anion exchangers or $\text{Na}^+ - \text{HCO}_3^-$, cotransporters. Tubular cells are also exposed to CO_2 in the extracellular fluid and will continue to generate H^+ even in the absence of filtered bicarbonate. This H^+ is then buffered by other buffers in the glomerular filtrate including HPO_4^{2-} and, to a lesser extent, creatinine. Strong acids, (e.g., H_2SO_4) with low pK_a values will dissociate in the urine (pH range 5–8), and be buffered whereas weaker acids may be excreted intact. In the presence of alkalosis, cellular transporters and their function may be reversed so that H^+ secretion occurs on the basolateral membrane and HCO_3^- on the brush border of tubular cells resulting in alkaline urine.

Classically, the final mechanism by which the kidney can excrete H^+ is by generation of ammonium (NH_4^{2+}) from metabolism of glutamine by glutaminase (Figure 2), a process that is stimulated by low pH and increased PCO_2 . Excretion of H^+ as part of ammonium accounts for approximately 70 mmol day^{-1} , increasing several-fold (albeit over a period of days) in the face of an acid load. Whether this is truly a urinary buffer is the subject of some debate as ammonium (NH_4^{2+}) is generated directly from glutamine rather than accepting additional protons. There are alternative mechanisms for the role of NH_4^+ in overall acid–base homeostasis that involve the liver. After being pumped into the glomerular filtrate, NH_4^+ may be reabsorbed by the tubule and used by the liver to synthesize urea, generating free H^+ ions. Thus, there is no net loss of H^+ . Thus, the overall role of NH_4^+ in acid–base balance is dependent on the balance between tubular reabsorption of NH_4^+ and the hepatic synthesis of urea. The latter function may also be directly influenced by extracellular pH.

Liver and Bone

The liver also plays additional roles in acid–base balance that may be underestimated. For example, the liver metabolizes lactate and ketoacids; the rate of metabolism is dependent on pH (e.g., ketogenesis is suppressed at low pH) and may be exceeded at higher concentrations of lactate or in liver disease. The synthesis of urea from ammonium and carbon dioxide (see Section on Kidneys) results in genesis of two protons, and is reduced in the presence of acidosis. Some buffering also occurs in bone due to slow exchange of bone calcium carbonate for extracellular phosphate.

Measurement of Urinary Acid Excretion

Urinary pH can be measured by commercially available ‘dipsticks’ or by using a pH meter on a fresh sample of urine. Loss

of CO_2 or the production of NH_4^+ from urea-splitting organisms in infected urine will alter the pH with time. The excretion of fixed acid can be determined by chemical titration of urine to pH 7.4, and is commonly termed ‘titratable’ acidity. The amount of NH_4^+ is usually estimated from the difference between the most abundant cation (Na^+ , K^+) and anion (Cl^-) concentrations in the urine.

Effects of Acid–Base Disturbance

In addition to the adaptive changes occurring in acidosis, a range of metabolic and pathophysiological changes occur; alkalosis tends to produce opposite but milder effects. Metabolism of carbohydrate is altered; both glycolysis and gluconeogenesis are inhibited in the liver. Delivery of oxygen to the tissues is increased by the reduced ability of hemoglobin to retain oxygen in an acid environment (the Bohr effect). Consciousness is impaired, leading to coma in severe cases. However, the most important effects from a clinical perspective are cardiovascular: vasodilatation occurs in peripheral tissues, cardiac contractility is impaired resulting in reduced blood pressure and, when severe, in reduced tissue perfusion. These adverse effects of acidosis contribute to the high mortality of conditions like septic shock. Conversely, in critically ill patients, often after surgery, severe alkalosis may be equally or more dangerous, particularly in the event of cardiac arrest, where resuscitation is extremely difficult.

Abnormalities in Acid–Base Balance

Disturbances in acid–base balance are classified either as ‘acidosis’, indicating an excess of H^+ ions in the blood (reduced pH) or ‘alkalosis’, indicating the opposite. In practice, acidosis is often the more common, varied, and serious problem, although profound alkalosis can be life threatening in the critically ill. Disturbances in acid–base balance are usually labeled according to their origin. For example, respiratory acidosis reflects a primary problem in gas exchange with impaired excretion of CO_2 , whereas metabolic acidosis reflects over-production of fixed acid or loss of bicarbonate. Compensation refers to the body’s ability to offset the primary problem. Thus, the response to a primary metabolic acidosis is to increase excretion of CO_2 ; respiratory compensation. In a primary respiratory acidosis, increased H^+ is secreted by the kidney with increased bicarbonate generation; metabolic compensation. If the pH returns to normal the problem is said to be ‘fully compensated’ whereas most disturbances tend to only be partially compensated (Table 3). Additionally in some circumstances, a mixed respiratory and metabolic acidosis may be present (e.g., septic shock in a patient with underlying respiratory disease affecting gas exchange).

Metabolic Acidosis

The main causes of metabolic acidosis are excessive acid production, inappropriate urinary loss of bicarbonate or failure of the kidney to excrete fixed acid. Although the

Table 3 Changes in blood and ECF during acid–base disturbance, the mechanism and degree of compensation

Problem	$[H^+]$	HCO_3^-	PCO_2	Compensation
<i>(a) Metabolic</i>				
Acidosis	↑	1°↓	2°↓	Partial respiratory
Alkalosis	↓	1°↑	2°↑	Partial respiratory
<i>(b) Respiratory</i>				
Acidosis	↑	2°↑	1°↑	Complete renal
Alkalosis	↓	2°↓	1°↓	Complete renal

↑, increase; ↓, decrease; 1°, primary; 2°, secondary.

Henderson–Hasselbalch equation provides mathematical information concerning the equilibrium of bicarbonate species, in practice it provides little information regarding the nature of the underlying cause of the acid–base disorder and the concept of ‘anion gap’ is useful in assessing cause of metabolic acidosis. This is derived from the principle of electroneutrality and is calculated thus (eqn [6]):

$$([Na^+] + [K^+]) - ([Cl^-] + [HCO_3^-]) \quad [6]$$

The anion gap represents an artificial disparity between the concentrations of these cations and anions routinely measured in clinical practice, therefore signifying concentration of unmeasured anions such as proteins (the most important in healthy subjects), sulfate, phosphate, and other unmeasured anions. The normal anion gap is 10–18 mmol l⁻¹ although recent calculations using more sensitive measurements estimate this as 6–12 mmol l⁻¹. This concept has limitations but is useful for dividing metabolic acidoses into those characterized by an increased anion gap as a marker of excess generation of organic acids and those with a normal anion gap due to decreased excretion of acid or external losses of bicarbonate. There are exceptions to this rule for example, the acidosis of chronic renal failure but nonetheless, it remains a useful concept in clinical practice. Classification of the causes of metabolic acidoses according to presence of an increased or normal anion gap is shown in Table 4.

Diabetic Ketoacidosis

The absence of pancreatic insulin secretion in insulin-dependent diabetes results in increased plasma glucose and reduced tissue uptake and utilization of glucose. In place of glucose, there is increased utilization of nonesterified fatty acid (NEFA) as an alternative source of energy that is metabolized to acetyl coenzyme A (acetyl-CoA). Under normal circumstances this substance is further metabolized in the liver *via* the tricarboxylic acid (TCA) cycle to carbon dioxide and water. In diabetic crises this cycle cannot accommodate the excess acetyl-CoA that is instead converted to acetoacetic acid, which can be further reduced to β-hydroxybutyric acid or decarboxylated to acetone. These metabolites are known as ‘ketone bodies’ and their accumulation results in metabolic acidosis. In diabetic ketoacidosis, homeostatic compensation is to increase ventilation and CO₂ excretion, leading to the characteristic pattern of Kussmaul breathing.

Table 4 Causes of metabolic acidoses according to the presence of an increased or normal anion gap

Increased anion gap	Normal anion gap
Ketoacidosis	Decreased renal acid excretion
Diabetic	Distal renal tubular acidosis
Starvation	
Alcoholic	
Inborn enzyme defects of metabolism	
Lactic acidosis	Loss of alkali Diarrhea Ureterosigmoidostomy (urinary conduit)
Renal failure	Increased renal bicarbonate loss Proximal renal tubular acidosis Acetazolamide Renal tubular damage
Intoxication Salicylates Methanol	Increased HCl production Ammonium chloride ingestion Increased catabolism of lysine, arginine
Ethylene glycol Paraldehyde	

Lactic Acidosis

Reduced tissue perfusion, or perfusion that is inadequate to meet the metabolic demands of tissues (such as exercising muscle), results in an inadequate supply of oxygen and a change from oxidative metabolism (the end products of which are CO₂ and H₂O) to anaerobic metabolism. The end product of anaerobic glycolysis is lactic acid, which is normally metabolized (to CO₂ and H₂O) by the liver or used in synthesis of glucose (gluconeogenesis). The normal plasma lactate concentration is less than 1 mmol l⁻¹ but may increase 10-fold in extreme exercise. When the ability to metabolize lactate is exceeded, either by increased production, or reduced delivery to the liver (in, for example, circulatory shock) or in the presence of impaired liver function, accumulation results in metabolic acidosis. Thus, lactic acidosis may occur in a variety of conditions, including both overt circulatory shock and conditions leading to circulatory shock (for example, septic shock), severe diabetic ketoacidosis, as a consequence of drugs (e.g., the oral hypoglycemic agent metformin that inhibits gluconeogenesis and lactate transport), chronic liver disease and poisoning (including ethanol and methanol).

Excess Bicarbonate Loss

The secretion of acid into the stomach is neutralized by intestinal alkaline secretions. It follows that excessive loss of pure intestinal secretions (e.g., due to an enteric fistula) may lead to acidosis. A more common circumstance is a urinary diversion procedure such as an ileal conduit where the ureters

are implanted into an isolated loop of intestine, which is then externalized (a 'urinary conduit'). The delivery of urine rich in chloride to the isolated intestine leads to exchange of Cl^- for HCO_3^- , and thence to excessive loss of HCO_3^- in the conduit, resulting in metabolic acidosis. Historically ureterosigmoidostomies performed for similar clinical indications tends to provoke a more severe metabolic acidosis by the same mechanism.

There is also a group of conditions known as renal tubular acidosis (RTA). These are mostly inherited but may be acquired, for example as a consequence of recurrent infection. There are two major forms – proximal and distal – reflecting the site of the tubular defect in the nephron. In distal tubular RTA (Type 1) H^+ secretion is impaired resulting in impaired H^+ excretion, whereas in proximal RTA (Type 2) HCO_3^- reabsorption is impaired (usually as part of multiple tubular abnormalities) leading to net loss of bicarbonate. Both cause acidosis, with low pH and hypokalemia as a result of increased distal tubular H^+/K^+ exchange. The precise causes of these conditions are not known but is likely to reflect genetic defects on individual transporter subtypes, for example those of the Na^+/H^+ exchanger (Figure 1).

A third form of RTA (Type 4), also called hyperkalemic RTA, is the result of generalized transport abnormalities of the distal tubule. Electrolyte transport of sodium, chloride, and potassium are impaired. This form differs from classical distal and proximal RTA because there are high (not low) levels of potassium in the blood. Type 4 RTA occurs with deficiency of the hormone aldosterone or when the kidneys fail to respond to it. Aldosterone acts *via* the kidneys to control levels of sodium, potassium, and chloride in the blood. Type 4 RTA also occurs when tubular transport of electrolytes such as sodium, chloride, and potassium is impaired due to an inherited disorder or use of certain drugs (e.g., spironolactone, nonsteroidal anti-inflammatory drugs or angiotensin-converting enzyme inhibitors) and in diseases which alter kidney structure and function (e.g., diabetic nephropathy, sickle cell disease, urinary tract obstruction, lupus, or amyloidosis). Treatment is directed at the underlying cause; in aldosterone deficiency, fludrocortisone, a mineralocorticoid is used.

Renal Failure

In progressive renal failure, renal clearance of all substances is progressively impaired, reflecting progressive loss of individual nephron function. Reduced excretion of fixed acid leads to bicarbonate consumption in extracellular fluids and to acidosis. Tubular recovery of HCO_3^- may also be impaired (see *Excess bicarbonate loss*), as may production of tubular NH_4^+ , and be associated with over-production of urea in the liver, although this is unlikely to be a major concern to excess urea production in clinical practice.

Drugs and Other Causes

Many drugs can cause metabolic acidosis, generally in overdose. A classic example is aspirin (acetylacetic acid). Lactic acidosis is also associated with oral hypoglycemic agents (specifically metformin, used in treatment of noninsulin

dependent diabetes), paracetamol, alcohol, and ethylene glycol (antifreeze) poisoning.

Compensation

The body's response to metabolic acidosis is a compensatory increase in ventilation to excrete excessive CO_2 , restoring equilibrium in the Henderson–Hasselbalch equation (eqn [5]). This respiratory compensation is usually incomplete, resulting in pH values or H^+ concentrations at, or marginally outside, the limits of 'normal' (Table 3). Complete compensation depends on renal excretion of excess H^+ , or resolution of the underlying condition.

Treatment

Treatment of metabolic acidosis is essentially that of the underlying condition: correction of tissue hypoxia in lactic acidosis; correction of fluid depletion and insulin therapy in diabetic ketoacidosis; dialysis in renal failure. Rapid correction of pH can be achieved by administration of intravenous sodium bicarbonate if necessary with the caveat that in a mixed respiratory and metabolic acidosis caution should be exerted as sodium bicarbonate can increase CO_2 levels. The treatment of chronic metabolic acidosis (e.g., in chronic renal failure or RTA) may be achieved by the administration of oral sodium bicarbonate. In uremia the prescription of a low protein diet will reduce acid load and may prevent uremic symptoms.

Metabolic Alkalosis

Metabolic alkalosis may be caused either by excessive loss of acid or intake of alkali. The latter may be iatrogenic or factitious, with the excessive intake of prescribed antacids (such as sodium bicarbonate for heartburn or peptic ulcer disease) – the 'milk-alkali' syndrome. The loss of acid-rich gastric secretions in severe vomiting for example, in cases of gastric outlet obstruction (due to pyloric stenosis, or a consequence of peptic ulcer disease), also leads to alkalosis. Administration of alkali only causes significant metabolic alkalosis when the absorption is considerable and sustained such as transfusions of citrated blood or sodium bicarbonate therapy for metabolic acidosis due to circulatory arrest in the presence of oliguric renal failure. Compensation is by reducing ventilation to promote retention of CO_2 and thus balance the Henderson–Hasselbalch equation. Treatment is usually of the underlying condition. In the case of severe metabolic alkalosis in critically ill patients with associated coma or arrhythmia where alternative treatment (hemodialysis or filtration) has failed or is contraindicated, administration of intravenous hydrochloric acid *via* a central vein can be beneficial.

Respiratory Acidosis

Impaired ventilation, reduces CO_2 excretion, increases PaCO_2 and thus lowers pH. This may occur acutely or chronically. Causes of respiratory acidosis include factors that interfere

with the neurological ‘drive’ for respiration (e.g., head injury, cardiac arrest, opiate, and anesthetic drugs), diseases of the respiratory muscles (e.g., poliomyelitis, Guillain–Barré syndrome), or primary lung diseases (acute-pulmonary edema or pneumonia; chronic bronchitis, emphysema). In acute conditions, pH may fall dramatically, whereas in chronic conditions, such as chronic lung disease, pH is generally nearer normal. In chronic conditions complete compensation occurs in the kidney where elevated PaCO_2 levels are offset by the increased generation of bicarbonate and excretion of fixed acid by the kidney, to balance the Henderson–Hasselbalch equation.

Respiratory Alkalosis

Respiratory alkalosis occurs as a result of inappropriately increased ventilation and increased excretion of CO_2 . This may occur as a transient response to pain or hysteria. Such stimuli tend to be short-lived and can be offset by analgesia, sedation or short-term re-breathing of expired air. Additional causes include the early phases of aspirin poisoning (where the respiratory centers are activated), hypoxia, sepsis, hepatic failure, stroke, and other conditions affecting brainstem respiratory control centers. Most causes of respiratory alkalosis are short-term and, although adaptive responses would be expected to require excretion of bicarbonate to balance the Henderson–Hasselbalch equation, resolution usually occurs with treatment of the underlying condition.

Transporter Mechanisms: Physiology and Pathophysiology

Developments in molecular biology have led to major improvements in our understanding of the physiology, and pathophysiology of renal tubular function. It is now possible to subdivide the various types of renal tubular acidosis for example, by the precise biochemical defect rather than simply the tubular location. Thus, distal (or type 1) RTA may be a consequence of impaired distal tubular H^+ excretion, either due to increased permeability to H^+ or to impaired secretion, the latter – in turn – being a consequence of a variety of defects that include carbonic anhydrase type 2 deficiency, mutations in anion transport protein AE1, or deficiency of collecting-duct proton transport ATPase. Although specific

knowledge of the molecular defect is not necessary to either diagnose or manage these disorders, it is likely that future classification of acid–base disorders will change to recognize the underlying defect.

See also: Amino Acids: Chemistry and Classification; Metabolism. Dehydration. Diabetes Mellitus: Classification and Chemical Pathology. Diarrheal Diseases. Eating Disorders: Bulimia Nervosa. Infection: Nutritional Management in Adults. Ketosis. Lung Diseases. Nutritional Aspects of Bone. Potassium. Sodium: Physiology

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Contents

Adaptation

Balance

Adaptation

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Abbreviations

BMR	Basal metabolic rate
DIT	Diet-induced thermogenesis
NEAT	Non-exercise activity thermogenesis

Pc	Partitioning characteristic
SNS	Sympathetic nervous system
SPA	Spontaneous physical activity

Glossary

Basal metabolic rate Basal metabolic rate (BMR), the largest component (60–75%) of daily energy expenditure for most individuals, is measured under standardized conditions, that is, in an awake subject lying in supine position, in a state of physical and mental rest in a comfortable warm environment, and in the morning in the postabsorptive state, usually 10–12 h after the last meal. These conditions are referred to as ‘basal’ as they should reflect the energy needed for the work of vital functions (maintaining electrolyte equilibrium across cell membranes, cell and protein turnover, respiratory and cardiovascular functions, etc.).

Diet-induced thermogenesis Heat production increases following the consumption of a meal (i.e., the thermic effect of food) and may also increase on a high plane of nutrition (classically termed ‘luxusconsumption’). These two forms of thermogenesis related to food have been regrouped under the term diet-induced thermogenesis (DIT), and are often divided into an ‘obligatory’ component (related to the energy costs of absorption and metabolic processing of nutrients or the energy cost of tissue synthesis during overfeeding) and a ‘facultative’ component, which is partly as a result of the sensory aspects of foods and partly from the stimulation of the sympathetic nervous system.

Isometric work In the context of energy expenditure, this is often referred to as isometric thermogenesis. It is due to increased muscle tension; no physical work is done in the physical sense. The differences in energy expenditure in a person who is lying, sitting, or standing are mainly due to changes in muscle tone.

Nonexercise activity thermogenesis Nonexercise activity thermogenesis (NEAT) is defined as the energy expended for all physical activities other than volitional exercise and sports activities. NEAT is therefore not limited solely to spontaneous physical activity but also includes energy expended for ‘voluntary’ occupational and leisure-time activities.

Spontaneous physical activity Nonresting energy expenditure can be classified into voluntary and involuntary physical activities. The voluntary physical activity comprises volitional activities such as exercise and sports as well as occupational (e.g., going to work and performing work duties) and leisure activities (e.g., gardening). The involuntary physical activity comprises spontaneous and subconscious fidgeting and posture maintenance, and is referred to as spontaneous physical activity (SPA). SPA is a component of ‘nonexercise activity thermogenesis’.

Energy Adaptation: Beyond Mass Action

The regulation of body weight results from a complex integration of genetic, behavioral, and physiological factors that are conveyed through numerous hormonal, metabolic, and neural

signals. In view of how tightly body weight is regulated in the face of widely varying levels of food intake and energy expenditure, it is clear that robust autoregulatory feedback control systems must operate to precisely match energy intake and energy expenditure in order to achieve long-term energy balance.

In fact, there is a built-in stabilizing mechanism in the overall homeostatic system for body weight. Any imbalance between energy intake and energy requirements would result in a change in body weight, which in turn, will alter the maintenance energy requirements in a direction which will tend to counter the original imbalance and would hence be stabilizing. The system thus exhibits 'dynamic equilibrium.' For example, an increase in body weight is predicted to increase metabolic rate (on the basis of the extra energy cost for synthesis and subsequent maintenance of extra lean and fat tissues), which will tend to produce a negative energy balance and hence a subsequent decline in body weight toward its set or preferred value. Similarly, a reduction in body weight would result in a reduction in metabolic rate due to the loss of lean and fat tissues, which will tend to produce a positive balance and hence a subsequent return toward the 'set' or 'preferred' weight. But in reality, the homeostatic system is much more complex than this simple effect of 'mass action,' because the efficiency of metabolism (or metabolic efficiency) may also alter in response to the alterations in body weight. Indeed, subjects forced to maintain body weight at a level 10% above their initial body weight showed an increase in daily energy expenditure even after adjusting for changes in body weight and body composition. Conversely, in subjects maintaining weight at a level 10% below the initial body weight, daily energy expenditure was also lower after adjusting for losses in weight and lean tissues. These compensatory changes in energy expenditure (~15% above or below predicted values) reflect changes in metabolic efficiency that oppose the maintenance of a body weight that is above or below the 'set' or 'preferred' body weight.

Energy Adaptation: Interindividual Variability

These experiments of forced changes in body weight have also revealed that there is a large interindividual variability in the ability to readjust energy expenditure, with some individuals showing little or no evidence for altered metabolic efficiency, whereas others reveal a marked capacity to decrease or increase energy expenditure through alterations in metabolic efficiency. Indeed, the most striking feature of virtually all experiments of human overfeeding (lasting from a few weeks to a few months) is the wide range of individual variability in the amount of weight gain per unit of excess energy consumed. Some of these differences in the efficiency of weight gain could be attributed to interindividual variability in the gain of lean tissue relative to fat tissue (i.e., variability in the composition of weight gain), but most is in the ability to convert excess calories to heat, that is, in the large interindividual capacity for diet-induced thermogenesis (DIT). A detailed reanalysis of data from about 150 human beings participating in various overeating experiments conducted between 1965 and 1999 suggested that at least 40% of these overfed subjects must have exhibited an increase in DIT, albeit to varying degrees. Furthermore, it has been established from overfeeding experiments in identical twins that genes play an important role in variability in metabolism and underlie such susceptibility to weight gain and obesity. Conversely, a role for genotype in human variability both in the composition of weight loss (i.e., ratio of lean to fat tissue), as well as in

enhanced metabolic efficiency (i.e., adaptive reduction in thermogenesis) during weight loss, has been suggested from studies in which identical twins underwent slimming therapy with a very low-calorie diet. Taken together, it is evident that in addition to the control of food intake, differences in the composition of weight changes (via partitioning between lean and fat tissues) and in metabolic efficiency (via adaptive thermogenesis) also play an important role in the regulation of body weight and body composition, and that the magnitude of these adaptive changes is strongly influenced by the genetic make-up of the individual.

Adaptive Thermogenesis at Rest and During Movements

The quantitative assessment of adaptive thermogenesis in the regulation of body weight and body composition is, however, hampered by difficulties in pin-pointing which component(s) of energy expenditure could be contributing importantly to the changes in metabolic efficiency. As depicted in **Figure 1**, energy expenditure in the resting state is measured as basal metabolic rate (BMR) or as thermic effect of food. In response to overnutrition or undernutrition, the changes in resting energy expenditure (after adjusting for changes in fat-free mass (FFM) and fat mass) and in the thermic effect of food (as percentage of calories ingested) can be quantified; they reflect changes in metabolic efficiency and hence in adaptive changes in thermogenesis. Decreases in mass-adjusted BMR in response to weight loss and increases in mass-adjusted BMR in response to weight gain have often been demonstrated in humans and other mammals, and hence reflect the operation of adaptive changes in thermogenesis in the compartment of resting energy expenditure. By contrast, any change in heat production from what is generally clustered under nonresting energy expenditure (**Figure 1**) is more difficult to quantify. The efficiency of muscular contraction during exercise is low (~25%), but that of spontaneous physical activity (SPA) – including fidgeting, muscle tone, and posture maintenance, and other low-level physical activities of everyday life – is even lower because these essentially involuntary activities comprise a larger proportion of isometric work which is simply thermogenic. Because actual work done on the environment during SPA is very less compared with the total energy spent on such activities, the energy cost associated with SPA has been referred to as movement-associated thermogenesis or SPA-associated thermogenesis. As SPA is essentially subconscious and hence beyond voluntary control, a change in the 'level' or 'amount' of SPA in a direction that defends body weight also constitutes autoregulatory changes in energy expenditure. In this context, an increase in the amount of SPA in response to overfeeding or a decrease during starvation also constitute adaptive changes in thermogenesis.

Spontaneous Physical Activity

To date, the most direct evidence that changes in SPA contribute to autoregulatory changes in energy expenditure in humans is derived from data obtained from the eight men and

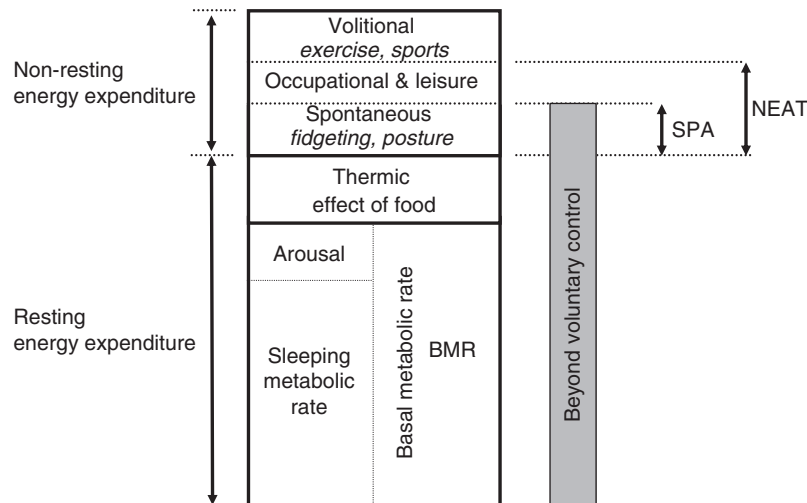


Figure 1 Components of energy expenditure contributing to voluntary and involuntary control of energy expenditure (see text for details); SPA, spontaneous physical activity; NEAT, nonexercise activity thermogenesis. Reproduced from Dulloo AG, Seydoux J, and Jacquet J (2004) Adaptive thermogenesis and uncoupling proteins: A reappraisal of their roles in fat metabolism and energy balance. *Physiology and Behaviour*. 83(4): 587–602.

women who were participating in the Biosphere 2 experiment, a self-contained ecologic ‘miniworld’ and prototype planetary habitat built in Arizona, USA. As a result of an unexpected shortage of food, their losses in body weight (8–25%) over a 2-year period was found to be accompanied by a major reduction in SPA, which like their reduced energy expenditure, persisted several months after the onset of weight recovery and disproportionate recovery of fat mass. Whether interindividual variability in the ‘amount’ of SPA during overfeeding contributes to variability in resistance or susceptibility to obesity has also been the focus of a few human studies of energy expenditure. The importance of SPA-associated thermogenesis in human weight regulation was in fact underscored by the findings that even under conditions where subjects are confined to a metabolic chamber, the 24-h energy expenditure attributed to SPA (as assessed by radar systems) was found to vary between 100 and 700 kcal day⁻¹, and to be a predictor of subsequent weight gain. This notion has gained considerable support from the findings that more than 60% of the increase in total daily energy expenditure in response to an 8-week overfeeding period could be attributed to SPA, and that interindividual variability in energy expenditure associated with SPA – an important component of nonexercise activity thermogenesis (NEAT) – was the most significant predictor of the resistance or susceptibility to obesity. Furthermore, in addition to the suppression of thermogenesis in the compartment of resting energy expenditure, reduced SPA and NEAT may play a role in the conservation of energy in response to caloric restriction, and are hence counteractive to the efficacy of slimming regimens.

Efficiency of Muscle Work

Although changes in SPA are certainly an adaptive response to undernutrition or overnutrition, there is however no consistent evidence to suggest that SPA is always the major component in

adaptive changes in nonresting energy expenditure. In fact, in the experiments of forced changes in weight where subjects maintained body weight at 10% above or below their habitual body weight, the autoregulatory increases or decreases in nonresting energy expenditure could not be explained by the amount of time spent in physical activity. Instead, changes in muscle work efficiency could account for one-third of the change in daily energy expended in physical activity. These findings are consistent with other reports of an increase in skeletal muscle work efficiency (i.e., decreased thermogenesis) after experimentally induced weight reduction or in chronically undernourished subjects.

Interactions between Resting and Nonresting Energy Expenditure

It must be emphasized that the separation of adaptive thermogenesis between resting and nonresting is artificial, given the possibilities of interactions across these two compartments of energy expenditure. For example, energy expenditure during sleep, which is generally nested under ‘resting’ energy expenditure, also comprises a ‘nonresting’ component due to spontaneous movement (or SPA) occurring during sleep, the frequency of which seems to be highly variable between individuals. Furthermore, nonresting energy expenditure or NEAT could also include heat production resulting from the impact of physical activity (exercise or SPA) on postabsorptive metabolic rate or postprandial thermogenesis. There is in fact some evidence that relatively low-intensity exercise can lead to potentiation of the thermic effect of food and that the effect of physical activity on energy expenditure can persist well after the period of the physical activity (postexercise or post-SPA stimulation of thermogenesis). Reduction in postexercise stimulation of metabolic rate has also been put forward as a mechanism for energy conservation in individuals who are considered to be chronically energy deficient since childhood.

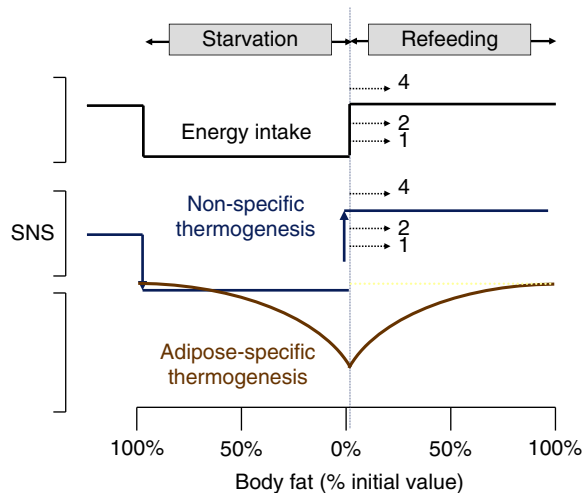


Figure 2 Schematic representation of the concept of two distinct control systems underlying adaptive thermogenesis during prolonged starvation and subsequent refeeding. One control system, which is a direct function of changes in the food energy supply, responds relatively rapidly to the energy deficit. Its effector mechanisms are suppressed early during the course of starvation, and on refeeding they are restored relatively rapidly as a function of energy re-availability (levels 1–4), and are activated further if hyperphagia occurs during refeeding (level 4). Because the efferent limb of this control system is primarily under the control of the sympathetic nervous system (SNS) whose functional state is dictated by overlapping or interacting signals arising from a variety of environmental stresses including food deprivation, deficiency of essential nutrients, excess energy intake, and exposure to cold or to infections, it is referred to as the ‘nonspecific’ control of thermogenesis, and is likely to occur primarily in organs/tissues with a high specific metabolic rate (e.g., liver, kidneys, and brown adipose tissue). The other control system, by contrast, is independent of the functional state of the SNS, has a much slower ‘time constant’ by virtue of its response ‘only’ to signals arising from the state of depletion/repletion of the fat stores; it is therefore referred to as the control system operating through an ‘adipose-specific’ control of thermogenesis. Although suppression of this ‘adipose-specific’ thermogenesis during starvation and during refeeding leads to energy conservation, the energy thus spared during refeeding is directed specifically at the replenishment of the fat stores, resulting in an accelerated fat recovery – a phenomenon that could contribute to the disproportionately rapid rate of fat relative to lean-tissue recovery during refeeding after substantial fat stores depletion. Adapted from Dulloo AG and Jacquet J (2001) An adipose-specific control of thermogenesis in body weight regulation. *International Journal of Obesity* 25(supplement 5): S22–S29, with permission from Nature.

Thus, any change in metabolic efficiency in resting or non-resting state that would tend to ‘attenuate energy imbalance’ or to ‘restore body weight and body composition’ toward its ‘set’ or ‘preferred’ value constitutes adaptive changes in thermogenesis.

Autoregulation of Body Weight and Body Composition

From a system physiology standpoint, the available evidence, based on classic longitudinal studies of starvation, refeeding,

and overfeeding, suggests that the adaptive mechanisms for optimal survival in an environment of famine-and-feast are embodied in three distinct autoregulatory control systems: the control of partitioning between protein and fat (the two main energy-containing compartments in the body), and two distinct control systems underlying adaptive changes in thermogenesis – as depicted conceptually in Figure 2. One control system is a direct function of changes in the food energy supply and responds relatively rapidly to the energy deficit. Its effector mechanisms are suppressed early during the course of starvation, and on refeeding they are restored relatively rapidly as a function of energy reavailability, and are activated further if hyperphagia occurs during refeeding, which could hence account for increased DIT. Because the efferent limb of this control system – which is primarily under the control of the sympathetic nervous system (SNS) – is dictated not only by the dietary energy supply but also by a variety of other environmental factors such as diet composition, specific nutrient deficiencies, ambient temperature, psychological stress, and so on, it is referred to as the ‘nonspecific’ control of thermogenesis. By contrast, the other control system has a much slower ‘time constant’ by virtue of its response only to signals arising from the state of depletion/repletion of body fat stores; it is therefore referred to as the control system operating through an ‘adipose-specific’ control of thermogenesis. The definitions of these two control systems underlying adaptive thermogenesis are thus made on the basis of their differential commands – either deriving solely from the state of adipose tissue fat stores or not.

A Compartmental Model

An overall integration of these autoregulatory control systems in the regulation of body weight and body composition during a cycle of weight loss and weight recovery is discussed with the help of a schematic diagram presented in Figure 3. This diagram embodies the findings that the control of body energy partitioning between protein and fat is an individual characteristic, that is, individuals vary in their partitioning characteristic (P_c) during weight loss and weight recovery, and takes into account the two distinct control systems for adaptive thermogenesis which can operate independently of each other.

During starvation, the control of partitioning determines the relative proportion of protein and fat to be mobilized from the body as fuel – the individual’s P_c being dictated primarily by the initial body composition. The functional role of the control of partitioning is to meet the fuel needs of the individual in such a way that the energy-reserve component in both the fat and protein compartments (i.e., the part that can be lost without death or irreversible damage) would reach complete depletion simultaneously – a strategy that ensures the maximum duration of survival for a given individual during long-term food scarcity. Furthermore, the energy conserved resulting from suppressed thermogenesis is directed at reducing the energy imbalance, with the net result that there is a slowing down in the rate of protein and fat mobilization in the same proportion as defined by the P_c of the individual. Indeed, the fact that the fraction of fuel energy derived from protein (i.e., the P ratio) remains relatively constant during

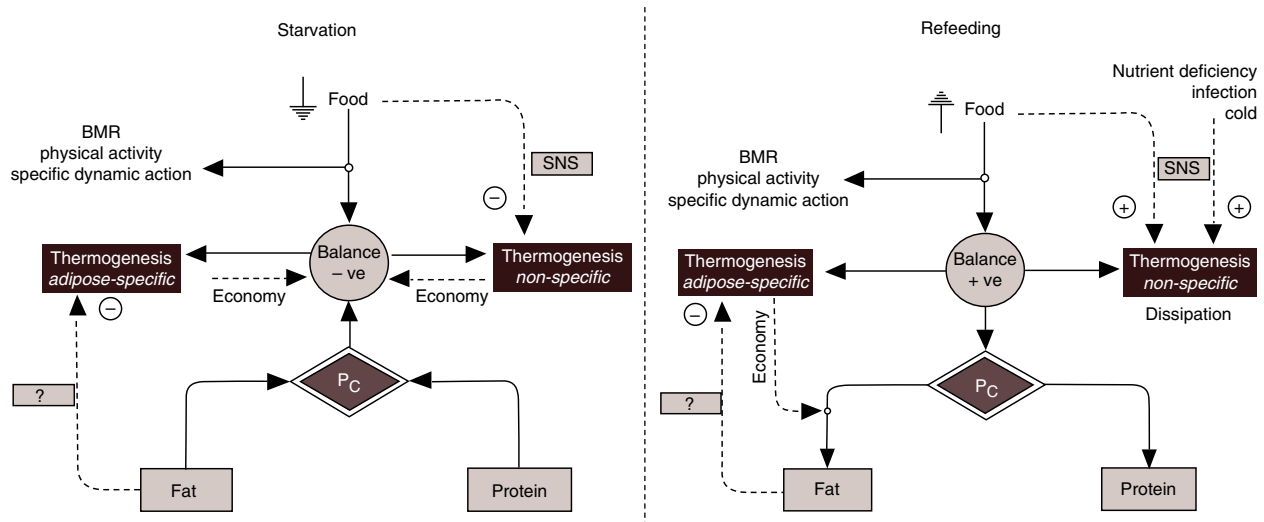


Figure 3 Schematic representation of a compartmental model for the regulation of body weight and body composition during a cycle of weight loss (prolonged starvation) and weight recovery (refeeding). In this model, the two distinct control systems underlying adaptive thermogenesis – the ‘nonspecific’ control and the ‘adipose-specific’ control – are integrated with the more ‘basal’ control of partitioning between the body fat and protein compartments as determined by the partitioning characteristic (P_C) of the individual; see text for details. Adapted from Dulloo AG and Jacquet J (2001) An adipose-specific control of thermogenesis in body weight regulation. *International Journal of Obesity* 25(supplement 5): S22–S29, with permission from Nature.

the course of prolonged starvation, albeit in normal-weight humans, implies that the control system underlying suppressed thermogenesis is directed at sparing neither specifically protein nor specifically fat, but at sparing both protein and fat compartments simultaneously. During starvation, therefore, the functional role of both control systems underlying suppressed thermogenesis is to reduce the overall rate of fuel utilization (i.e., for energy conservation directed at sparing both lean and fat tissues).

During refeeding, the control of partitioning operates in such a way that protein and fat are deposited in the same relative proportion as determined by the P_C of the individual during starvation, and this serves to reestablish the individual's prestarvation capacity for survival during long-term food scarcity. Furthermore, the increased availability of food leads to the rapid removal of suppression on the ‘nonspecific’ (SNS-mediated) control of thermogenesis. By contrast, the suppression of thermogenesis under ‘adipose-specific’ control is only slowly relieved as a function of fat recovery, such that the energy that continues to be spared is directed specifically at the replenishment of the fat stores. The net effect is that fat is deposited in excess of that determined by the P_C of the individual, thereby contributing to the disproportionate rate of fat relative to lean-tissue recovery. This phenomenon of catch-up fat (rather than catch-up of lean tissue) is often observed – both in adults after severe weight loss due to food unavailability and disease, and in infants and children recovering from protein-energy malnutrition and early growth arrest. A role for thrifty (energy conservation) mechanisms driving this catch-up fat phenotype has been proposed as a key feature in the link between fetal or neonatal growth retardation, catch-up growth and risks for development of type 2 diabetes and cardiovascular diseases later in life.

Biological Significance

Such an adaptive phenomenon that accelerates the restitution of fat stores rather than to divert the energy saved toward compensatory increases in body protein synthesis (an energetically costly process) would have survival value in ancestral famine-and-feast lifestyle. This is because, by virtue of the fact that body fat has a greater energy density and a lower energy cost of synthesis/maintenance than protein, it would provide the organism with a greater capacity to rapidly rebuild an efficient energy reserve, and hence to cope with recurrent food shortage. Thus, the functional role of the ‘adipose-specific’ control of thermogenesis during weight recovery is to accelerate specifically the replenishment of the fat stores whenever food availability is increased after a long period of food deficit and severe depletion of body fat stores. It provides an alternative mechanism to recover survival capacity ‘in the absence of hyperphagia.’ But equally important for the survival of mammals during weight loss and weight recovery is the need to retain the capacity to increase heat production (i.e., to activate thermogenesis) in response to a number of other environmental stresses namely: (1) for increased thermoregulatory needs in cold environments, (2) for the generation of fever during exposure to infections, or (3) for increased heat production as an adaptation to nutrient-deficient diets. The necessity to increase DIT in the face of nutrient-deficient diets probably had evolutionary survival advantage of ‘homeostatic waste’ because it enables individuals to overeat relatively large quantities of poor-quality food in order to obtain essential nutrients without the deposition of excess nonessential energy, as fat. Excessive weight gain would be a hindrance to optimal locomotion, hunting capabilities, and the ability to fight or flee. It has been proposed that DIT may have evolved as a means of regulating the

metabolic supply of essential nutrients (protein, minerals, and vitamins) with only a secondary role in regulating energy balance and body weight. Whatever the exact functional significance of DIT, it is clear that in a context of weight recovery, an elevated efficiency for catch-up fat can be shown to persist even under conditions of hypermetabolism (a net increase in thermogenesis) induced by hyperphagia or nutrient-deficient diets. To explain this apparent paradox, the model presented in Figure 3 provides a structural framework that illustrates how suppressed 'adipose-specific' thermogenesis that results in enhanced fat deposition during refeeding – and that is postulated to occur in the skeletal muscle – persists under conditions when the 'nonspecific' control of thermogenesis is activated in organs/tissues recruited by the SNS (liver, kidneys, heart, and brown adipose tissue). Such differentially regulated control systems for thermogenesis may thus have arisen during the course of mammalian evolution as dual-adaptive processes that can satisfy the need for energy conservation during weight loss, or for catch-up fat during weight recovery, even under environmental stresses when SNS-mediated activation of heat production has equally important survival values.

Energy Adaptation During a Longitudinal Human Study of Weight Fluctuations

The existence and operation of this dual-control system for adaptive thermogenesis is consistent with the temporal changes of BMR and body composition during the unique longitudinal study of semistarvation, refeeding, and subsequent overfeeding

in men participating in the Minnesota Experiment. The pattern of changes in food intake and body weight, together with kinetics of altered thermogenesis (assessed as changes in BMR adjusted for FFM and fat mass, and expressed as a percentage of baseline BMR value) are presented in Figure 4.

During the 'phase of weight loss,' the operation of the two control systems for adaptive thermogenesis is suggested by the fact that reduction in thermogenesis is 'biphasic' in nature, with an initial rapid reduction in adjusted BMR at week 4, corresponding to 10% of baseline BMR, followed by a slower reduction in adjusted BMR, corresponding to 20% and 25% of baseline BMR at weeks 12 and 24, respectively. At latter time points during starvation (at S12 and S24), the magnitude of reduced adjusted BMR was found to be associated with the reduction in fat mass – i.e., greater the degree of depletion of the fat stores, the greater the suppression of thermogenesis.

During the 'phase of weight recovery,' the operation of the two control systems for thermogenesis is also suggested by the following:

1. the relation between the degree of depletion of fat stores and suppressed (adipose-specific) thermogenesis persists at week 12 of restricted refeeding, at which time point (R12) the mean adjusted BMR is still approximately 10% below baseline BMR level, the body fat is 80% recovered, whereas body weight and FFM recoveries are less than 50%, and
2. after withdrawal of the dietary restriction during the subsequent period of *ad libitum* refeeding, the development of hyperphagia is accompanied by a prompt (perhaps SNS-mediated 'nonspecific') increase in thermogenesis, as judged by increases in adjusted BMR corresponding to

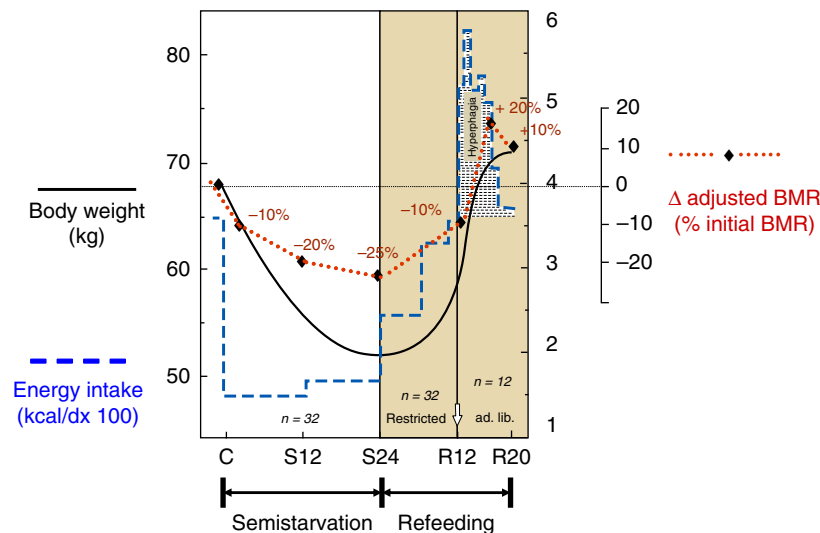


Figure 4 Pattern of changes in body weight, food intake, and adaptive thermogenesis during the various phases of the longitudinal 'Minnesota Experiment' of human semistarvation and refeeding. The changes in adaptive thermogenesis at the various 'time points' are assessed as changes in basal metabolic rate (BMR) after adjusting for changes in fat-free mass (FFM) and fat mass, and expressed as a percentage of the baseline (control, C) BMR level. C=end of control (baseline) period; S12 and S24=weeks 12 and 24 of semistarvation, respectively; R12 and R20=weeks 12 and 20 after onset of refeeding. Drawn from data of Keys A, Brozek J, Henschel A, Mickelson O, and Taylor HL (1950) *The Biology of Human Starvation*. Minneapolis: University of Minnesota Press, and Dullloo AG and Jacquet J (1998) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: A role for feedback signals from fat stores. *American Journal of Clinical Nutrition* 68: 599–606.

approximately 20% of baseline BMR at week 14 of refeeding.

It is also noticeable that by week 20 after the onset of refeeding (at R20), when FFM has been almost 100% recovered and body fat had overshot baseline (prestarvation) level by > 70%, the adjusted BMR remains significantly higher (by approximately 10%) above baseline BMR despite the fact that hyperphagia is no longer present. This postoverfeeding sustained elevation of thermogenesis is consistent with a feedback mechanism existing between thermogenesis and body fat – i.e., the result of an activated ‘adipose-specific’ control of thermogenesis, which may well have contributed to the subsequent slow return of body weight toward the baseline level after the phase of fat overshooting.

It should be noted that this study only enabled analysis of adaptive changes in thermogenesis in the BMR compartment, because no measurements were performed pertaining to the thermic effect of food or to the energy cost of physical activity. Nonetheless, the authors observed that there was a profound decrease in SPA of the subjects, particularly during weeks S12 and S24 of semistarvation, thereby suggesting that adjustments in energy expenditure occurred importantly in both resting and nonresting energy expenditure.

Energy Adaptation and Susceptibility to Leanness and Fatness

In addressing the issue of energy adaptation in human susceptibility to leanness and fatness, it must be underlined that even in individuals that maintain a relatively stable lean body weight over decades, there is no ‘absolute’ constancy of body weight over days, weeks, and years. Instead, body weight tends to fluctuate or oscillate around a mean constant value, with deviations from a ‘set’ or ‘preferred’ value being triggered by events that are cultural (weekend parties, holiday seasons, etc.), psychological (stress, anxiety, or emotions), and pathophysiological (ranging from minor health perturbations to more serious disease states). Very short-term day-to-day changes in body weight have a standard deviation of approximately 0.5% of body weight, whereas longitudinal observations over periods of between 10 and 30 years indicate that individuals experienced slow trends and reversal of body weight amounting to between 7% and 20% of mean weight. In such a dynamic state within which weight homeostasis occurs, it is likely that long-term constancy of body weight is achieved through a network of regulatory systems and subsystems through which autoregulatory changes in food intake, body composition, and energy expenditure are interlinked.

The above-described autoregulatory control systems – operating through adjustments in energy partitioning and through the two distinct control systems underlying thermogenesis – can play a crucial role in attenuating and correcting deviations of body weight from its ‘set’ or ‘preferred’ value. The extent to which these adjustments are brought about is dependent on the environment (e.g., diet composition), and is highly variable from one individual to another, largely because of the previous nutritional status of the individual and because of genetic variations. They probably conferred varying

capacities to defend the body’s protein and fat stores in an ancestral hunter-gatherer lifestyle of famine-and-feast, but now underlie our varying metabolic susceptibilities to fatness in societies where palatable foods are abundant all year round. The resultant subtle variations between individuals in energy partitioning and in adaptive thermogenesis can, over the long term, be important in determining constancy of body weight in some and in provoking drift toward obesity in others.

Furthermore, the adaptive responses to starvation, so far discussed in the context of experimentation in normal-weight (lean) individuals, also persist in individuals in whom obesity has developed spontaneously, and contribute to the defense of the obese state once acquired. In fact, longitudinal studies of obese humans losing weight in response to therapeutic slimming also indicate that these subjects show a reduction in BMR (even after adjustments for losses of lean and fat tissues) as well as in SPA, during both during the dynamic phase of weight loss and subsequent weight maintenance. These findings support the notion that suppressed thermogenesis in response to food deprivation is a factor that reduces the efficacy of therapeutic regimens, and contributes to obesity relapse. Furthermore, because the initial body composition (percentage fat) is the most important determinant of energy partitioning between lean and fat tissue (i.e., P_c of the individual) during weight loss and weight recovery, higher the percentage body fat (i.e., the more obese the individual), lower the fraction of energy mobilized as protein, and hence greater the propensity to mobilize fat during weight loss and to subsequently deposit fat during recovery. The low P_c of the obese, coupled with sustained (adipose-specific) suppression of thermogenesis in response to their relative state of body fat depletion, will contribute to the relapse of obesity.

Concluding Remarks

There is considerable interindividual heterogeneity concerning the compartments and subcompartments of energy expenditure in which energy adaptations via adaptive changes in thermogenesis might be occurring. There may hence be considerable interindividual differences in metabolic strategies to conserve energy through suppressed thermogenesis or to dissipate excess energy through DIT. Long-term energy balance requires the precise matching between energy intake and energy expenditure, and in dynamic systems, alterations in metabolic efficiency that corresponds to even only a few percent of daily energy expenditure can significantly impact body composition and body fat stores over time.

Acknowledgment

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See also: Body Composition. Energy: Balance. Energy Metabolism. Obesity: Treatment. Weight Management: Weight Cycling/Weight Change; Weight Maintenance

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Balance

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Introduction

To maintain physiologic functions, the human body continuously expends energy by oxidative metabolism. This energy is used to maintain chemical and electrochemical gradients across cellular membranes for the biosynthesis of macromolecules such as proteins, glycogen, and triglycerides, and for muscular contraction. Another part of the energy is lost as heat because of the inefficiency of metabolic transformations. Ultimately all the energy produced by the organism is dissipated as heat.

The energy expended by an individual can be assessed by two different techniques: indirect and direct calorimetry. The term indirect calorimetry stems from the fact that the heat released by chemical processes within the body can be indirectly calculated from the rate of oxygen consumption ($\dot{V}O_2$). It is the rate of adenosine triphosphate (ATP) utilization that determines the overall rate of substrate oxidation and therefore $\dot{V}O_2$. With the exception of anaerobic glycolysis, ATP synthesis is coupled with substrate oxidation. Because there is a proportionality between $\dot{V}O_2$ and ATP synthesis, and because each mole of ATP synthesized is accompanied by the production of a given amount of heat, one understands the rationale of using $\dot{V}O_2$ measurement to calculate heat production within the body.

Since indirect calorimetry measures the heat released by the oxidative processes and direct calorimetry assesses the heat dissipated by the body, a relationship exists between the two: For a subject in resting conditions, the difference between metabolic heat production and heat dissipation represents the body heat balance (Figure 1). The heat production from oxidative processes is equal to the sum of the nonevaporative components (radiant heat exchange + convective + conductive heat transfer) plus the evaporative heat transfer. To ensure the equality of the equation, an additional term representing the rate of storage of body heat must be included: heat production = heat losses \pm heat storage.

Heat storage can be positive when excess heat is gained, resulting in a rise in internal body temperature. Heat storage can be negative when excess heat is lost, resulting in a cooling of the body. The rate of heat storage can be estimated from the body weight, the specific heat capacity of the body (which depends upon body composition), and the rate of change in internal body temperature. In practice, this calculation remains somewhat uncertain as the changes in temperature within the body are not uniformly distributed within each tissue.

Under most environmental conditions, heat is lost by all channels (i.e., radiative + convective + conductive + evaporative). However, except during immersion in water, the rate of heat gain or loss by conduction constitutes a small proportion of the total heat loss (typically 3%). Heat can be lost by convection (air currents) but it can also be gained in very hot conditions such as in a desert characterized by high movement of hot air.

Energy Balance: Definition

Overall energy balance is given by the following equation:

$$\text{Energy balance} = \text{energy intake} - \text{energy expenditure.}$$

Thus, if the total energy contained in the body (as fat, protein, and glycogen) is not altered (i.e., in a state of energy equilibrium) energy expenditure is equal to energy intake.

If the intake and expenditure of energy are not equal, to ensure the equality of the equation an additional term (Δ energy) representing the rate of energy storage or mobilization of body must be included: A change in body energy content will occur, in state of negative energy balance resulting in the utilization of the body's energy stores (glycogen, fat, and protein) or vice versa in state of positive energy balance resulting in an increase in body energy stores, primarily as fat.

The difference between the concepts of energy balance and heat balance is presented in Figure 1.

Model of Energy Balance: A Dynamic State

There are multiple reciprocal direct and indirect influences of energy intake on energy expenditure and vice versa: for example, energy intake influences resting energy expenditure by increasing postprandial and dietary-induced thermogenesis, whereas changes in energy expenditure via a modification of physical activity are susceptible to influence energy intake to reequilibrate energy balance. To ensure an accurate regulation of body stores, a double control is essential (Figure 2). Body weight and body composition are not invariant with time, but small corrections of both input and output from day-to-day or week-to-week ensure energy homeostasis (Figure 2). When attempting to explain the actual responses in energy balance and weight regulation in real life, we need to recognize that several factors may be operating at once on both sides of the energy balance equation. Compensatory adjustments occur in both intake and output, so unraveling the importance of one or other adjustment is not easy in a man.

Gross and Metabolizable Energy

The traditional way of measuring the energy content of food-stuffs is to use a 'bomb calorimeter' in which the heat produced when a sample of food is combusted (under high pressure of oxygen) is measured. When the food is combusted, it is completely oxidized to water, carbon dioxide, and other incompletely burned elements. The total heat liberated (expressed in kilocalories or kilojoules) represents the gross energy value or heat of combustion of the food. The heat of combustion differs between carbohydrates, proteins, and fats. There are also important differences within each category of

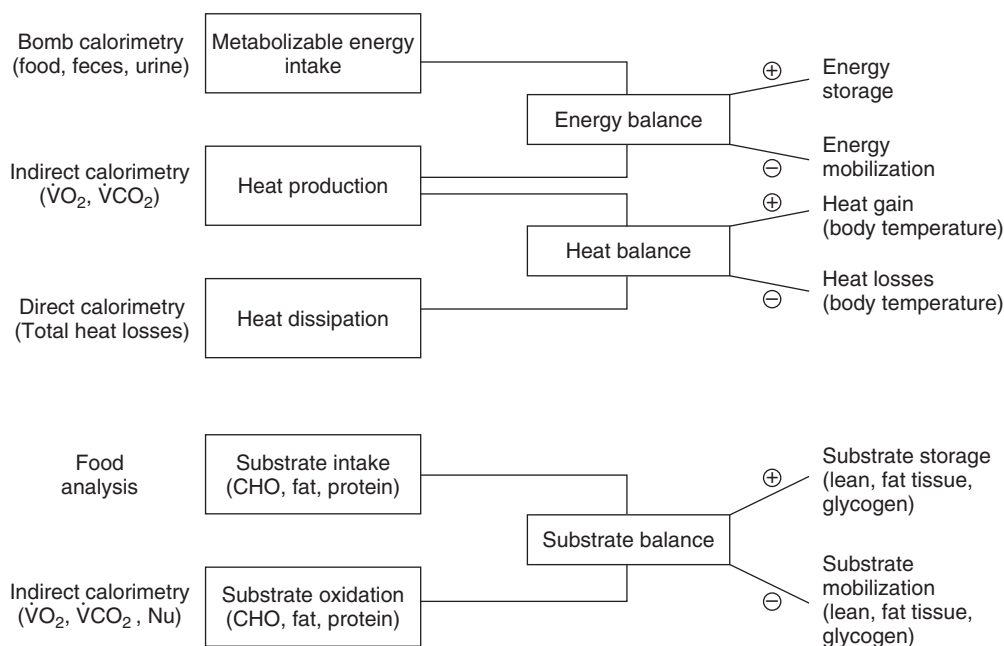


Figure 1 Heat balance, energy balance, and substrate balance: three different concepts.

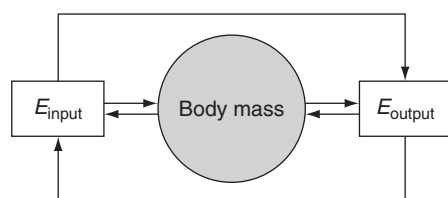


Figure 2 Simple model of energy (E) balance. Long-term maintenance of body weight through the regulation of energy balance is achieved through a highly complex network of regulatory systems operating through changes in food intake, in energy expenditure, and body energy content (i.e., change in body composition).

macronutrient. The gross energy yield of sucrose, for example, is 16.5 kJ g^{-1} , whereas starch yields 17.5 kJ g^{-1} . The energy yield of butterfat is 38.5 kJ g^{-1} and of lard 39.6 kJ g^{-1} . These values have been rounded off to give 17.3 kJ g^{-1} for carbohydrates rich in starch and poor in sugar, 39.3 kJ g^{-1} for average fat, and 23.6 kJ g^{-1} for mixtures of animal and vegetable proteins.

However, the gross energy value of foodstuffs (Table 1) does not represent the energy actually available to the body, since no potentially oxidizable substrate can be considered available until it is presented to the cell for oxidation. None of the foodstuffs are completely absorbed; therefore, some energy never enters the body and is excreted in feces. However, digestibility of the major foodstuffs is high; on the average, 97% of ingested carbohydrates, 95% of fats, and 92% of proteins are absorbed from the intestinal lumen.

In the body, the tissues are able to oxidize carbohydrate and fat completely to carbon dioxide and water, but the oxidation of protein is incomplete, and results in the formation

of urea and other nitrogenous compounds, which are excreted in the urine. Determination of both the heat of combustion and the nitrogen content of urine indicates that approximately 33.0 kJ g^{-1} of urine nitrogen is equivalent to 5.2 kJ g^{-1} of protein because 1 g urinary nitrogen arises from $\sim 6.25 \text{ g}$ protein. This energy represents metabolic loss and must be subtracted from the 'digestible' energy of protein to obtain metabolizable energy (ME).

With a mixed diet, rich in carbohydrates and fibers, the ME of food is approximately 90% of the gross energy (heat of combustion) (Figure 3). The remaining 10% is mainly due to unabsorbed energy (fecal energy losses) and urinary excretion of metabolites.

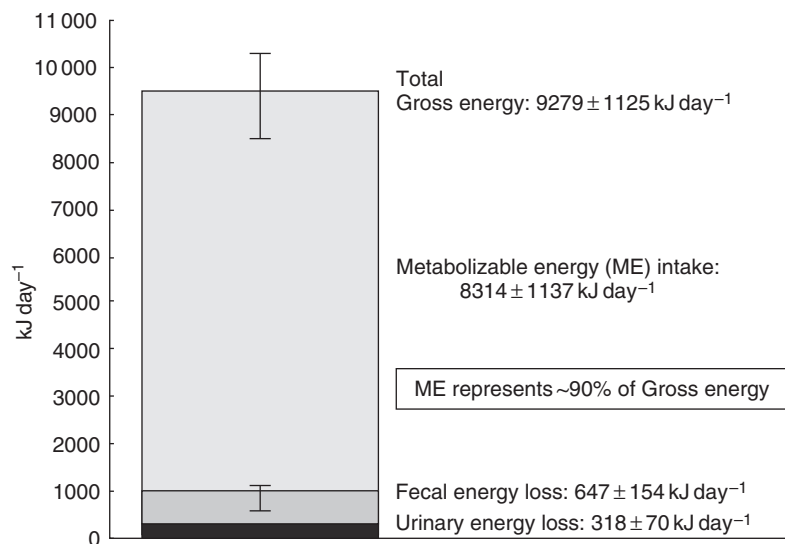
The collection of representative samples of all food eaten combined with the collection of urine and feces for a week (i.e., complete nutritional balance) is technically difficult and cumbersome in practice. The pioneer investigator Atwater developed in the early twentieth century a practical approach for calculating, rather than measuring, the ME of a diet based on its composition of carbohydrates, fat, and proteins. A specific calorimetric factor was developed according to the digestibility and absorption of each macronutrient and the loss of energy in urine (measured by nutrition balance technique). These are the so-called 'Atwater factors.' The ME values for the three substrates and their derivation are shown in Table 1.

Total Energy Expenditure and its Components

Classically we consider energy expenditure as being made up of three components: the energy spent for basal metabolism (or basal metabolic rate (BMR)), the energy spent on physical activity, and the increase in resting energy expenditure in response to a variety of stimuli (in particular food, cold, stress,

Table 1 Metabolizable energy (ME) and Atwater's factors

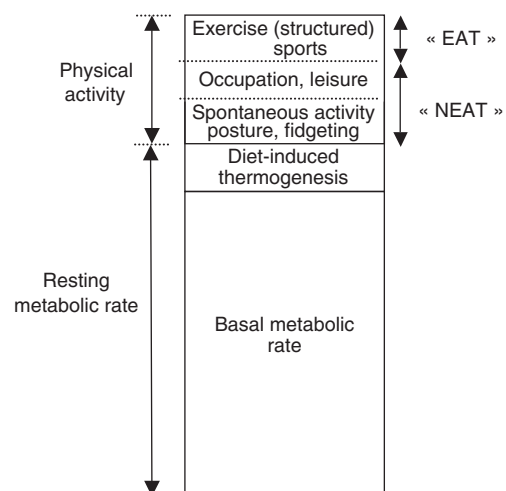
Nutrient	Gross energy in kJ g^{-1} (kcal g^{-1})	Percentage absorbed (Atwater's values)	Digestible energy in kJ g^{-1} (kcal g^{-1})	Urinary loss in kJ g^{-1} (kcal g^{-1})	Metabolizable energy in kJ g^{-1} (kcal g^{-1})	Atwater's factor (kcal g^{-1}) ^a
Starch	17.5 (4.2)	99	17.3 (4.15)	–	17.3 (4.15)	4
Glucose	15.6 (3.75)	99	15.4 (3.7)	–	15.4 (3.7)	4
Fat	39.1 (9.35)	95	37.1 (8.88)	–	37.1 (8.88)	9
Protein	22.9 (5.47)	92	21.1 (5.04)	5.2 (1.25)	15.9 (3.8)	4
Alcohol	29.8 (7.1)	100	29.8 (7.1)	Trace	29.8	7

^aValues are rounded off.**Figure 3** Gross and metabolizable energy intakes of a mixed, high-carbohydrate diet. Calculation of metabolizable energy for 10 women on a high-carbohydrate, high-nonstarch polysaccharide (NSP) diet for a period of 7 days.

and drugs). Additional components are depicted in **Figure 4**. It includes subcomponent of physical activity partitioned into structured exercise (such as sport) and nonexercise activity thermogenesis (NEAT).

Basal Metabolic Rate or Resting Metabolic Rate

This is the largest component of energy expenditure accounting for between half to three-quarters of daily energy expenditure. It is measured under standardized conditions, that is, in an awake subject lying in the supine position, in a state of physical and mental rest in a comfortable warm environment, and in the morning in the postabsorptive state, usually 10–12 h after the last meal. There is an arbitrary distinction between RMR and BMR in the literature. RMR may be considered equivalent to BMR if the measurements are made in postabsorptive conditions. It seems difficult to partition RMR into various subcomponents because the metabolic rates of individual organs and tissues are hard to assess in humans

**Figure 4** The components of total energy expenditure in a person with moderate physical activity. EAT, exercise activity thermogenesis; NEAT, non-exercise activity thermogenesis.

under noninvasive experimental conditions. BMR can vary up to $\pm 10\%$ between individuals of the same age, gender, body weight, and fat-free mass (FFM), suggesting that genetic factors are also important. Day-to-day intraindividual variability in BMR is low in men (coefficient of variation of 1–3%) but is greater in women because the menstrual cycle affects BMR. In both women and men, sleeping metabolic rate is lower than BMR by 5–10%, the difference being explained by the effect of arousal. BMR is known to be depressed during starvation.

The major part of the whole-body RMR stems from organs with high-metabolic activity such as the liver, kidneys, brain, and heart, although these account for a small proportion of the total body weight (5%). Per unit body weight, the kidneys, and heart have a metabolic rate more than twice as high as the liver and the brain. In contrast, the metabolic rate of muscle per unit body weight is nearly 35 times lower than that of the heart and kidneys. Since the proportion of muscle to non-muscle changes with age from birth to adulthood, the RMR per unit body weight is not constant with age. The tissue with the lowest metabolic activity per unit body weight is adipose tissue, which accounts for only 4% of the whole-body RMR in nonobese subjects. Calculations show that this value can increase up to 10% or more in obese subjects with a large excess in body fat. Skin and intestines (which have a relatively large protein mass and protein turnover), as well as bones and lungs, also contribute significantly to RMR.

Numerous studies have demonstrated that a major factor explaining the variation in RMR between individuals is FFM. FFM is a heterogeneous component that can be partitioned into muscle mass and nonmuscle mass. Unfortunately, there is no simple and accurate way to assess these two subcomponents. Owing to the larger variation between individuals in fat mass, as compared to FFM, and because in grossly obese women fat mass can represent a nonnegligible component of total RMR, the prediction models for RMR that include both FFM and fat mass explain significantly more variance in RMR than FFM alone. In addition, age, sex, and family membership are additional factors that should be taken into account.

The effects of gender on RMR are explained by differences in body composition. Caution should be used when comparing RMR expressed per kilogram FFM in men and women, because the composition of FFM is influenced by gender. The muscle mass of men is greater than that of women and this tends to give a lower value of RMR per kilogram FFM in men when compared to that of women. This is explained by a greater component of tissue with a low metabolic rate (resting muscle) in men than in women.

In clinical work, where body composition is difficult to assess, body weight, gender, and age can be used to estimate BMR and RMR, bearing in mind that many important determinants of RMR, in addition to body size, have been identified.

As an example, the equation based on the results of the Medicine Institute (USA) can be presented:

$$\text{Women: MR (kcalJ}^{-1}) = 8.60 \times \text{Weight (kg)} + 402 \\ \times \text{Height (m)} - 2.67 \times \text{age (years)} + 247$$

$$\text{Man: MR (kcalJ}^{-1}) = 10.1 \times \text{Weight (kg)} + 456 \\ \times \text{Height (m)} - 3.8 \times \text{age (years)} + 293$$

If body composition is measured, then the following equation, based on FFM only, is often used:

$$\text{RMR (kcalJ}^{-1}) = 302 + 22.3 \times \text{fat} - \text{free mass (kg)}$$

Note that the latter is sex independent.

Thermic Effect of Food or Postprandial Thermogenesis, Nonshivering Thermogenesis

The energy expenditure increases significantly after a meal. The so-called thermic effect of food is mainly due to the energy cost of nutrient absorption and storage. The total thermic effect of food represents ~10% of the total energy intake in sedentary subjects. The thermic effect of nutrients mainly depends on the energy costs of processing and/or storing the nutrient. Expressed as a percentage of the energy content of the nutrient, values of 8%, 2%, 20–30%, and 22% have been reported for glucose, fat, protein, and ethanol, respectively.

Glucose-induced thermogenesis mainly results from the cost of glycogen synthesis and substrate cycling. Glucose storage as glycogen requires 2 mol ATP mol⁻¹. In comparison with the 38 mol ATP produced on complete oxidation of glucose, the energy cost of glucose storage as glycogen corresponds to 5% (or 2/38) of the energy content of glucose stored. The thermic effect of dietary fat is very small; an increase in 2% of its energy content has been described during pure fat ingestion or IV infusion of an emulsion of triglyceride. This modest increase in resting energy expenditure suggests that fat is used very efficiently. It is explained by the ATP consumption in the process of free fatty acid reesterification to triglyceride. The thermic effect of proteins is the highest of all macronutrients (20–30% of the energy content of proteins). Protein synthesis requires much energy due to the high cost of peptide bonds synthesis and biochemical processing, which represents ~25% of the energy content of amino acids.

Nonshivering thermogenesis represents the increased heat production due to a rise in sympathetic nervous system activity, particularly in brown adipose tissue (BAT). It has been demonstrated in adult human beings chronically exposed to extreme temperatures. However, recent morphological and scanning studies have raised the possibility that BAT in humans may not be as rare as once believed. Main depots in man occur primarily in the supraclavicular and neck regions, with some additional locations in the axillary and paravertebral regions, of normal individuals.

A systematic study on the presence, distribution, and activity of BAT in lean and obese men during exposure to mild cold temperature (16 °C) has recently been made in Holland (Van Marken Lichtenbelt *et al.*, 2009). Positron-emission tomography was used to detect BAT activity, which was observed in 96% of the whole groups but only under mild cold and not under thermoneutral conditions. Both body-mass index and percentage of body fat had significant negative correlations with BAT suggesting that the leaner the subjects the more the BAT activity. These BAT depots express uncoupling protein 1 (UCP1), a carrier protein that is located in the mitochondrial innermembrane of brown adipocytes. In BAT, energy derived from metabolic fuels is much more

dissipated in a process that is facilitated by proton leak thereby diminishing the net efficiency of ATP production by releasing more heat. Regulated proton leak is mediated by UCP1. These findings have generated interests into approaches to activate BAT for obesity management.

Energy Expenditure Due to Physical Activity

The energy spent on physical activity depends on the type and intensity of the physical activity and on the time spent in different activities. Physical activity is often considered to be synonymous with 'muscular work,' which has a strict definition in physics (force \times distance) when external work is performed in the environment. During muscular work (muscle contraction), the muscle produces three to four times more heat than mechanical energy so that useful work costs more than muscle work. There is a wide variation in the energy cost of any activities both within and between individuals. The latter variation is due to differences in body size and in the speed and dexterity with which an activity is performed. To adjust for differences in body size, the energy cost of physical activities are expressed as multiples of BMR. These generally range from 1 to 5 for most activities, but can reach values between 10 and 15 during intense exercise. In terms of daily energy expenditure, physical activity accounts for 15–40% of total energy expenditure (TEE) but it can represent up to 70% of daily energy expenditure in an individual involved in heavy manual work or competition athletics. However, for most people in industrialized societies the contribution of physical activity to daily energy expenditure is relatively small.

The energy expenditure associated with everyday life activity, which excludes structured exercise, is called NEAT. It plays a pivotal role in the regulation of human energy metabolism and body weight regulation. In practice, NEAT includes, for example, the energy expended moving around, walking to work, typing, and fidgeting (Figure 4).

NEAT can be measured by the so-called factorial approach: each component of NEAT is quantified, and total NEAT is calculated by summing up these components.

NEAT varies substantially between people of similar size in part because of the substantial variation in the amount of spontaneous physical activity they perform. Obesity is generally associated with low NEAT; the NEAT deficit in obesity is not negligible: Obese individuals stand and ambulate significantly less and seat more than their lean sedentary counterparts.

The effect of body weight in average women (~ 60 kg) on energy expenditure is illustrated in Figure 5. The relationship is slightly curvilinear because of differences in body composition in terms of leanness and fatness. RMR is shown as a baseline value.

Just as described above for a specific activity, it has been customary to express TEE relative to RMR (TEE/RMR or TEE/BMR) to offset the large variation in RMR among subjects of different body weight and body composition. This quotient is called physical activity level (PAL) and reflects multiples of RMR. A PAL of 1.5 indicates that TEE is 50% greater than RMR over 24 h.

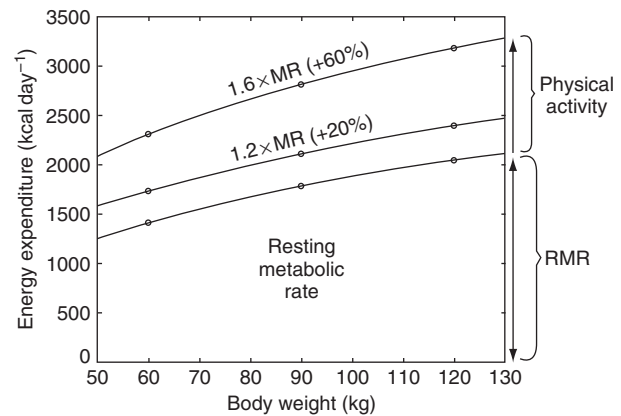


Figure 5 Effect of body weight on total energy expenditure at two levels of physical activity in young women. A physical activity level (PAL) of 1.2 represents minimal physical activity compatible with health, whereas a value of 1.6 represents a 'medium' level of physical activity.

Macronutrient Balance, Energy Balance, and Storage

Since macronutrients (carbohydrate, fat, protein, and alcohol) are the sources of energy, it is logical to consider energy balance and macronutrient balance together as the opposite side of the same coin.

There is a direct relationship between energy balance and macronutrient balance, and the sum of individual substrate balance (expressed as energy) must be equivalent to the overall energy balance. Thus:

$$\begin{aligned} \text{carbohydrate balance} &= \text{exogenous carbohydrate} \\ &\quad - \text{carbohydrate oxidation} \end{aligned}$$

$$\text{protein balance} = \text{exogenous protein} - \text{protein oxidation}$$

$$\text{lipid balance} = \text{exogenous lipid} - \text{lipid oxidation}.$$

It follows that Δ substrate balance $\equiv \Delta E$ balance. Fat balance is closely related to energy balance (Figure 6).

Provided the respiratory quotient is known, indirect calorimetry also allows an estimation of the macronutrient oxidation rates in the whole body in addition to energy expenditure.

Energy stores (constituted mainly of fat stores) are big as a proportion of the food intake ($2000 \text{ kcal day}^{-1}$, mixed diet in a 60-kg nonobese woman with 25% body fat). The total energy stored is about 90 times total daily energy intake: typically fat stores are 175 times daily fat intake, protein 133 times daily protein intake, and carbohydrate only 1.3 times daily carbohydrate intake (Figure 7).

Energy Imbalance and Body Weight

Positive energy balance leads to body weight gain and negative energy leads to body weight loss. There is no fixed relationship between these two variables so that relatively small energy retention can be accompanied by large body weight gain and vice versa. The confounding factor is the associated water storage.

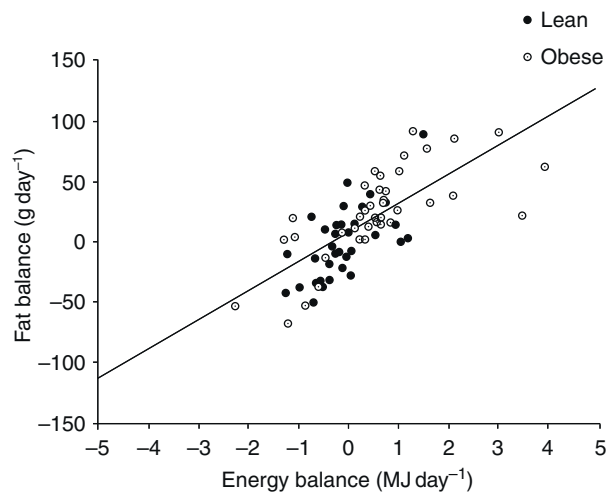


Figure 6 Relationship between energy balance and fat balance in lean and obese individuals. Note that at zero energy balance fat balance is zero. At an excess or deficit of 4.2 MJ day^{-1} (1000 kcal) the fat imbalance (approximately $100 \text{ g day}^{-1} = 900 \text{ kcal day}^{-1}$) accounts for more than 90% of the magnitude of energy balance. Reproduced from Schrauwen P, Lichtenbelt WD, Saris WH, Westerterp KR (1998) Fat balance in obese subjects: Role of glycogen stores. *American Journal of Physiology* 274: E1027–E1033.

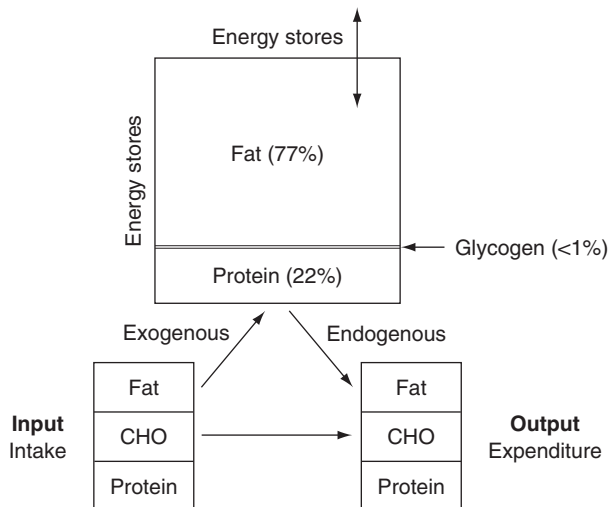


Figure 7 Macronutrient (substrate) stores versus macronutrient intakes.

Long-term fluctuations in fat stores will be reflected in body weight. There is a difference in the energy value of fat mass and FFM, the latter including the glycogen–water pool and the protein–water pool.

Energy density of the tissue stored (or the substrate pool stored) represents an indicator of the composition of tissue stored or mobilized. It is defined as the total calories per gram of substance. It is approximately 8 kcal g^{-1} for adipose tissue compared to the fat value (triglyceride) of 9 kcal g^{-1} . This lower former value is due to the fact that fat is diluted out by the small amount of water (5–10%) and proteins the adipose

tissue contains. As explained previously, the energy density of the glycogen–water pool is low, approximately 1 kcal g^{-1} , because glycogen (4.2 kcal g^{-1}) is associated with approximately three times its weight of water.

Let us take an energy imbalance of say 1000 kcal . The body weight change will be approximately eight times lower (i.e., $\approx 125 \text{ g}$) if fat is stored in adipose tissue, as compared to glycogen stored (under the form of glycogen–water pool) in liver and muscles ($\approx 1000 \text{ g}$ as an extreme). In other words, rapid weight gain (or weight loss) means little fat storage despite what the layman thinks. Day-to-day energy imbalance is generally accommodated by water retention due to changes in carbohydrate storage and sodium intake.

In real life, it is more reasonable to consider that the reserve is composed of a mixture in different proportions of fat and glycogen. If approximately half of the energy imbalance is accounted for by fat and half by glycogen storage, the energy density will be $4\text{--}5 \text{ kcal g}^{-1}$. With the imbalance value described above, it will generate a body weight change of $400\text{--}500 \text{ g}$.

Timescale of Energy Balance

Any regulated function varies within a certain window that is largely determined by the limits for survival.

Because large variations in body fat can be observed both between and within individuals, it could therefore be argued that body weight is a poorly regulated variable as compared to body temperature and blood pH.

Short-term day-to-day energy imbalance is mostly accommodated by rapid changes in carbohydrate balance, whereas over a prolonged period of time, positive energy balance is mostly expressed as fat storage since carbohydrate stores are small.

By contrast, the fact that the body weight of certain individuals varies in a very narrow range over years/decades, in spite of large day-to-day fluctuations in the amount of food consumed and in the level of physical activity, may suggest that body weight is accurately regulated in these individuals.

A critical feature of any regulated system is that disturbance of the regulated variable results in compensatory responses that tend to attenuate the disturbance and to restore the system to its 'set' or 'preferred' value or initial state.

Peripheral hormonal signals contribute to the control of energy balance. Leptin (Greek leptos meaning 'thin') plays a key role in regulating energy intake and energy expenditure, and hence energy balance (Figure 8), although many additional factors are known to be involved in the complex regulation of energy balance. It is one of the most important hormones, produced in white adipose tissue, the level of circulating leptin being directly proportional to the total amount of fat in the body. Leptin is not only involved in energy balance, appetite, and body weight control, but also in fat and carbohydrate metabolism, as well as reproduction. Leptin has many specific receptors both centrally (hypothalamus) and peripherally in the skeletal muscle, lungs, and kidneys.

Excess weight gained during experimental overeating (or during pregnancy) is progressively lost, and many individuals return close to their initial body weight.

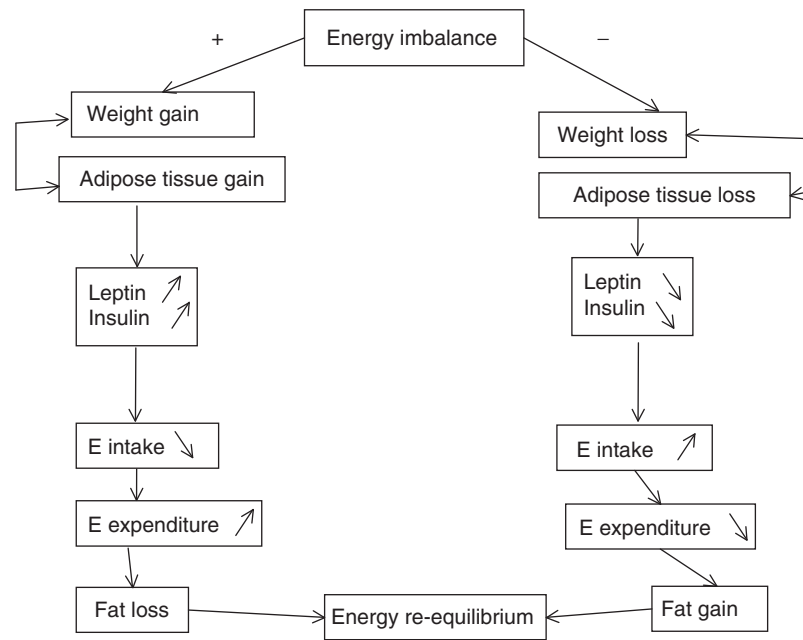


Figure 8 The cascade of events after a prolonged situation of energy imbalance, mediated by a change in circulating leptin (and insulin) ultimately contributing to a restoration of energy balance.

It seems important to highlight three key features of energy balance and weight regulation.

First, the energy balance varies from day-to-day because human beings cannot match energy intake and energy expenditure on a day-to-day basis. The changes in daily energy intake and expenditure are not necessarily synchronized. Positive energy balance on one day may not be spontaneously fully compensated by negative energy balance on the subsequent day, so that it is important to consider the overall energy balance regulation over a prolonged period of time. Near equality of intake and expenditure most often appears over 1–2 weeks.

Note that in real life, it is difficult to accurately track energy input and energy output, the former being much more uncertain than the latter in particular in obese individuals. Longer measurements are difficult to conduct and impractical because of cumulative errors. Nevertheless, over months and years, total metabolizable energy intake and TEE must be very close in any individual whose body weight and body composition have remained relatively constant.

Second, this matching of long-term energy intake and energy expenditure must be very accurate in certain individuals, because a theoretical error (imbalance) of only 1% between energy input and energy output, if constant and persistent over several years (which in fact is unlikely), will lead to a gain or loss of approximately 10 kg in the first decade.

Third, even in adults who apparently maintain a stable body weight over months, years, and decades, there is in reality no ‘absolute’ constancy of body weight per se. Instead, body weight tends to fluctuate or oscillate around a constant mean value, with small or large deviations being triggered by factors such as season and/or cultural factors (weekend parties, holiday seasons), psychology (stress, depression, anxiety, or emotions), and pathophysiological

factors (ranging from minor health perturbations to serious diseases).

Very short-term day-to-day changes in body weight have a standard deviation of less than 1% of body weight, whereas longitudinal observations over periods of between 10 and 30 years indicate that individuals experience slow upward trends and/or reversal of body weight amounting to between 7% and 20% of mean body weight.

To understand the regulation of a system, it must be subjected to perturbation. Excess food intake during overfeeding or deficit in food intake during underfeeding disrupts the balance system. How does the organism react metabolically?

Overfeeding Studies

In a perfectly regulated system, any increase in energy intake should be offset by a change in energy expenditure of the same magnitude and direction. However, a 100% efficient adaptive process would obviously be counterproductive, since this would signify that an increase in energy storage (required during nutritional rehabilitation) or an increase in energy mobilization (required for decreasing body weight) would be very limited. Adaptation to energy imbalance only occurs at the cost of increasing (or decreasing) body weight. In fact, excess energy intakes result in an increased metabolic turnover and energy flux through the mechanism of adaptive thermogenesis. The efficiency of energy storage is not constant and depends on several factors including the magnitude of energy imbalance and the composition of the surfeit energy fed, as well as endogenous factors. As shown in **Figure 9**, the energy expenditure increases during acute overfeeding, an evidence of the ‘flexibility’ of the metabolism.

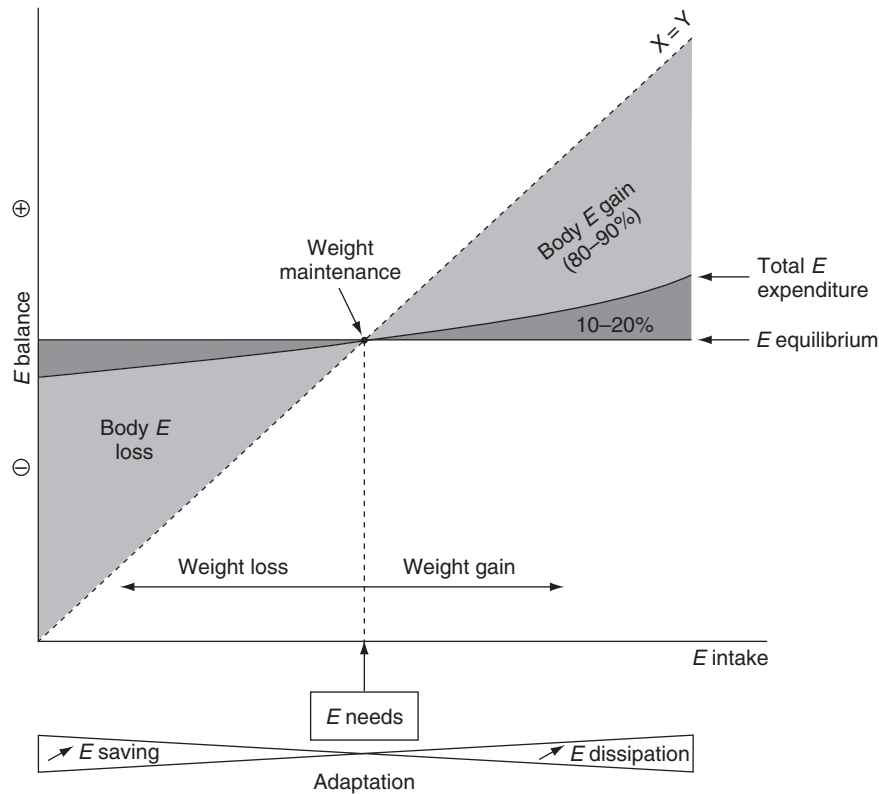


Figure 9 Energy balance in underfeeding (below maintenance) and overfeeding (above maintenance) conditions. E, energy.

Underfeeding Studies

Analysis of underfeeding experiments shows that the decrease in energy expenditure has three components. First, if energy intake is decreased the thermic effect of feeding (approximately 10% of energy intake) is similarly decreased. Second, there is an adaptive decrease in metabolic rate during the first week, related in part to a decrease in sympathetic activity. The magnitude of this decrease is significantly related to the initial metabolic rate, and is usually approximately 5–8%. Third, there is a decrease in metabolic rate related to the weight lost: most investigators find a decrease in 10–12 kcal per day per kg weight loss. The effect of all three processes is that a person who lost weight from, say, 100–70 kg (a 30% reduction in weight) would experience approximately a 15% reduction in energy requirements for weight maintenance. Thus, a decrease in energy intake causes a reduction in body weight but, provided the decrease is not too great, a new equilibrium will be reached at which the reduced requirement will be satisfied by the reduced intake, and body weight will stabilize. Taken together, we can conclude that the efficiency of energy utilization is lower in overfeeding than in underfeeding conditions because substrate storage in tissues is energetically costly (ATP needs), whereas the process of energy mobilization requires little energy. In the former situation excess energy must be dissipated.

Adaptive changes in thermogenesis do attenuate the impact on energy balance of excessive or insufficient food consumption (as compared to requirement). The magnitude of adaptive

thermogenesis varies as a function of the nature of excess substrates fed (protein is higher than carbohydrate and fat).

Energy Expenditure is Less Effective than Food Intake as a Control Mechanism of Energy Balance

It should be stressed that the relationship between the change in energy intake below and above energy equilibrium and energy storage is not quite linear, indicating an increased net efficiency of energy utilization below energy maintenance and a decreased net efficiency of energy utilization above energy equilibrium (Figure 9).

Dynamics of Energy Balance with Overfeeding and Underfeeding (Figure 10)

To understand the dynamic aspect of energy balance while overfeeding is of the utmost importance because as mentioned previously the system is not invariant. Continuous increase in energy intake above energy requirement will lead to a gradual gain in body weight. The size of the energy imbalance will progressively diminish with time as weight is gained. The reason for this is that the expansion of FFM and fat mass (adipose tissue) will be accompanied by a rise in energy metabolism. A new equilibrium in weight is eventually reached after adaptation of each component of TEE, that is, RMR, diet-induced thermogenesis, and the increasing

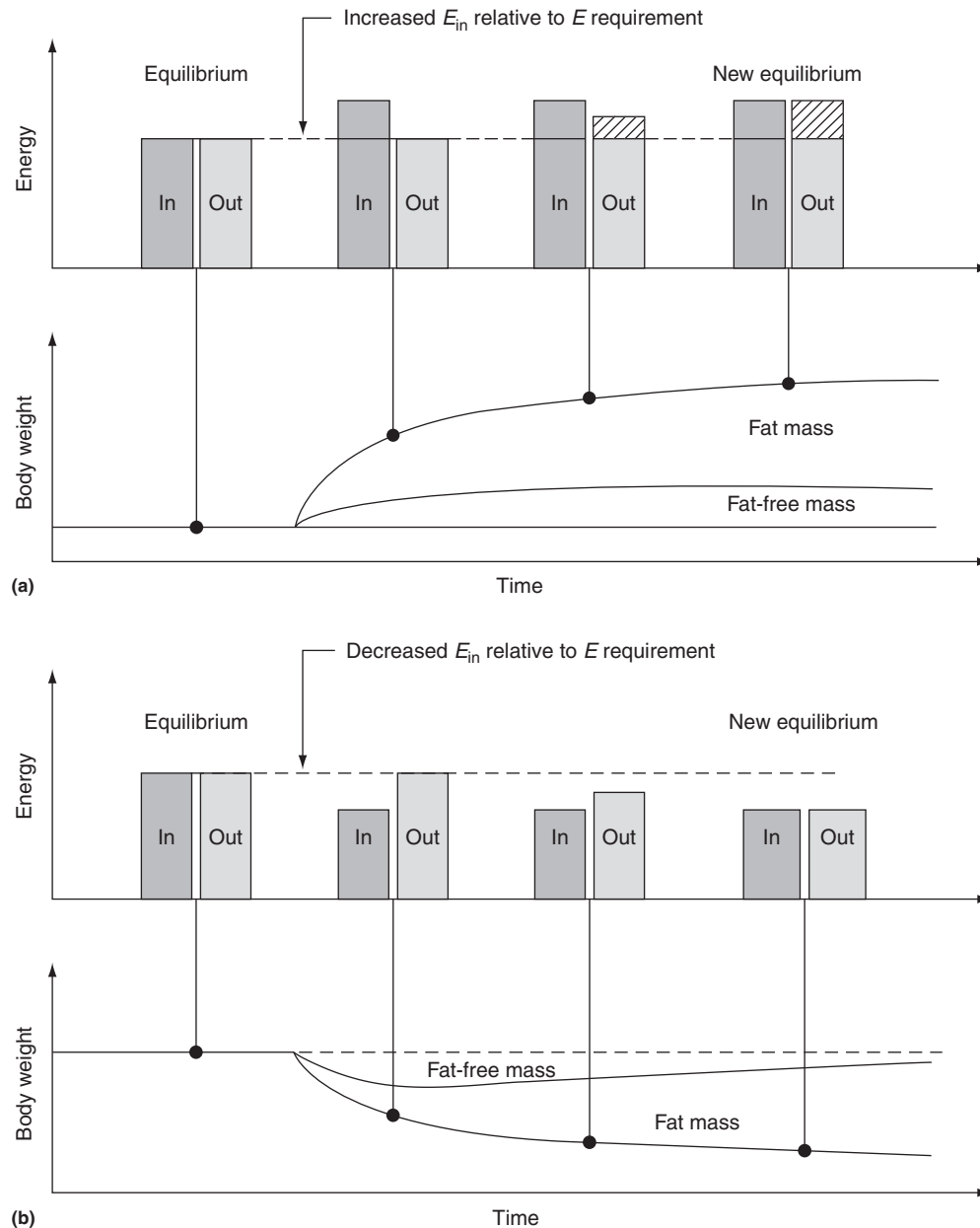


Figure 10 Dynamic change in energy balance following a step and steady increase (a) (or decrease) (b) in energy intake. The time required to reach a new equilibrium in energy balance is very long (years) and depends on the initial energy imbalance, the magnitude of adaptation of energy expenditure in response to change in energy intake, and on the factors related to the subject (obesity vs leanness). The figure shows that the static energy balance as such tells us nothing about the absolute level of energy intake and expenditure (see initial and final balance).

energy cost of supporting a heavier body weight. Note that each kilogram of excess body weight increases TEE by approximately $20\text{--}25 \text{ kcal day}^{-1}$, and $10\text{--}12 \text{ kg day}^{-1}$ when RMR is considered.

Let us take the following practical example: small increase in daily intake, for example, of $100\text{--}200 \text{ kcal}$, will induce small increases in body weight with its associated rise in energy expenditure as the mass of lean tissue increases. If these changes occur on a daily basis, month-by-month, and if after 3–5 years the adult is still eating $200 \text{ kcal day}^{-1}$ more than at

baseline, they will now be heavier, and have a higher energy expenditure, and will come into energy balance; therefore, they cease to gain more weight.

Summary

Energy balance is the difference between ME intake and TEE. It is strongly related to macronutrient balances, and the sum of the individual substrate balances, expressed as energy, must be

equivalent to the overall energy balance. Energy in foods is provided by carbohydrate, proteins, fats, and alcohol; only 5–10% is lost through the feces and urine. The energy available to the body, called 'ME', is on average 17 kJ g^{-1} of carbohydrate, 17 kJ g^{-1} of protein, 37 kJ g^{-1} of fat, and 29 kJ g^{-1} of alcohol. These figures vary slightly according to the type of carbohydrate, protein, or fat in the diet. The energy used in the body, or energy expenditure, is classically assessed by indirect calorimetry. It involves measuring the oxygen consumption and carbon dioxide production by an individual. Short-term regulation of energy balance is poor, but (in most people) long-term regulation is more accurate. The exact mechanism remains unknown. During long periods of energy imbalance, the weight gained (or lost) is initially glycogen associated with water with a low-energy density ($\sim 1.0 \text{ kcal g}^{-1}$). If the imbalance continues, after a week the tissue gained (or lost) is a mixture of mostly fat, water, and protein and the energy density of weight change progressively increases to $\sim 7 \text{ kcal g}^{-1}$. Undernutrition leads to a decrease in energy expenditure. Part of the decrease in metabolic rate is related to weight loss. In overfeeding, although some of the excess energy intake will be stored in adipose tissue, there are compensatory adaptive increases in the components of energy expenditure.

See also: Amino acids: Metabolism. Energy: Adaptation; Metabolism; Requirements. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Fats and Oils

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ENERGY EXPENDITURE

Contents

Doubly Labeled Water
Indirect Calorimetry

Doubly Labeled Water

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Glossary

Body composition Partitioning of body tissues in terms of percentage of fat versus fat-free mass (typically excluding bone).

Doubly labeled water (DLW) A combination of two stable isotopes, typically deuterium and oxygen-18, used as a tracer in water.

Indirect calorimetry The measurement of a chemical produced during the oxidation of fuel in the body

versus directly measuring the heat released during that oxidation.

Respiratory Exchange Ratio (RER) The ratio of CO₂ output and O₂ input that occurs during respiration.

Total energy expenditure The sum of resting metabolic rate, the thermic effect of meals, and physical activity energy expenditure.

Chemically defined, water is composed of one oxygen atom bonded to two hydrogen atoms. As we know, this molecule has unique physical and chemical properties that are vital to support life. The molecule is polar, having a slightly negative end and a slightly positive end. This characteristic causes water to easily dissolve other polar molecules and also creates surface tension, capillary action, and minor electrical conductivity. From the largest mammal to the smallest bacteria, the existence of life on Earth depends on water. Water also fills cells, providing cell volume and intracellular structural support. Without water, organisms would not have a means to store genetic information as water's polarity is what causes DNA to coil in a helical shape. In multicellular organisms, water is used as a transport medium for hydrophilic molecules between cells because cell membranes are permeable by water. Energy production taking place within cells in the form of photosynthesis and cellular respiration requires water as a chemical reactant. Water also provides the essential functions of lubrication and transport for nutrients and waste. In these roles and many others, water is central to life.

Water is usually the largest component of the four basic molecular entities that together comprise the body: protein, lipid, mineral, and water. Because these are the only basic components, measurement of any of these can provide considerable insight into an individual's body composition. Moreover, nutritionists generally combine protein, water, and mineral into a single compartment that is termed fat-free mass. This allows an investigator to describe the body in terms of fat and fat-free mass, which is useful in determining body energy stores and in assessing the health status of an individual in terms of malnutrition and obesity. Several methods are available to determine fat or fat-free mass. Dual-energy X-ray absorptiometry (DXA or DEXA) uses X-rays to differentiate fat mass, fat-free mass, and bone mass. Underwater weighing is a way to measure body volume by displacement. This procedure helps to calculate body density, and from density the body fat percentage can be estimated using predetermined calculations. However, one of the oldest and still commonly used methods is to measure total body water (TBW). Water is found only in the fat-free compartment because fat is anhydrous. In humans and other mammals, TBW has been found to be relatively constant at approximately 73% of the body's fat-free mass. Thus, a measured TBW allows us to

[†]Deceased.

calculate fat mass = body mass - (TBW/0.73). The common method for measuring TBW is isotope dilution, which is based on the principle of dilution using a small amount of tracer with a known volume to measure a large unknown volume.

The principle of dilution can be illustrated by analogy. Consider a highly concentrated dye in a small volume will have a very dark appearance. If this is diluted in a large beaker of water, the mixture will have a lighter color (**Figure 1(a)**). If the amount of the concentrated dye added into the small beaker is known and the concentration of the dye in the larger mixture is measured, then the volume of the diluting water in the larger beaker can be calculated. Isotope dilution uses the same principle, but instead of a dye, an isotope of water is used. TBW, or more correctly, isotope dilution space (N), which is slightly larger than TBW due to exchange with solids, is calculated as $N = \text{dose}/(\text{isotope concentration})$. For this purpose, isotopes of interest in a water molecule typically include ^2H (deuterium) and ^{18}O , which are stable (nonradioactive) isotopes, and ^3H (tritium), which is a radioactive isotope. Because stable isotopes do not undergo nuclear decay, they do not release harmful radiation; yet, as isotopes, they act very much like their parent element in chemical processes. Stable isotopes occur in nature and make up a certain percentage of the total existing elements. Because we ingest these daily with no risk to radiation, they constitute a safe option for a tracer.

These isotope-labeled water molecules, however, do not remain in body water after TBW is measured. Water is constantly being lost from the body. This loss, if unchecked, further demonstrates the importance of water for human life. A loss of 2% bodily fluids will cause a dry mouth, headaches, and decreased urine production. A loss of 5% bodily fluids will further cause increased heart rate, respiration, fatigue, muscle cramps, and even further decreased urine production. Severe dehydration, a loss of 10% bodily fluids or more after several days without water, will cause seizures, loss of vision, high pulse, muscle spasms, and eventually death. Repletion

through drinking and food moisture along with the required electrolytes replaces lost water. In normal daily living, this loss and replacement occur well before dehydration occurs and create a dynamic state in which part of the human water pool turns over on a daily basis. This daily loss of labeled water and replacement with unlabeled water further dilutes the isotopically labeled body water. Returning to the analogy of the dye solution, the mixture color will become lighter with each loss of labeled water and replacement with unlabeled water (**Figure 1(b)**).

A little-appreciated fact is that the turnover of water labeled with both the stable isotopes, ^2H and ^{18}O can be used to measure not only TBW but also how much energy a person releases every day. The covalent bonds in a water molecule can be easily broken; thus, the fate of hydrogen and oxygen in body water differs. Hydrogen is excreted almost exclusively as water, but oxygen can be excreted as both water and carbon dioxide.

The labeling of water with the two isotopes ^2H and ^{18}O led to the use of the name doubly labeled water (DLW). These labeled water molecules mix readily with unlabeled water and can therefore follow all the same physiological and biochemical pathways in the body. Lifson was the first who noted that the oxygen in body water exists in equilibrium with carbon dioxide. The ^{18}O is thus excreted as both water and carbon dioxide; thus, the turnover rate of ^{18}O is greater than ^2H , which is almost exclusively excreted as water. Because CO_2 is a product of energy metabolism, we can use the difference between the two turnover rates to calculate the total CO_2 output. This creates the opportunity for the measurement of energy expenditure.

The DLW method is a form of indirect calorimetry because it measures a product produced during the oxidation of fuel in the body instead of directly measuring the heat released during that oxidation. This form of indirect calorimetry has become the gold standard for the measurement of energy metabolism in free-living animals. The isotope dilution allows for the dynamic measurement of each of the water isotopes

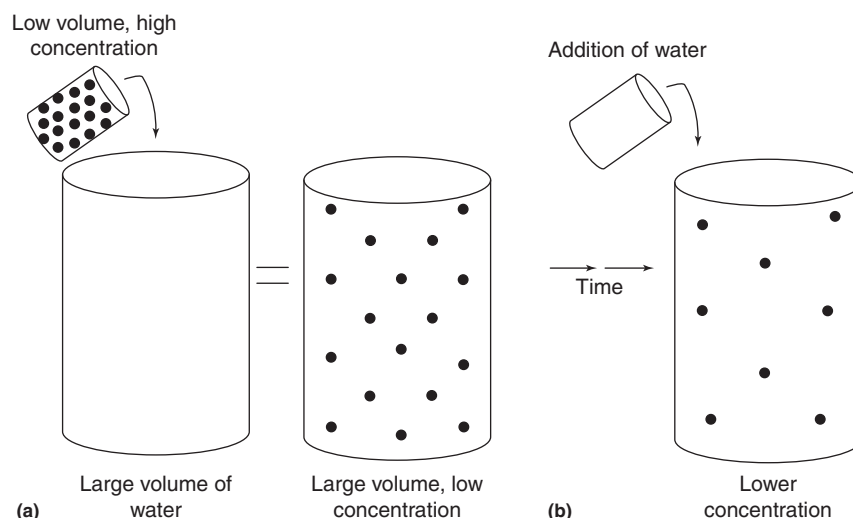


Figure 1 (a) Adding a concentrated dye to a beaker of water is analogous to a person adding a small amount of isotopic tracer to their body's water pool. (b) Adding water to the mixture created in (a) is analogous to that person then ingesting water over time and further diluting the mixture.

over time. Because ^2H exits the body as water, it is possible to calculate the water elimination rate: $r\text{H}_2\text{O} = N_{\text{D}}k_{\text{D}}$, where N_{D} is the deuterium dilution space and k_{D} is the fractional turnover rate of $^2\text{H}_2\text{O}$, which equals $(\ln[D]_2 - \ln[D]_1)/(t_2 - t_1)$. Similarly, ^{18}O 's elimination rate can be calculated: $r\text{H}_2\text{O} + 2r\text{CO}_2 = N_{\text{O}}k_{\text{O}}$, where N_{O} is the ^{18}O dilution space and k_{O} is the fractional turnover rate of H_2^{18}O . However, note that the oxygen turnover rate is the sum of both the water turnover and the carbon dioxide production rates, and therein lies the key to the energy expenditure calculation. The CO_2 production is realized as the difference between the two isotope elimination rates and is utilized in the calculation of heat production or energy expenditure. This can be expressed by combining and rearranging the two previous equations into $r\text{CO}_2 = 1/2[\text{TBW}(k_{\text{O}} - k_{\text{D}})]$, where TBW is the average of the two dilution spaces N_{O} and N_{D} . However, this equation is not exact because it does not take into account isotope exchange and isotope fractionation. A small amount of both ^2H (4.1%) and ^{18}O (0.7%) exchange with nonaqueous atoms of their parent element on other compounds and are therefore not part of the TBW pool. Incorporating these factors into the equation transforms it into $r\text{CO}_2 = (\text{TBW}/2) \times (1.007k_{\text{O}} - 1.041k_{\text{D}})$. Additionally, isotope fractionation occurs when the isotopically labeled CO_2 or water (in the form of water vapor) leaves the body at rates different from non-labeled water. At 37°C , the isotope fractionation rates are as follows: 0.946 for deuterium and 0.991 for ^{18}O between water and water vapor, and 1.038 for ^{18}O between water and CO_2 . This can be interpreted as the isotopes' abundance in the gaseous form being present either that much more or less than in the liquid form in body water. Thus, a more accurate human equation combining both fractionation and exchange can be condensed to $r\text{CO}_2 = 0.455 \times \text{TBW} (1.007k_{\text{O}} - 1.041k_{\text{D}})$.

The initial equilibration of the two isotopes after ingestion and subsequent elimination from the body provides the basis for the total energy expenditure calculation. Because the isotopic analysis is for aqueous samples, various body fluids (urine, saliva, and plasma) can be used. Urine is the primary fluid of choice as it is easily acquired in sufficient quantities and requires minimal cleaning steps to prepare for analysis. The procedure starts with the participant in the fasted state and providing a baseline sample, after which the dose is administered. Three more samples are obtained typically over 3–6 h post dosing depending on the experimental protocol and specific population characteristics. At this point the subject is free to leave the testing center and resume their normal lifestyle and experimental protocol. Urine samples are again required from 7 to 14 days after the dose was administered, preferably at the same time of the day that the post dose samples were obtained. This minimal sampling protocol is the methodologically important attribute of the DLW method. All other methods of calorimetry require frequent or continuous sample collection. The DLW method turns the human body into a metabolic recorder. By measuring the tracer concentrations in the body at the start and end of a one- or two-week metabolic period, the amount of tracer that left the body can be calculated. This provides a measure of the total water flux and CO_2 flux through the body during that period, which, when divided by the number of days, yields the average daily flux.

To calculate total energy expenditure (TEE), one more piece of information must be filled in. The respiratory exchange ratio (RER) is the ratio of CO_2 output and O_2 input that occurs during respiration. The RER can give us an idea of what type of substrate (fat vs carbohydrate) is being used to produce energy on a cellular level. The RER can be estimated based on diet. For example, the typical Western diet of 30–35% of energy from fat yields a food quotient of approximately 0.86. When the subject is close to the energy balance, the RER is assumed to equal the food quotient and this is used as an estimate of the RER in the standard indirect calorimetric equations for calculating energy expenditure from CO_2 production. The following modified Weir equation is the simplest and thus the most commonly used: $\text{TEE} (\text{kcal day}^{-1}) = 22.4 \times r\text{CO}_2 \times (1.10 + 3.90\text{RER})$, where 22.4 is the gas volume constant in l mol^{-1} , $r\text{CO}_2$ is derived from the tracer measurements as discussed above, and RER is estimated from diet or diet plus any change in body fat and protein stores.

Instrumentation

Unlike radio isotopes, that can be detected by the amount or the form of electromagnetic radiation they produce, stable isotopes must be differentiated and identified by their mass. Several types of mass spectrometers are currently available that separate and measure isotope concentrations based on its mass-to-charge ratio (m/z), where m is the mass of the ion and z is its corresponding charge. In basic terms, a sample must first be introduced into the instrument in the gas phase, ionized, separated by m/z , and then detected. Briefly, magnetic sector mass analyzers create and then accelerate ions through a magnetic field. Because all ions have equal initial kinetic energies, they are separated by their velocities through the tube as a lighter isotope will follow a more curved path than the heavier isotope. The quadrupole mass analyzer, which is more commonly used, contains four parallel rods that use a combination of a dc and an ac voltage at radio frequencies to guide ions of selected mass toward the detector. Ions in the sample whose m/z ratios are not selected by the detector simply collide with the rod and are rendered inert. Although these instruments may be more compact and less expensive to purchase and operate, for the purposes of analyzing DLW, the isotope ratio mass spectrometer (IRMS) is used. This is due to the superior accuracy and precision for isotope ratio analysis of the light elements. The use of IRMS allows investigators to use lower concentrations of isotopic tracer in their protocols. Typically, 10% atom percent excess (APE) rather than 95–99% APE normalized ^{18}O is mixed with 99% APE $^2\text{H}_2\text{O}$ for dosing, thus making the application of the DLW method more cost efficient, typically ranging from \$125 to \$250 per subject. In this context APE is the percentage of water molecules that contain the heavy stable greater than that present in natural water. This fact alone has led to the use of this type of analysis in human experimental trials than any other. The sample is introduced into the IRMS as a pure gas (CO_2 or H_2) which is then ionized by electron impact under vacuum and accelerated from the source as an ion beam (Figure 2). The ionized gas sample is then propelled through an evacuated flight tube, during which the ions are separated by a magnetic field. The

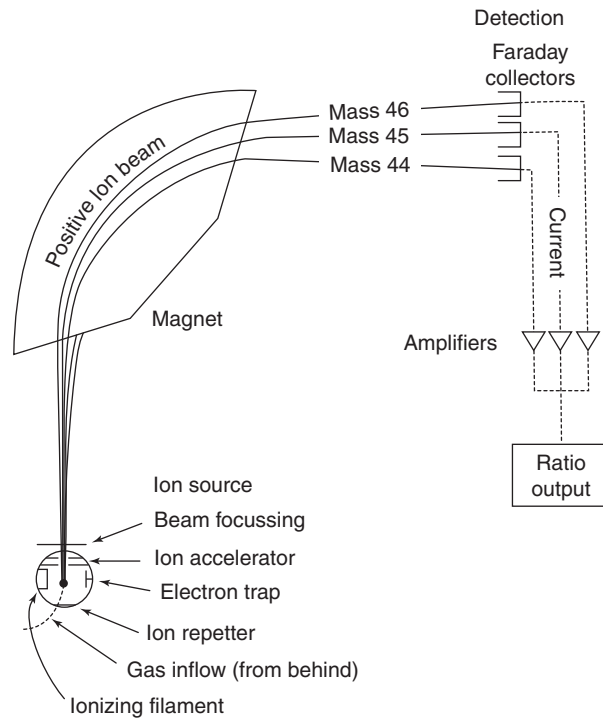


Figure 2 Schematic of an IRMS measuring CO₂ at the US Geological Survey (http://en.wikipedia.org/wiki/Isotope_ratio_mass_spectrometry).

ion flight for each gas species is then terminated on impact with a Faraday cup or collector. An array of these cups are configured to measure the current for each of the charged species of a gas and the isotope ratio is then measured for each sample and then compared with a reference of known isotope abundance, typically being either CO₂ or H₂.

Traditionally the introduction of the sample is through a dual-inlet system. This allows an unknown to be measured against a known reference and the isotope abundance expressed using the permil unit (‰). The permil value is essentially a ratio of the isotopic ratios for each of the two gases. Originally introduced by geochemists to express isotope abundances for natural water samples collected from around the world, permil is calculated as follows: $d = ((R_R + R_S) / R_R) * 1000 ‰$, where R_S is the ratio of the minor (heavy) to the major (light) isotope for the sample and R_R is the same for the reference. For example, the isotope ratio for an unknown sample of carbon dioxide enriched with ¹⁸O is calculated by measuring the mass-to-charge ratio of 46/44 (a molecule of ¹²C-¹⁶O-¹⁸O $m/z = 46$ over that of a molecule ¹²C-¹⁶O-¹⁶O $m/z = 44$). This is also done for the reference gas and the two ratios are used to calculate the permil value for the unknown. These values are then adjusted to express results relative to an international standard. Similarly, isotope ratios can be measured by introducing the gas sample into the IRMS via a continuous flow system. This method utilizes a noninterfering carrier gas (typically He) to transport the sample into an ion source. Once in the source, the sample with the carrier is subjected to the process of isotope analyses. The detector selects the mass of the sample and therefore the carrier is not detected. Other analytical methods also exist that are used for

the analysis of isotopically stable water. Fourier transform infrared spectrometry (FTIR) is a technique that utilizes the absorption of infrared light to measure the deuterium content of a water sample. By identifying the vibration energy of the O–H and O–²H bonds, the concentration of deuterium can be calculated, but FTIR does not provide the same precision for isotope abundance measures and thus requires the use of a larger and more costly dose of DLW. The recently introduced cavity ring-down (CRD) spectroscopy has an analytic precision close to that of IRMS, but costs less than IRMS and is easier to operate. CRD instruments measure the decay time for a pulse of laser light as it is absorbed by a gas sample in an optical cavity. An advantage of CRD is its ability to measure both ²H₂O and H₂ ¹⁸O simultaneously, potentially increasing user throughput and reducing operating costs.

Uses for the Doubly Labeled Water Method

When introduced as a novel method more than 50 years ago, the doubly labeled dosage was large, rendering the method expensive to measure the total energy expenditure. The improvements in measuring isotope abundances, however, have reduced the dose requirements and DLW is now used to measure energy expenditure in thousands of humans, making it the 'gold standard' method against which others are validated. However, one of the most significant roles DLW has played has been its influence in revising the dietary reference intake energy requirements for humans. Historically, energy balance and its requisite intake criteria have been based on dietary intake data provided largely by various self-report questionnaires. A recent review of various dietary intake methods established that, regardless of the type of dietary survey instrument used, participants regularly underreported their caloric intake by anywhere from 10 to 35%. Thus, when the most recent dietary reference standards were revised using DLW-measured energy requirements instead of dietary intake estimates, the energy requirements of healthy individuals established by national and international organizations have been corrected upward. Based on the DLW data, adult human energy intake requirements were correctly increased by 10% compared with estimated energy requirements published before 1990. An exception is the energy requirement for infants and neonates during the first two years of life, which were corrected downward by 10–15%.

As obesity has taken on epidemic and global status, it is not surprising that DLW has been essential in evaluating its impact and studying its etiology. Of particular interest was the finding that obese individuals had greater energy expenditure than their age- and height-matched lean counterparts. This was contrary to the popular hypothesis of 1990s that obesity developed in individuals due to a low energy expenditure as compared with their corresponding age- and height-matched lean counterparts. However, using DLW for the assessment of their energy requirements, investigators concluded that the rapid increase in human obesity is largely an effect of increases in dietary intake and not solely a product of lower energy expenditure (Figure 3).

In contrast to the epidemic of obesity DLW has also been used to measure the energy expenditures of extreme physical

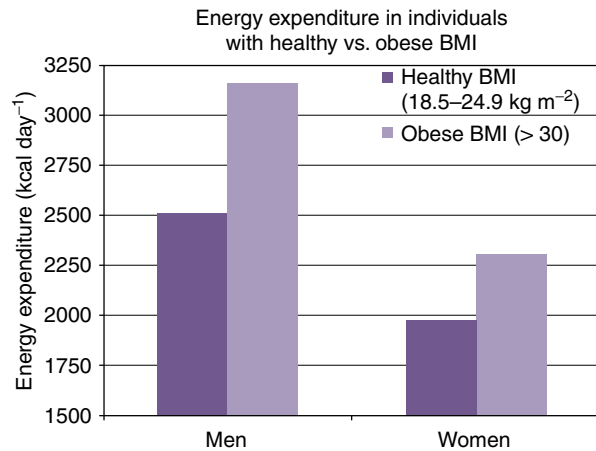


Figure 3 Comparison of energy expenditure among men and women in individuals with healthy vs obese BMI as measured by DLW. Data are from more than 2000 accumulated subjects in studies performed in the USA.

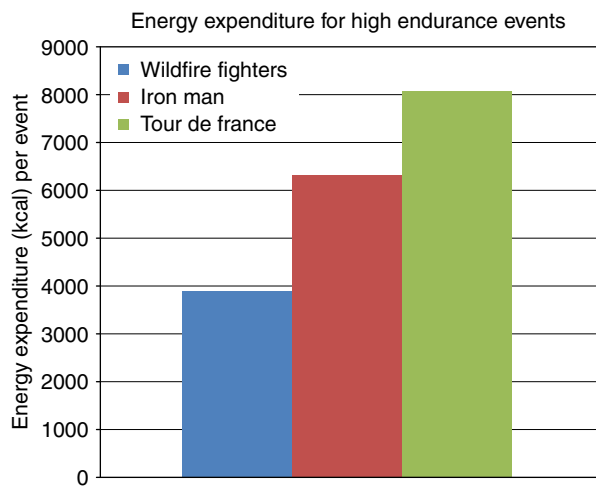


Figure 4 Energy expenditures of three high-endurance activities as measured by DLW. Data are from publications listed in the Further Reading section. See Ruby *et al.*, Saris *et al.*, and personal communication from Brent Ruby.

and endurance events. One advantage inherent to the DLW method is the ability to measure caloric expenditure while allowing subjects to remain in a free-living state by essentially turning each individual into their own indirect calorimeter. Energy requirements for a variety of high-intensity physical events are usually measured using the DLW method. For example, cyclists during the Tour de France, tri-athletes in international ironman competitions, and firefighters engaged in arduous wildfire suppression have all utilized DLW (Figure 4). Additionally, this method has been used to define the caloric and hydration requirements of those individuals who have participated in rapid ascent mountain climbing, the Iditarod race, open ocean self-propulsion, as well as various military training exercises of the US Special Forces.

Not constrained to only a single physical event, nutritional epidemiologists have used DLW to track body composition

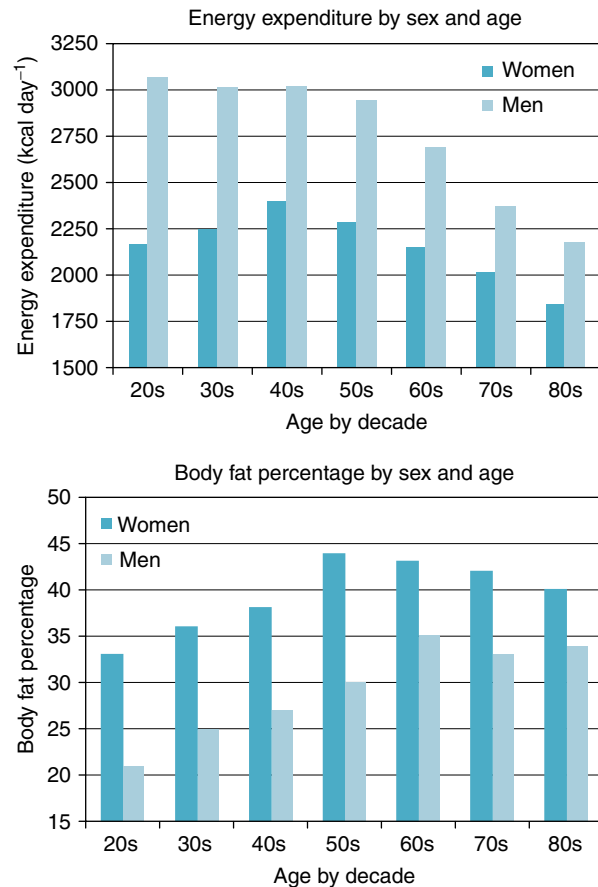


Figure 5 Comparison between men and women of the effects of age on body composition and energy expenditure. Data are from more than 2000 accumulated subjects in studies performed in the USA.

changes and energy expenditures in groups of individuals across countries, continents, societies, and economic conditions. Recently, researchers have analyzed energy expenditure from two groups of women from different countries with very different lifestyles. Urban African-Americans were compared with rural Nigerians in an attempt to understand the impetus of the American obesity epidemic. Although the Americans weighed more than their African counterparts by nearly 60 pounds, their activity energy expenditure (AEE = TDEE – REE – (0.1 × TDEE), where TDEE is total daily energy expenditure, 0.1 is the constant for the thermic effect of food, and REE is resting energy expenditure) was not significantly different compared with the Nigerians. For this comparison of American and Nigerian women, the AEE was adjusted for the differences in the weight and age of the women from the two countries. These findings again suggest that diet rather than a decline in physical activity appears to be the main contributor to the current rapid rise in the prevalence of obesity in Americans. Additionally, the DLW technique can be utilized *via* meta-analysis to corroborate or refute long-held hypotheses. Combining more than 2000 DLW study participants, the effects of age and sex on body composition and energy expenditure can now be analyzed. These data show the interactions between age, fatness, and energy expenditure for men and women (see Figure 5).

The DLW method has been an important tool for researchers in a variety of applications and has made an impact on the way we understand the human body. As obesity continues to increase globally, the use of DLW to measure body composition and energy metabolism will become even more paramount to health-related research in the future. In countries subjected to the dual burden of obesity and undernutrition, the DLW method has also become an important tool in the study of undernutrition and repletion.

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Relevant Website

http://www-pub.iaea.org/MTCD/publications/PDF/Pub1370_web.pdf
IAEA Human Health Series No.3: Assessment of Body Composition and Total Energy Expenditure in Humans Using Stable Isotope Techniques.

Indirect Calorimetry

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Glossary

Bicarbonate The chemical form of carbon dioxide when dissolved in body water.

Body water The sum of all water within and between tissue of the body.

Doubly labeled water A method to measure energy expenditure over one to weeks based on the elimination of stable isotope labeled water from body water.

Energy Expenditure The sum of energy expended while at rest, to digest and metabolize food, and during all external work (i.e., physical activity).

Indirect calorimetry Estimation of the metabolic heat produced by measuring differences of oxygen and carbon dioxide in the inspired and expired air.

Metabolic cart A common term for a portable, table mounted indirect calorimeter.

Respiratory Exchange Ratio (RER) or Respiratory Quotient (RQ) The ratio of the amount of carbon dioxide produced to the amount of oxygen consumed, used to calculate rates of carbohydrate versus fat used to support energy metabolism.

V_{CO_2} Rate of CO_2 production, often expressed in ml (milliliters) min^{-1} .

V_{O_2} Rate of O_2 consumption, often expressed in ml (milliliters) min^{-1} .

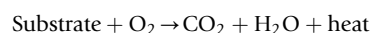
Introduction

All living organisms require a source of energy for survival. Among animals, this energy is provided in the form of chemical energy in the nutrients they consume, which are converted to other forms of energy through respiration. This conversion is subject to the same laws of thermodynamics that govern all energy systems. The first law of thermodynamics states that energy can neither be created nor destroyed; it can only be exchanged from one system to another. Hence, the chemical energy consumed in the form of food is converted into mechanical energy for work performed by the body, thermic energy for maintenance of body temperature, or stored as chemical energy in tissues such as fat, protein, or a small fraction as carbohydrates (glycogen). This conservation of energy can be stated mathematically as

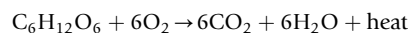
$$\text{Energy}_{\text{in}} = \text{Energy}_{\text{work}} + \text{Energy}_{\text{heat}} \pm \text{Energy}_{\text{stored}}$$

The sum of energy converted to work and heat is defined as metabolism. Although metabolism constitutes thousands of chemical reactions occurring at the same time throughout the body that cannot be individually measured, their sum can be measured as either the sum of work+heat energy or, in the absence of any measurable work, the rate of heat production by the body. This is based on the assumption that all cellular events ultimately result in heat production.

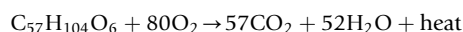
Calorimetry is defined as the measurement of heat production ('calor'=heat, 'metry'=process of measuring). Thus, the process of measuring heat produced by the body during oxidative combustion of macronutrients is a form of calorimetry. At the cellular level, each macronutrient is ultimately combined with oxygen to produce carbon dioxide, water, and heat as described by the following general equation:



These chemical reactions are similar to those that would be observed if the nutrient were combusted in a flame, except the reaction in the body is an enzymatic process that does not produce a flame. Still, the chemical reaction is the same; O_2 is a necessary substrate and CO_2 an end product of metabolism. The term 'indirect' calorimetry is used when heat production is calculated by measuring rates of oxygen consumption (V_{O_2}) and carbon dioxide production (V_{CO_2}) over time. Once V_{O_2} and V_{CO_2} have been determined, the Weir equation (Table 1) or similar equations can be used to calculate the rate of energy expenditure. In addition, the ratio of V_{CO_2}/V_{O_2} is used to calculate the percentage of carbohydrate versus fat being used to support energy expenditure. This ratio is commonly called the respiratory quotient (RQ), although the term respiratory exchange ratio (RER) is often used when applied to a whole body measurement. For example, one molecule of sugar (glucose) breaks down as follows:



In this reaction, six molecules of CO_2 are produced and six molecules of O_2 are consumed. Thus, the ratio of CO_2 produced to O_2 consumed has a value of 1.0. Similarly, when one molecule of fat (tripalmitin) is broken down completely, the chemical reaction is



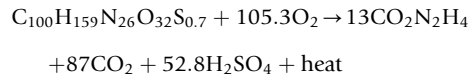
When this molecule of fat is completely oxidized, 57 molecules of CO_2 are produced whereas 80 molecules of O_2 are consumed (RER=0.71). The third macronutrient, protein, is more difficult to describe on a chemical basis because a protein is made from a mixture of amino acids, and for each dietary protein the number and composition of amino acids differ. The breakdown of the average dietary protein,

Table 1 Formulae for calculation of energy expenditure

Variable	Formula
V_{O_2} (ml min ⁻¹)	$= V_i (FiO_2) - V_e (FeO_2)$
V_{CO_2} (ml min ⁻¹)	$= V_e (FeCO_2) - V_i (FiCO_2)$
Respiratory exchange ratio (RER or RQ)	$= V_{CO_2} / V_{O_2}$
Full weir equation (TEE, kcal min ⁻¹)	$= (0.0039 V_{O_2} \text{ ml min}^{-1}) + (0.0011 V_{CO_2} \text{ ml min}^{-1}) - (2.2 \times \text{urinary } N_2 \text{ g min}^{-1})$
Abbreviated weir equation (TEE, kcal min ⁻¹)	$= (0.0039 V_{O_2} \text{ ml min}^{-1}) + (0.0011 V_{CO_2} \text{ ml min}^{-1})$

V_{O_2} =rate of O_2 consumption, V_{CO_2} =rate of CO_2 production, V_i =volume of inspired air per minute, V_e =volume of expired air per minute, F_i =fraction of inspired O_2 or CO_2 , F_e =fraction of expired O_2 or CO_2 .

however, can be described by the following chemical reaction:



In this example, 87 molecules of CO_2 are produced whereas 105.3 molecules of O_2 are consumed when one molecule of protein is oxidized (RER=0.83). Although this RER value is intermediate between carbohydrate and fat, protein is unique among the three energy substrates because it is the only one to contain nitrogen (N_2). As such, urinary N_2 can be assayed to obtain an estimate of protein oxidized by an individual. Combining this with the knowledge that the average protein is 16% N_2 by weight (1 g urinary N_2 =6.25 g protein), it is possible to use the previous chemical relationship to calculate V_{O_2} and V_{CO_2} resulting from the oxidation of protein. Subtracting protein V_{O_2} and V_{CO_2} from the total respiratory gas exchange measurements yields a nonprotein V_{O_2} , nonprotein V_{CO_2} , and nonprotein RER that are used to calculate the nonprotein metabolic rate using the equations found in Table 1. However, urinary N_2 is often not measured and therefore results from indirect calorimetry often use the abbreviated Weir equation to calculate rates of energy expenditure (Table 1), which assumes protein oxidation supports 12% of total energy expenditure.

Physiologic RER values generally range between 0.71 (100% fat oxidation) and 1.00 (100% carbohydrate oxidation). Thus, an RER of 0.85 reflects approximately 50% carbohydrate and 50% fat oxidation supporting energy expenditure. A healthy individual consuming an average mixed diet of carbohydrate, fat, and protein will likely have an RER between 0.80 and 0.85. For example, assume a healthy male consumes 350 ml O_2 min⁻¹ and produces 298 ml CO_2 min⁻¹ on average over a period of 24 h. He will be expending 1.7 kcal min⁻¹ (using the abbreviated Weir equation in Table 1). This equals a 24 h energy expenditure of approximately 2448 kcal day⁻¹ (1.7 kcal \times 1440 min). An RER above 1.00 usually indicates whole body *de novo* lipogenesis (endogenous synthesis of fatty acids). High intensity exercise will also result in an RER above 1.00 as the accumulation of lactic acid in the blood is buffered by the body bicarbonate pool, resulting in additional CO_2 output.

The first calorimeters developed in the early 1800s directly measured the rate of body heat production of an animal in a thermally isolated chamber ('direct' calorimetry). Although this technique is both an accurate and precise method for measuring energy expenditure, the disadvantages and difficulties of use make it unsuitable for most clinic and research

applications. A detailed account of the history and techniques of calorimetry can be found in the further reading section at the end of the article. The purpose of the following text is to describe laboratory and field indirect calorimetry techniques commonly used in most clinic and research settings.

Laboratory Methods

Whole Body Indirect Calorimetry

The advent of indirect calorimetry was a significant event in the history of animal and human nutrition. In whole body indirect calorimetry a person is kept in a small sealed room, often referred to as a 'metabolic chamber,' which is ventilated with a constant, measured supply of air. It is a setting similar to a person's habitual living environment and hence a more applicable measurement of energy expenditure. The chambers in use today are typically furnished with a bed, a chair, desk or table, toilet, sink, TV/computer, telephone, and an airlock (also called a pass-through) used for passing food and biological samples between subjects and study personnel. Two main types of whole body indirect calorimetry systems exist.

Closed-circuit indirect calorimetry involves the recirculation of the same air through the sealed chamber. The recirculated air is kept breathable by removing CO_2 produced by the subject and replacing O_2 consumed by the subject. The replacement of O_2 is controlled by continuously monitoring the change in the volume of the gas in the closed breathing circuit. As the subject consumes O_2 , a sensor detects the decrease in volume and a signal is sent to an external source to release constant calibrated pulses of O_2 back into the system to restore the original values. The rate of O_2 consumption is measured by recording the amount of O_2 that is added to the air during recirculation. The CO_2 produced by the subject is removed from the recirculated air by an absorber attached to the system and is measured from the increased weight of the absorber (Figure 1).

Open-circuit indirect calorimetry involves a system in which both ends of the breathing system are open to the atmosphere (Figure 2). Outside air is drawn into the chamber at a constant flow rate, which produces a slight negative pressure inside the chamber. At the same time, the composition of this outside air is measured by CO_2 and O_2 gas analyzers. Well-mixed expired chamber room air is drawn out at a constant flow rate and analyzed for O_2 and CO_2 composition. The difference in O_2 and CO_2 composition of the outside air and chamber room air is used to calculate the energy expenditure

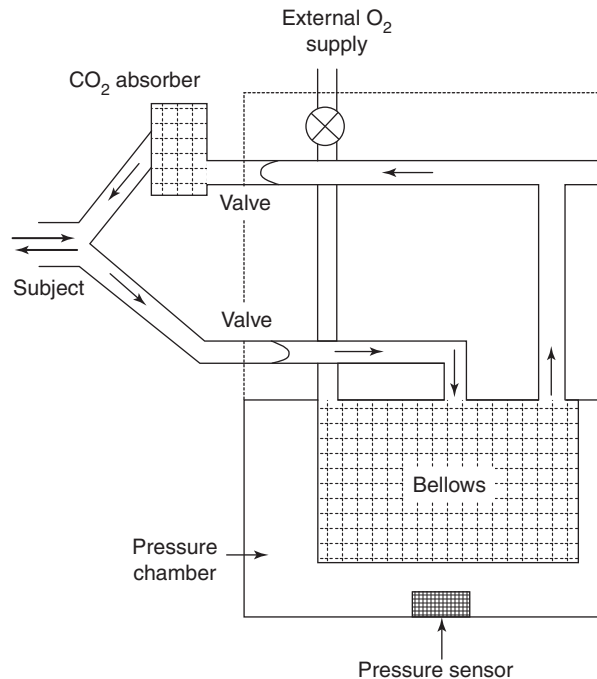


Figure 1 Closed-circuit metabolic chamber in which the subject's oxygen consumption is measured to calculate the corresponding energy expenditure. The change in volume of air in the system is constantly monitored by the sensors and a measured quantity of oxygen is added back to the system. Carbon dioxide is taken out of the recirculated air by a CO₂ absorber.

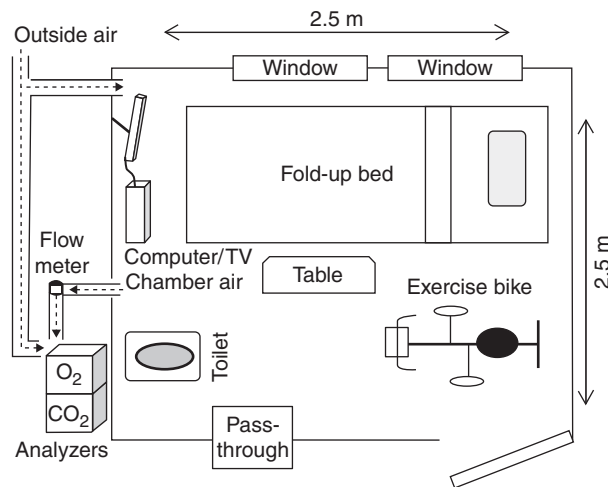


Figure 2 Open-circuit metabolic chamber.

and macronutrient oxidation of the subject using the equations in Table 1.

The concept of indirect calorimetry is often applied on a scale smaller than a whole room in many research and clinic settings. Many smaller indirect calorimeters have been developed over the years. The instrumentation used for each varies in complexity and the degree to which they restrict the subject's movement.

Metabolic Carts

Metabolic cart is a common name for a semiportable respiratory gas analyzer that has been made small enough to be placed on a cart with wheels so that it can be rolled to different locations within a building. Two designs are generally available: the ventilated hood and the mouthpiece system.

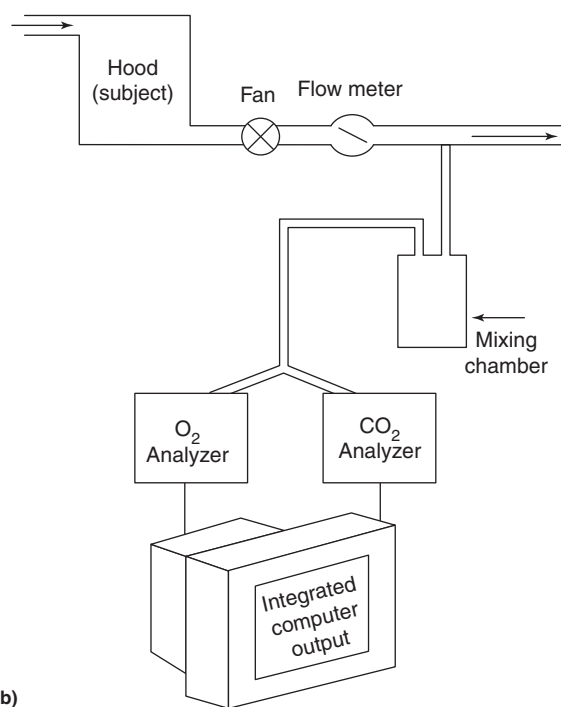
The ventilator hood system is an open-circuit indirect calorimeter that usually consists of a pliable plastic or rigid Perspex hood placed over the subject's head with a latex or thin plastic apron providing a rough seal around the neck or chest. These allow air to be drawn across a subject's face while in a reclining or lying position. For longer term measurements, ventilated plastic tents are available that cover all or part of the patient's bed. Because these hoods operate on a suction principle, a tight seal of the hood is not required. For field measurements, whole body transparent plastic ventilated boxes have been used successfully in infants. Many of the ventilatory hoods are constructed by researchers from the components according to the requirements of their study. The components include a pump, a flowmeter, and a means of regulating the airflow. Samples of the air drawn from the hood can be directed to gas analyzers, which are usually connected in series to the hood. Respiratory gas exchange is calculated from the difference in O₂ and CO₂ concentration between the air entering and exiting the hood and from knowing the controlled airflow rate (Figures 3(a) and (b)).

Instruments have been developed to operate in adult and pediatric applications and differ with respect to flow rates and internal volume because metabolic rate, and hence gas exchange, of children is smaller than that of an adult. With adult and pediatric systems, the expired air enters a mixing chamber within the instrument to eliminate concentration variation resulting from inspiration and expiration before the sample enters O₂ and CO₂ sensor analyzers, which measure the concentration differences between the expired and inspired air. For state-of-the-art instruments, the data are input into a microprocessor providing minute-by-minute calculation of the O₂ consumption, CO₂ production, RER, and energy expenditure. These instruments are generally used for measurements of subjects at rest as part of nutritional studies of energy expenditure and macronutrient utilization. These units can also be connected to mechanical ventilators for use in hospitalized patients.

Mouthpiece systems are similar to ventilated hood systems in principle, but instead of placing a hood over the subject's head, the subject wears a mouthpiece connected to the analyzer and nose clips to prevent breathing through the nose. The mouthpiece is connected to a valve system that allows the subject to breathe in atmospheric air while directing the exhaled air to the gas analysis system. The expired breath is again subjected to analysis of O₂ and CO₂ concentration, but rather than passing the breath through a mixing chamber to smooth out the changes in concentration gradient of these gases from the start to end of an exhalation, the concentration profile is measured in real time along with the rate of gas flow from the exhalation. Again, the data are logged into a microprocessor for calculation of O₂ consumption and CO₂ production, but in this case the calculation is performed on a breath-by-breath basis. Results are averaged over time, usually provided as



(a)



(b)

Figure 3 (a, b) Ventilatory hood system showing a hood that is placed on the subject's head, a mixing chamber, and O_2 and CO_2 analyzers. A fan maintains a slight negative pressure in the hood to pull air into the chamber and also to prevent the escape of the expired air from the system. The air is mixed in the mixing chamber and is analyzed for oxygen and carbon dioxide by the respective analyzers. Results are calculated by the computer.

minute-by-minute averages of O_2 consumption, CO_2 output, and the rate of energy expenditure. The mouthpiece systems are generally used for studies of gas exchange and energy metabolism during exercise and provide a shorter measurement response time than the ventilated hood systems. The mouthpiece and nose clip used with some of the instruments make long time measurements highly cumbersome. Also, breathing through the mouthpiece often causes untrained subjects to involuntarily hyperventilate leading to inappropriate O_2 and CO_2 rates. It is also often difficult with

mask systems to obtain an airtight seal without excessive pressure at the site of contact with the mask and face.

Different types of metabolic carts or monitors are available that are designed for various applications ranging from nutrition to exercise science. Most have built-in gas analyzers and data processing computers, making them highly user-friendly, easy to use tools for measurement of energy metabolism. They generally provide accurate and reliable data but do require periodic calibration. Ventilated hood systems often use a combination of gases with known concentrations and weighed ethanol or methanol burns for such calibration, whereas breath-by-breath systems use a combination of large volumetric syringes and gases of known O_2 and CO_2 concentration (Figure 4).

Field Methods

As for whole body indirect calorimetry, ambulatory and portable systems measure the respiratory gas exchange with the V_{O_2} and V_{CO_2} measurements. Ambulatory methods and less refined laboratory methods often dispense with the measurement of CO_2 to avoid the need for two gas analyzers. The error incurred by assuming a CO_2 production rate is several percentage points, which researchers are prepared to compromise on. When only O_2 consumption is measured, however, it is not possible to compute macronutrient-specific oxidation rates. The accuracy of ambulatory and portable methods is generally between +4% and -2%. Field methods involve the collection of expired air over a fixed period of time as in the Douglas bag or small online analysis systems that sample inspired and expired air through a mouthpiece.

Douglas Bag/Tissot Tank

The Douglas bag method is a classical example of collection of expired air to measure energy expenditure in the field during both rest and physical activity. It consists of a gas-impermeable bag with a capacity of ~100 l or a Tissot tank suspended over water, which is used to collect and store the subject's expired air over a fixed, short time interval. A classic Douglas bag is made of either a rubber sheeting cemented between two layers of canvas or plastic material lined by PVC or aluminum with welded seams. The rubber bags are subject to slow leakage of CO_2 by diffusion, which is unavoidable, but PVC and metalized bags reduce this loss. If the bags are filled to capacity and analyzed with 20 min of collection, the effects of diffusion are minor. The subject wears a nose clip and mouthpiece or a face mask. Outside air or its equivalent is inhaled through the mouthpiece or mask containing a one-way valve and exhaled into a Douglas bag or Tissot tank for a precise period of time. It is important that the mouthpiece and connecting tubing provide minimal resistance to airflow, or the cost of breathing will increase the energy expenditure. Ambient temperature, barometric pressure, and relative humidity are recorded for converting values under conditions of standard temperature and pressure. The volume of air collected in the bag or tank is measured and a sample of exhaled air is obtained to measure the O_2 and CO_2 concentrations using gas analyzers. The

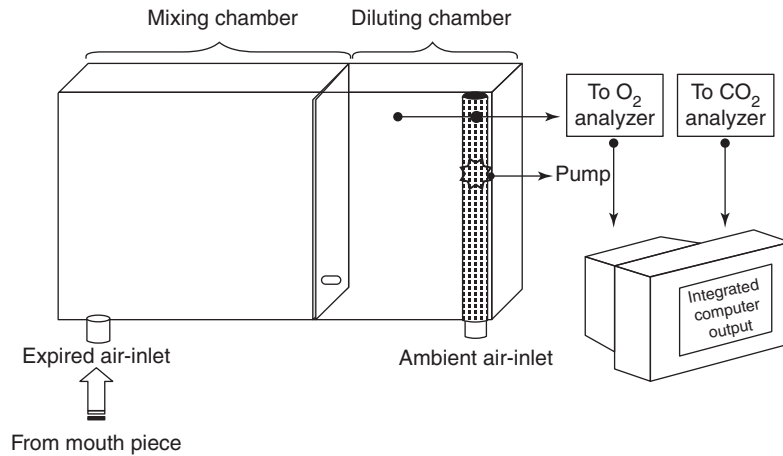


Figure 4 Metabolic unit measuring both O₂ consumption and CO₂ production rates during rest and exercise. In this type of system, expired air is diluted using ambient air before being analyzed by the respective analyzers.

volume of oxygen consumed and carbon dioxide exhaled are calculated by analyzing the gas from the Douglas bag for the precise time period during which it was collected. This method is relatively simple and inexpensive yet gives reliable results. It is suitable only for short durations of field measurement, and wearing the mask and nose clip for the whole duration of the study may be cumbersome, interfere with daily activities, and is socially undesirable to the subject. Spirometers were used in the past for measurement of the volume of the respired air. With the advent of continuous flow electronic analyzers and superior gas flowmeters, spirometers are now rarely used. Ambulatory methods also consist of a mouthpiece incorporating light action-sensitive but robust one-way gas valves, corrugated tubes, and three-way taps. The volume of air respired and the relative concentrations of O₂ and CO₂ in the expired air are measured using O₂ and CO₂ gas analyzers. These small analyzers have replaced the Haldane system or microScholander chemical gas analyzers, which used reagents to absorb the CO₂ and O₂, with the weight of absorbents measured before and after the gases were absorbed.

Max Plank/Kofranyi–Michaels Respirometer

A Max Plank respiration gas meter is a small, compact, and lightweight backpack-mounted respirometer. It combines a gas volume meter and a sampling device for continuous sampling of each breath of expired air. The Max Plank respirometer consists of a dry, bellow-type gas meter for measuring the total volume of expired air during activity. The subject breathes through a low-resistance valve and the expired volume is monitored. A measured quantity of expired air is removed continuously (0.3% or 0.6%) by an aliquoting device to be sent to a small butyl rubber bag. This rubber sampling bag can be connected directly to the oxygen analyzer, eliminating the need for transfer of samples to gastight syringes for analysis. The respirometer is suitable for flow rates between 15 and 50 l min⁻¹ or for period of 110 min on a slow flow rate and 55 min on a faster rate. It is smaller, more compact, and lighter than the Douglas bag apparatus and can be used in

studies involving light to moderate physical activity. Although the system has a low resistance, at higher ventilation rates the resistance increases substantially and hence cannot be used in higher flow rate scenarios. It is also seen that this can be used in studies of shorter duration only. Owing to the use of mouthpiece and nose clip, prolonged usage may cause discomfort to the subjects.

Telemetry Systems

The K2 system was the first of a series of portable systems that consists of a soft face mask with a turbine flowmeter attached to it. A transmitter and battery are attached to a chest harness, which transmits signals to a receiver unit. The flowmeter measures the rate of airflow, calculates the volume of expired air per minute, and counts the number of expirations per minute. A small capillary tube passes through to the transmitter unit, which contains an electrochemical gas analyzer used to measure the concentration of oxygen in expired air. The signals from this analyzer are transmitted to the receiver unit by the portable transmitter unit. The receiver unit processes the data and prints it in a desired format. The electrochemical gas analyzer is a polarographic electrode. It has a membrane through which oxygen permeates into an electrolyte solution generating an electrical impulse proportional to the rate of oxygen permeation through the membrane. Because these systems are portable and easy to use, they have many potential uses in exercise science studies and rehabilitation medicine. They allow a breath-by-breath pulmonary gas exchange measurement while still being very light and portable, enabling a direct field assessment of human performance and cardiopulmonary limitations. The low-resistance flowmeter allows a wide range of oxygen flow rates to be measured, though these systems face the issue of air leakage from the face masks when subjects are made to exercise at high intensities. The measurement durations usually are limited to 1–5 h. The polarographic electrode membrane is known to have a short life span and hence monitoring of the usage of the instrument is essential. If CO₂ concentrations are essential for a study, this is not a good instrument to use (Figure 5).

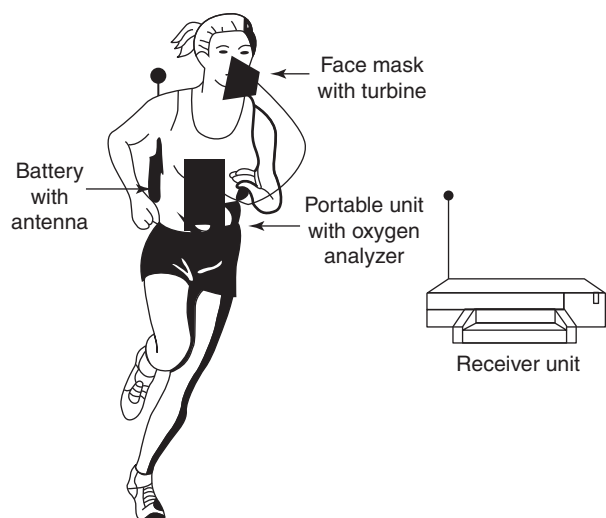


Figure 5 Telemetry system with a face mask attached to a turbine flowmeter, a transmitter, and a receiver unit. The flowmeter measures the rate and volume of airflow and the expiratory cycles per minute. The expired air is analyzed for oxygen concentration by an oxygen analyzer in the transmitter unit. The transmitter then transmits the signals to the receiver unit, which integrates the data and prints the results.

Tracer Methods of Indirect Calorimetry

A third category of techniques have gained popularity among investigators during the past two decades. These techniques provide a measure of CO_2 production through the use of dilution techniques using isotopic tracers.

Labeled Bicarbonate

A constant infusion-labeled bicarbonate method is useful in estimating the net CO_2 production and hence energy expenditure in animals and humans. This method is based on an isotopic dilution technique whereby the administered label is diluted by the CO_2 produced endogenously by the body. The extent of this isotope dilution is used to measure the rate of CO_2 production and is used to estimate the energy expenditure of the individual. A microinfusion of ^{13}C or ^{14}C labeled bicarbonate is given to an individual and the specific activity or enrichment of his or her physiological fluids, especially breath or urine, are measured to estimate the rate of label elimination and hence the rate of endogenous CO_2 . Thus, variation in the endogenous CO_2 production rate will be reflected in the dilution of the body pool and consequently in the breath samples. These measurements are accurate when energy expenditures are measured over a longer duration of time (> 1 day), but are subject to effects of label sequestration over shorter periods. Sequestration refers to trapping, or fixation, of the label in tissues that utilize bicarbonate/ CO_2 for their metabolic functions. Shorter duration of collection of breath samples requires a correction for the fraction of label that is sequestered. This is based on the assumption that similar amounts of label are sequestered in various individuals. When breath samples are collected over longer durations, the sequestration is often assumed to be negligible.

Some investigators have used a bolus bicarbonate administration rather than the continuous infusion. These investigators measured the rate at which the label concentration decreases with time as a measure of CO_2 turnover and the initial concentration as a measure of the body's bicarbonate pool size. Taken together, these provided a measure of energy expenditure during a short period of constant physical activity.

Doubly Labeled Water (DLW)

This is an isotope dilution technique, discussed in detail in the next article, wherein deuterium and heavy oxygen-labeled water (DLW) are given to individuals and timed urine samples are collected to measure the elimination rates of ^2H and ^{18}O in the urine. ^2H label from DLW mixes with the body water and is eliminated as water in the urine. Similarly, ^{18}O label from DLW is eliminated as water, but it is also utilized in bicarbonate synthesis and hence is also eliminated in the breath as CO_2 . The difference in turnover rates of isotopic ^2H -H and ^{18}O -labeled water is proportional to CO_2 production. DLW is technically a method of indirect calorimetry even though respiratory gas exchange measurements across the lung are not made. Energy expenditure, oxygen consumption, water intake, and metabolic water production can be calculated using standard indirect calorimetry equations with an estimated RER. Unlike the majority of the other methods, the DLW method provides a measure of average energy expended over a period of 3–21 days, which provides a better estimate of habitual free-living energy expenditure.

Summary

Indirect calorimetry is a noninvasive, reliable, and valuable tool in assessing energy expenditure and fuel utilization by the body. Scientists from multiple disciplines have used it to measure energy expenditure, establish nutrient requirements, measure physical fitness, and evaluate macronutrient utilization during exercise and rest. Clinicians have used indirect calorimetry to optimize the nutritional support in metabolic disorders and to quantify the energy requirements of patients. Indirect calorimetry has such universal appeal because animals and humans derive their energy for sustenance by transforming the chemical energy from nutrients consumed to heat through respiration, and their existence depends on their ability to balance energy intake and expenditure.

See also: Energy Expenditure: Doubly Labeled Water. Energy Requirements. Starvation and Fasting: Biochemical Aspects. Weight Management: Weight Maintenance

Further Reading

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ENERGY METABOLISM

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Glossary

Coenzyme A loosely bound nonprotein compound required for an enzyme function. Frequently derived from vitamins, for example, nicotinamide adenine dinucleotide (NAD⁺) derived from niacin.

Enzyme Protein compound that acts as a biological catalyst to speed up the rate of a chemical reaction, without itself being permanently changed.

Ketone bodies Soluble compounds produced from the catabolic metabolism of fatty acids, and some amino acids, mainly in the liver. Ketone bodies can be transported to other tissues which have limited capacity for fat and protein oxidation and metabolized for energy when glucose is limited.

Metabolism The sum of anabolic (synthetic) chemical reactions that require energy and catabolic chemical reactions, which break down large organic molecules into smaller molecules, thereby releasing energy.

Oxidation and reduction Reduction is the gain in electrons by an ion, atom or molecule and therefore a change in their oxidation status or overall charge. Oxidation is the reverse, a loss in electrons by an ion, atom or molecule. These reactions are paired as redox reactions when one substance is reduced and another oxidized, such as occurs in the hydrogen electron transfer chain in mitochondria (see below).

Cellular Respiration and Adenosine Triphosphate (ATP)

Cellular respiration can be defined generally as the process by which chemical energy is released during the oxidation of organic molecules. If it requires oxygen it is called aerobic respiration, whereas if it takes place in the absence of oxygen it is anaerobic respiration.

Organic molecules, usually carbohydrate or fat are broken down by a series of enzyme-catalyzed reactions. Many of these reactions release a small amount of energy that is channeled into molecules of a chemical nucleotide called adenosine triphosphate or ATP (**Figure 1**).

ATP is the standard unit in which the energy released during respiration is stored. ATP is an instant source of energy within the cell. It is mobile and transports energy to wherever energy-consuming processes are occurring within the cell. The energy is released by the dephosphorylation of ATP to adenosine diphosphate (ADP), which can then be rephosphorylated to ATP thorough coupling to the processes of respiration. ATP is found in all living cells and can be thought of as a universal energy transducer.

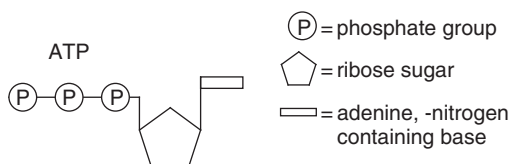
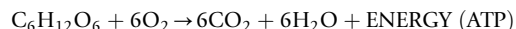


Figure 1 Structure of adenosine triphosphate (ATP).

The principle metabolic fuel is glucose and there are three stages in its oxidation to carbon dioxide, water, and energy; captured as ATP. This process can be summarized very simply by the following equation:



In the first stages of glycolysis and the tricarboxylic acid cycle, glucose, and other metabolic fuels are oxidized, linked to the chemical reduction of coenzymes (nicotinamide adenine dinucleotide (NAD⁺), flavin adenine dinucleotide (FAD), and flavin mononucleotide (FMN)). In the final stage, of oxidative phosphorylation via the hydrogen electron transfer chain, ATP is synthesized from ADP and phosphate using energy released from the oxidation and recycling of the reduced coenzymes (**Table 1**). Thus the oxidation of metabolic fuels is tightly coupled to energy consumption and the production of ADP from ATP in energy consuming processes (**Figure 2**).

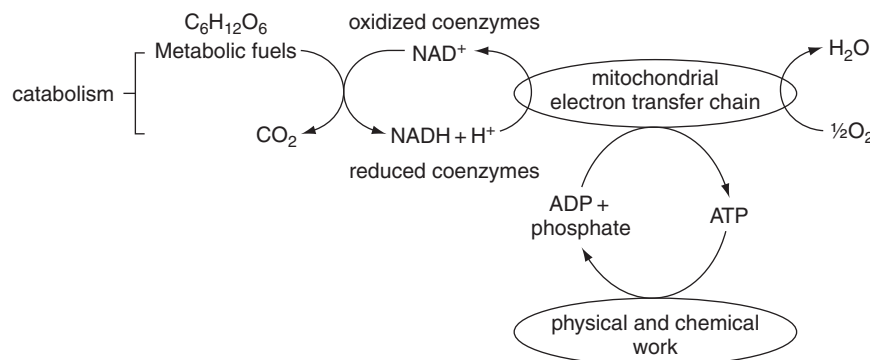
Glycolysis

The main substrate for glycolysis is glucose. Glycolysis does not require oxygen and is important for the direct production of ATP when oxygen is limiting, i.e., in rapidly contracting muscle. Glycolysis results in the splitting of glucose – a 6 carbon (6C) compound – into two molecules of pyruvic acid (3C), which in the cytoplasmic solution becomes pyruvate (**Figure 3**). Pyruvate can enter the mitochondrion and be metabolized by oxidative decarboxylation to CO₂, or if oxygen is unavailable it can be further metabolized to lactic acid

Table 1 The three principle stages in the production of ATP from 1 molecule of glucose

Metabolic pathway	Where	O ₂ required?	Net ATP or reduced coenzymes/glucose	Products
Glycolysis	Cytoplasm	Anaerobic	Net gain 2 ATP	Glucose → 2 pyruvate
Pyruvate → acetyl-CoA	Mitochondrial matrix	Aerobic	2 NADH + H ⁺	2 Pyruvate → 6CO ₂
TCA cycle	Mitochondrial matrix	Aerobic	2 NADH + H ⁺ 2 GTP → 2 ATP 8 NADH + H ⁺ 2 FADH ₂	
Electron transfer chain (oxidative phosphorylation)	Mitochondrial crista and primary particles	Aerobic	12 NAD ⁺ + 2 FAD → 38 ^a ATP	12H ₂ + 6O ₂ → 6H ₂ O

^aThe exact net gain in the number of ATP produced from the oxidation of the reduced coenzymes NADH + H⁺ and FADH₂ can vary dependent on the mechanism used to transport them across the crista membrane in the mitochondria, the site of oxidative phosphorylation.

**Figure 2** Linkage between ATP utilization in physical and chemical work and the oxidation of metabolic fuels.

resulting in the regeneration of NAD⁺ from NADH + H⁺, thus allowing glycolysis to continue in the absence of oxygen. Red blood cells lack mitochondria and therefore glycolysis is the only source of energy metabolism. Thus red cells can only metabolize glucose or other simple sugars and not fats or proteins. Red cells produce lactate that is excreted into the blood. Lactate is primarily metabolized back to pyruvate in the liver, where it is mostly used for the synthesis of glucose (gluconeogenesis), which is essentially the reverse of glycolysis, except for the irreversible reaction of phosphoenolpyruvate (PEP) to pyruvate. Hence in the liver pyruvate is converted back to PEP via oxaloacetate (**Figure 3**). This cycling of lactate and pyruvate is known as the Cori cycle.

Other sugars such as fructose and galactose can be fed into glycolysis at different points and then metabolized in the same way as glucose to pyruvic acid.

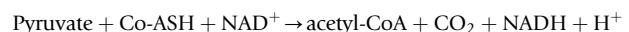
The pentose-phosphate shunt (sometimes also known as the hexose-monophosphate pathway) is when glucose-6-phosphate is metabolized via an alternative route to glycolysis to generate pentose phosphates to be used as components in DNA and RNA nucleotides. Alternatively, pentose phosphates can be returned into the glycolytic pathway by conversion back to fructose-6-phosphate or glyceraldehyde-3-phosphate. Another purpose of this shunt is the production of NADPH + H⁺ from NADP⁺, which is the required coenzyme for fat synthesis (lipogenesis). Reduced NADP⁺ is also required for the reduction and recycling of oxidized glutathione, an important intermediate in antioxidant defense and in the

generation of the respiratory burst, used to kill parasites ingested by macrophages, a type of white blood cell.

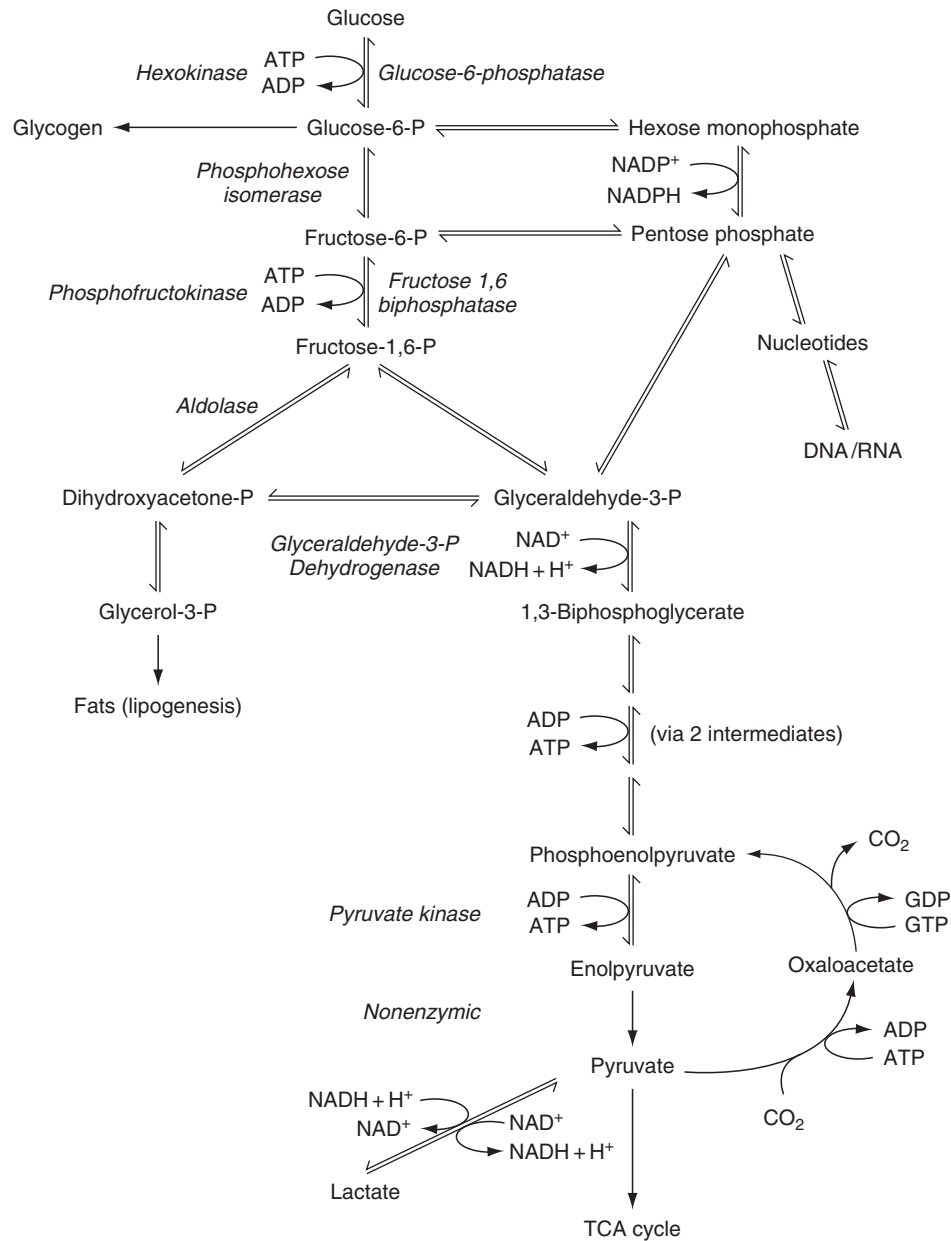
Tricarboxylic Acid Cycle (also known as the TCA Cycle, Citric Acid Cycle, or Krebs's Cycle)

The tricarboxylic acid cycle (TCA cycle) is located in the mitochondrial matrix and is a common metabolic pathway for all fuels, and is responsible for the production of the majority of the reduced coenzymes used for the generation of ATP in the electron transfer chain. It also plays a central role in the interconversion of fuels and metabolites. The TCA cycle participates in gluconeogenesis from amino acids and lactate during fasting between meals and longer term in starvation. TCA cycle intermediates are the source of most of the non-essential amino acids such as aspartate and glutamate. It is also involved in the conversion of carbohydrates to fat for storage after a carbohydrate rich meal.

Pyruvate (3C) from glycolysis is oxidatively decarboxylated to acetyl-CoA (2C) in the mitochondria, catalyzed by the multienzyme complex, pyruvate dehydrogenase, and the coenzyme A (Co-ASH):



Pyruvate dehydrogenase requires several coenzymes derived from vitamins, including thiamine, niacin (NAD), riboflavin (FAD), and pantothenic acid (a component of



$$\text{net} = 2 \text{ NADH} = 6 \text{ ATP} + \text{net } 2 \text{ ATP}$$

Figure 3 Glycolysis and its interactions with other metabolic pathways.

CoA). Deficiencies in any of these vitamins can affect energy metabolism, as evidenced by the increased cellular pyruvate and cardiac and skeletal muscle weakness in beri-beri caused by thiamine deficiency. Pyruvate dehydrogenase catalyzes a central reaction in carbohydrate metabolism and therefore its activity is regulated through multiple pathways.

Acetyl-CoA can be produced from pyruvate, but also from fatty acids released from fat stores and from amino acids released from proteolysis of protein tissue, which can be converted to acetyl-CoA or TCA cycle intermediates.

In the beginning of eight enzymatic reactions acetyl-CoA (2C) combines with oxaloacetate (4C), forming citrate (6C)

and releasing the CoA for further reactions with pyruvate to acetyl-CoA. A cycle of reactions follows in which two molecules of CO₂ are released and three molecules of NAD + H⁺ and one of FADH₂ are produced along with one molecule of guanosine triphosphate (GTP) (equivalent to ATP). At the end of the cycle oxaloacetate is regenerated and able to react again with another molecule of acetyl coenzyme A, and so the cycle continues (**Figure 4**).

In the electron transfer chain each NAD + H⁺ yields approximately 3 ATP and FADH₂ yields 2 ATP. Thus each rotation of the TCA cycle produces approximately 12 ATP (3 NAD + H⁺ ≈ 9ATP + 1 FADH₂ ≈ 2ATP + 1GTP).

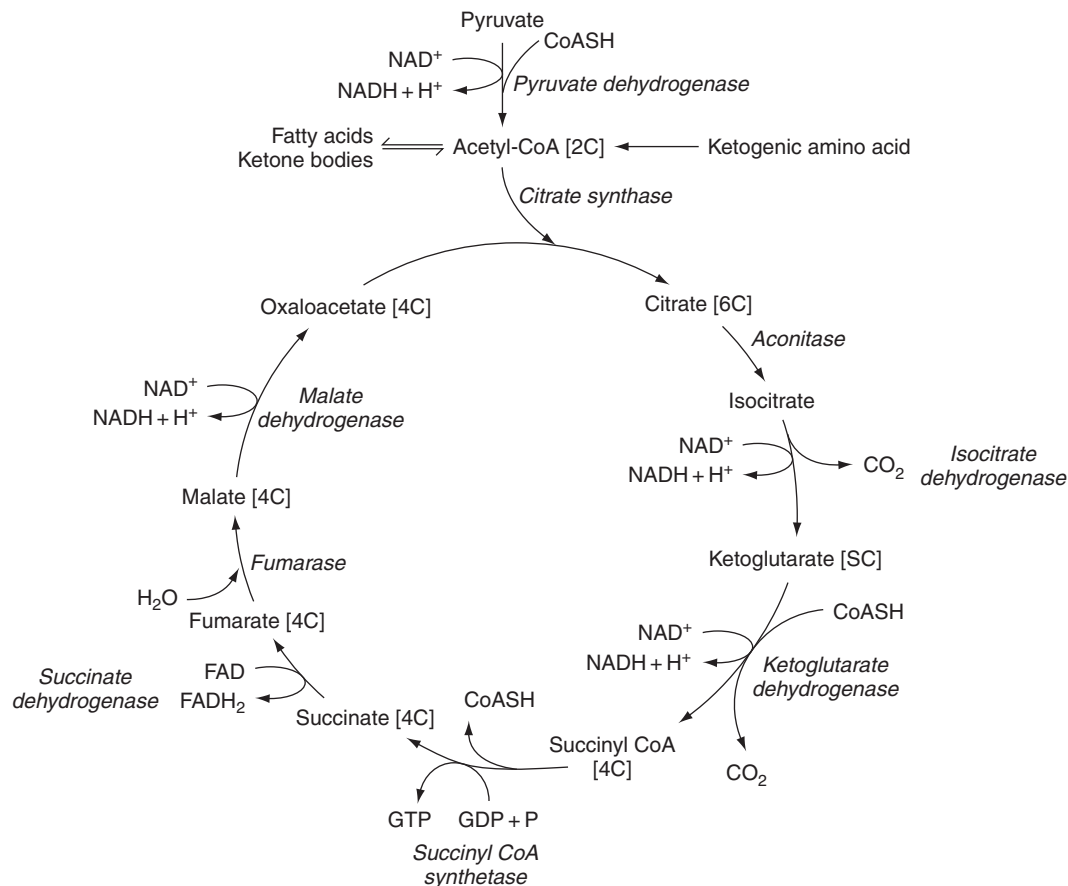


Figure 4 The oxidative decarboxylation of pyruvate and the tricarboxylic acid cycle.

As two molecules of acetyl-CoA are formed from one glucose molecule, the TCA cycle rotates twice for each molecule of glucose respired, producing a net of 24 ATP (Table 1).

The Electron Transfer Chain (Oxidative Phosphorylation)

Oxidative phosphorylation occurs in the crista of mitochondria, formed by invaginations of the inner mitochondrial membrane (Figure 5). The hydrogen accepted by NAD⁺ and FAD during glycolysis and the TCA cycle is oxidized to water by molecular oxygen accompanying phosphorylation of ADP → ATP. This is achieved by phosphorylation of ADP being coupled with a series of redox reactions whereby the hydrogen ions (H⁺) and their electrons (e⁻) are passed along a chain of intermediate carriers in the crista membrane of the mitochondria (Figure 6), each intermediate being reduced by the proceeding one and in turn reducing the next one and hence itself being oxidized. The chain consists of a flavoprotein and a ubiquinone (coenzyme Q), both are hydrogen carriers, then followed by a series of cytochromes that are carriers of electrons only. Finally, at the end of the chain is cytochrome oxidase which catalyzes the formation of water from hydrogen ions, electrons, and molecular oxygen. Unlike the other cytochromes, cytochrome oxidase contains copper (Cu²⁺) in a prosthetic group instead

of Fe³⁺ (in the form of a heme molecule) and this final stage can be inhibited by the irreversible binding of cyanide to the Cu²⁺ preventing it from accepting electrons and therefore terminating the entire hydrogen electron transfer chain and hence all aerobic respiration. This is the basis of the toxicity of cyanide and several other substances.

When the hydrogen from NADH + H⁺ or FADH₂ is passed from ubiquinone to the first cytochrome, the hydrogen dissociates into a hydrogen ion (proton) and an electron. The proton is excreted into the crista space, whereas the electron carries on down the chain of cytochromes. This creates a proton gradient across the crista membrane. In the last step, in the reduction of molecular oxygen to water, the hydrogen protons are obtained not from the hydrogen excreted into the crista space, but from the mitochondrial matrix from the dissociation of water (H₂O ↔ H⁺ + OH⁻) hence maintaining a proton gradient across the crista membrane. The resulting movement of protons from the crista space to the matrix through the transmembrane stalk of the primary particle drives the multienzyme complex of ATP synthase. It is the energy of the flow of the protons that provides the energy required for the synthesis of ATP in a manner analogous to a water mill where the flow of water can be used to turn a motor, to grind wheat, or generate electricity.

The oxidation of FADH₂ and NADH + H⁺ is normally tightly coupled to the phosphorylation of ADP → ATP as the phosphorylation of ADP cannot occur unless there is a proton

A. Representation of a cross section through a mitochondrion

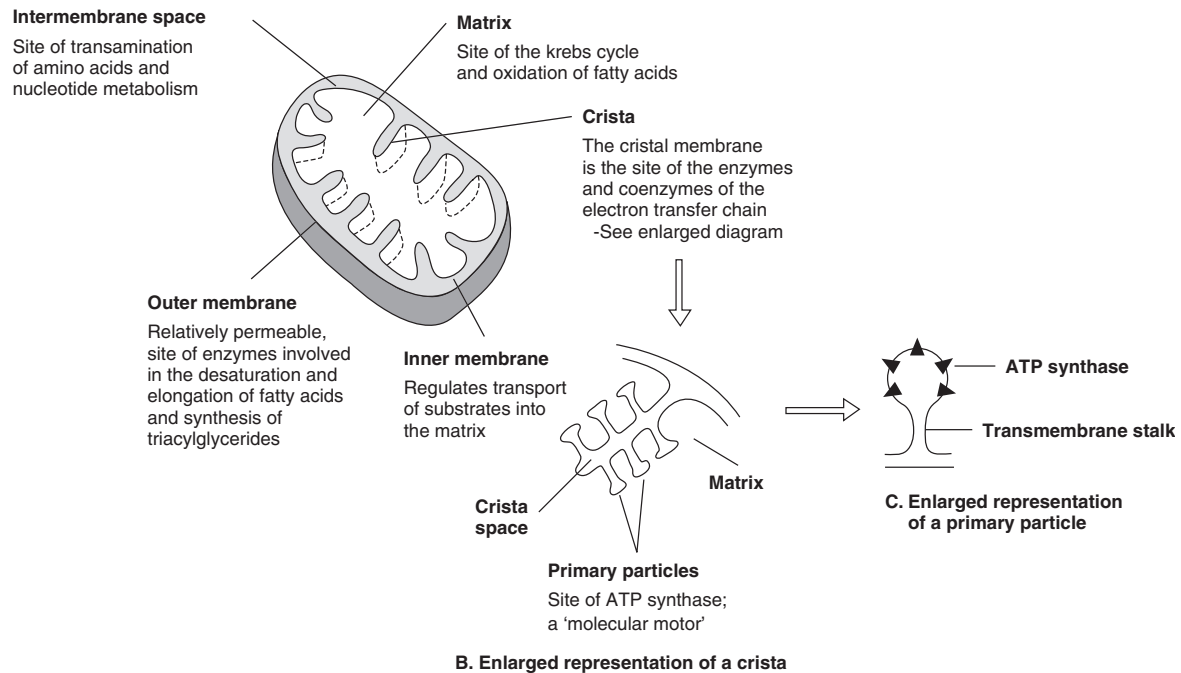


Figure 5 Structure and related functions of a mitochondrion.

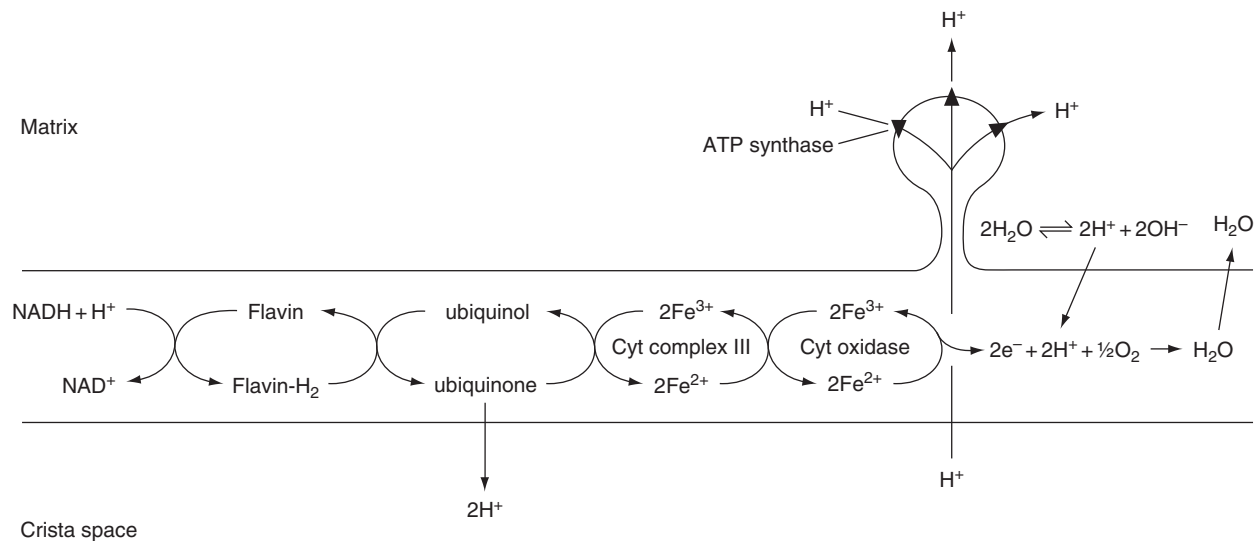


Figure 6 Overview of the electron transfer chain.

gradient across the crista membrane of the mitochondria, resulting from the processes in the electron transfer chain. Metabolic fuels can only be oxidized if there is NAD^+ and FAD available to be reduced. If there is no ADP because it has all been phosphorylated to ATP, protons cannot move down the concentration gradient across the crista membrane through the 'water mill' of ATP synthase because it will not turn if there is no ADP to bind to it. Once a critical concentration of protons is reached in the crista space it is no longer possible to extrude any more protons from the oxidation of FADH or $\text{NAD} + \text{H}^+$

during the transfer of hydrogen from ubiquinone to the first cytochrome. Hence the whole chain stops and no more oxidized NAD^+/FAD is available for glycolysis or the TCA cycle.

The uncoupling of the electron transfer chain from the production of ATP can only occur if protons move down the concentration gradient across the crista membrane through routes independent of ATP synthase. In some circumstances and tissues, uncoupling proteins can be expressed in the crista membrane, which allow protons to flow down the concentration gradient into the mitochondrial matrix, without

passing through the ATP synthase and therefore not generating ATP, but resulting in the generation of a large amount of heat energy. This is the basis of nonshivering thermogenesis, thought to occur mostly in brown adipose tissue in babies by the uncoupling protein thermogenin. In adults it is not clear what importance brown adipose tissue has compared with other uncoupling proteins in muscle and other tissues. In addition to maintenance of body temperature, uncoupling proteins may also be important in overall energy balance and body weight.

Energy Metabolism of Other Nutrients

The TCA cycle and pyruvate are central in the metabolism of carbohydrate, fat, and protein in the fed and fasting state. Pyruvate can have three main fates depending on the metabolic circumstances. It can be a substrate for gluconeogenesis, or it can undergo oxidative decarboxylation to acetyl-CoA and either enter the TCA cycle, or be used for fatty acid synthesis.

Acetyl-CoA can be made from carbohydrates via pyruvate, from fatty acids via β -oxidation in the mitochondrial matrix or from the proteolysis of proteins to amino acids, some of which are converted to acetyl-CoA.

There are three main stores of metabolic fuels: Triacylglycerols in adipose tissue, glycogen as a carbohydrate reserve in liver and muscle and protein as a source of amino acids which can be oxidized via the TCA cycle or used as a substrate for gluconeogenesis.

Overview of Fat Metabolism

β -oxidation of Fatty Acids and Ketogenesis

Fats (triacylglycerides) are stored mainly in adipose tissue. Lipolysis breaks down fats into the constituent fatty acids and glycerol. Fatty acids can be oxidized via the β -oxidation pathway in the mitochondrial matrix. In this process a cyclical series of reactions removes the last two carbon atoms from the carboxyl end of the fatty acyl-CoA with the addition of another CoA to form a new fatty acyl-CoA that is two carbon atoms shorter plus acetyl-CoA. In muscle the acetyl-CoA is metabolized via the TCA cycle to produce reduced coenzymes for the production of ATP. In the liver it is shunted largely to the synthesis of ketone bodies (ketogenesis), which like glucose are exported for use in other tissues.

On the outer face of the mitochondrial membrane fatty acids are esterified to CoA to form fatty acyl-CoA, which cannot enter the matrix of the mitochondria, the site of the enzymes for β -oxidation. This function is performed by the carnitine shuttle. On the outer mitochondrial membrane, fatty acyl is transferred onto carnitine to form fatty acyl-carnitine that is transported across the inner and outer mitochondrial membranes on a countercurrent transporter system, in exchange for transporting free carnitine into the intermembrane space. Once in the matrix, the fatty acyl is esterified to CoA, thus releasing free carnitine. There is no dietary requirement for carnitine as it is readily synthesized from the amino acids lysine and methionine.

Most tissues have a limited capacity for β -oxidation. However, the liver can produce large amounts of acetyl-CoA by β -oxidation and can then convert some of these into four carbon ketone bodies that can be easily transported to other tissues for use as a metabolic fuel. Acetoacetate is formed by the combination of two acetyl-CoA and the removal of the CoA molecules. This is unstable and undergoes a nonenzymic reaction to acetone, which is poorly metabolized, most of it being excreted in urine and exhaled air. Hence most of the acetoacetate is reduced to β -hydroxybutyrate before being released from the liver. β -hydroxybutyrate is metabolized by extra-hepatic tissues by adding a CoA via succinate-CoA to form succinate and acetoacetyl-CoA which is then broken down into two acetyl-CoA by β -ketothiolase and CoA (Figure 7).

Synthesis of Fatty Acids and Triacylglycerides

The majority of fatty acids are supplied by the diet, but many tissues are capable of *de novo* synthesis, including the liver, brain, kidney, mammary glands, and adipose tissue. The *de novo* synthesis of fatty acids occurs in conditions of excess energy intake.

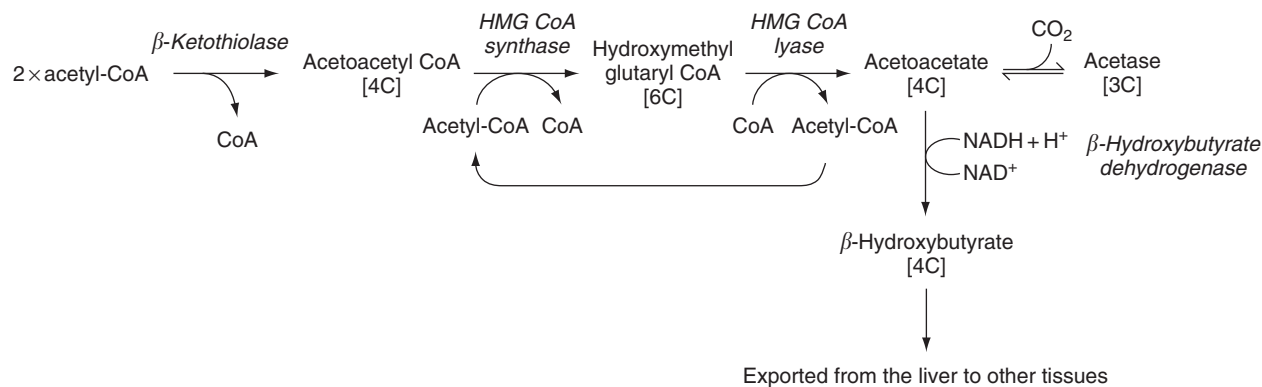
Fatty acid synthesis occurs in the cytosol, but is essentially the reverse of β -oxidation of fatty acids (although it employs a separate set of enzymes), whereby fatty acids are synthesized from the successive additions of 2C acetyl-CoA, followed by reduction.

Acetyl-CoA is formed in the mitochondrial matrix, but it cannot pass across the mitochondrial inner membrane. Hence the source of acetyl-CoA for fatty acid synthesis is from citrate, which can pass out of the mitochondria, whereas with CoA it is cleaved to produce acetyl-CoA and oxaloacetate. The oxaloacetate is returned indirectly to the mitochondrial matrix via its oxidation to pyruvate, which is linked to the generation of reduced NADP, required for fatty acid synthesis. Once in the mitochondrial matrix the pyruvate is converted back to oxaloacetate and thus returned into the TCA cycle.

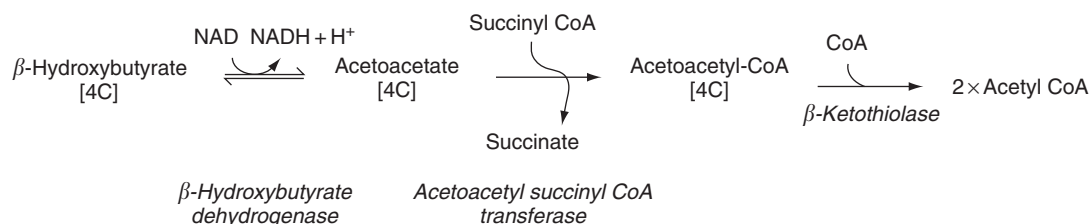
The first step in fatty acid synthesis is the carboxylation of acetyl-CoA to malonyl-CoA, followed by the addition of a series of malonyl-CoA units by a complex series of reactions via the multienzyme complex, fatty acid synthase. The carboxylation of acetyl-CoA to malonyl-CoA catalyzed by acetyl-CoA carboxylase is regulated by the hormones insulin and glucagon that affect the activity of the enzyme catalyzing this reaction. Hence in the fed state insulin increases the activity of the enzyme, whereas in the fasting state glucagon decreases its activity. Also, malonyl-CoA inhibits the uptake of fatty acids into the mitochondria via acetyl carnitine-CoA, so that when fatty acid synthesis is occurring, β -oxidation is inhibited by limiting the supply of substrate into the mitochondria.

Storage of fatty acids in adipose tissue can only occur when glycolysis is activated in the fed state as the source of glycerol in adipose tissue is from blood glucose entering glycolysis and DHA-P being removed to be converted to glycerol-3-P.

During fatty acid synthesis, desaturase enzymes can introduce double bonds to make mono- and polyunsaturated fatty acids. However, these enzymes cannot introduce double



(a) Ketogenesis in the liver from acetyl-CoA



(b) Extrahepatic metabolism of β-hydroxybutyrate (Ketone bodies) to acetyl-CoA

Figure 7 Production (a) and metabolism (b) of ketones produced from acetyl-CoA.

bonds after C10 and this is why there is a requirement for the essential FA's linoleic acid (n-6) and linolenic acid (n-3), which can then be converted to the long chain polyunsaturated fatty acids—arachidonic acid and eicosapentaenoic acid which are important metabolic precursors.

Protein Metabolism

After a meal there is an increase in the synthesis of tissue protein from absorbed amino acids and the increased availability of metabolic fuel to provide ATP for protein synthesis. During fasting some of the relatively labile protein laid down in response to a meal can be mobilized during fasting and the amino acids used both as a metabolic fuel, and as a source of TCA cycle intermediates for gluconeogenesis (Figure 8).

After removal of the nitrogen containing amino group of amino acids their carbon skeletons can have five different fates: gluconeogenesis (gluconeogenic amino acids only), converted into ketone bodies via acetyl-CoA (ketogenic amino acids), fully oxidized to CO₂ and H₂O, converted into fat or glycogen for storage or used as a precursor for a wide range of important biomolecules.

Gluconeogenesis

The brain can normally only metabolize glucose as an energy source. Therefore, it is very important to maintain relatively constant levels of circulating glucose. Under normal circumstances, glycogen serves as a source of blood glucose as free fatty acids from adipose tissue and ketone bodies from the liver are

used preferentially as metabolic fuels by muscle and some other tissues. However, this would still lead to the exhaustion of glycogen reserves within 12–18 h. Hence the formation of glucose from noncarbohydrate sources 'gluconeogenesis' becomes important.

Glucose can be formed from the gluconeogenic amino acids and from glycerol released from the lipolysis of triacylglycerides in adipose tissue. Amino acids can enter the TCA cycle as intermediates and be converted to oxaloacetate, the excess of which can then be removed and metabolized to phosphoenolpyruvate carboxylase (PEP) and then by a process the reverse of glycolysis be converted to glucose. Glycerol can be converted to an intermediate in the glycolytic pathway and therefore undergo gluconeogenesis if required.

Amino acids that can only be metabolized to acetyl-CoA cannot undergo gluconeogenesis because acetyl-CoA cannot be converted back to pyruvate and the inclusion of more acetyl-CoA will not generate a net increase in oxaloacetate, which can be removed from the cycle. Hence fatty acids and ketones that are broken down into acetyl-CoA also cannot be used for gluconeogenesis.

There are three enzymes in gluconeogenesis that are different to those in glycolysis and the relative activity of these compared to the equivalent glycolytic enzymes is tightly controlled by hormones, hence controlling whether glycolysis or gluconeogenesis is the dominant pathway.

Glycogen Metabolism

The red blood cells and the brain have an absolute requirement for glucose for energy metabolism. Glucose is absorbed

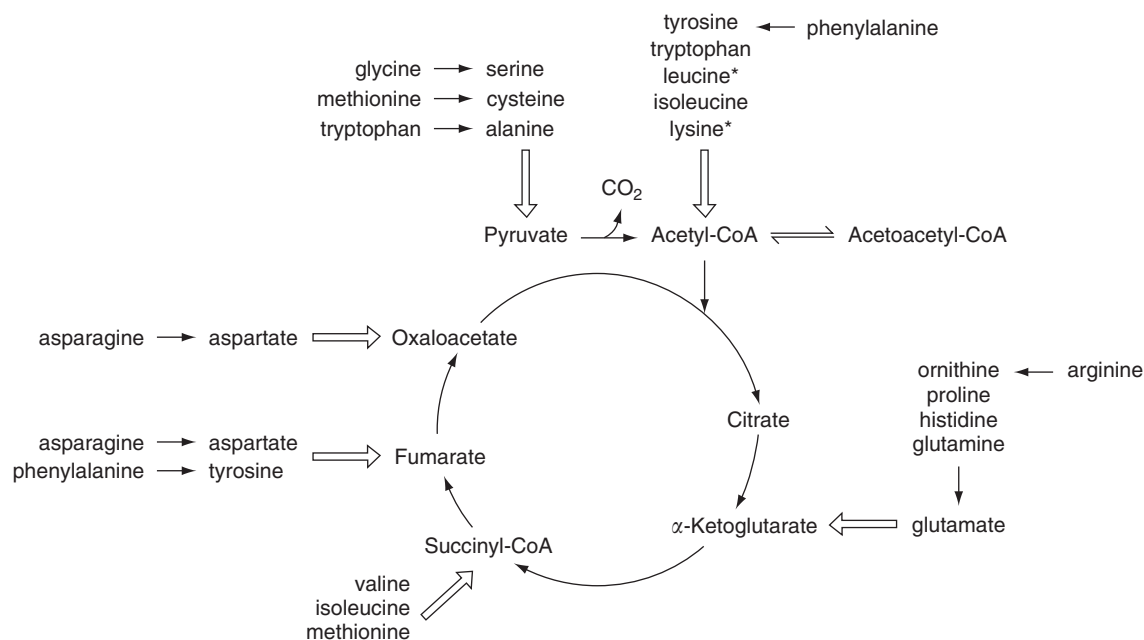


Figure 8 Entry of amino acid carbon skeletons into the TCA cycle. Adapted from Bender DA (2008) *Introduction to Nutrition and Metabolism*, 4th edn. Boca Raton: CRC Press, with permission from Taylor and Francis.

Table 2 A summary of relative importance of different metabolic pathways in intermediary metabolism in different tissues

Tissue	Principle catabolic and anabolic pathways
Brain	25% basal O ₂ consumption Metabolizes glucose only, except after prolonged starvation when it can adapt to uptake and metabolize ketones
Blood	Mature red blood cells have no mitochondria: energy from anaerobic glycolysis: glucose → lactate
Muscle	Preferentially metabolize fatty acids and ketones produced from the liver. Anaerobic glycolysis of glucose from glycogen stores Aerobic respiration of glucose from glycogen or fatty acids/ketones
Liver	Mostly amino acid oxidation for generation of ATP Most important tissue for maintaining blood glucose by gluconeogenesis from amino acids and lactate (via Cori cycle) and glycerol and also from breakdown of glycogen stores Fatty acid synthesis and synthesis of lipoproteins for transport Production of ketones into circulation Site of the pentose-phosphate pathway for generation of NADPH + H ⁺
Adipose tissue	Designed for the storage of fat Can synthesize fat from glucose
Kidneys	Gluconeogenesis Amino acid oxidation for ATP generation

from the intestines only for 2–3 h after a meal and therefore there must be another source of glucose to maintain a constant blood glucose level. When blood glucose levels rise after a meal, the liver can uptake large amounts of glucose, where it is converted to glucose-6-phosphate, which can be used to synthesize glycogen (glycogenesis). When glycogen stores are full the glucose-6-phosphate can enter glycolysis or be used to synthesize glycerol for the formation of fat. When blood glucose levels decrease, during fasting between meals, glycogen is broken down in the liver and glucose

released (glycogenolysis). During the fasting state glycogen is broken down by the removal of glucose units as glucose-1-phosphate from many ends of the molecule. This is then isomerized to glucose-6-phosphate. Only the liver can release free glucose as muscle tissue lacks glucose-6-phosphatase. The free glucose released by the liver is for use by the brain and red blood cells.

The glucose-6-phosphate released in the muscle tissue from glycogen can enter directly into glycolysis for energy production by the muscle. Alternatively, it can be metabolized to

pyruvate and then transaminated to alanine that is exported from the muscle to the liver, where it can be used as a substrate for gluconeogenesis.

Table 2 shows the relative importance of energy metabolic pathways in different tissues of the body.

See also: Amino Acids: Metabolism. Carbohydrates: Regulation of Metabolism

Further Reading

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ENERGY REQUIREMENTS

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Glossary

BMR Basal metabolic rate: The rate of oxygen uptake at rest in the fasting and thermo-neutral state.

MET Metabolic equivalent is a unit of energy expenditure typically used by those concerned with sports where 1 MET is approximately 1 kcal min⁻¹.

NEAT Non-exercise activity thermogenesis. This refers to the cost of energy involved in spontaneous activity rather than deliberate exercise.

PAL Physical activity level: The ratio of the total energy expenditure on a 24 h basis divided by the BMR expressed over the same time period.

PAR Physical activity ratio: This is the total energy cost when active divided by the measured or predicted BMR.

Definition of Energy Requirements

The term energy requirement usually refers to an estimation of the amount of food energy needed by an individual or population under normal or specific circumstances. The amount of energy required is that needed to match the body's demands for maintaining all its functions, processing food, and engaging in all forms of physical activity. Additional energy will be needed when children are growing, when women are pregnant or breast feeding, when recovering from illness, or when exposed to cold. The amount of food energy needed by an individual also depends on his/her size: If he/she is overweight the food needs will be greater than if they are underweight for the same level of physical activity.

The Difficulty of Estimating Energy Requirements by Measuring Food Intake

It is often assumed that the assessment of food energy needs can simply be made by measuring an individual's current intake. However, detailed studies show that daily intake varies quite markedly, not only for social and other reasons but also because the body has a complex system of short, medium, and longer term regulatory mechanisms for subconsciously adjusting appetite and satiety in response to changes in the body's energy stores. Appetite in children also varies in anticipation or response to growth spurts and young premenopausal women also display a cyclical shift in food intake linked to the hormonal changes occurring during the menstrual cycle. Thus, even measuring a week's food intake accurately may not reflect the overall average food needs of somebody over say a period of a month. In many countries the seasonal demands for physical work and the availability of

food may also impose an additional complication when trying to estimate an individual or group's overall food needs.

Using food intake measures to assess food requirements is also mistaken because, as repeatedly shown, substantial errors are involved in measuring food energy intake. People usually forget to include everything they have eaten and drunk and their estimate of the amount and type of food eaten is usually inadequate for any reasonably accurate estimate to be made. In affluent weight-conscious societies, adults who are overweight also tend to underreport their true intake. In poorer societies underreporting is also common, particularly when there is great social pressure to distribute the food preferentially to children or men in the household. Therefore, traditional methods for measuring food intake, for example, by questioning people about their intake the previous day/month/year often leads to marked underestimates of their true habitual energy intake. Descriptions of foods often do not adequately describe the ingredients even if very good food composition tables are available.

An Assessment of Food Needs by Estimating or Measuring Energy Use

The problem encountered in estimating food energy requirements by attempting to measure typical food intakes for different groups in the population was clearly recognized 30 years ago. A Food and Agriculture Organization/World Health Organization/United Nations University (FAO/WHO/UNU) expert group in 1985 therefore set about estimating energy needs by collating studies that had measured the rates of energy use by children and adults both at rest and when engaged in the different forms of physical activity. The variation in physical activity is less than that of food intake so the

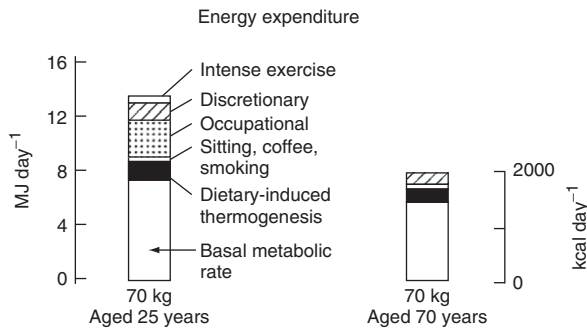


Figure 1 Different components of energy expenditure in a young man and the impact of ageing. Note: the energy expenditure falls mainly because of a decline in physical activity. There is also a fall in the BMR but this is predominantly reflecting a reduction in the lean tissues, i.e., fat-free mass, in the elderly.

large data set available on adults at rest and at work in different parts of the world was collated. The paucity of data in children presented a greater problem and detailed metabolic studies of the energy cost of growth had to be calculated by studying children growing under metabolic ward conditions with accurate measurements of all their food intake and urine and fecal output when they were growing at different rates. Estimates of what this tissue growth meant in terms of accumulated lean and fat tissue energy had to be made from a variety of body compositional measurements.

The estimation of energy needs was rationalized as shown in **Figure 1**. This framework was based on new detailed studies of adults in whole body calorimeters capable of measuring minute-by-minute energy expenditure if need be for several days with differences from day to day of only approximately 1% that also included the measurement errors of the instruments. This constant rate of energy output was only possible if the physical activity and food intake of the individuals was strictly controlled.

Factors Affecting Metabolic Rate

The process of oxidation involves a series of enzymatically controlled biochemical reactions leading eventually to the combination of oxygen with the carbon and hydrogen components of the body's fuels thereby yielding carbon dioxide and metabolically derived water. The incompletely oxidized nitrogen is excreted as urea, which is synthesized by the liver and excreted by the kidneys. The intermediate steps in the metabolism of the body's fuels are linked biochemically to drive the generation of phosphate-containing organic molecules, such as adenosine triphosphate (ATP), which in turn serve as the direct energy sources for all the body's cell activities, including the synthesis of complex molecules, the maintenance of tightly controlled ionic gradients in the cell, and the excretion of ions and molecules outside the cell. Thus, the oxygen being taken up by the lungs reflects the tissue metabolism of the fuels needed to regenerate the ATP used up in either biochemical 'internal' work or mechanical external work undertaken by the body's muscles. The rate at which the

body burns its own stored fuels in the fasted, resting, and relaxed state, i.e., in the basal state in a warm room, is called the basal metabolic rate (BMR). This varies with the age of the individual, mainly because of the varying sizes of metabolically very different organs at different ages. Thus, a child has a relatively large brain, liver, and intestine with a higher metabolic rate per kilogram of body weight than a more muscular adult. Body fat cells are metabolically active but contain a substantial amount of inert fat, so that the larger fat mass of a woman results in a lower BMR per unit body weight than a man but if the oxygen uptake is calculated in terms of the metabolically active fat-free mass, then her metabolic rate is the same. As men and women age, they tend to lose lean tissue and store extra fat, so that the BMR on a weight basis falls with age.

Equations can now be used to estimate a group's BMR from their sex, age, and body weight (**Table 1**). The kilojoule (kJ) is the standard measure, and 4.184 kJ corresponds to 1 kcal, which was originally defined in energy terms as that required to increase the temperature of 1 g of pure water by 1 °C from 14.5 to 15.5 °C. There is a range of BMR amounting to $\pm 20\%$ of the mean value at each weight. Thus, in a young 25-year-old woman of 55 kg, the anticipated mean BMR is 5460 kJ (1305 kcal) day⁻¹ but could vary under normal conditions from 4448 to 6473 kJ day⁻¹. The differences in BMR of different individuals of the same weight in part reflects differences in their proportion of lean to fat tissues. Thus, the BMR per unit fat-free mass varies by 12–15% rather than by $\pm 20\%$ as for weight. Approximately 40% of the BMR variation between sexes and the age of individuals may be explained by differences in the size of the body organs, for example, liver, intestine, and muscle, but there is a residual difference between individuals who seem to be explicable only in terms of differences in the rate at which every organ of the body metabolizes its fuel. This is controlled principally by the circulating concentration of thyroid hormones. Adults with an above-average level of circulating thyroid hormones tend to have a higher but still normal BMR. Smokers' BMRs are approximately 5% above normal perhaps because of activation of the sympathetic nervous system. Young women show a swing in BMR that is at its lowest in the late follicular phase of the menstrual cycle, just before ovulation. On ovulation, the basal body temperature rises rapidly by approximately 0.5 °C and the BMR immediately increases but rises further to a peak in the later luteal phase. This metabolic swing of $\pm 5\%$ is independent of changes in food intake, but the recognized 5–10% fall in intake during the follicular phase with a similar rise in the luteal phase may accentuate the hormonally dependent swing in metabolism. The effects of contraceptives that inhibit ovulation and the subsequent rise in basal temperature are not well documented. The previous day's food intake does not affect the BMR unless there has been substantial overeating. However, the mixture of fuels combusted during fasting is influenced by the proportion of the previous 3–4 days' intakes derived from carbohydrate; much of the glucose from glycogen is metabolized in the fasting state if carbohydrate intake was previously high. When glycogen stores in the liver near exhaustion, the body switches to using body fat with a small fall in carbon dioxide output; a carbohydrate-rich diet induces a slightly higher fasting metabolic

Table 1 Equations for estimating BMR from body weight (kg)*(a) The Schofield equations used by FAO/WHO/UNU (2004)*

Age (years)	Males (MJ day^{-1})	Females (MJ day^{-1})
<3		BMR = $0.244 \text{ kg} - 0.130$ BMR = $0.085 \text{ kg} + 2.033$
3–9.9	BMR = $0.255 \text{ kg} - 0.226$ BMR = $0.0949 \text{ kg} + 2.07$	BMR = $0.056 \text{ kg} + 2.898$ BMR = $0.062 \text{ kg} + 2.036$
10–17.9	BMR = $0.74 \text{ kg} + 2.754$	BMR = $0.034 \text{ kg} + 3.538$ BMR = $0.0386 \text{ kg} + 2.875$
18–29.9	BMR = $0.063 \text{ kg} + 2.896$	BMR = $0.0410 \text{ kg} + 2.610$
30–59.9	BMR = $0.048 \text{ kg} + 3.653$	
60–74	BMR = $0.0499 \text{ kg} + 2.930$	
75 +	BMR = $0.035 \text{ kg} + 3.434$	

(b) BMR Equations as derived by Henry, et al. (2005) and used by the UK SACN working party (2009)

Gender	Age (years)	BMR (MJ day^{-1})		
		Coefficient weight (kg)	Coefficient height (m)	Constant
Males	<3	0.118	3.59	-1.55
	3–10	0.0632	1.31	1.28
	10–18	0.0651	1.11	1.25
	18–30	0.0600	1.31	0.473
	30–60	0.0476	1.26	-0.574
	>60	0.0478	1.26	-1.070
Females	<3	0.127	2.94	-1.2
	3–10	0.0666	0.878	1.46
	10–18	0.0393	1.04	1.93
	18–30	0.0433	2.57	-1.180
	30–60	0.0342	2.1	-0.0486
	>60	0.0356	1.76	0.0448
			Coefficient weight (kg)	Coefficient height(m)
			28.2	859
			15.1	313
			15.6	266
			14.4	313
			11.4	541
			11.4	541
			30.4	703
			15.9	210
			9.40	249
			10.4	615
			8.18	502
			8.52	421
				Constant
				-371
				306
				299
				133
				-137
				-256
				-287
				349
				462
				-282
				-11.6
				10.7

Note: The BMR values for infants and children are no longer used to calculate energy requirements, but provide an indication of the likely values. The adult data are those from the original Schofield, *et al.* analyses with the data for 60 years and older adults being derived from both the Schofield data and further information provided to the UK.

Source: Reproduced from Department of Health (1991) Dietary reference values for food energy and nutrients for the United Kingdom. *Report on Health and Social Subjects*, vol. 41. London: HMSO.

rate probably because of a slight induction of thyroid metabolism by dietary carbohydrates.

The BMR falls by 2–5% when individuals transfer to live in a tropical warm environment; in uninsulated houses, seasonal BMR increases of 5–10% on winter are seen as in Japan before World War II. The BMR formulae shown in [Table 1](#) ignore any temperature effects. The observed lower BMR of some people living in the tropics may also reflect the effects of malnutrition. Poor nutrition can have an immediate and long-term effect in lowering the BMR. After 4 days of semistarvation the BMR falls and after 2 weeks the BMR is approximately 15% lower as thyroid metabolism changes and the body's organs become more efficient. More prolonged or severe semistarvation induces a progressive loss of the body lean tissues as well as fat, and the BMR therefore continues to decline in proportion to the loss of lean tissues. Body weight can eventually stabilize at a new low level and, if the physical activity is also reduced, semistarved volunteers can come back into energy balance on 50% of their initial intake. However, this requires a 40% loss of weight and marked lethargy if energy balance is to be preserved on such a low intake.

The constancy of individual's BMR under normal circumstances allowed global data on BMR to be used for developing prediction equations ([Table 1\(a\)](#)) for FAO/WHO/UNU expert committees. Subsequently greater databases have been collected ([Table 1\(b\)](#)) albeit on potentially more malnourished adults globally.

The Components of Metabolic Rate

Traditionally, the metabolic rate is divided into three components: the BMR, postprandial thermogenesis, and physical activity that can be classified in different ways as in [Figure 1](#). The BMR usually amounts to 50–60% of an individual's total energy expenditure and postprandial thermogenesis to 10% used for the metabolic cost of processing, i.e., eating, absorbing, transporting, and storing food. The remaining energy is used for physical activity.

Postprandial Thermogenesis

The surge in oxygen uptake after a meal, known as postprandial thermogenesis, has also been described as the specific dynamic action of food, dietary-induced thermogenesis or the thermic effect of feeding. The last term is particularly favored by animal nutritionists. Its total effect is difficult to measure accurately because the measurements have to be done at complete rest for up to 10 h or more. Thus, the BMR is measured after a 14-h fast. The maximum oxygen uptake occurs after ingesting protein equivalent to approximately 30% of the protein's energy; glucose induces a 5–10% effect and fat only a 2–5% effect, consistent with its slow absorption by the lymphatic tissue; alcohol induces a variable 0–8% effect. Some dietary components increase metabolism: caffeine equivalent to two cups of tea induces a 1–3% increase and spices, as in Indian curry, by up to 25%. Exercise moderately amplifies the response to a standard meal, so that the combined effect of exercise and food is greater than the sum of the oxygen uptake

after each separate stimulus. However, the effect amounts to only 2% of total energy expenditure.

Differences in postprandial energy expenditure have been sought as an explanation for the propensity of some individuals and animals to obesity. Results are often conflicting because of daily variations observed in individuals. A proportion of obese subjects have a reduced metabolic response to a meal; this effect may prove to depend on the degree of abdominal insulation because the response is reduced if volunteers are swathed in insulation to reduce the abdominal heat loss, thereby increasing the temperature of the blood entering and leaving the liver. This seems to reduce the stimulus to body metabolism. Lactating mothers (and pregnant women) have a lower postprandial thermogenesis that returns to normal after they have stopped breast-feeding. Smoking and postprandial thermogenesis interact synergistically so the thermic output after a meal is enhanced. The small postprandial response during lactation is consistent with that observed in many species of animal in which brown adipose tissue is used as the organ for modulating heat production as a mechanism to maintain body temperature. However, this organ is normally not very active in humans although recent analyses with new scanning techniques have shown that its activity does continue in adults and small seasonal changes in BMR are reflected in changes in brown fat metabolism. So postprandial thermogenesis as well as the response to cold in adults may involve brown adipose activity.

Prolonged overfeeding can produce a marked BMR and postprandial response providing the intensity of overfeeding (especially with carbohydrate) is high. Thus, progressive overconsumption of 6.3 MJ (1500 cal) day⁻¹ leads to a 33% increase in daily energy expenditure. Nevertheless, this apparent mechanism for dissipating excess energy is limited because energy is stored, two-thirds as fat the rest as lean tissue. The majority of the increased metabolism is accounted for the cost of fat synthesis from carbohydrate, although the human capacity to transform carbohydrate into fat is limited, preference being given to the selective storage of the fat component of the ingested energy.

Physical Activity

The energy cost of physical activity can be predicted but an analysis of activity patterns on a minute-by-minute basis used to be required. Children are often very active, making a detailed analysis difficult because each activity needs to be linked to its energy cost. Weight-bearing movement and antigravitational moves, for example, walking up a hill with a load, are particularly energetic. The simplest way of estimating individual costs for specific tasks involves the use of extensive tables listing the energy cost of different movements in children and adults. For simplicity, these can be expressed as a ratio of the BMR, because, in this way, differences between the sexes and individuals of varying size are removed. [Table 2](#) illustrates how this is achieved. The physical activity ratio (PAR), i.e., energy cost in relation to BMR, is the cost of the activity. Those in sports medicine also now call this unit cost a metabolic equivalent (MET), where one MET is equal to the resting metabolic rate of approximately 4.2 kJ(1 kcal) min⁻¹.

Table 2 An example of the calculation of the energy requirements as PALs for adults with a moderately active lifestyle

Main daily activities	Time allocation (h)	Energy cost (PAR)	Time (h) × Energy cost	Mean PAL (multiple of 24-h BMR)
Sleeping	8	1	8.0	
Personal care (dressing, showering)	1	2.3	2.3	
Eating	1	1.5	1.5	
Standing, carrying light loads ^a (waiting on tables, arranging merchandise)	8	2.2	17.6	
Commuting to/from work on the bus	1	1.2	1.2	
Walking at varying paces without a load	1	3.2	3.2	
Low intensity aerobic exercise	1	4.2	4.2	
Light leisure activities (watching TV, chatting)	3	1.4	4.2	
Total:	24		42.2	42.2/24 = 1.76

^aComposite of the energy cost of standing, walking slowly and serving meals, or carrying a light load.

The PAR is the minute-by-minute ratio of the total cost of the activity to the estimated or measured BMR which is taken as 1. If this PAL is applied to a female population, 20–25 years old, with mean weight of 57 kg and mean BMR of 5.60 MJ day⁻¹ (1338 kcal day⁻¹), TEE = 1.76 × 5.60 = 9.86 MJ (2355 kcal), or 173 kJ (41 kcal) kg⁻¹ day⁻¹.

Table 3 Classification of lifestyles in relation to the intensity of habitual physical activity, or PAL for (a) children as set out by the UK SACN working party draft report in 2009 and (b) adults of either sex by the FAO/WHO/UNU Technical group in 2004 and by the SACN working party draft report in 2009

Category	PAL values		
(a) Children	25th Percentile	Median	75th Percentile
Aged 1–3 years	1.36	1.40	1.45
> 3 to < 10 yrs	1.43	1.58	1.70
10–18 years	1.68	1.75	1.86
(b) Adults of both sexes	FAO 2004	SACN 2009	
Sedentary or light activity lifestyle	1.40–1.69	25th Centile: 1.49	
Active or moderately active lifestyle	1.70–1.99	Median: 1.63	
Vigorous or vigorously active lifestyle	2.00–2.40	75th centile: 1.78	

The adult percentile values in the SACN report are based on double-labeled water measurements in two large US studies so reflect the PALs of a very sedentary society whereas the FAO/WHO/UNU report took account of data from global sources including low-income countries where manual work is still common.

Source: Reproduced from FAO (2004) Human energy requirements. *Report of a Joint FAO/WHO/UNU Expert Consultation Food and nutrition Technical series 1. Report of a Joint FAO/WHO/UNU Expert Consultation Rome, 17–24 October 2001*. Rome: FAO.

If the average energy requirement of the individual is to be estimated, account must be taken of the different types of work involved throughout the year. There are now internationally recognized questionnaires for assessing individuals' physical activity patterns but new methods of checking on these with sophisticated sensitive accelerometers worn by the individual, with a standardization of their energy equivalence from measures of oxygen uptake, show that these questionnaires are very inaccurate. Accelerometers are increasingly used to measure the pattern and energy equivalence of daily activities in population studies.

The ratio of the total daily energy expenditure to the BMR is designated the physical activity level (PAL). Physical activity is of general health benefit so it is desirable that the overall PAL of individuals should be 1.75 or more which requires at least 60 min of moderately vigorous activity daily. However, in sedentary societies 30 min of moderately vigorous exercise three times a week benefits muscular tone and physical fitness. This then improves cardiovascular health and insulin sensitivity and therefore is likely to limit the development of type 2 diabetes and cardiovascular disease in susceptible individuals.

Table 3 provides a listing of PAL values for adults of all ages according to their activity patterns. First, one estimates the BMR by knowing the sex, age, and bodyweight of individuals (see **Table 1**). Given this BMR figure in megajoules per day, multiply by the PAL value shown in **Table 3**, and the energy needs can be estimated. Individuals vary in their energy needs by 10% at equivalent weights and activity levels, so that an individual's needs cannot be predicted very accurately unless his or her BMR is measured and account taken of their personal lifestyle i.e., activity pattern. Nevertheless, the total energy expenditure of an individual child or adult is remarkably consistent from day to day, varying by only 1–2% provided that food intake and physical activity are meticulously standardized, and account is taken in women of the stage of the menstrual cycle.

Nonexercise Activity Thermogenesis (NEAT)

Recently new techniques of assessing physical activity by using multiple accelerometers stitched into a body suit have shown that individuals vary markedly in their physical activity response to overfeeding with sometimes a marked increase in

energy expenditure as they spontaneously move more. This substantially accounts for differences in the amount of body fat gained when a group of people are overfed and go into positive energy balance. In practice, given the interplay of regulatory changes in both energy expenditure through NEAT, dietary thermogenesis and BMR, as well as the complex control of food intake, most people until their last few decades remain in approximate energy balance on a yearly basis. The development of obesity only arises because of a consistent discrepancy between the physiological controlled intake and expenditure, which may amount to only 1–2% of the average daily energy intake. However, this consistent discrepancy produces a 2–5-kg weight change in a year.

Measures of total energy expenditure estimated over 2–3 weeks can be obtained by the use of the double-labeled water technique, which relies on the difference in labeling of urine or saliva with the two heavy isotopes of water, deuterium and ^{18}O . The differential dilution of deuterium and ^{18}O in urinary water is monitored over a 2–3-week period following a single oral dose of D_2^{18}O . The ^{18}O content is diluted more rapidly than the deuterium because the oxygen in water exchanges rapidly with the body's bicarbonate pool, which is turning over rapidly as carbon dioxide is produced by tissue metabolism. Thus, the difference in dilution rates of ^{18}O and deuterium provides a measure of the rate of carbon dioxide production. The technique is expensive and difficult to perform analytically but very convenient for the subject being studied, because only single daily or occasional urine or saliva specimens are needed over the period of observation.

This method is increasingly used, and now allows a new perspective to be taken of both children's and adult's energy

requirements and the D_2^{18}O method together with estimates of growth costs have been used to compile new and lower estimates of energy needs than were originally estimated by the old factorial method.

Age-related changes in energy needs are important not only in childhood but also in adults for different reasons. Figure 1 gives an indication of the decline in energy needs during adult life. This results from the atrophy of the lean tissues, which may be related to the fall in physical activity. Lack of exercise is therefore a handicap because it directly reduces energy expenditure, and it may also lead to a slow shrinkage of tissues, such as muscle, thereby producing a long-term fall in metabolism at rest. There may also be up to a further 5% fall in the rate of tissue metabolism. Thus, unless people adapt their intake extraordinarily well to this progressive decline in energy output, energy storage, weight gain and overweight, or even obesity are inevitable as people age.

Extra Energy Costs of Growth, Pregnancy and Lactation

The cost of growth amounts to $10\text{--}25\text{ kJ g}^{-1}$ of new tissue deposited; the value being higher if fat with little lean tissue is laid down. A newborn has a high energy requirement of approximately 460 kJ kg^{-1} with a cost of weight gain amounting to 26 kJ g^{-1} , but by 1 year of age, the total daily requirement has fallen to approximately 335 kJ kg^{-1} as growth slows with growth now costing 10 kJ g^{-1} . A breast fed baby has an energy requirement which is approximately 10% lower than bottle fed babies but overall figures are given in Table 4. Without

Table 4 Average energy requirements of infants (breast and bottle fed) and of children up to the age of 18 years based on FAO/WHO/UN analyses (2004)

Infants (age in months)	Energy requirement (kJ kg day^{-1})			Children (age in years)	Weight (kg)	PAL	Energy requirement (kJ kg day^{-1})	Weight (kg)	PAL	Energy requirement ($\text{kJ kg}^{-1}\text{ day}^{-1}$)
	Boys	Girls	Mean							
					Boys			Girls		
1	475	445	460	1.1–2	11.5	1.45	345	10.8	1.44	335
2	435	420	430	2.1–3	13.5	1.46	350	13.0	1.44	337
3	395	395	395	3.1–4	15.7	1.45	334	15.1	1.46	320
4	345	350	345	4.1–5	17.7	1.50	322	16.8	1.50	309
5	340	345	345	5.1–6	19.7	1.54	312	18.6	1.54	299
6	335	340	340	6.1–7	21.7	1.58	303	20.6	1.58	290
7	330	330	330	7.1–8	24.0	1.61	295	23.3	1.62	279
8	330	330	330	8.1–9	26.7	1.65	287	26.6	1.65	267
9	330	330	330	9.1–10	29.7	1.68	279	30.5	1.68	254
10	335	330	335	10.1–11	33.3	1.72	270	34.7	1.73	242
11	335	330	335	11.1–12	37.5	1.77	261	39.2	1.77	229
12	335	330	335	12.1–13	42.3	1.81	252	43.8	1.78	217
				13.1–14	47.8	1.84	242	48.3	1.78	206
				14.1–15	53.8	1.86	233	52.1	1.76	197
				15.1–16	59.5	1.86	224	55.0	1.74	189
				16.1–17	64.4	1.85	216	56.4	1.73	186
				17.1–18	67.8	1.84	210	56.7	1.72	185

Note: The energy requirements of infants were derived from double-labeled water measurements of total energy expenditure to which was added the age-specific energy deposited during growth, taking into account the different proportions of lean and fat tissue laid down during infancy. The children's requirements were estimated from quadratic equations relating body weight to total energy expenditure of girls and boys measured separately, or from estimates of total energy expenditure based on calibrated heart rate recordings. Again, the energy deposited as growth was added to give requirements expressed on a weight basis to allow adjustments for children of different weights at each age. The values for energy requirements of children and adults as reassessed by the 2009 UK SACN working party are somewhat lower than these values which apply globally.

sufficient energy, a child will fail to grow, but the causes of growth failure usually relate to a deficiency of other nutrients or to infection, rather than to a lack of dietary energy. Adolescents, particularly boys, who are physically very active, may have a high demand for energy. However, the actual cost of even rapid growth rates at this age is modest.

Traditionally, pregnancy is considered, incorrectly, a time of great demand for food. Good nutrition is extremely important and a weight gain in pregnancy of approximately 12 kg for a woman of normal height and weight is considered appropriate for reducing the risk of maternal and fetal complications and preterm and low birth weight babies. With a weight gain of 12 kg, increases in maternal BMR amount to 5%, 10%, and 25% in the first, second, and third trimester of pregnancy, respectively. In practice, the intensity of physical activity often declines, particularly in late pregnancy, and some enhanced metabolic efficiency seems to occur. Thus, the rise in total energy expenditure amounts to only 1%, 6%, and 17% in the three trimesters. So the need for additional energy as such is small amounting to 85, 350, and 1300 kJ day⁻¹ (20, 85, and 310 kcal day⁻¹) for sequential trimesters and in practice this means that a pregnant woman needs to increase her food intake by approximately 1.5 MJ day⁻¹ (360 kcal day⁻¹) in the second trimester, and 2.0 MJ day⁻¹ (475 kcal day⁻¹) in the third trimester. However, new analyses show that women who enter pregnancy overweight or even obese need to gain much less weight to produce a normal-sized baby. If they gain too much weight they are in danger of gestational diabetes with major long-term consequences for both mother and baby; larger babies programmed for future overweight and obesity are also more likely. Obese pregnant women produce more babies with congenital abnormalities and they would be better to gain less than half the normal expected 12 kg during pregnancy.

In lactation the extra energy needed to cover the energy in the milk and the cost of producing it is now estimated at 1.4 MJ in the first 6 months of lactation and 1.7 MJ day⁻¹ in the second 6 months. Part of the energy derives from the extra maternal fat stored during pregnancy; the average woman loses 0.8 kg month⁻¹ but mothers also compensate by eating as they become hungry when breastfeeding. During lactation there are no significant changes in BMR, efficiency of work

performance, nor in total energy expenditure and in most societies women resume their usual level of physical activity in the first month postpartum or shortly thereafter.

Convalescent patients who need to put on weight need extra food, but the cost of this weight gain amounts to between 20 and 40 MJ per kg⁻¹ weight gain. If 1 kg is gained per month, the extra food needed therefore amounts to approximately 1 MJ day⁻¹.

See also: Energy: Adaptation; Balance. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Energy Metabolism. Weight Management: Approaches; Weight Cycling/Weight Change; Weight Maintenance

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FAMINE

Causes, Consequences, and Responses

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There are so many hungry people, that God can not appear to them except in the form of bread. – Mahatma Gandhi

Famines in History

Famine has afflicted humankind, shaping its demography and history from antiquity. Records of famine in ancient Egypt during the third millennium BC are depicted in *bas-relief* on the Causeway of the Pyramid of Unas in Saqqara. During a famine from drought in Egypt in the second millennium BC (Middle Kingdom) that stretched to Mesopotamia, Biblical accounts describe the devastation wrought on the land and society and means by which Joseph predicted and managed its consequences. The fall of the Roman Empire followed repeated food shortages and famines from 500 BC to 500 AD. China experienced some 1828 famines, or nearly one per year, from 108 BC to 1911 AD. The ranks of the Crusades in the eleventh and twelfth centuries swelled in response to assurance of food. The storming of the Bastille and French Revolution followed decades of periodic rises in flour and bread prices that had caused widespread hunger and hardship, and hundreds of ‘food riots.’

Recurrent famine motivated the settling of the New World. The Great Irish Famine in the late 1840s caused one and a half million deaths and an equal number of migrations, mostly to America. Decades of Russian famines following crop failures in the late nineteenth century resulted in waves of immigrants to the United States. Repeated famines led to the overthrow of Czarist Russia that ushered in the Bolshevik Revolution in the early twentieth century. Using food deprivation to wage class warfare and crush the Cossack revolution in the 1930s, Stalinist policies led to the starvation and death of 3.5 million Ukrainians. In China, multiple famines throughout the nineteenth century reportedly led to over 50 million deaths that continued throughout the first half of the twentieth century. Maoist communism rose to power in the 1940s understandably amidst promises of land reform and freedom from chronic hunger and periodic famine. However, collectivization

of private farms and irrational rural industrialization schemes coupled with monopolistic control of food grain movement, purchase and access, abusive taxation, and repressive policies against the peasantry left China mostly food insecure throughout the 1950s and primed for what has turned out to be the worst single famine in human history, in 1959–60. During this period an estimated 30 million people perished, in absence of worldview and reaction, following the secretive, cultist policy failures of Mao’s ‘Great Leap Forward.’ Famine was notorious on the Indian subcontinent through the mid-twentieth century, with the two final famines both occurring in Bengal, in 1943 toward the end of British rule and again in Bangladesh (formerly East Bengal) in 1974–75. An India free from overt famine over the past half-century, despite continuing chronic undernutrition, has been attributed, in part, to the country’s economic rise, relative peace, and democratic and popular processes that have included political accountability and a flourishing free press; lessons still lost on some modern states. In North Korea, for example, effects of repeated floods in the late 1990s that ruined crops, combined with isolation, a collapsed centralized economy, and politicization and diversion of already insufficient international food aid from those most in need led to a famine of devastating proportion.

Famines in the late twentieth century have inflicted heavy loss of life in Africa, especially in the Greater Horn (i.e., Ethiopia, the Sudan, and Somalia). At least one modern regime’s demise, that of Emperor Haile Selassie in 1974, followed famine. Famines of seemingly increased complexity in Africa have resulted from deteriorating crop production associated with steady rainfall decline, failures in development and commerce, repressive and corrupt governance, and armed conflict leading, at times, to outright anarchy. Tragically, famines over the past 30 years have occurred at a time in human history when general understanding of causes and consequences of famine, and a global ability to monitor antecedents and intervene to avert mass starvation, disease, and death have never been greater. A most recent example was

in the Horn of Africa in mid-2011, where anarchy and drought precipitated famine in two regions. Yet, with conflict, especially internal civil war, rising as the decisive and yet unpredictable trigger of modern famine, stable governance with democratic processes (e.g., free press, people's participation, and fair trade) is increasingly recognized to be one of the most important means for its prevention. History has increased awareness and understanding of the need for a stable, peaceful, and equitable political economy to guide the developing world away from famine in the twenty-first century.

Definition

Definitions of famine vary, but all contain the necessary elements of widespread inaccessibility to food, leading to mass numbers of starved individuals. Importantly, lack of access is not equivalent to nonavailability of food within a region, as famines have been known to occur amidst food stocks sufficient to feed the afflicted population. More comprehensive definitions of famine may include elements of time-dependency (e.g., steady, continuous erosion of or sudden collapse in food available for consumption), partial causation (e.g., due to natural calamity, armed conflict, or convergence of other complex causal events), class (e.g., affecting certain ethnic, geographic, economic, or occupational groups more than others), and health consequence on a population scale (e.g., accompanied by severely comprised nutrition, epidemics of disease, and high mortality), or other population responses (e.g., social breakdown, mass migration, and adoption of destructive coping strategies). Although poverty-stricken communities tend to view famine as a continuum of increasing loss and oppression that typically begins long before mass casualty, formal 'external' definitions tend to invoke thresholds or shocks involving sudden inflections in trends for events that afflict large numbers of people. These may include spikes in prices of staple grains, levels of violence, destitution, mortality from starvation and infectious disease, and migratory movement. Threshold events distinguish famine from endemic, chronic food deprivation that results from extreme poverty, political corruption, developmental neglect, and food insecurity and that leads to chronic, high rates of malnutrition, disease, and mortality. Yet, these factors are ones that, often when acting together, predispose underserved populations of the developing world to risk of famine. Such conditioning factors are antecedent causal elements that require more continuous, sensitive, and specific indicators to detect as well as a set of longer term economic, political, and developmental solutions to prevent. In defining famine in a region, the United Nations presently sets three threshold conditions: (1) A severe food shortage that affects at least 20% of households, (2) wasting malnutrition that affects 30% or more of the population, and (3) a crude death rate exceeding two deaths per 10 000 people day⁻¹. Whether continuous and evolving or more sudden, unleashed famine – where such thresholds have been transgressed by masses of people – is catastrophic, distinct, and a human tragedy of unparalleled proportion.

Causes

Starvation is a matter of some people not having enough food to eat, and not a matter of there being not enough food to eat. – Amartya Sen

Large numbers of people starve during famine, which is usually followed by epidemics of lethal infectious diseases. Typically, a plethora of forces or conditions act within society to deprive people of food to survive. General food decline in a population may be an important factor, but it is neither necessary nor sufficient as a cause, as amply revealed by critical treatises of numerous famines over the past two centuries. This has led analysts to recognize that famines are complex, often with many ('component') causes that vary in their attribution, depending on the classes of society affected, and their timing, severity, duration, and degrees of interaction. The constellation of causes and potential solutions of famine can be examined from ecological, economic, social and public health perspectives, each offering different insights into the ecology of famine. Although each view is valid and informative, none is complete or mutually exclusive, making it necessary to integrate diverse perspectives to understand the complexity of famine and approaches to its prevention. In offering an epidemiologic overview, there appear to be at least three dominant causes of famine that have emerged from the nineteenth and twenty-first centuries that are relevant to understanding modern famine causation (**Figure 1**): (1) Market failure, (2) armed conflict, and (3) failure in central planning. A 'new variant famine' hypothesis, developed from observations in Africa over the past decade, proposes that the human immunodeficiency virus (HIV) epidemic, though itself not directly responsible, overlays and exacerbates virtually every metric by which famine is defined. Importantly, none is a sole acting cause and, therefore, for each one there are other antecedent factors, sometimes operative for years before, as well as concurrent and late-acting components that together may lead to famine.

Market Failure

Market failure famines occur when free, competitive market forces, driven by agriculture, transportation, communication, and trade, enabled by an abiding government, fail to assure minimal entitlement to food, either directly (through subsistence) or via trade for a large sector of society. Following Amartya Sen, entitlement failure is an economic phenomenon, broadly defined, in which individuals and households are unable to obtain sufficient amounts of food through all available legal means (cash, labor, skills, credit, and other assets that comprise 'endowment') at the market's existing terms of exchange (costs of securing sufficient amounts of food). Combinations of loss of endowment and adverse shifts in the conditions of exchange (e.g., spikes in grain prices) can lead to certain classes of society being severely deprived of food. Component causes that lead to market failure-driven famine are complex, interacting over an extended time (**Figure 2**). Causes acting at various times in the pathway to market failure can be numerous, including long-term trends in climate change or shorter term events such as drought,

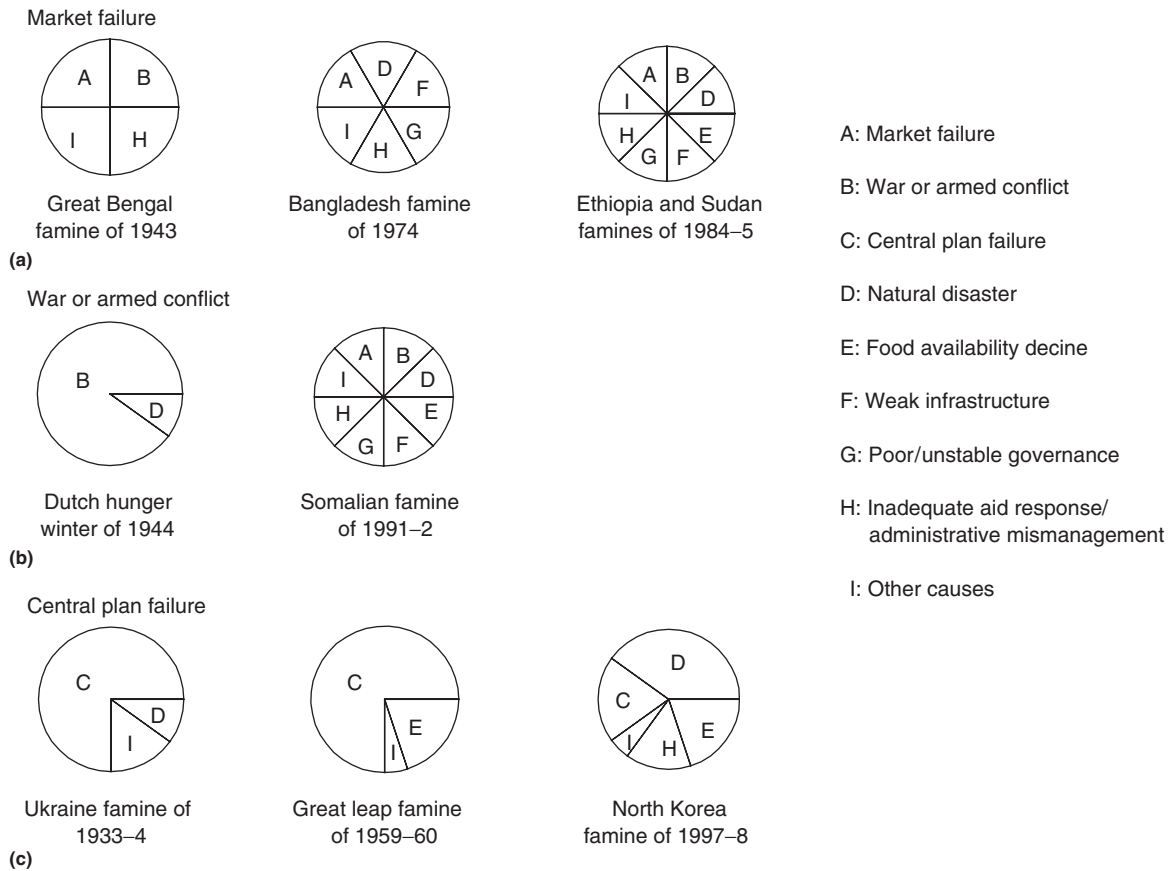


Figure 1 Complex causal networks of selected modern famines, stratified by a dominant cause. Each pie illustrates a complete cause; each wedge illustrates an assumed, essential component cause, without any one of which famine would not occur. Inclusion of causes based on literature reviews; sizes of pie slices are subjective based on descriptions in the literature: A: Market failure – Loss of direct or trade entitlement through a combination of (1) increased food prices due to food shortage from decreased agricultural production or importation, hoarding, and speculation or other market forces leading to unfavorable terms of exchange plus (2) loss of means to command food through cash, labor, credit, and other assets (endowment) by vulnerable groups of society; B: War or armed conflict – Declared or internal; through siege, blockade, or other expression of force, during a time course leading up to and concurrent with famine; C: Central plan failure – Occurring within centrally planned states lacking democratic processes, notably in the twentieth century communist states, with directives that disrupt infrastructure, productivity, economic well-being and access to food through heavy taxation, extraction of food grains and livestock, with restricted movement of food stocks outside of free-market dynamics, leading to starvation of masses; D: Natural disaster – Climatological and environmental catastrophes including floods, or single, repeated or chronic droughts; E: Food availability decline – Food shortage resulting from poor crop production, lack of trade, poor food transport, storage and marketing systems; F: Weak infrastructure – inadequate systems of finance, credit, roads, communications, agricultural production, including irrigation or flood protection systems; G: Poor/unstable governance – Weak and ineffective forms of governance, including anarchy; H: Inadequate aid response/administrative mismanagement – Inadequate national or international counter-famine measures, including employment or food procurement policies as well as withheld, slow, ineffectual, or insufficient relief; I: Other causes – a catch-all ‘causal complement’ to those listed above, of interacting prefamine and intrafamine sociological, governmental, environmental, and market forces that render each famine unique. Reproduced from Rothman K and Greenland S (1998) *Modern Epidemiology*. Philadelphia: Lippincott-Raven. 7–28, with permission from LWW.

excessive floods, pestilence, or other causes that collapse crop yield. These, along with reduced food imports, inefficient transport, rudimentary marketing infrastructures, can lead to national or, more often, regional declines in food availability, inflationary grain market responses to speculation and hoarding, ineffectual trade policies, political instability and corrupt governance, market depressions with year-round or seasonal job losses and depletion of assets of the poor (endowment). Prior or present conflict can destabilize markets and contribute to such types of famine.

Famines that can be classified as ones primarily of market failure include the Great Irish Famine from 1844 to 1848,

which was triggered by a potato blight that stripped the country of the only staple that Irish peasantry could afford to grow on their small parcels of land. Peasants who did grow other staple grains had to sell them to pay rent to landlords. However, during these same years, there were substantial exports of wheat, barley, oats, and animal products by land-owners to English markets. Food did not enter the local Irish markets because the peasants lacked effective demand.

Market or entitlement failures marked the last two famines of the twentieth century in South Asia: The Great Bengal Famine of 1943 and the Bangladesh (formerly Bengal) Famine of 1974–75 (Figure 1). The 1943 famine, during which some

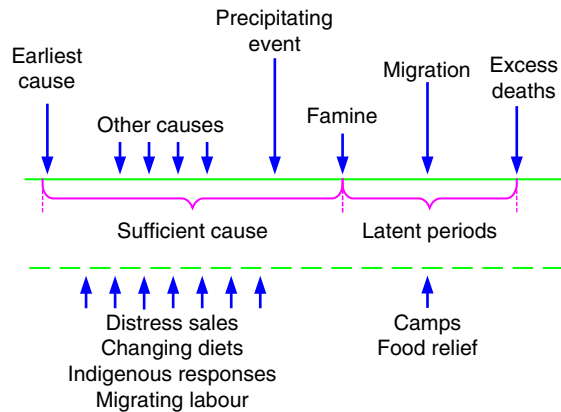


Figure 2 A model depicting actions of individual, or component, causes that can lead to a sufficient cause of famine, and societal, indigenous responses to famine predominantly caused by market failure. Famine may be latent or delayed from external view until migrations or excess deaths occur. Government relief is typically a late response to famine.

3 million people are estimated to have died, was originally judged by a Famine Inquiry Commission to be due to a shortage in rice supply. However, a seminal in-depth analysis years later by Sen showed that the famine occurred in a year during which rice production in Bengal was only 5% lower than the average of the previous 5 years. It was also a year when most economic indicators of Bengal were showing a 'boom' in growth due to World War II. Rural food stocks were being procured by the government to support military needs, subsidize rations for civil servants, and stabilize general prices of rice in Calcutta, which drove up the price of rice in rural areas. This practice, coupled with 'boat blockade' and 'rice denial' policies imposed in regions along the Bay of Bengal for reasons of defense, left certain low wage-earning rural classes (agricultural workers, day laborers, artisans, and fishermen) disentitled, and unable to acquire enough food for their own survival.

In Bangladesh, at least 100 000 people died in 1974–75 in a famine that followed an unusually severe flood. During the several years leading up to the famine there were events that brought the country to a highly vulnerable state, including a devastating cyclone and tidal wave, a civil war that led to the country's independence, and a series of partial crop failures, all superimposed on preexisting high burdens of malnutrition, disease, underdevelopment, and ensuing political chaos. The flood in the middle of 1974 was expected to destroy much of the major *aman* rice to be harvested a few months later. In anticipation of impending rice shortage, rural traders began to hoard grains in early September of that year causing rice prices to spike across the country's rural markets in a contagious pattern (Figure 3). Rice prices remained at approximately twice the normal price for months thereafter, even after it became evident that the presumed poor harvest was, in fact, a normal one. Thus, total and per capita aggregate grain supplies in Bangladesh remained at about average levels throughout the famine. Local area food deficits and hoarding of grains by traders led to the observed points of inflection in the price of



Figure 3 Consecutive maps of a contagious spread of spikes in the price of rice in local markets throughout rural Bangladesh from (a) late August 1974 through (h) the end of October 1974 during a flood-associated period of a famine that reportedly killed from 100 000 up to 1 million persons. Reproduced from Seaman J and Holt J (1980) Markets and famines in the third world. *Disasters* 4(3): 283–297, with permission from Wiley.

rice throughout the country that caused the entitlements of rural wage earners to collapse, initiating a famine that resulted in extremely high mortality and massive migrations to urban centers in search of relief.

The Horn of Africa has been wracked by famine or famine-like conditions, leading to what have become classically defined as 'complex emergencies' for much of the past four decades. Aggregate food shortage has appeared to play a more variable and, at times, prominent role in recent famines in the eastern Horn. In Ethiopia, Sudan, Eritrea, and Somalia, large tracts of land are drought prone, average annual rainfall has been declining since the 1930s, and robust, indigenous farming and animal husbandry practices have been weakened as agricultural land has increasingly been used for growing export crops. In the Ethiopian famine of 1972–75, in which over 100 000 people died, national crop production dropped to only ~7% below normal levels, a decline that, like in Bengal in 1943 and 1974, would not have been expected to trigger a famine. However, crop production had been severely below normal in Wollo Province, where the famine began. Although the famine subsequently spread to other areas of the country, a reluctance by the government to formally recognize the famine and excessive delays in mobilizing and targeting

food aid within country (whether from national or international stocks) were deemed responsible for unleashing a famine that, based on national stocks, should have been averted. Famines during 1982–85 in Ethiopia and in the Sudan appeared to be more closely tied to gradual declines in national food security during the preceding decade. These trends were exacerbated by repressive governments enacting policies resulting in civil wars and severely deteriorating economic conditions, aggravated by weak international food aid responses.

Armed Conflict

A second major class of famine comprises those precipitated or triggered by declared war or armed insurgency, leading to a siege or food blockade by a foreign power (e.g., Allied blockade of Germany in 1915–18; Nazi blockade of Holland precipitating the Dutch Winter Famine of 1944–45, and the Nazi siege of Leningrad in 1942–44) or, as occurring more in recent years, severe civil war that disrupts normal markets as well as emergency food delivery systems (e.g., the Somali civil war and famine of 1991–92). Armed conflict can incapacitate or destroy a country's ability to govern, develop, produce, and feed itself domestically or through food aid, as scores of people become displaced, destitute, starve, and die from severe malnutrition and epidemic illness. The famine in Somalia in the early 1990s exemplifies the rapid emergence of military conflict as a precipitating cause of famine. With significant transfers of weaponry to rogue vigilante groups and increased deployments of land mines in other poor, warring countries in recent years, civil violence and lawlessness also pose a major hindrance to the effective provision of short-term relief during the acute phase of famine and to subsequent economic recovery.

Failure in Central Planning

A third class of modern famine, distinct from the other two, has resulted from failure by intent, indifference, ignorance, or incompetence of a centrally planned state to adequately provide food to all sectors of society. Examples of this third type of famine in the twentieth century include those induced by notorious policies of Stalin in Soviet Russia in the 1920s and 1930s. In an effort to achieve rapid industrial growth, Stalin waged class warfare among rural peasantry, abolished economic incentives, collectivized farms into massive (inefficient) production units, and merged villages into socialist agrotowns, seized and exported grain for foreign exchange to fuel industrialization, restricted population movements across municipalities, and brutally suppressed all opposition. Agricultural production plummeted across regions of Russia leading to disastrous shortages (e.g., by 40% in some areas), further intensifying state seizures of food grain, especially in the grain-belt region of the Ukraine where Stalin sought to crush a nationalist revolt by forcibly extracting available foodgrains from the population, and inducing the worst famine in Russian history. Between 1930 and 1937, it was estimated that nearly 15 million peasants died, of whom 7–8 million died in the Ukraine in 1933–34.

Under communist rule imposed by Mao Zedong, China experienced in 1959–60 the worst recorded famine in human history that left an estimated 30 million people dead. The Great Leap Forward Famine was provoked through a causal chain of centrally planned policy steps during the preceding decade, modeled after Stalin, that was motivated by ill-conceived goals to 'leap forward' by achieving agricultural sufficiency and superiority through massive agricultural collectivization and the formation of huge peasant communes, and rapid rural industrialization schemes through crash programs to increase steel production. The plight of tens of millions rural peasants was tightly controlled by the state through brutal force, terror, propaganda, and state control of grain production, procurement, and taxation motivated by a blind faith among civil servants in the vision and leadership of Mao. As a result of fabricated inflation of grain production figures, driven by a zeal to demonstrate success, China, during the peak of famine mortality in the countryside in 1960 became a net exporter of more than a million metric tons of grain, mimicking Stalinist Russia. Thus, in addition to events immediately leading to famine, some component causes contributing to the centrally planned Great Leap Forward Famine can be traced back through the previous one to three decades and to influences beyond the borders of China.

Communist North Korea's inability to avert the famine of 1997–98 is a recent example of a central planning failure, conditioned by chronic food insecurity over the previous decade and precipitated by poorly timed, torrential rains and floods in 1995–96 and drought in 1997. However, some causal elements related to how slowly and secretly the isolationist government responded, actions of governance that date back to the Korean War, remnants of Cold War politics and consequent politicization of food aid.

New Variant Famine Hypothesis

This hypothesis, although still being established from emerging data, postulates that crises situations may be exacerbated to famine status by unfamiliar vulnerabilities imposed by new stresses that occur, for example, with the Acquired Immuno-deficiency Syndrome (AIDS) epidemic. Communities and households that have lost working-aged adults to AIDS-related illness or death are less productive, are burdened by an increased dependency ratio (children plus older persons/working-age adults) and greater responsibilities placed on older, feeble adults to look after food production, other household assets, and care. Further, additionally expended resources on medical treatment coupled with increased nutritional needs of large numbers of AIDS patients in communities, leave families less able to cope or able to cope with otherwise routine societal food stresses.

AIDS has been thought to play a role in the severe food crisis in Southern Africa in 2002 (Malawi, Lesotho, Zimbabwe, etc.). In Zambia, drought-prone districts with higher rates of AIDS infections had much lower levels of agricultural productivity and in areas that were poor to start with, asset holdings were compromised where HIV rates were higher. Nevertheless, although predisposing, no famine has yet been directly attributed to HIV.

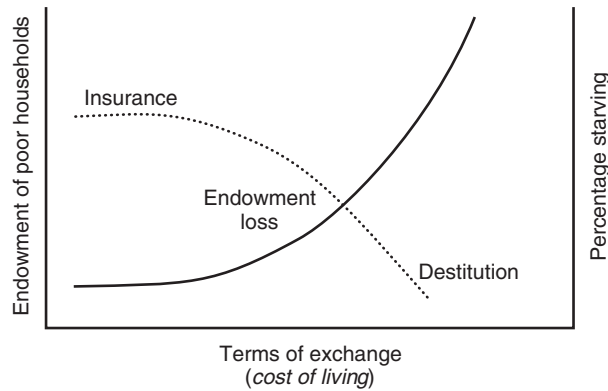


Figure 4 Illustration of collapse in entitlement. As endowment of the poor decreases toward a state of destitution with increasingly severe (costly) terms of exchange for food, the risk of starvation and famine increases.

Coping Strategies

In cultures where food shortage or inaccessibility to large sectors of society is chronic, and threat of famine periodic, there exist indigenous responses that enable the local populace to cope, protect their entitlement, and minimize the risk of starvation as terms of exchange for food deteriorate (illustrated as a concept in [Figure 4](#)). A first line of responses may be viewed as 'insurance' against uncertainty; these are activities that can reduce loss of endowment, such as restructuring the mix of crops grown or pastoral practices in ways that insulate against drought- or flood-induced shortages. Examples include planting more robust crops, dispersing crops across a wider area, staggering plantings, or increasing livestock diversity and mobility. Food preservation practices and dietary changes to include less commonly eaten foods can initially increase the size and diversity of the food base. As terms of exchange become worse, coping mechanisms aimed at survival increasingly cost households their endowment. These responses include working longer and at different jobs for lower wages, migrating far from home to find marginal work, reducing meal frequency, consuming the next planting's seeds, and substituting or expanding consumption of 'famine foods,' of low nutritional quality. At first these may include unusual tubers, leaves, flowers, and other plants. Household assets such as pots, utensils, watches, and small animals are increasingly sold as, eventually, are larger assets such as bullock carts, bicycles, and draft animals. Land mortgage or sales transactions become more numerous. With indebtedness and destitution, petty crime increases, child abandonment is common; famine foods may include tree bark, ground bone, and rodents; suicide and cannibalism may ensue.

Defined in terms of coping strategies, the distinction between crisis and famine is considered to be the point when families start compromising 'future security for present survival.' An indicator of severe entitlement loss in a community is the livestock-to-grain price ratio in local markets. Normally this ratio is of a figure that reflects the greater asset value of livestock than grain. However, it may invert as the cost of grain and feeding animals and the level of animal wasting all continue to rise, such that, at a peak of famine vulnerability, large

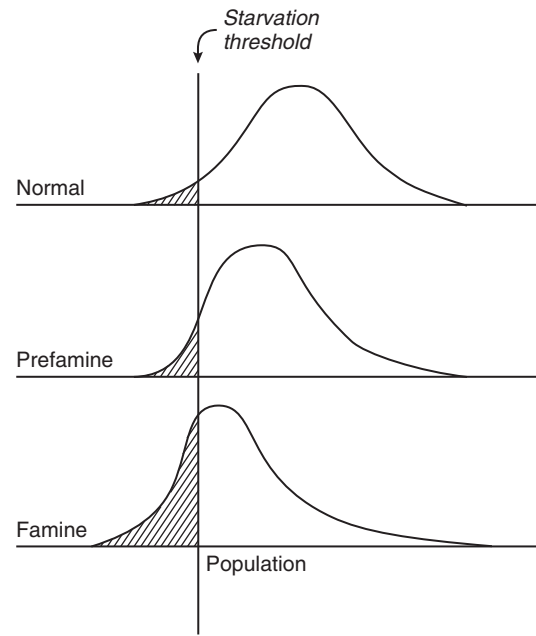


Figure 5 Shifting of a high-risk, undernourished population toward increased starvation during prefamine and famine conditions, particularly those most vulnerable. Truncated left tail area reflects hypothetical effects of coping strategies that prevent starvation. Right skew reflects polarizing of wealth, with some sectors profiting from famine.

numbers of animals may be sold at very low prices relative to the costs of grain.

Viewed over time, famine is a continuum. As household and community entitlement erodes for increasing numbers due to both deteriorating conditions of exchange and endowment loss, destitution and starvation become more likely. [Figure 5](#) depicts a hypothetical shift in distribution of starving individuals in a poor population exposed to increasing risk of famine, where under usual conditions a small proportion of individuals routinely face the threat of starvation and wasting malnutrition (top panel). During periods of high or repeated stress, such as those of prolonged drought and internal conflict, whereas the population faces less food security, coping mechanisms continue to protect most vulnerable groups from abject starvation, even as they near such a 'threshold' amidst inevitable losses of human and economic asset (middle panel). During severe distress of famine, entitlement has collapsed for the most vulnerable classes of society, pushing large numbers of persons into a state of starvation, leaving them destitute and migrating or dying (bottom panel). However, not all individuals starve. Some segments of society lose little or no economic ground, or benefit considerably from the plight of others who must sell property or other assets, work for reduced wages, or borrow at high interest to avoid starvation during famine, leaving them deep in debt. Still other segments, particularly those trading in famine relief goods and services, stand to gain large profits throughout the famine and recovery periods (depicted by the right skew). Postfamine, the economic landscape is nearly always one of greater polarization of wealth and an increase in size and vulnerability of society's poor and destitute. Peri-urban slums typically remain swollen following famine as a result of permanent migration.

Government and International Responses

Famine through the ages has invoked, from law-abiding governments, preventive actions and relief responses in the face of imminent catastrophe. In Genesis, Pharaoh's grain taxes during years of plenty were aimed at relieving dwindling food stores during famine. During China's Eastern Chou and Ch'in dynasties of the third century BC, and in India over 2000 years ago, steps that were taken to prevent or relieve famine included disaster reporting procedures, cropping alterations, grain distribution, feeding kitchens, tax remissions, vulnerable group relocation, and public works construction to facilitate irrigation, food shipment, or flood control. In the sixteenth-century England, to counter inflationary effects of speculative grain hoarding, the Tudor First Book of Orders called for enforced extraction and marketing of private grain stocks as a way to control staple prices and thwart famine. Policy response can also amount to inaction. The Great Irish Famine from 1844 to 48 evoked a different response from the British Government, a flawed 'laissez-faire' policy intending to allow market forces to equilibrate on their own to meet local food needs, a course that never materialized as entitlement collapsed among Irish peasantry. However, learning from a century of repeated famine, 'Famine Codes' emerged in British India in 1880 that called for massive public works coupled with food distribution and feeding centers for vulnerable groups, which served as the core famine relief policy on the subcontinent for more than a half century and have continued to guide famine relief efforts to the present day.

Today, modern preventive response by international agencies and governments can be informed and guided by surveillance systems with regional, national, and local data collection mechanisms. Examples, available online, are the Famine Early Warning System (FEWS) that functions across sub-Saharan Africa, supported by the US Agency for International Development over the past two decades and the Global Information Early Warning System (GIEWS) managed by the Food and Agricultural Organization of the United Nations (FAO). The primary aim of surveillance is to detect worsening conditions in high-risk populations in sufficient time to permit effective preventive or preemptive action. The task is a 'tall order' given widespread, often complex component causes that must converge in certain ways to cause famine, against a usual plethora of endemic risk factors. With early, adequate, and effective response serving as the criterion of success, modern surveillance has so far failed to prevent famine. In part, this may reveal a basic epidemiologic dilemma: Against a background of profound, widespread economic and nutritional need throughout the developing world, including numerous prefamine but intact situations arising under surveillance, famine is a rare event. For example, the global financial crisis that erupted in 2008, causing enormous hardship and intensified undernutrition, failed to unleash famine. Thus, even with presumed high sensitivity and specificity, a low predictive value of indicators stemming from infrequent occurrence makes action to prevent a particular famine unlikely given the enormous political and financial resources required to mount an adequate prevention strategy.

Perhaps the most effective preventive action relates to enacting a development agenda that recognizes and seeks to

strengthen productivity and well-being of population groups in famine-prone areas. These can include boosting infrastructural, commercial, education, agricultural, and other inputs into priority areas that improve long-term economic conditions.

Preemptive government policies are directed toward relieving a prefamine condition once it becomes apparent. Setting up FEWs that monitor climatic, agricultural, population mobility, economic and nutritional indicators is considered preemptive in that such information is intended to identify high-risk trends so that corrective action could be taken before famine becomes imminent. Normally, early warning surveillance is only possible in high-risk countries with significant international assistance. Another example is a government making large purchases of food on the international market and releasing the commodities through ration shops, food for work, and other programs that do not disrupt the local food economy but stabilize local grain market prices instead as a means to prevent speculation throughout the period of high risk.

Lagged or relief-oriented responses comprise emergency responses to acute and enormous need that typically are enacted after famine begins and its harsh consequences already evident in a population. These actions, usually in coordination with major international relief and donor agencies, are typically intended to relieve acute suffering and death and promote the rehabilitation of those masses who have survived to migrate, and reach encampments. By definition, lagged responses represent policy failure for governments intending to minimize the destruction, malnutrition, and mortality of famine.

See also: Hunger. Nutritional Surveillance: Developing Countries. Refugees: Nutritional Implications. Starvation and Fasting: Biochemical Aspects

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FATS AND OILS

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Glossary

ω -3 Fatty acid Polyunsaturated acyl chain with a carboxyl group at one end and a methyl group at the other end. First double bond is three carbons from the methyl end of the acyl chain. Of the major dietary ω -3 fatty acids, carbon chain lengths range from 18 to 22 carbons. Vegetable sources include soybean and canola oils (α -linolenic acid (ALA, 18:3)); animal sources include fish (eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6)). Dietary ω -3 fatty acids, particularly EPA and DHA, are associated with decreased cardiovascular disease risk.

Atherosclerotic plaque Accumulation of lipid, primarily cholesterol, in blood vessel walls. Impedes blood flow.

Dietary cholesterol Fat-soluble compound found in animal fats, particularly eggs. Steroid ring structure that is important for cell membrane fluidity and lipid transported in the blood. Serves as a substrate for steroid hormones, bile acids, and vitamin D. In cholesterol-sensitive individuals, high intake of dietary cholesterol is associated with increased plasma cholesterol concentrations.

Dietary fat Macronutrient, which provides 9 kcal g⁻¹. Hydrophobic component of food.

Lipoproteins Spherical particles with a hydrophilic surface composed primarily of phospholipid and protein, and hydrophobic core composed primarily of triglyceride and cholesteryl ester. Particles facilitate the transport of lipid in the aqueous milieu of the blood stream.

Monounsaturated fatty acid Acyl chain with a carboxyl group at one end and a methyl group at the other end. One carbon-carbon double bond in the acyl chain. Usually liquid at room temperature. Dietary monounsaturated fats are associated with either null or decreased cardiovascular disease risk. Common dietary sources are canola and olive oils.

Polyunsaturated fatty acid Acyl chain with a carboxyl group at one end and a methyl group at the other end. Two or more carbon-carbon double bonds in the acyl chain. Usually liquid at room temperature. Dietary polyunsaturated fatty acids are associated with decreased cardiovascular disease risk. Common dietary sources include soybean, corn, safflower, and sunflower oils.

Saturated fatty acid Acyl chain with a carboxyl group at one end and a methyl group at the other end. No carbon-carbon double bonds in the acyl chain. Usually solid at room temperature. Dietary saturated fats are associated with increased cardiovascular disease risk. Common dietary sources include meat and dairy fat.

Trans fatty acid Polyunsaturated acyl chain with a carboxyl group at one end and a methyl group at the other end. At least one double bond is in the *trans*, rather than *cis*, configuration. There are two main sources of dietary *trans* fatty acids: animal fat and partially hydrogenated fat. Dietary *trans* fatty acids are associated with increased cardiovascular disease risk.

Dietary fat is a macronutrient that has historically engendered considerable controversy and continues to do so. Contentious areas include optimal amount and type for cardiovascular disease risk reduction, and role in body weight regulation.

Dietary Fats and Oils – The Good, Bad, and Ugly

Dietary fats and oils are unique in modern times in that they have good, bad, and ugly connotations. The aspects of dietary fat that are classified as good include serving as a carrier of soluble vitamins (vitamins A, D, E, and K), enhancing the bioavailability of fat-soluble bioactive substances (e.g., absorption of fat-soluble micronutrients), providing essential substrate for the synthesis of metabolically active compounds (e.g., essential fatty acids for eicosanoid synthesis), providing critical structural components (e.g., cell membranes and lipoprotein particles), preventing carbohydrate-induced hypertriglyceridemia, and serving

an energy-dense form of reserve metabolic fuel (triglyceride). The aspects of dietary fat that are classified as bad include serving as a reservoir for fat-soluble toxic compounds. The aspects of dietary fat that are classified as ugly include providing a concentrated form of metabolic fuel in times of excess and contributing saturated and *trans* fatty acids that promote atherosclerotic plaque formation, the underlying cause of heart disease, stroke, and phlebitis.

Lipids – In Food and in the Body

Fatty Acids

Fatty acids are the basic components of larger lipid compounds or serve as substrates for bioactive molecules. They are composed of an acyl (hydrocarbon) chain with a methyl and a carboxyl group at either end. The majority of fatty acids have

an even number of carbons. The range of chain lengths for common fatty acids is broad, 12–22 carbons, although shorter- and longer-chain fatty acids occur naturally. The predominant fatty acids, in the human body and food, are depicted in **Table 1**. In addition to chain length, fatty acids differ from each other with regard to the number, type, and location of double bonds. Fatty acids with no double bonds

are referred to as saturated, with one double bond as mono-unsaturated, and with two or more double bonds as poly-unsaturated (**Figure 1**).

The double bonds within unsaturated fatty acids can be in either the *cis* (hydrogen atoms on same side of the acyl chain) or *trans* (hydrogen atoms on opposite sides of the acyl chain) conformation. The presence of a *cis* relative to a *trans* double bond results in a greater bend or kink in the hydrocarbon chain. This kink impedes the fatty acids from aligning (packing together). In a cell membrane, this results in increased fluidity; in food, it results in oils that are liquid or fats that are soft at room temperature. The vast majority of fatty acids occur in the *cis* conformation. Two fatty acids with the same number of carbons and double bonds, and position of double bonds, but with at least one double bond differing in conformation, are referred to as geometric isomers (e.g., oleic acid (18:1*cis*) and elaidic acid (18:1*trans*)) (**Figure 2**).

Fatty acids also vary with regard to the location of the double bonds within the acyl chain. Fatty acids with the same number of carbons and double bonds, and conformation of the double bonds, but having different double bond locations within the acyl chain, are referred to as positional isomers (e.g., oleic acid (18:1*cis*) and elaidic acid (18:1*trans*)) (**Figure 2**). The most common positional isomers differ in the location of the double bonds from the methyl end of the acyl chain. Fatty acids in which the first double bond occurs at the third or the sixth carbon are termed ω -3 (*n*-3) or ω -6 (*n*-6)

Table 1 Common fatty acids

Code	Common name
Saturated	
12:0	Lauric acid
14:0	Myristic acid
16:0	Palmitic acid
18:0	Stearic acid
Monounsaturated	
16:1 <i>n</i> -7 <i>cis</i>	Palmitoleic acid
18:1 <i>n</i> -9 <i>cis</i>	Oleic acid
18:1 <i>n</i> -9 <i>trans</i>	Elaidic acid
Polyunsaturated	
18:2 <i>n</i> -6,9 all <i>cis</i>	Linoleic acid
18:3 <i>n</i> -3,6,9 all <i>cis</i>	α -Linolenic acid
18:3 <i>n</i> -6,9,12 all <i>cis</i>	γ -Linolenic acid
20:4 <i>n</i> -6,9,12,15 all <i>cis</i>	Arachidonic acid
20:5 <i>n</i> -3,6,9,12,15 all <i>cis</i>	Eicosapentaenoic acid
22:5 <i>n</i> -3,6,9,12,15 all <i>cis</i>	Docosapentaenoic acid
22:6 <i>n</i> -3,6,9,12,15,18 all <i>cis</i>	Docosahexaenoic acid

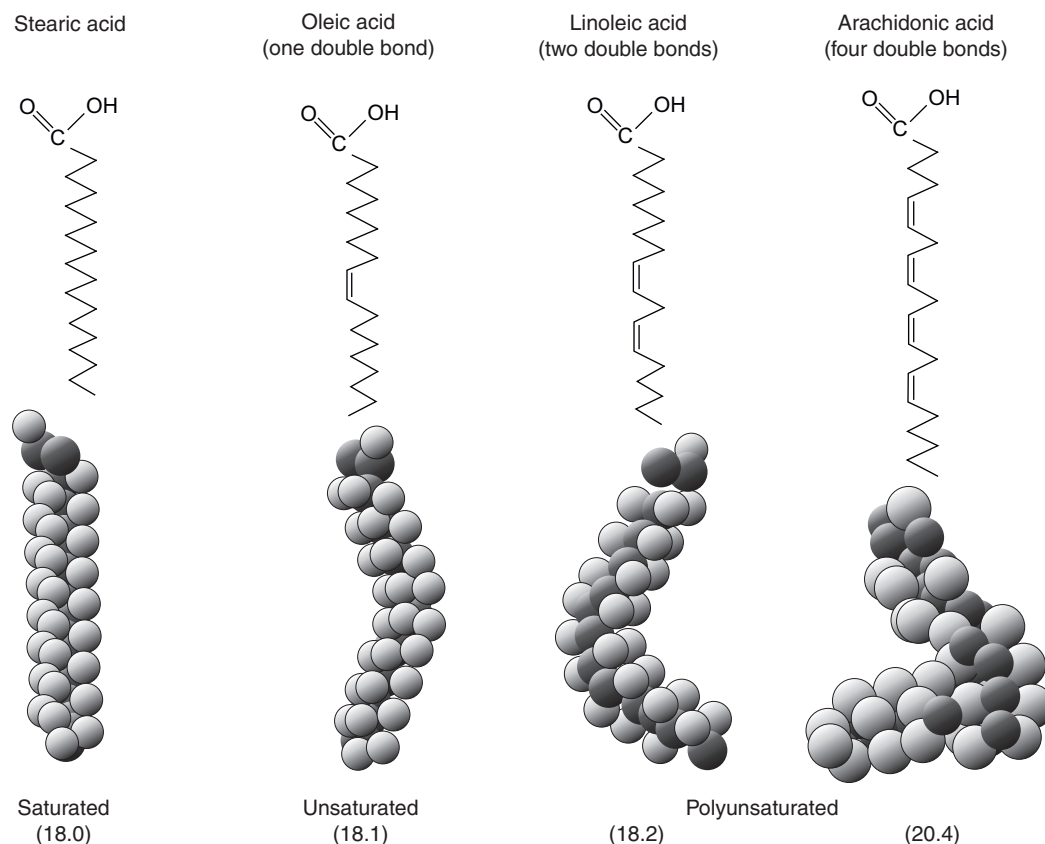


Figure 1 Structures of saturated, monounsaturated, and polyunsaturated fatty acids.

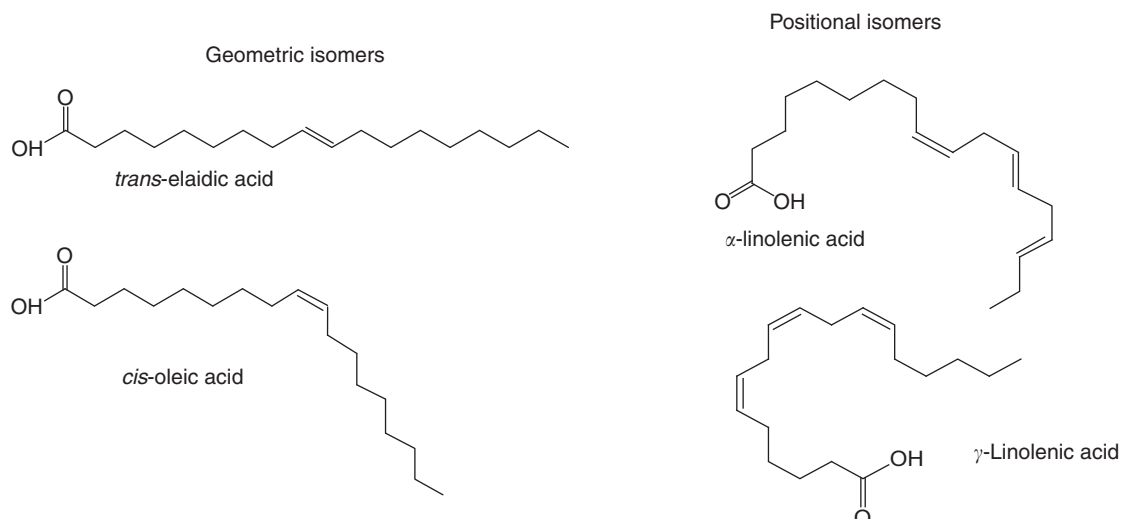


Figure 2 Examples of fatty acids geometric isomers (*trans*-elaidic acid and *cis*-oleic acid) and positional isomers (α -linolenic acid and γ -linolenic acid).

fatty acids, respectively (e.g., α -linolenic acid (18:3 n -3) and γ -linolenic acid (18:3 n -6)).

Most double bonds occur in a nonconjugated sequence, that is, a single carbon atom with single carbon-carbon bonds separates the carbons making up the double bonds. Some double bonds occur in the conjugated form, without an intervening carbon separating the double bonds. Conjugated double bonds tend to be more reactive chemically (e.g., more likely to become oxidized). Enzymes that metabolize fatty acids distinguish among both geometric and positional isomers. The metabolic products of different fatty acid isomers, especially positional isomers, have different and at times opposing biological effects.

Some fatty acids are classified as essential. An essential nutrient is a nutrient that the body cannot synthesize or cannot synthesize in amounts adequate to meet requirements. Linoleic acid (18:2) and fatty acids that can be derived from linoleic acid (Figure 3), such as arachidonic acid (20:4), are classified as essential fatty acids. Their essentiality is due to an inability of humans, in contrast to plants, to introduce a double bond after the ninth carbon in a fatty acyl chain (from the carboxyl end).

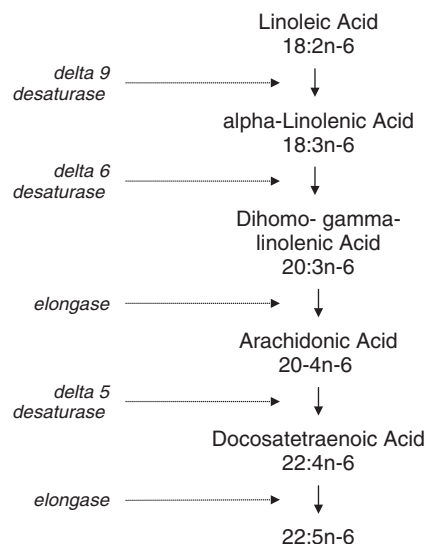


Figure 3 Metabolism of linoleic acid.

Triacylglycerol (Triglyceride)

Triacylglycerol is the major form of dietary lipid in fats and oils, whether derived from plants or animals. Triacylglycerol is composed of three fatty acids esterified to a glycerol molecule (Figure 4). The physical properties of the triacylglycerol are determined by the specific fatty acids esterified to the glycerol moiety and the actual position the fatty acids occupy. Each of the three carbons comprising the glycerol molecule allows for a stereochemically distinct fatty acid bond position: *sn*-1, *sn*-2, and *sn*-3. A triacylglycerol with three identical fatty acids is termed a simple triacylglycerol. These are exceedingly rare in nature. A triacylglycerol with two or three different fatty acids is termed a mixed triacylglycerol and makes up the bulk of the

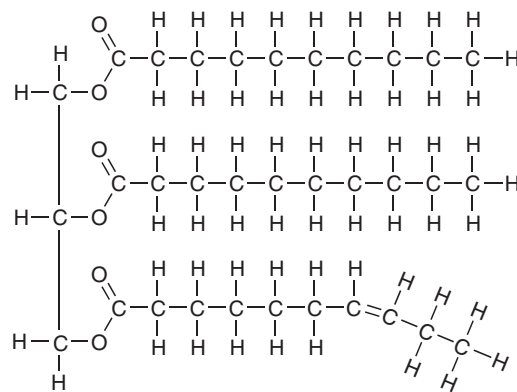


Figure 4 Triacylglycerol molecule.

fat. The melting point of a triacylglycerol is determined by the physical characteristics and position of the fatty acids esterified to glycerol – their chain length; number, position, and conformation of the double bonds; and the stereochemical position.

Approximately 90% of the molecular weight of triacylglycerol is accounted for by the fatty acids. The fatty acid profile of the diet is reflected, in part, in the fatty acid profile of the adipose tissue triacylglycerol, particularly for essential fatty acids. Such data have been used to approximate long-term food intake patterns of humans.

Mono- and diglycerides have one and two fatty acids, respectively, esterified to glycerol. In nature, they occur only in trace amounts. They are primarily intermediate products of triacylglycerol digestion, clearance from the bloodstream, or intracellular metabolism. They are used as emulsifiers in processed food.

Once consumed, triacylglycerol is hydrolyzed into free fatty acids and monoglycerides in the small intestine prior to absorption. Once these compounds enter the intestinal cell, they are used to resynthesize triacylglycerol. This lipid is then incorporated into nascent triglyceride-rich lipoprotein particles, termed chylomicrons, for subsequent introduction into peripheral circulation. Chylomicrons are secreted directly into the lymph before entering the blood stream. Once in circulation, triacylglycerol is hydrolyzed before crossing the plasma membrane of peripheral cells. The primary enzyme that hydrolyzes triacylglycerol in plasma is lipoprotein lipase. Lipoprotein lipase hydrolyzes triacylglycerol into free fatty acids and 2-monoacylglycerol. The enzyme is attached to the luminal surface of capillary endothelial cells via a highly charged membrane-bound chain of heparin sulfate-proteoglycans. The ability of lipoprotein lipase to bind both the chylomicron particle and the cell surface ensures the cellular uptake of free fatty acids that are generated from the hydrolysis. Once inside the cell, free fatty acids can be oxidized to provide energy, metabolized to biologically active compounds, or resynthesized into triacylglycerol for storage as a potential reservoir for fatty acids for subsequent use.

Phospholipid

There are only trace amounts of phospholipid in dietary fats and oils. However, because the fatty acids in fats and oils provide substrate for the synthesis of phospholipid in the body, this subtype of fat is important to discuss. Phospholipid is a critical component of all cells, both plant and animal. It is composed of two fatty acids esterified to the *sn*-1 and *sn*-2 positions and a moiety frequently referred to as a polar head group to the *sn*-3 position of glycerol, the latter group via a phosphate bond (Figure 5). Phospholipid molecules are amphipathic, that is, there are both hydrophobic and hydrophilic domains in the molecule. The two fatty acids confer hydrophobic properties and the polar head group confers hydrophilic properties. The specific fatty acids esterified to the glycerol backbone tend to be unsaturated fatty acids. The different polar head groups, most commonly phosphorylcholine, phosphorylserine, phosphorylinositol, and phosphorylethanolamine, result in phospholipids that

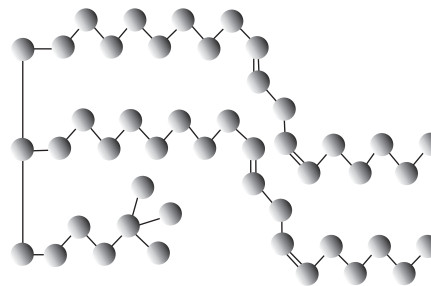


Figure 5 Phospholipid molecule (phosphatidylcholine).

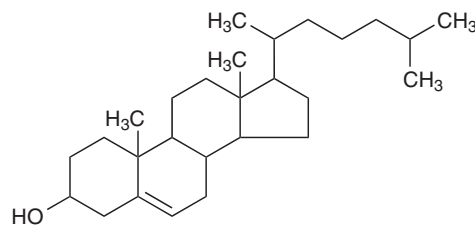


Figure 6 Cholesterol molecule.

vary in size and charge. Because of their amphipathic nature, phospholipids serve as the major structural component of cellular membranes and, in so doing, also serve as a reservoir for metabolically active unsaturated fatty acids. In the small intestine, because of their amphipathic properties, they play an important role in facilitating the emulsification and absorption of fat and fat-soluble vitamins. On the surface of lipoprotein particles, they provide a critical component in the packaging and transport of lipid in circulation. In cells, they form bilayers that serve as the plasma membrane and intracellular membranes.

Cholesterol

Dietary sources of cholesterol are limited to foods of animal origin. Cholesterol is an amphipathic molecule that is composed of a steroid nucleus and a branched hydrocarbon tail (Figure 6). Cholesterol occurs naturally in two forms: non-esterified (free cholesterol) and esterified (cholesteryl ester). If esterified, the fatty acid is linked to cholesterol at the number 3 carbon of the sterol ring.

Free cholesterol is a component of cell membranes and, along with membrane phospholipid fatty acid, determines membrane fluidity. Cholesterol intercalates into the phospholipid bilayer restricting motility of the fatty acyl chains and hence decreases fluidity. Free cholesterol is critical for normal nerve transmission. It makes up approximately 10% (dry weight) of total brain lipids. Cholesterol is a precursor of steroid hormones (e.g., estrogen, testosterone), vitamin D, adrenal steroids (e.g., hydrocortisone, aldosterone), and bile acids. This latter property is exploited in certain approaches to decrease plasma cholesterol concentrations by preventing the resorption of bile acids (recycling), hence forcing the liver to use additional cholesterol for bile acid synthesis and in so doing, creating an alternate mechanism for cholesterol net excretion.

The receptor-mediated cellular uptake of cholesterol from plasma lipoprotein particles is critical in maintaining intracellular and whole-body cholesterol homeostasis. Once internalized, intracellular free cholesterol can have three metabolic effects. It inhibits the activity of 3-hydroxy 3-methylglutaryl CoA (HMGCoA) reductase, the rate-limiting enzyme in endogenous cholesterol biosynthesis. This property serves to decrease the intracellular rate of cholesterol biosynthesis commensurate with the uptake of cholesterol from extracellular sources (plasma lipoproteins), thereby minimizing intracellular accumulation. Intracellular free cholesterol inhibits the synthesis of receptors that take up lipoproteins containing apoproteins B100 or E from the plasma, thereby limiting the amount of additional cholesterol taken up by the cell. Intracellular free cholesterol also increases the activity of acyl CoA cholesterol acyltransferase (ACAT), the intracellular enzyme that converts free cholesterol to cholesteryl ester. A high level of intracellular free cholesterol is cytotoxic, whereas cholesteryl ester is a highly nonpolar molecule and coalesces to form lipid droplets within the cell, preventing interaction with intracellular components and subsequent detrimental effects.

Free cholesterol can be esterified intracellularly, as indicated, by ACAT. ACAT uses primarily oleoyl CoA as substrate, resulting primarily in cholesteryl oleate. Free cholesterol can also be esterified in plasma by lecithin cholesterol acyltransferase (LCAT). LCAT uses phosphatidylcholine as a substrate, resulting primarily in the products cholesteryl linoleate and lysolecithin. Cholesteryl ester is less polar than free cholesterol and this difference dictates how the two forms of cholesterol are handled, as mentioned above, intracellularly, and also as noted below, in the blood stream.

Approximately one-third of cholesterol in plasma circulates as free cholesterol and approximately two-thirds as cholesteryl ester. Cholesterol in circulation is carried on all subclasses of lipoprotein particles: both intestinally derived chylomicrons and hepatically derived very low density lipoprotein, intermediate-density lipoprotein, low-density lipoprotein (LDL), and high-density lipoprotein (HDL). Free cholesterol is incorporated into the phospholipid monolayer surface of lipoprotein particles, whereas cholesteryl ester is incorporated into the core of the lipoprotein particle. The majority of the cholesterol in circulation is carried on LDL particles. Cholesteryl ester is the major component of atherosclerotic plaque. In the arterial wall, cholesteryl ester is either derived from the infiltration of lipoprotein-associated cholesteryl ester resulting

from LCAT activity or synthesized *in situ* as a result of ACAT activity, depending on the mode of entry. The fatty acid profile of the cholesteryl ester in arterial plaque can provide some hint as to its source.

Historically, dietary cholesterol has been associated with increased cardiovascular disease risk. However, within the range currently consumed and on the basis of more recent data indicating that dietary fat type has a greater effect on cardiovascular disease risk indicators than dietary cholesterol, the emphasis has shifted.

Other Sterols

Fats and oils derived from plants contain a wide range of phytosterols, compounds structurally similar to cholesterol. The difference between phytosterols and cholesterol is related to their side-chain configuration and steroid ring double bonds. The most common dietary phytosterols are β -sitosterol, campesterol, and stigmasterol (Figure 7). In contrast to cholesterol, phytosterols are absorbed only in trace amounts. For this reason, plant sterols have been used therapeutically to reduce plasma cholesterol concentrations. They compete with cholesterol for absorption, hence effectively reduce cholesterol absorption efficiency.

Dietary Fats and Oils

Fatty Acid Profile of Common Dietary Fats

Dietary fats and oils come from both the animal and plant sources, primarily in the form of triacylglycerol. The fatty acid profile of commonly consumed dietary fats varies considerably (Figure 8). In general, fats of animal origin tend to be relatively high in saturated fatty acids, contain cholesterol, and are solid at room temperature. Oils of plant origin tend to be relatively high in unsaturated fatty acids (monounsaturated and polyunsaturated) and are liquid at room temperature. Notable exceptions include plant oils, termed tropical oils (e.g., palm, palm kernel, coconut oils), and partially hydrogenated fat. Tropical oils are high in saturated fatty acids but remain liquid at room temperature because they contain a high proportion of short-chain fatty acids. Partially hydrogenated plant oils are relatively high in *trans* fatty acids due to chemical changes induced during processing.

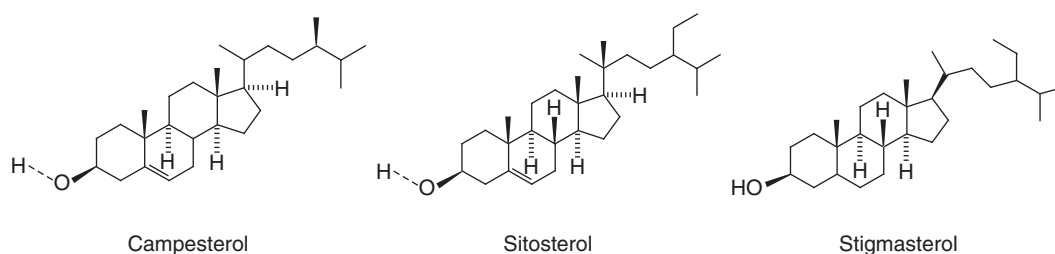


Figure 7 Plant sterols and stanols.

Major Contributors of Dietary Saturated, Monounsaturated, and Polyunsaturated Fatty Acids and Cholesterol

The major types of dietary fats and oils are generally broken down on the basis of animal and plant sources. The relative balance of animal and plant foods is an important determinant of the fatty acid profile of the diet. However, with the increasing prominence of processed, reformulated, and genetically modified foods, it is becoming difficult to predict the fatty acid profile of the diet on the basis of the animal versus plant distinction.

According to the National Health and Nutrition Examination Survey (NHANES) recall data, the 10 major dietary sources of saturated fatty acids in the US diet are regular cheese, whole milk, regular ice cream, 2% low-fat milk, pizza with meat, French fries, Mexican dishes with meat, regular processed meat, chocolate candy, and mixed dishes with beef (Table 2). Hence, regular dairy products contribute the majority (~16%) of saturated fatty acids in the diet, and the top 10 sources contribute ~30% of the saturated fatty acids consumed. The increased prevalence of fat-free and low-fat dairy products provides a viable option with which to encourage a population-wide decrease in saturated fat intake. To put the value of decreasing population-wide intakes of saturated fat into perspective, it has been estimated that the isocaloric replacement of 5% of energy from saturated fatty acids with complex carbohydrate, on average, would reduce total cholesterol concentrations by 10 mg dl⁻¹ (0.26 mmol l⁻¹) and LDL cholesterol by 7 mg dl⁻¹ (0.18 mmol l⁻¹). For a person at moderately high risk of developing cardiovascular

disease with total cholesterol concentration of 220 mg dl⁻¹ (5.69 mmol l⁻¹) and LDL cholesterol concentration of 140 mg dl⁻¹ (3.62 mmol l⁻¹), such a dietary modification would decrease total and LDL cholesterol concentrations by 4.5% and 5%, respectively. Each 1% decrease in total cholesterol concentrations has been associated with a 2% reduction in the incidence of coronary heart disease. Using this example, such a difference would theoretically translate into a 9% decrease in cardiovascular disease risk. However, it is important to note that decreasing the saturated fatty acid content of the diet should not necessarily be done by displacing fat with carbohydrate. As will be discussed in the next section, the quantity of dietary fat, relative to carbohydrate and protein, also impacts on blood lipid concentrations and lipoprotein profiles. Current data suggest that displacing saturated fatty acids with polyunsaturated fatty acids would result in the greatest decrease in cardiovascular disease risk.

The 10 major dietary sources of monounsaturated fatty acids in the US diet are French fries, regular processed meat, regular cookies, regular miscellaneous snacks, pizza with meat, regular salad dressing, regular cheese, Mexican dishes with meat, sausage, and mixed dishes with beef (Table 2).

The 10 major dietary sources of *n*-6 polyunsaturated fatty acids in the US diet are regular salad dressing, regular white bread, regular mayonnaise, French fries, regular cake, regular cookies, mixed dishes with chicken and turkey, regular miscellaneous snacks, regular potato chips, and fried fish (Table 2). The distribution of polyunsaturated fatty acids among commonly consumed foods is wide.

The 10 major dietary sources of cholesterol in the US diet are fried eggs, regular eggs including scrambled eggs, mixed dishes with eggs, mixed dishes with beef, whole milk, regular cheese, fried fish, mixed dishes with chicken and turkey, lean cut meat, and regular processed meat (Table 2). Eggs or foods high in eggs contribute ~30% of the cholesterol intake.

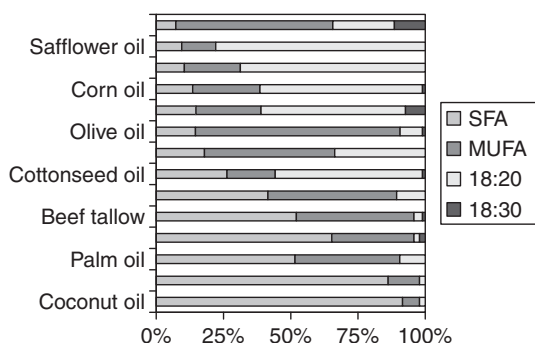


Figure 8 Relative composition of common dietary fats.

Dietary Fat and Cardiovascular Disease Prevention

Quantity of Dietary Fat

When considering the percentage of energy contributed by dietary fats and oils (amount of fat) and cardiovascular disease prevention and management, there are two major factors to

Table 2 Ten major sources of saturated, monounsaturated, and polyunsaturated fatty acids, and cholesterol in the US diet^a

Saturated fatty acids	Monounsaturated fatty acids	Polyunsaturated fatty acids	Cholesterol
Cheese, regular	Fried potatoes	Salad dressing, regular	Eggs, fried
Whole milk	Processed meat, regular	White bread, regular	Eggs, regular, including scrambled
Ice cream, regular	Cookies, regular	Mayonnaise, regular	Eggs, mixed dishes
2% milk	Snacks, regular	Fried potatoes	Beef, mixed dishes
Pizza with meat	Pizza with meat	Cakes, regular	Whole milk
Fried potatoes	Salad dressing, regular	Cookies, regular	Cheese, regular
Burritos/tacos with meat	Cheese, regular	Chicken/turkey, mixed dishes	Fish, fried
Processed meat, regular	Burritos/tacos with meat	Snacks, regular	Chicken/turkey, mixed dishes
Chocolate candy	Sausage	Potato chips, regular	Beef, lean/trimmed
Beef, mixed dishes	Beef, mixed dishes	Fish, fried	Processed meat, regular

^aRanks of food for adults older than 19 years from the NHANES recall data 1999–2000.

consider: impact on body weight and plasma lipoprotein profiles. The potential relationship with body weight is important because overweight and obesity are strongly associated with elevated lipid and lipoprotein concentrations, blood pressure, dyslipidemia, and type 2 diabetes. These factors are all associated with increased cardiovascular disease risk. With respect to plasma lipoprotein profiles, the focus is usually on triglyceride and HDL cholesterol concentrations or total cholesterol/HDL cholesterol ratios.

When body weight is maintained at a constant level, decreasing the total fat content of the diet, expressed as a percentage of total energy, and replacing it with carbohydrate frequently results in an increase in triglyceride concentrations, decrease in HDL cholesterol concentrations, and less favorable (higher) total cholesterol/HDL cholesterol ratios. Low HDL cholesterol concentrations are an independent risk factor for cardiovascular disease. Very low fat diets are of particular concern to individuals with glucose intolerance and excess body weight who have a predisposition to low HDL cholesterol and high triglyceride concentrations or those individuals classified as having metabolic syndrome (having three or more of the following: abdominal obesity, elevated triacylglycerol concentrations, low HDL concentrations, hypertension, elevated fasting glucose concentrations). Because of these findings, the Adult Treatment Panel of the National Cholesterol Education Program (NCEP) revised their guidelines in 2001 from recommending a diet with less than 30% of energy as fat to a diet with 25–35% of energy as fat. About that same time, the American Heart Association and the USDA/HHS 2000 Dietary Guidelines for Americans changed their recommendations to shift the emphasis from a general recommendation to limit intakes of total and saturated fat to limit saturated and *trans* fat. These modifications have been echoed in more recent updates of these guidelines.

With respect to the quantity of dietary fats and oils and body weight, comprehensive reviews of the long-term data have concluded that even a relatively large downward shift in dietary fat intake, approximately 10% of energy, results in only modest weight loss, 1 kg, over a 12-month period in normal-weight individuals and 3 kg in overweight or obese individuals. A recent 2-year intervention study has concluded that there is no advantage, with respect to weight loss or CVD risk indicators, of diets with different proportions of fat, carbohydrate, and protein. The major determinant of successful weight loss was adherence to the protocol, including attendance to group meetings.

Quality of Dietary Fat

Early evidence demonstrated that diets relatively high in saturated fatty acids increase plasma total cholesterol concentrations. Subsequent work demonstrated that this elevation in total cholesterol concentrations is contributed to by increases in both LDL and HDL cholesterol concentrations, the former more so than the latter. More recent work has indicated that the effect of saturated fatty acids on plasma lipoprotein concentrations and cardiovascular disease risk is modified by the macronutrient balance of energy intake. Displacing saturated fatty acids with unsaturated fatty acids, monounsaturated or polyunsaturated fatty acids, lowers both LDL and HDL

cholesterol concentrations, polyunsaturated to a greater extent than monounsaturated. Displacing saturated fatty acids with carbohydrate elevates triglyceride and lowers HDL cholesterol concentrations. Observational data indicate that dietary patterns low in saturated fatty acids and high in polyunsaturated fatty acids are associated with the lowest cardiovascular disease risk.

Quantitatively, α -linolenic acid (ALA, 18:3 n -3) is the most abundant n -3 fatty acid in the diet. Two other n -3 polyunsaturated fatty acids, sometimes referred to as the very long chain n -3 fatty acids, eicosapentaenoic acid (EPA, 20:5 n -3) and docosahexaenoic acid (DHA, 22:6 n -3), are present in smaller amounts. EPA and DHA intakes are associated with decreased cardiovascular disease risk. The relationship of ALA and cardiovascular disease risk is tenuous. Although humans have the ability to elongate and desaturate ALA to form EPA and subsequently DHA, the capacity is low and current recommendations are to consume preformed EPA and DHA. Predominant dietary sources of ALA include soybean and canola oils (Figure 8). The major source of EPA and DHA is fish, specifically dark flesh fish such as salmon and mackerel.

Trans fatty acids occur naturally in meat and dairy products as a result of anaerobic bacterial fermentation in ruminant animals. *Trans* fatty acids are formed as a result of partial hydrogenation of vegetable oils. Partial hydrogenation, in addition to converting some *cis* double bonds into *trans* double bonds, also saturates some double bonds and causes migration of double bonds along the acyl chain; in sum, this results in multiple geometric and positional isomers. Oils are partially hydrogenated to increase viscosity (change a liquid oil into a semiliquid or solid) and extend shelf life (decrease susceptibility to oxidation). *Trans* fatty acid intake, regardless of the source, is associated with elevated LDL cholesterol concentrations and cardiovascular disease risk. Major contributors of dietary *trans* fatty acids are commercially baked products, animal products, traditional margarines and shortenings, and commercially fried foods. Mandatory inclusion of *trans* fatty acid content on Nutrient Facts Panels and bans on the use of partially hydrogenated fats in cities and towns resulted in secular decreased intake.

Composition of Dietary Fats

Types of fat relatively high in saturated fatty acids include butterfat (62%), beef tallow (50%), tropical oils (coconut 87%, palm kernel 81%, palm oil 49%), and lard (39%) (Figure 8). The content of cholesterol in these fats is 33, 14, 0, and 12 mg tablespoon⁻¹, respectively. Types of fat relatively high in monounsaturated fatty acids include canola oil (56%), olive oil (73%), and peanut oil (46%). Types of fat relatively high in polyunsaturated fatty acids include soybean oil (51%), corn oil (58%), safflower oil (74%), and sunflower oil (66%). Vegetable oils do not naturally contain cholesterol.

Dietary Guidance

There are multiple sources of dietary guidance with respect to fats and oils. In general, current recommendations are to consume a diet moderate in total fat (25–35% of energy) and

rich in fruits and vegetables, whole-grain products, low-fat and nonfat dairy products, legumes, fish, and lean meats. Liquid vegetable oils are recommended in place of other types of fats (animal fat, partially hydrogenated fat). Important with any type of dietary guidance, especially when it is intended to shift dietary intakes, is to take into consideration availability, price, and personal preference, including regional, cultural, and religious dietary patterns.

Summary

Dietary fats and oils have both positive and negative attributes with respect to health outcomes. This makes determining optimal dietary recommendations difficult. Fats and oils are made up primarily of triacylglycerol. The fatty acid profile of the triacylglycerol dictates the physical properties of the fat. During fatty acid biosynthesis, humans are unable to insert a double bond above the ninth carbon of the acyl chain. For this reason, linoleic acid and fatty acids derived from linoleic acid are classified as essential; hence, these must be consumed preformed. Animal fats are the major contributors of dietary saturated fatty acids. Vegetable oils, such as canola and olive, and animal fats, are the major contributors of dietary mono-unsaturated fatty acids. Vegetable oils, such as safflower, sunflower, and corn oils, are the major contributors of dietary polyunsaturated fatty acids. Foods of marine origin are major contributors of *n*-3 fatty acids. Partially hydrogenated fat and, to a less extent, animal fats are major contributors of *trans* fatty acids. Dietary patterns high in polyunsaturated and low in saturated fatty acids have been associated with optimal health outcomes. Very long chain *n*-3 fatty acids have been independently associated with reduced risk of developing cardiovascular disease. *Trans* fatty acids have been associated with elevated cardiovascular disease risk. Dietary fatty acid intakes are determined by the sum of individual food choices. Current

general dietary recommendations from major health advocacy organization recommend moderate-fat diets rich in fruits and vegetables, whole-grain products, low-fat and nonfat dairy products, legumes, fish, and lean meats. Such a dietary pattern, while accommodating personal preferences, is consistent with a dietary pattern predicted to minimize chronic disease risk.

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FATTY ACIDS

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Health Effects of Omega-6 Polyunsaturated Fatty Acids

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Abbreviations

AA	Arachidonic acid – 20:4 <i>n</i> -6	HDL	High-density lipoprotein
ALA	α -Linolenic acid – 18:3 <i>n</i> -3	HETE	Hydroxyeicosatetraenoic acid
COX-2	Cyclooxygenase-2	LA	Linoleic acid – 18:2 <i>n</i> -6
DGLA	Dihomo- γ -linolenic acid – 20:3 <i>n</i> -6	LDL	Low-density lipoprotein
DHA	Docosahexaenoic acid – 22:6 <i>n</i> -3	LT	Leukotriene
DiHETE	Dihydroxyeicosatetraenoic acid	LXA₄	Lipoxin A ₄
DPA	Docosapentaenoic acid – 22:5 <i>n</i> -3	LXB₄	Lipoxin B ₄
EET	Epoxyeicosatrienoic acids	<i>n</i>-6	Omega-6
EFA	Essential fatty acid	<i>n</i>-3	Omega-3
EPA	Eicosapentaenoic acid – 20:5 <i>n</i> -3	PG	Prostaglandin
GLA	γ -Linolenic acid – 18:3 <i>n</i> -6	PUFA	Polyunsaturated fatty acid
		VLDL	Very low density lipoprotein

Glossary

Arachidonic acid (20:4*n*-6) A 20 carbon omega-6 fatty acid which is a precursor of eicosanoids.

Eicosanoids A family of signaling molecules made by oxidation of twenty-carbon essential fatty acids; they include the prostaglandins, thromboxanes, leukotrienes and epoxyeicosatrienoic acids.

Essential fatty acids Fatty acids that the body cannot manufacture and that may cause nutritional deficiency if not supplied through diet. The two essential fatty acids for humans are α -linolenic acid and linoleic acid.

Linoleic acid (18:2*n*-6) The precursor fatty acid of the *n*-6 fatty acids, and the primary dietary *n*-6 fatty acid comprising greater than 97% of total *n*-6 fatty acid intake.

Omega-3 (*n*-3) polyunsaturated fatty acid A class of polyunsaturated fatty acids characterized by the presence of two or more *cis*-double bonds, with the position of the first double bond three carbon atoms from the methyl end of the molecule.

Omega-6 (*n*-6) polyunsaturated fatty acid A class of polyunsaturated fatty acids characterized by the presence of two or more *cis*-double bonds, with the position of the first double bond six carbon atoms from the methyl end of the molecule.

Polyunsaturated fatty acids Fatty acids in which more than one double bond exists within the molecule.

α -Linolenic acid (18:3*n*-3) The precursor fatty acid of the *n*-3 fatty acids.

Introduction

The omega-6 (*n*-6) fatty acids have the potential to influence a number of chronic diseases and disorders. This article focuses on the effects of *n*-6 fatty acids in relation to cardiovascular disease and atherosclerosis.

Structure, Function, and Nutritional Requirements

The *n*-6 fatty acids are a class of polyunsaturated fatty acids (PUFAs) characterized by the presence of two or more *cis*-double bonds, with the position of the first double bond six carbon atoms from the methyl end of the molecule. The general formula of *n*-6 fatty acids is $\text{CH}_3(\text{CH}_2)_4(\text{CH}=\text{CHCH}_2)_x(\text{CH}_2)_y\text{COOH}$. Linoleic acid [*cis*-9, *cis*-12-octadecadienoic acid, 18:2*n*-6, LA] and α -linolenic acid [*cis*-9, *cis*-12, *cis*-15-octadecatrienoic acid, 18:3*n*-3, ALA] are the precursor fatty acids of the *n*-6 and omega-3 (*n*-3) fatty acids, respectively.

LA is the primary dietary *n*-6 fatty acid comprising greater than 97% of total *n*-6 fatty acid intake. The average daily intake of LA for adults older than 19 years is approximately 11 g or 4.4% of energy in Australia, and 14.8 g and 6.7% of energy in the United States. In contrast, the average intake of ALA is approximately 1.1 g day^{-1} or 0.6% of energy. LA and ALA cannot be made by mammals and are therefore termed essential fatty acids (EFAs). In addition, mammals are unable to interconvert LA and ALA, or any of the *n*-6 and *n*-3 fatty acids, because mammalian tissues do not contain the necessary desaturase enzyme. Plant tissues and plant oils are rich sources of LA. ALA is also present in plant sources such as green

vegetables, flaxseed, canola, and some nuts. Once consumed in the diet, LA can be converted via chain elongation and desaturation to γ -linolenic acid (GLA, 18:3*n*-6), dihomo- γ -linolenic acid (DGLA, 20:3*n*-6), and arachidonic acid (AA, 20:4*n*-6) (Figure 1). The same enzymes involved in elongation and desaturation of the *n*-6 fatty acids are common to the omega-3 (*n*-3) series of fatty acids (Figure 1). Thus, ALA can be converted to eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3). In humans, however, the conversion is relatively inefficient and gender dependent. The major source of dietary EPA and DHA is fish, seafood, and marine oils, although small quantities of *n*-3 fatty acids, particularly of docosapentaenoic acid (DPA, 22:5*n*-3), are derived from meat and poultry.

The *n*-6 and *n*-3 fatty acids are metabolically and functionally distinct and often have important opposing physiological functions. Indeed, the balance of EFA is important for good health and normal development. Historically, human beings evolved on a diet in which the ratio of *n*-6 to *n*-3 fatty acids was approximately 1:1. In contrast, Western diets have a ratio of approximately 15:1. Evidence for this change in diet through history comes from studies on the evolutionary aspects of diet, modern-day hunter-gatherers, and traditional diets. Modern agriculture has led to a substantial increase in *n*-6 fatty acids at the expense of *n*-3 fatty acids, which has resulted in excessive consumption of *n*-6 fatty acids by humans.

The *n*-6 EFAs have two main functions. First, they act as structural components of membranes forming the basis of the phospholipid component of the lipid bilayer of plasma membranes in every cell in the body, thus providing a membrane impermeable to most water-soluble molecules. The

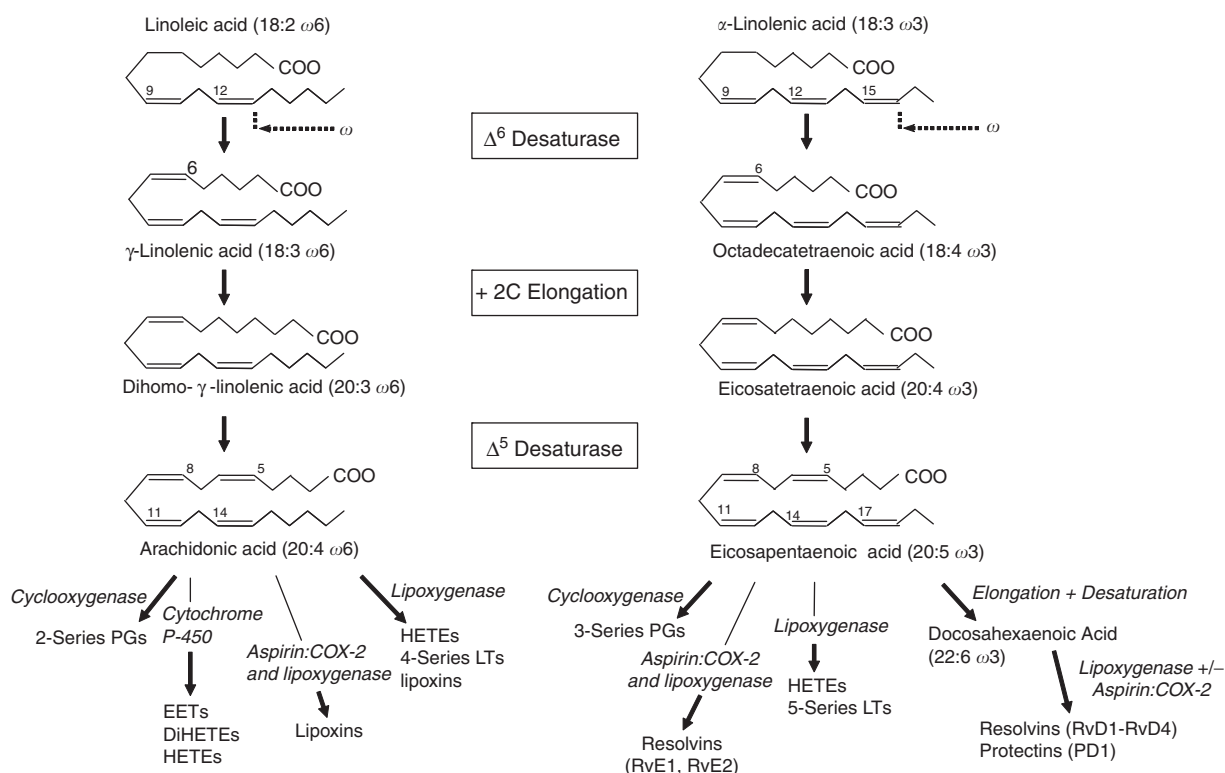


Figure 1 Essential fatty acid metabolism.

length and degree of saturation of the fatty acids determine how the phospholipid molecules pack together and consequently affect membrane fluidity, signal transduction, and the expression of cellular receptors. The second role of *n*-6 fatty acids is to act as precursors to the eicosanoids (Figure 1). The eicosanoids are a family of 'hormone-like' compounds including prostaglandins (PGs), leukotrienes (LTs), and hydroxy- (HETEs), dihydroxy- (DiHETEs), and epoxy- (EETs) fatty acids. Eicosanoids, however, are distinct from most hormones in that they act locally, near their sites of synthesis, and they are catabolized extremely rapidly. Thus, they are considered to be locally acting hormones. The eicosanoids modulate renal and pulmonary functions, vascular tone, and inflammatory responses. The enzymes involved in AA metabolism include the cyclooxygenases and lipoxygenases, which yield the two-series PGs and four-series LTs, respectively. Lipoxygenases also utilize AA for the formation of the hydroxyeicosatetraenoic acids (HETEs). A third pathway for the utilization of AA involves the cytochrome P-450 enzymes found in the liver, kidney, lung, intestines, heart, small blood vessels, and white blood cells. AA metabolized via cytochrome P-450 yields epoxyeicosatrienoic acids (EETs), dihydroxyeicosatetraenoic acids (DiHETEs), as well as HETEs. The cytochrome P-450 metabolites play an important role as paracrine factors and second messengers in the regulation of pulmonary, cardiac, renal, and vascular function and modulate inflammatory and growth responses. Finally, AA can be utilized by a pathway comprising the interaction of aspirin with cyclooxygenase-2 (COX-2) and lipoxygenase to generate a family of compounds called the lipoxins. COX-2 is an inducible form of the cyclooxygenase enzyme activated during inflammation. The lipoxins (LXA₄ and LXB₄) are potent anti-inflammatory mediators. They can also be derived from the action of lipoxygenase in the absence of aspirin-COX-2 (Figure 1).

Endothelial Function, Atherosclerosis, and Cardiovascular Disease

The vascular endothelium is the most important organ controlling vascular function and consists of a single layer of epithelial cells lining blood vessels. Its primary function is to regulate vascular tone, but it plays a critical role in modulating coagulation and fibrinolysis, inflammation, smooth muscle cell proliferation, and macrophage function. Many of these functions are regulated through the release of various mediators including eicosanoids. There is multiple and close interaction of the endothelial cells with circulating cells, smooth muscle cells, and macrophages. There is also evidence that endothelial dysfunction precedes clinically apparent atherosclerosis.

Atherosclerosis is an inflammatory disease involving multiple cellular and molecular responses that lead to an alteration in vascular function and structure, and the development and progression of cardiovascular disease. Atherosclerosis is characterized by degenerative changes, deposition of cholesterol, proliferation of smooth muscle cells, involvement of a range of circulating proinflammatory cells, and fibrosis. Resulting atheromatous plaques cause narrowing of arteries and increase the likelihood of thrombosis and occlusion. When

this occurs in the coronary arteries, the outcome is myocardial infarction with possible death.

Eicosanoids: Relevance to Endothelial Function, Thrombosis, Inflammation, and Atherosclerosis

In general, the eicosanoids derived from AA have potent prothrombotic and proinflammatory activity. In contrast, the eicosanoids derived from EPA have reduced biological activity and are less prothrombotic and proinflammatory. Eicosanoid production is generally tightly controlled through homeostatic mechanisms. However, eicosanoid production can be significantly altered in situations in which endothelial dysfunction, atherosclerosis, and plaque rupture, or various thrombotic or inflammatory conditions are present.

Prostaglandins and Leukotrienes

Prostaglandins have a central role in the regulation of platelet aggregation and vascular tone. In this regard, two of the major prostaglandins derived from AA are thromboxane A₂, produced in platelets, and prostacyclin I₂, produced in endothelial cells. Thromboxane A₂ promotes platelet aggregation and blood vessel constriction, whereas prostacyclin I₂ has opposite effects. An increase in availability of EPA can decrease platelet thromboxane A₂ and increase thromboxane A₃, the latter having considerably less physiological activity. EPA supplementation also stimulates formation of prostacyclin I₃, whereas prostacyclin I₂ is unaffected. Prostacyclin I₃ and prostacyclin I₂ are equipotent in their biological activity. The net result following intake of *n*-3 fatty acids is a shift in the thromboxane/prostacyclin balance toward a reduced prothrombotic state.

Leukotriene B₄ is a potent inflammatory mediator produced by neutrophils from 20:4*n*-6 at the site of injury. Leukotriene B₄ is also a powerful chemotactic factor responsible for attracting neutrophils to the site of injury. Leukotriene B₅, which is produced from EPA, has significantly lower biological activity. Therefore, an increased availability of EPA has the potential to reduce inflammation.

Fatty Acid Intake and Eicosanoids

The concentration of the eicosanoid precursor fatty acids both circulating and in tissues depends on dietary intake. DGLA and AA can be obtained from animal meat and fat, and by desaturation and chain elongation of LA. The major dietary source of EPA and DHA is fish and seafood. EPA can also be obtained indirectly from ALA, although desaturation and chain elongation of ALA appears to be a less important pathway in humans.

Only the free form of the fatty acid precursors of eicosanoids can be utilized by the enzymes for conversion to the biologically active metabolites. However, the amount of precursor free fatty acid in the cytoplasm and circulating is usually low and so too is basal eicosanoid formation. Furthermore, basal eicosanoid formation may depend on dietary and adipose tissue fatty acid composition. The amount of

eicosanoid precursor free fatty acids is controlled to a large extent by incorporation and release from cellular phospholipids. Which eicosanoids are produced during stimulated synthesis may depend on membrane fatty acid composition as well as the cell type involved. Dietary fatty acid composition, therefore, has the potential to affect basal and stimulated synthesis of eicosanoids and influence endothelial function, and thrombotic and inflammatory responses.

***n*-6 Fatty Acids and Risk of Cardiovascular Disease**

Evidence that differences in *n*-6 fatty acid intake can influence cardiovascular disease risk derives from several sources. Population studies may provide useful data for establishing optimal intakes of *n*-6 fatty acids. However, valuable information on the potential mechanisms and effects of these fatty acids is derived from randomized controlled studies focusing on their impact on thrombosis, inflammation, endothelial function, and other cardiovascular risk factors.

Cardiovascular Disease: Population Studies

The incidence of cardiovascular disease within populations with either very high or very low intakes of *n*-6 fatty acids may provide some indication for optimal intakes of *n*-6 fatty acids. Within populations with low *n*-6 fatty acid intakes (< ~3%), there would appear to be a benefit of having a higher *n*-6 fatty acid intake on cardiovascular disease risk reduction. These observations suggest that very low *n*-6 fatty acid intakes increase the risk for cardiovascular disease. The presence of EFA deficiency in a significant proportion of such populations may explain the increased risk. Several populations, including the Israelis, Taiwanese, and !Kung bushmen in the African Kalahari desert, have high to very high intakes of *n*-6 fatty acids. The contribution of *n*-6 fatty acids to total energy intake is approximately 10% in the Israelis and Taiwanese and approximately 30% in the !Kung bushmen. Rates of cardiovascular disease are low in the Taiwanese, where dietary *n*-6 fatty acids are obtained mainly from soybean oil, and estimated to be very low in the !Kung bushmen, where dietary *n*-6 fatty acids were obtained mainly from the monongo fruit and nut. In the Taiwanese, the soybean oil is refined but is accompanied by a diet rich in antioxidant polyphenols, notably from tea, fruits, and vegetables. In the !Kung bushmen, the oil is unrefined and is therefore likely to contain a range of phytochemicals. There is, however, a high prevalence of cardiovascular disease in the Israeli population, where *n*-6 PUFAs are obtained largely from refined sources. These observations suggest that a high *n*-6 fatty acid intake can be compatible with low risk of cardiovascular disease, but the dietary context may be very important. Given that *n*-6 fatty acids are susceptible to lipid peroxidation, high *n*-6 fatty acid intake may increase risk for cardiovascular disease when consumed against a background diet low in antioxidants. The potential impact on eicosanoid metabolism remains uncertain.

Several factors may need to be considered in the interpretation of the results of population studies. (1) The effect of LA on atherosclerosis and cardiovascular disease may depend

on the background intake in the population being studied. (2) Any relationships observed may be confounded by intake of other foods from which LA derives. (3) LA may have differential effects on aspects of the etiology of cardiovascular disease, including endothelial function, thrombosis, arrhythmia, and atherosclerosis.

Thrombosis

Dietary fatty acids influence thrombosis by altering the activity and function of endothelial cells, platelets, and other circulating cells: effects that can be mediated, in part, by alterations in eicosanoid metabolism. Replacement of dietary saturated fatty acids with unsaturated fatty acids, including *n*-6 fatty acids, generally lowers the risk of thrombosis and cardiovascular disease. Furthermore, studies have shown that an increase in *n*-3 fatty acid intake can increase vasodilation, attenuate platelet aggregation, and alter circulating concentrations of factors involved in coagulation and fibrinolysis. The net effect of increasing *n*-3 fatty acid intake is a tendency toward reduced risk for thrombosis. These findings are supported by population studies demonstrating that *n*-3 fatty acids may reduce the risk of thrombosis. It remains uncertain whether the major factor influencing these functions is the absolute increase in *n*-3 fatty acids, or the relative proportions of *n*-6 and *n*-3 fatty acids in the diet and cell membranes. There is evidence, however, that increased *n*-3 fatty acid intake may be more beneficial in populations consuming relatively small quantities of fish, which includes many Western populations.

Much of the evidence for a potential impact of *n*-6 fatty acids on thrombosis derives from research on platelet function. The role of platelets in thrombosis is established and the influence of fatty acid intake on platelet function has been assessed in many studies. Platelets play a part in thrombosis by adhering to, and aggregating at, the site of injury. Platelet reactivity and increased platelet activation may increase the risk of thrombosis. *In vitro* and *in vivo* studies assessing effects of *n*-6 fatty acids on platelet aggregation are inconsistent. To date there is little evidence that a high *n*-6 fatty acid diet in humans decreases platelet aggregation and some studies are suggestive of increased aggregation with high *n*-6 fatty acid diets, primarily in the form of LA. The effects of AA on platelet aggregation are also not clear. One of the main difficulties in interpreting these studies is the unresolved issue as to how the *in vitro* aggregation test reflects platelet function *in vivo*.

Inflammation

Inflammation is involved in many human diseases including cardiovascular conditions such as thrombosis, stroke, and atherosclerosis. Conditions associated with increased inflammation, such as inflammatory arthritis, dermatological conditions such as psoriasis and atopic dermatitis, chronic inflammatory bowel disease, autoimmune diseases, and bronchial asthma, appear to be beneficially influenced to a greater extent by *n*-3 fatty acids than by *n*-6 fatty acids.

Whether increased intake of *n*-6 fatty acids can exacerbate inflammation *via* increased production of proinflammatory

eicosanoids remains uncertain. Results of *in vitro* studies and intervention studies in humans are generally consistent with this theoretical potential of *n*-6 fatty acids to enhance inflammation, at least in comparison to *n*-3 fatty acids and probably omega-9 (*n*-9) monounsaturated fatty acids. The importance of absolute and relative intakes of *n*-6 fatty acids to inflammatory processes also remains unclear. The effects of changes in *n*-6 fatty acid intake on inflammatory processes may depend on the background dietary fatty acid intake, as well as proportional and absolute intake of *n*-3 fatty acids.

Resolution of inflammation involves the reduction or removal of inflammatory cells and debris from inflamed sites, enabling the return to homeostasis. This process, which was initially considered to be a passive process, is now known to be rapidly initiated after acute challenges by cellular pathways that lead to the synthesis of lipid mediators such as lipoxins, resolvins, and protectins. The lipoxins (LXA₄ and LXB₄) are derived from AA and have anti-inflammatory and proresolution properties (Figure 1). They are biosynthesized by the sequential action of lipoxygenases or via interaction of aspirin with COX-2 and lipoxygenase. Recently, a novel family of lipid mediators, the resolvins and protectins, which are derived from EPA and DHA, has also been described and implicated in the resolution of inflammation (Figure 1). Resolvins and protectins are local mediators that are generated during spontaneous resolution of inflammation.

Cholesterol and Lipoproteins

The major classes of circulating lipoproteins in human plasma are chylomicrons, very low density lipoproteins (VLDL), low-density lipoproteins (LDL), and high-density lipoproteins (HDL). High fasting plasma concentrations of LDL cholesterol and triglycerides – predominantly circulating as part of VLDL – and low plasma concentrations of HDL cholesterol, are associated with increased risk of cardiovascular disease. Dietary fatty acids can influence lipoprotein metabolism and therefore have the potential to influence atherosclerosis and cardiovascular disease risk. Most studies examining the effects of *n*-6 PUFAs on cholesterol metabolism have focused on LA.

It is now established that LDL cholesterol lowering reduces the risk of cardiovascular disease. In the fasting state, LDL is the major cholesterol-carrying lipoprotein in human plasma. The mechanisms through which raised plasma LDL cholesterol concentrations increase cardiovascular disease risk are not entirely understood, but oxidative modification of LDL is thought to be involved. An increase in LA intake results in a lowering of plasma LDL cholesterol concentrations and therefore has the potential to reduce cardiovascular disease risk. These effects may not be linear over the entire range of LA intake and most of the benefits appear to be gained by moving from lower (<2% of energy) to moderate (~4–5% of energy) intakes. In addition, the effects of dietary *n*-6 PUFAs are less than half that of lowering dietary saturated fatty acids. Therefore, if total fat intake is maintained, the LDL cholesterol lowering effects of increasing *n*-6 PUFA intake are greatly enhanced if saturated fatty acid intake is also decreased.

HDL cholesterol is inversely associated with cardiovascular disease risk. The mechanism by which HDL reduces

cardiovascular disease risk may involve reverse cholesterol transport and reductions in cholesterol accumulation in the arterial wall. Intakes of LA within the normal ranges of intakes in most populations do not appear to alter HDL cholesterol concentrations. However, very high intakes – more than 12% of energy – can lower HDL cholesterol concentrations.

Oxidative Stress

Several lines of evidence suggest that oxidatively modified LDL plays an important role in the development of atherosclerosis. Oxidative modification of LDL involves peroxidation of PUFAs. LDL particles enriched in PUFAs have been shown to be more susceptible to oxidative modification compared with LDL particles rich in monounsaturated fatty acids. Others have also suggested that a diet high in PUFAs may overwhelm the antioxidant defenses of cells. In particular, studies have shown that LA-enriched LDL is more prone to *in vitro* oxidation than oleic acid-enriched LDL. Concern also remains with respect to the potential for increased lipid peroxidation following *n*-3 fatty acids. However, much of the early literature relating to PUFAs and lipid peroxidation is based on indirect and nonspecific assays, including measurement of LDL oxidative susceptibility, which relies on the isolation of LDL from plasma. In this regard, the recent discovery of F₂-isoprostanes, which are nonenzymatic prostaglandin-like products of free radical peroxidation of AA, has allowed for the direct assessment of *in vivo* lipid peroxidation. There is now good evidence that quantitation of F₂-isoprostanes provides a reliable measure of *in vivo* oxidative stress. Using measurement of F₂-isoprostanes, recent data have demonstrated that *n*-3 fatty acids decrease oxidative stress. It has also been suggested that the concentration of PUFAs may be a more important factor affecting lipid peroxidation than the degree of unsaturation. Further research using better markers of lipid peroxidation is required before definitive statements can be made relating to the effect of *n*-6 fatty acids and indeed PUFAs in general, on oxidative stress.

Blood Pressure

The possible effects of dietary fatty acids on blood pressure have been explored in population studies and dietary intervention trials. Although a hypotensive influence of *n*-6 fatty acids was suggested in early clinical studies, this has not been confirmed in subsequent randomized controlled trials. In normotensive individuals, randomized controlled trials have shown no consistent effects on blood pressure with dietary modifications to change the intake of *n*-6 fatty acids. LA supplements have also produced either no change or reduction in blood pressure. Additionally, studies in hypertensives have shown no consistent effects of *n*-6 fatty acids on blood pressure. Current data suggest that *n*-6 fatty acids, when substituted for saturated fatty acids, may have some blood pressure lowering effect if part of complex dietary changes that include increases in fruit, nuts, and vegetable consumption and low-fat dairy products. These effects may also be enhanced by moderation of salt intake.

Conclusions

Diets low in *n*-6 fatty acids, principally LA, appear to be associated with an increased risk of cardiovascular disease. The results of studies examining the effects of LA on risk factors for atherosclerosis and cardiovascular disease are consistent with this observation. An increase in *n*-6 PUFA intake from a low to a moderate intake level, in conjunction with decreases in total and saturated fat intake, may beneficially influence lipoprotein metabolism, lower blood pressure, and reduce cardiovascular disease risk. Observations in populations with high *n*-6 PUFA intake indicate that high intakes of *n*-6 fatty acids (>10%) can occur together with low rates of cardiovascular disease and possibly also cancer. However, where antioxidant composition of the diet is low, there is the potential for increased risk of cardiovascular disease. An increased susceptibility of PUFAs to oxidative damage, particularly in the presence of low concentrations of protective antioxidants, may be an important factor. The source of *n*-6 PUFAs in the diet, refined versus unrefined, and the composition of the background diet, may therefore be important determinants of whether high *n*-6 fatty acid intake increases or decreases the risk of cardiovascular disease. In addition, the quantity of *n*-6 to *n*-3 fatty acids in the diet may also play an important role in determining cardiovascular risk.

Available data suggest that the relative proportion of all the classes of dietary fatty acids may be more important and relevant to cardiovascular risk reduction than any single class of fatty acids. The importance of *n*-6 fatty acids in relation to cardiovascular risk was recently summarized in an American Heart Association statement, which concluded that consumption of at least 5–10% of energy from *n*-6 fatty acids in the context of other appropriate lifestyle and dietary behavior may reduce the risk of coronary heart disease relative to low intake.

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Health Effects of Saturated Fatty Acids

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Glossary

Apoprotein The protein part of lipoproteins.

Coagulation The formation of a stable thrombus through the formation of fibrin.

Eicosanoids Bioactive molecules made from fatty acids with 20 carbon atoms.

Fibrinolysis The process of dissolving a thrombus by lysis of fibrin.

Lipoproteins Lipid–protein complexes that carry fats (e.g., triacylglycerol and cholesterol) in the blood stream.

Platelet aggregation The formation of a loose aggregate of platelets (thrombus) in the blood.

Fats and oils always consist of a mixture of fatty acids, although one or two fatty acids are usually predominant. **Table 1** shows the fatty acid composition of some edible fats rich in saturated fatty acids. In the Western diet, palmitic acid ($C_{16:0}$) is the major saturated fatty acid. A smaller proportion comes from stearic acid ($C_{18:0}$), followed by myristic acid ($C_{14:0}$), lauric acid ($C_{12:0}$), and short-chain and medium-chain fatty acids (MCEFA) ($C_{10:0}$ or less).

When discussing the health effects of the total saturated fat content of diets, this class of fatty acids has to be compared with some other component of the diet that provides a similar amount of energy (isoenergetic). Otherwise, two variables are being introduced: changes in total dietary energy intake and as a consequence, changes in body weight. Normally, an isoenergetic amount from carbohydrates is used for comparisons.

Cholesterol Metabolism

Lipoproteins and their associated apoproteins are strong predictors of the risk of coronary heart disease (CHD). Concentrations of total cholesterol, low-density lipoproteins (LDL) and apoprotein B are positively correlated with CHD risk; high-density lipoprotein (HDL) and apoprotein AI concentrations are negatively correlated. Controlled dietary trials have now demonstrated that the total saturated fat content

and the type of saturated fatty acid in the diet affect serum lipid and lipoprotein levels.

Total Saturated Fat Content of Diets

Using statistical techniques, results from independent experiments have been combined to develop equations that estimate the mean change in serum lipoprotein levels for a group of subjects when carbohydrates are replaced by an isoenergetic amount of a mixture of saturated fatty acids. The predicted changes for total LDL and HDL cholesterol, and triacylglycerols are shown in **Figure 1**. Each bar represents the predicted change in the concentration of that particular lipid or lipoprotein when a particular fatty acid class replaces 10% of the daily energy intake from carbohydrates. For a group of adults with an energy intake of 10 MJ daily, 10% of energy is provided by about 60 g of carbohydrates or 27 g of fatty acids.

A mixture of saturated fatty acids strongly elevates serum total cholesterol concentrations. It was predicted that when 10% of dietary energy provided by carbohydrates was exchanged for a mixture of saturated fatty acids, serum total cholesterol concentrations would increase by 0.36 mmol l^{-1} . This increase in total cholesterol will result from a rise in both LDL and HDL cholesterol concentrations. Saturated fatty acids will also lower fasting triacylglycerol concentrations compared with carbohydrates. Besides affecting LDL and HDL cholesterol concentrations, a mixture of saturated fatty acids also

Table 1 Composition of fats rich in saturated fatty acids

	Weight per 100 g of total fatty acids (g)								
	< C _{10:0}	C _{12:0}	C _{14:0}	C _{16:0}	C _{18:0}	C _{18:1}	C _{18:2}	C _{18:3}	Other
Butter fat	9	3	17	25	13	27	3	1	2
Palm kernel fat	8	50	16	8	2	14	2		
Coconut fat	15	48	17	8	3	7	2		
Palm oil			1	45	5	39	9		1
Beef fat			3	26	22	38	2	1	8
Pork fat (lard)			2	25	12	44	10	1	6
Cocoa butter				26	35	35	3		1

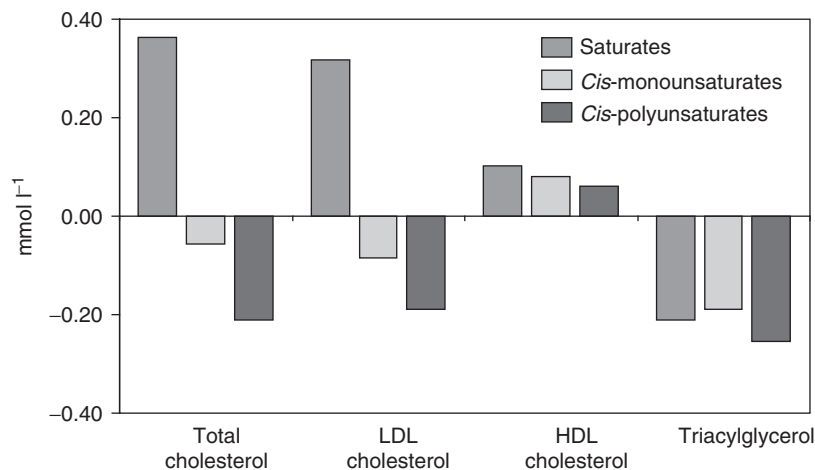


Figure 1 Predicted changes in serum lipids and lipoproteins when 10% of energy from dietary carbohydrates is replaced by an isoenergetic amount of saturated fatty acids. Adapted from Mensink RP, Zock PL, Kester AD, and Katan MB (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition* 77: 1146–1155, with permission from ASN.

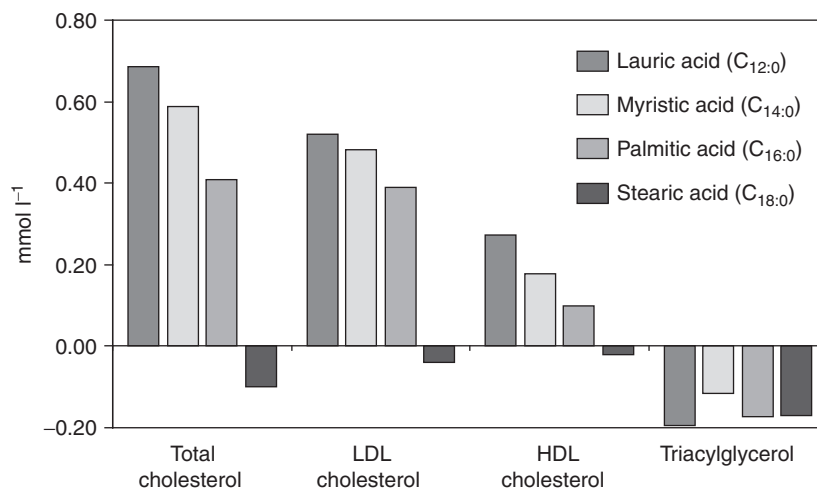


Figure 2 Overview of the effects of particular fatty acids on serum total, LDL, and HDL cholesterol concentrations when 10% of energy from dietary carbohydrates is replaced by an isoenergetic amount of a particular saturated fatty acid. Adapted from Mensink RP, Zock PL, Kester AD, and Katan MB (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition* 77: 1146–1155, with permission from ASN.

changes the concentrations of their associated apoproteins. In general, strong associations are observed between changes in LDL cholesterol and changes in apo-B, and between changes in HDL cholesterol and apo-AI.

The figure also shows that total and LDL cholesterol concentrations decrease when saturated fatty acids are replaced by unsaturated fatty acids. In addition, slight decreases of HDL cholesterol concentrations are then predicted.

Effects of Specific Saturated Fatty Acids

Cocoa butter raises total cholesterol concentrations to a lesser extent than palm oil does. This difference in the serum cholesterol-raising potency of two fats high in saturated fatty acids (see [Table 1](#)) shows that not all saturated fatty acids have

equal effects on cholesterol concentrations. [Figure 2](#) illustrates the effects of lauric, myristic, palmitic, and stearic acids on LDL and HDL cholesterol concentrations. Compared with other saturated fatty acids, lauric and myristic acids have the strongest potency to increase serum total and LDL cholesterol concentrations, and also HDL cholesterol concentrations. Effects of lauric acid on HDL cholesterol are stronger than those of myristic acid.

Stearic acid, a major fatty acid in cocoa butter, does not raise total, LDL and HDL cholesterol levels compared with carbohydrates. Also, MCFA have been reported not to raise LDL and HDL cholesterol concentrations compared with carbohydrates, but data are limited. Like carbohydrates, diets containing large amounts of MCFA increase fasting triacylglycerol concentrations compared with the other saturated fatty acids. However, such diets are the sole energy source only in

parenteral or enteral nutrition, or in sports drinks. Other saturated fatty acids have not been reported to raise triacylglycerol concentrations compared with each other, but lower triacylglycerol concentrations compared with carbohydrates.

Platelet Aggregation

Increased platelet aggregation may be an important risk marker for the occurrence of cardiovascular disease, and different types of fatty acids can modify platelet aggregation *in vitro*. However, reports of research on this topic are confusing. All measurements have their limitations, and it is not known whether measurement *in vitro* of platelet aggregation reflects the reality of platelet reactivity *in vivo*.

Many methods are available to measure platelet aggregation *in vitro*. Firstly, the blood sample is treated with an anticoagulant to avoid clotting of the blood in the test tube or in the aggregometer; many different anticoagulants are used, which all differ in their mechanism of action. Secondly, platelet aggregation can be measured in whole-blood, in platelet-rich plasma or (to remove the influence of the plasma constituents) in a washed platelet sample. Finally, the platelet aggregation reaction in the aggregometer can be initiated with many different compounds, such as collagen, Adenosine diphosphate (ADP), arachidonic acid, and thrombin. Platelet aggregation can also be studied by measuring the stable metabolites of the proaggregatory thromboxane A₂ (TxA₂), thromboxane B₂ (TxB₂), the stable metabolite of the antiaggregatory prostaglandin (prostaglandin: PGI₂), or 6-keto-PGF_{1α}.

Total Saturated Fat Content of Diets

Platelet aggregation and clotting activity of plasma were studied in British and French farmers, who were classified according to their intake of saturated fatty acids. A positive correlation was observed between thrombin-induced aggregation of platelet-rich plasma and the intake of saturated fatty acids. Aggregation induced by ADP or collagen, however, did not correlate with dietary saturated fat intake. In a follow-up study, a group of farmers consuming high-fat diets were asked to replace dairy fat in their diets with a special margarine rich in polyunsaturated fatty acids. Besides lowering the intake of saturated fatty acids, this intervention also resulted in a lower intake of total fat. A control group of farmers did not change their diets. After this intervention the thrombin-induced aggregation of platelet-rich plasma decreased when saturated fat intake decreased. Aggregation induced by ADP, however, increased in the intervention group. From these studies, it is not clear whether the fatty acid composition of the diets or the total fatty acid content is responsible for the changes in platelet aggregation. Furthermore, it is not clear if one should favor increased or decreased platelet aggregation after decreasing the saturated fat content of diets as effects did depend on the agonist used to induce platelet aggregation. Saturated fatty acids from milk fat have also been compared with unsaturated fatty acids from sunflower and rapeseed oils. Aggregation induced by ADP or collagen in platelet-rich plasma was lower with the milk fat diet than with either oil.

One of the mechanisms affecting platelet aggregation is alteration of the proportion of arachidonic acid in the platelet phospholipids. Arachidonic acid is a substrate for the production of the proaggregatory TxA₂ and the antiaggregatory PGI₂, and the balance between these two eicosanoids affects the degree of platelet activation. The proportion of arachidonic acid in membranes can be modified through changes in dietary fatty acid composition. Diets rich in saturated fatty acids increase the arachidonic acid content of the platelet phospholipids, but this is also dependent on the particular saturated fatty acid consumed (see below).

Diets rich in saturated fatty acids have also been associated with a lower ratio of cholesterol to phospholipids in platelet membranes, which may affect receptor activity and platelet aggregation. However, these mechanisms have been described from studies *in vitro* and on animals and have not adequately been confirmed in human studies.

Effects of Specific Saturated Fatty Acids

Diets rich in coconut fat have been reported to raise TxB₂ and lower 6-keto-PGF_{1α} concentrations in collagen-activated plasma compared with diets rich in palm or olive oils, indicating a less favorable eicosanoid profile. The main saturated fatty acids of coconut fat – lauric and myristic acids – did not, however, change collagen-induced aggregation in whole-blood samples compared with a diet rich in oleic acid. Also, diets rich in MCEA or palmitic acid did not change collagen-induced aggregation in whole-blood samples. Compared with a diet rich in a mixture of saturated fatty acids, a stearic acid diet increased collagen-induced aggregation in platelet-rich plasma. In addition, a decreased proportion of arachidonic acid in platelet phospholipids was demonstrated after a cocoa butter diet compared with a diet rich in butter fat. Changes in eicosanoid metabolite concentrations in urine, however, were not observed after either diet. These results are conflicting and it is debatable whether measurement *in vitro* of platelet aggregation truly reflects the situation *in vivo*.

Coagulation and Fibrinolysis

Processes involved in thrombus formation include not only those required for the formation of a stable thrombus (platelet aggregation and blood clotting), but also a mechanism to dissolve the thrombus (fibrinolysis). Long-term prospective epidemiological studies have reported that in healthy men factor VII coagulant activity (factor VIIc) and fibrinogen concentrations were higher in subjects who developed cardiovascular diseases at a later stage of the study. Factor VIIc in particular was associated with an increased risk of dying from cardiovascular disease. A high concentration of plasminogen activator inhibitor type 1 (PAI-1) indicates impaired fibrinolytic capacity of the plasma and is associated with increased risk of occurrence of coronary events.

Saturated fatty acids can affect the plasma activity of some of these coagulation and fibrinolytic factors and thus the prethrombotic state of the blood. However, the effects of saturated fatty acids on coagulation and fibrinolytic factors in humans, unlike effects on cholesterol concentrations, have

received little attention, and few well-controlled human studies have been reported. Also, regression equations derived from a meta-analysis, which predict the effects on coagulation and fibrinolytic factors of different fatty acid classes compared with those of carbohydrates, do not exist. Therefore, the reference fatty acid is dependent on the experiment discussed. In the epidemiological studies that have found associations between CHD risk and factors involved in thrombogenesis or atherogenesis, subjects were mostly fasted. Also, the effects of saturated fatty acids on cholesterol metabolism, platelet aggregation, and coagulation and fibrinolysis have been studied mainly in fasted subjects. It should be noted, however, that concentrations of some coagulation factors (e.g., factor VIIc) and fibrinolytic factors change after a meal.

Total Saturated Fat Content of Diets

Coagulation

Results of studies on the effects of low-fat diets compared with high-fat diets provide some insight into the effects of decreasing the saturated fat content of diets. However, in these studies multiple changes are introduced which makes interpretation of results difficult.

Figure 3 demonstrates that decreased factor VIIc levels were demonstrated in subjects on low-fat diets compared with those on high-saturated fat diets. In many of these studies, the low-fat diet provided smaller quantities of both saturated and unsaturated fatty acids and more fiber than the high-saturated fat diets. The combined results, however, suggest that, apart from a possible effect of dietary fiber, saturates increase factor VII levels compared with carbohydrates. Effects on other clotting factors are less clear. Measurements of markers of *in vivo* coagulation (e.g., prothrombin fragment 1 + 2) might have provided more information on the effect of saturates on blood coagulation, but were unfortunately not measured in most experiments.

Fibrinolysis

Effects of low- and high-fat diets on the fibrinolytic capacity of the blood have also been studied. A similar problem, as stated

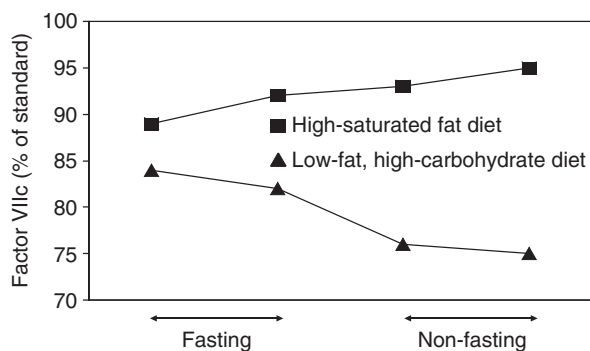


Figure 3 Effects of a high-saturated fat diet on fasting and postprandial factor VIIc activity. Adapted from Mensink RP, Zock PL, Kester AD, and Katan MB (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *American Journal of Clinical Nutrition* 77: 1146–1155, with permission from ASN.

before, is that multiple changes were introduced within a single experiment. Results of longer and shorter-term studies with dietary changes of total fat (decrease of saturated and unsaturated fatty acids contents) and increased fiber content indicate beneficially increased euglobulin fibrinolytic capacity of the blood. However, when the saturated fatty acid and fiber content of two diets were almost identical and only the unsaturated fatty acid content was changed, no significant differences of fibrinolytic capacity were observed.

Little is known about the relative effects on fibrinolytic capacity of saturated fatty acids compared with unsaturated fatty acids. It has been reported, however, that diets rich in butter fat decreased plasminogen activator inhibitor (PAI)-1 activity compared with a diet rich in partially hydrogenated soybean oil, but whether this is because of changes in the saturated acid or the trans fatty acid content is not clear from this study.

As for coagulation factors, the findings on the fibrinolytic effects of saturates are still inconclusive and need to be examined by more specific assays, measuring the activities of the separate fibrinolytic factors such as tPA and PAI-1.

Effects of Specific Saturated Fatty Acids

Coagulation

The interest in the effects of particular fatty acids on coagulation and fibrinolytic factors has increased because the observation that different saturated fatty acids raise serum lipids and lipoproteins in different ways (*see* Cholesterol metabolism). Although results are conflicting, some studies indicate that the most potent cholesterol-raising saturated fatty acids also increase factor VII activity.

Diets rich in lauric plus myristic acids compared with a diet rich in stearic acid also increase concentrations of other vitamin K-dependent coagulation proteins. In addition, this mixture of saturated fatty acids raised F1 + 2 concentrations, indicating increased *in vivo* turnover of prothrombin to thrombin. This agreed with a study in rabbits where increased F1 + 2 concentrations were associated with increased hepatic synthesis of vitamin K-dependent clotting factors.

Diets rich in certain saturated fatty acids (lauric acid and palmitic acid) and also diets rich in butter fat have been reported to raise fibrinogen concentrations, but increases were small.

Postprandially, increased factor VIIc concentrations have been demonstrated after consumption of diets rich in fat compared with fat-free meals (Figure 3). The response is stronger when more fat is consumed, but this occurs regardless of whether the fat is high in saturated or unsaturated fatty acids. Only meals with unrealistically high amounts of MCFA have been reported not to change factor VIIc levels in comparison with a meal providing a similar amount of olive oil.

Fibrinolysis

Increased PAI-1 activity of a palmitic acid-rich diet has been observed compared with diets enriched with oleic acid, indicating impaired fibrinolytic capacity of the plasma. However,

this was not confirmed by other experiments on the effects of particular saturated fatty acids (including palmitic acid), which did not indicate changes in fibrinolytic capacity of the blood, measured as tPA, PAI-1 activity, or antigen concentrations of tPA and PAI-1.

Conclusion

Saturated fatty acids as a group affect factors involved in cholesterol metabolism. Relative to the carbohydrate content of the diet, a decrease in saturated fat content induces a favorable decrease in serum total and LDL cholesterol concentrations, but unfavorably reduces HDL cholesterol concentrations. Both increasing and decreasing effects of saturates on platelet aggregation have been observed, as well as absence of effect, so results are inconsistent and difficult to interpret. Whether the beneficial effect of a diet low in saturated fat on the prethrombotic state of blood depends on the dietary fiber content is still unclear.

Of the saturated fatty acids, lauric and myristic acids have the strongest potency to raise total and LDL cholesterol concentrations. In addition, both of these saturated fatty acids raise HDL cholesterol levels. Palmitic acid raises total and LDL cholesterol levels compared with carbohydrates but is less potent than lauric and myristic acids. Stearic acid does not raise LDL and HDL cholesterol concentrations compared with carbohydrates. Lauric, myristic, and palmitic acids increase factor VII activity in a similar way, whereas the effects of MCFA and stearic acid seem limited.

See also: Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Coronary Heart Disease: Lipid Theory; Prevention. Fatty Acids: Metabolism

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Metabolism

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Glossary

Fatty acid An aliphatic hydrocarbon chain terminating in a carboxylic acid function.

Fatty acid activation Formation of fatty acyl-CoA *via* the attachment of coenzyme A to the fatty acid's carboxylic acid function *via* a thioester bond.

Fatty acid *de novo* synthesis Synthesis of a saturated, 16-carbon fatty acid from precursors typically derived from carbohydrate metabolism.

β -Oxidation Energy-yielding cyclic process in which the hydrocarbon chain of a fatty acid is shortened, typically by two carbon atoms per cycle.

Protein acylation The covalent modification of an amino acid residue in a protein by a fatty acid, most commonly a 14- or 16-carbon saturated fatty acid.

Introduction

Fatty acids (FAs) are hydrophobic molecules consisting of an aliphatic hydrocarbon chain terminating in a carboxylic acid moiety. Structurally, FAs are quite diverse. Although FAs containing 16–18 carbons are the most abundant in nature, the hydrocarbon chain can vary in length from two to more than 26 carbons. The carbon chain can be fully saturated, or contain one or more double bonds. Ingestion of dietary fats and oils is a major source of FAs for humans and most other animals. In addition, many physiologically important FAs can be synthesized *de novo* from metabolites derived from the catabolism of sugars and proteins.

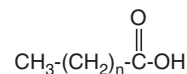
FAs in the human body serve two primary functions – they are an excellent source of metabolic energy, and they serve as building blocks for many diverse complex lipids. FAs are also precursors of bioactive molecules such as prostaglandins and other eicosanoids. An important covalent modification of proteins is the attachment of a 14- or 16-carbon FA. In addition, FAs and their coenzyme A derivatives have many metabolic regulatory roles.

Fatty Acid Nomenclature Conventions

In this article, FAs will be identified by their chain length, the number of double bonds present, and the position of the first double bond from the methyl end of the molecule. Thus 14:0 denotes a 14 carbon saturated FA, 16:1 ω 9 denotes a 16 carbon monounsaturated FA in which one double bond occurs nine carbons from the methyl end, and 20:4 ω 6 denotes a 20 carbon polyunsaturated FA in which the first of four double bonds is found six carbons from the methyl end. Unless otherwise noted, all double bonds are in the *cis* configuration and double bonds in polyunsaturated FAs are always separated by a single methylene (–CH₂–) group. The carboxyl carbon of any FA is carbon-1. The adjacent carbon is referred to as either carbon-2 or the α -carbon; the next is carbon-3 or the β -carbon, and so on. Some examples are shown in Figure 1.

Physical Properties of Fatty Acids

FAs are aliphatic organic acids with the fundamental structure



where n can range from zero to >26 . Thus, FAs range from the shortest, acetic acid (2:0), to the very long-chain FAs containing 26 or more carbon atoms (e.g., 26:0). Although FAs with an odd number of carbon atoms exist in nature, most common FAs have an even number. The most abundant FAs found in human lipids, as well as in dietary lipids, are the long-chain FAs 16:0 (palmitic acid) and 18:1 ω 9 (oleic acid) (Figure 1). The hydrophobic nature of the hydrocarbon chain of FAs containing more than eight carbons renders them quite insoluble in aqueous media. It has been estimated that for every two carbon increase in FA chain length, its solubility decreases 10 fold.

Owing to the poor solubility of the most abundant FAs, free (nonesterified) FAs are often found associated with binding or transport proteins. Serum albumin has at least six binding sites for FAs and is the primary transporter of these molecules through the bloodstream. Several low molecular weight intracellular FA binding proteins (FABPs) have also been identified. Free FAs can also associate with lipophilic cellular and organellar membranes; however, concentrations of these nonesterified compounds in membranes is typically very low.

Fatty Acid Activation

FAs are generally nonreactive unless first 'activated' by thioesterification to coenzyme A (CoA). Activation is catalyzed by acyl-CoA synthetases (ACS; E.C. 6.2.1.x) *via* a bi uni uni bi ping-pong mechanism. In the first half-reaction, an acyl-adenylate intermediate is formed, with the release of inorganic

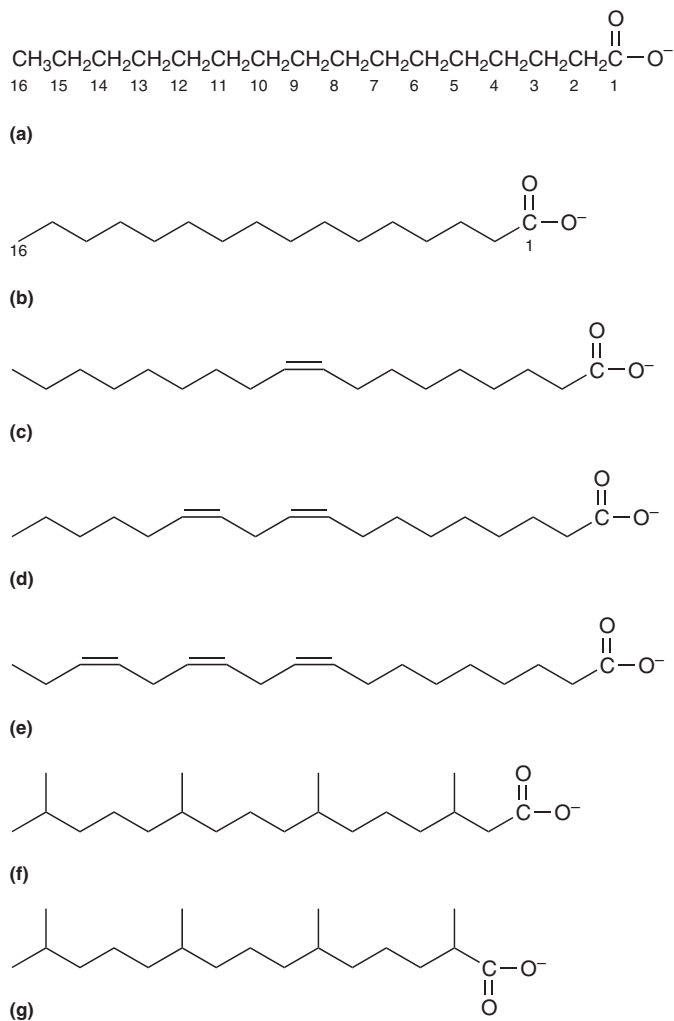
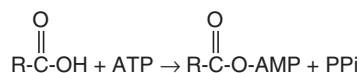
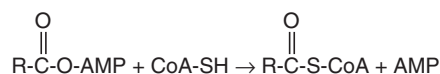


Figure 1 Fatty acid structure and nomenclature. (a) Chemical formula and carbon numbering system for a 16-carbon saturated FA (16:0). (b) Schematic representation of 16:0. (c) A monounsaturated FA, 18:1 ω 9, showing the double bond nine carbons from the methyl end (carbon 18). (d) The essential ω 6 FA, 18:2 ω 6 (linoleic acid), where the first double bond is found six carbons from the methyl end. The two double bonds are separated by a methylene ($-\text{CH}_2-$) group. (e) The essential ω 3 FA, 18:3 ω 3 (α -linolenic acid), where the first double bond is found three carbons from the methyl end. (f) Phytanic acid, a dietary β -methyl-branched-chain FA (3,7,11,15-tetramethyl 16:0). The methyl group on carbon-3 prevents this FA from degradation by β -oxidation. (g) Pristanic acid (2,6,10,14-tetramethyl 15:0) is the product of phytanic acid α -oxidation, in which a single carbon (carbon 1) is lost. The methyl group on carbon-2 does not preclude subsequent degradation by β -oxidation.

pyrophosphate (PPi):



Pyrophosphatases rapidly cleave PPi, effectively preventing reversal of this reaction. In the second half-reaction, CoA displaces AMP to form the acyl-CoA:



The thioester bond between the acyl moiety and CoA is a high-energy bond that facilitates subsequent participation of the FA

in metabolic pathways. Humans have more than 25 ACSs that differ in their tissue expression, subcellular location, and FA chain length preference.

Mitochondrial Fatty Acid β -Oxidation

To recover their stored energy, FAs must be oxidized. Quantitatively, the most important energy-yielding degradation pathway is mitochondrial β -oxidation (Figure 2). In the fed state, triacylglycerol in circulating lipoproteins delivers FAs to tissues; hydrolysis by lipoprotein lipases in the capillary endothelium releases FAs for cellular uptake. In the fasted state, FAs are released from adipocytes and delivered to tissues bound to serum albumin. Because of their hydrophobic nature, FAs can traverse the plasma membrane by simple

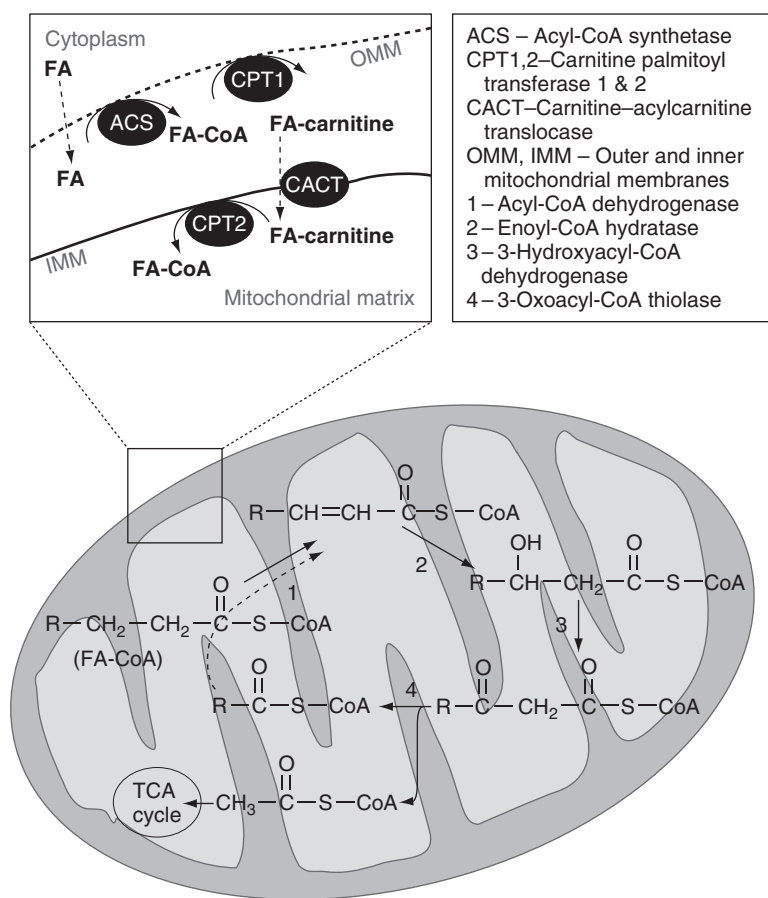
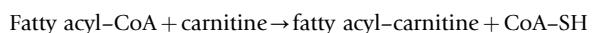


Figure 2 Mitochondrial fatty acid β -oxidation pathway. Long-chain FAs are activated, converted to carnitine esters, transported across the inner mitochondrial membrane, and re-converted to their CoA thioester once in the mitochondrial matrix. Four sequential mitochondrial enzyme reactions shorten the fatty acyl-CoA by two carbons, which are released as acetyl-CoA. The shortened fatty acyl-CoA can undergo additional cycles of degradation until the entire carbon chain has been converted to acetyl-CoA units. FADH₂ and NADH, produced in reactions 1 and 3, respectively, can enter the electron transport chain for ATP production. Acetyl-CoA enters the tricarboxylic acid (TCA) cycle, yielding additional NADH and FADH₂ for ATP production. Mitochondrial β -oxidation is the primary pathway for recovering the energy stored as triacylglycerol, or 'fat'.

diffusion; however, proteins such as **CD36**, **GOT2**, and **SLC27A1-6** have also been proposed to play a role in membrane FA transport. (Official Symbols for genes/proteins are shown in bold typeface throughout this article; other commonly used abbreviations are in normal typeface).

ACS activity toward long-chain FA substrates is present in the outer mitochondrial membrane. However, fatty acyl-CoAs do not readily traverse biological membranes such as the inner mitochondrial membrane. A highly sophisticated transport system has evolved that allows for tight regulation of FA entry into the mitochondrion (**Figure 2**). Carnitine palmitoyl transferase 1 (**CPT1**), located on the inner aspect of the outer mitochondrial membrane, catalyzes a transesterification reaction:



Carnitine:acylcarnitine translocase (**CACT**; **SLC25A20**), located in the inner mitochondrial membrane, carries the fatty acyl-carnitine inside the mitochondrion in exchange for a free carnitine molecule. **CPT2**, located inside the mitochondrion,

then catalyzes the reversal of the **CPT1** reaction. Thus the concerted actions of **CPT1**, **CACT**, and **CPT2** effectively translocate fatty acyl-CoA across the inner mitochondrial membrane. Entry of FAs into the mitochondrion is tightly regulated primarily at the **CPT1** step by malonyl-CoA. This intermediate in FA synthesis (*see* Fatty Acid *de novo* Synthesis, below) is a potent inhibitor of **CPT1**.

The four primary enzyme activities of mitochondrial β -oxidation act on intramitochondrial fatty acyl-CoA by: (1) dehydrogenation (acyl-CoA dehydrogenase), (2) hydration (enoyl-CoA hydratase), (3) dehydrogenation (3-hydroxyacyl-CoA dehydrogenase), and (4) cleavage (3-oxoacyl-CoA thiolase). The products are fatty acyl-CoA that has been shortened by 2 carbons, acetyl-CoA, FADH₂ (from reaction 1) and NADH (from reaction 3). FADH₂ and NADH directly enter the electron transport chain, yielding approximately 5 ATP molecules. Acetyl-CoA can be further degraded to CO₂ and water by the tricarboxylic acid cycle, yielding additional ATP molecules. Importantly, the entire β -oxidation process can be repeated multiple times using the shortened fatty acyl-CoA as substrate until the entire carbon skeleton of the FA has been

degraded to 2-carbon acetyl-CoA units. Complete oxidation of one molecule of 16:0 (β -oxidation + tricarboxylic acid cycle) yields more than 160 ATP molecules.

Depending on the acyl chain length, the first enzymatic step of mitochondrial β -oxidation is carried out by either short-, medium-, or very long-chain acyl-CoA dehydrogenase (ACADS, ACADM, and ACADVL, respectively). Long-chain acyl-CoA dehydrogenase (ACADL) functions in rodents but not in humans. Instead, VLCAD acts on long-chain acyl-CoAs in most human tissues whereas another dehydrogenase, ACAD9, performs this function in brain. For medium- to long-chain fatty acyl-CoAs, the three subsequent β -oxidation reactions are catalyzed by mitochondrial trifunctional protein (HADHA), whereas three separate enzymes (ECHS1, HADH, and ACAA2) are needed for oxidation of short-chain acyl-CoAs. Genetic defects in essentially all of the mitochondrial β -oxidation enzymes, as well as the carnitine transport system, are known. If unrecognized, these FA oxidation disorders are often fatal, and are a recognized cause of sudden infant death syndrome (SIDS).

Some tissues, for example skeletal muscle, completely oxidize FAs to CO_2 and water. Others, for example liver, only partially oxidize FAs, using the acetyl-CoA product for biosynthetic needs. In particular, liver utilizes intramitochondrial acetyl-CoA for the synthesis of ketone bodies, acetoacetate, and β -hydroxybutyrate (Figure 3). Ketone bodies can be oxidized by all tissues except liver and provide an alternative fuel source during starvation. In particular, brain and nerve, which do not derive energy from FA oxidation, can

oxidize ketone bodies. During prolonged starvation, increased ketone body utilization spares the brain's requirement for glucose.

Peroxisomal Fatty Acid β -Oxidation

Like mitochondria, peroxisomes contain pathways for the β -oxidation of FAs that yield a chain-shortened FA-CoA, acetyl-CoA (or propionyl-CoA), NADH, and FADH_2 (Figure 4). Unlike mitochondria, peroxisomes do not contain an electron transport chain or tricarboxylic acid cycle and thus FA degradation is not directly coupled to energy production. Typically, peroxisomes degrade FA substrates that cannot be catabolized in mitochondria. Peroxisomes are indispensable for the degradation of saturated very long-chain FAs (VLCFA; containing more than 22 carbon atoms), which are neurotoxic if allowed to accumulate. Detoxification *via* several cycles of peroxisomal β -oxidation decreases VLCFA chain-length to 8–10 carbons, after which they are converted to carnitine derivatives, exit peroxisomes, and translocate to the mitochondrion for further catabolism. Degradation of xenobiotic fatty acyl-like compounds (e.g., sulfur-substituted FAs and many nonsteroidal anti-inflammatory drugs) also takes place in peroxisomes. Oxidation of dicarboxylic acids (from the diet or from ω -oxidation) or 2-methyl-branched-chain FAs (from the diet or from α -oxidation of phytanic acid) also occurs in peroxisomes.

FAs enter peroxisomes *via* an unknown mechanism that is distinct from the mitochondrial CPT1/CACT/CPT2 pathway.

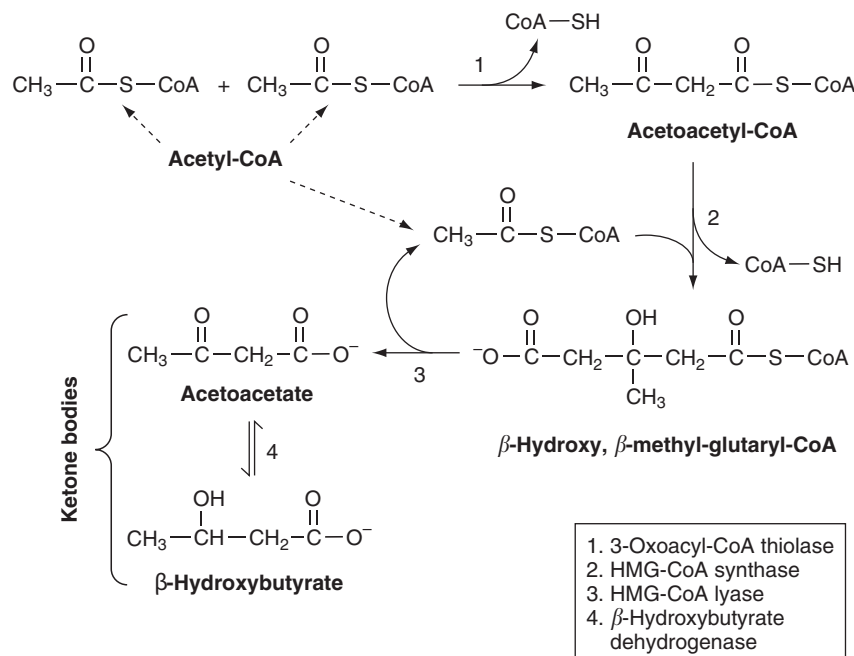


Figure 3 Ketone body synthesis. In the mitochondrion of liver hepatocytes, acetyl-CoA derived from β -oxidation is converted to 'ketone bodies', primarily acetoacetate and β -hydroxybutyrate, rather than enter the tricarboxylic acid cycle. Two molecules of acetyl-CoA condense in a reversal of the last β -oxidation reaction (3-oxoacyl-CoA thiolase). The product, acetoacetyl-CoA, condenses with another molecule of acetyl-CoA, yielding β -hydroxy, β -methyl-glutaryl-CoA (HMG-CoA), a reaction catalyzed by HMG-CoA synthase. Cleavage of HMG-CoA by HMG-CoA lyase yields acetoacetate, regenerating one molecule of acetyl-CoA. Acetoacetate is reversibly reduced to β -hydroxybutyrate *via* the NAD-dependent enzyme β -hydroxybutyrate dehydrogenase. These ketone bodies can traverse the inner mitochondrial membrane, eventually reaching the bloodstream for ultimate utilization by brain and other tissues.

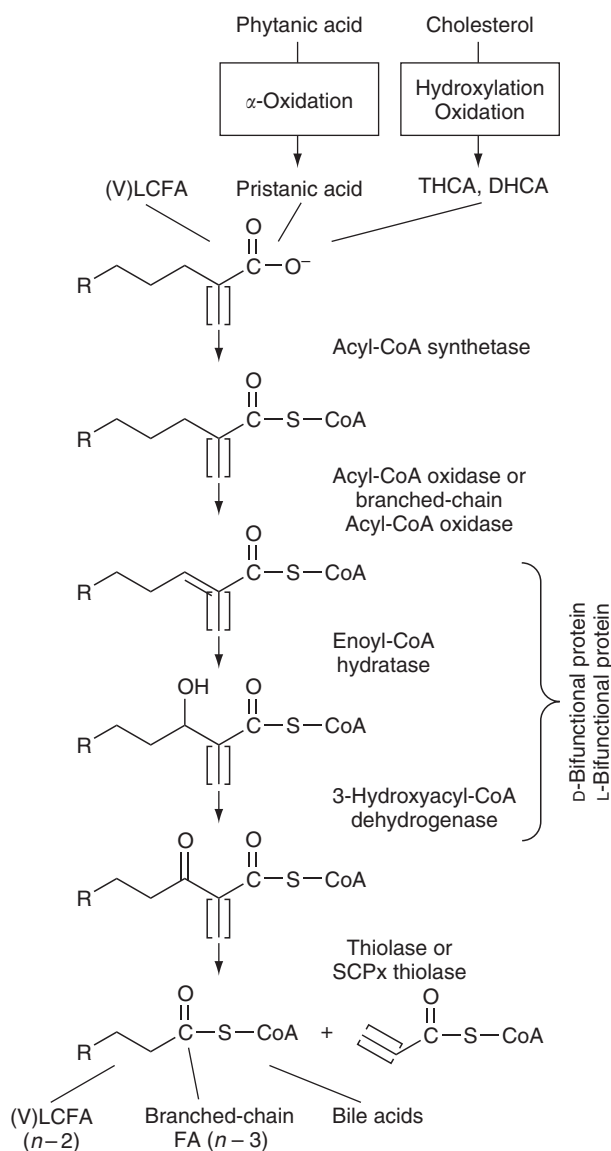


Figure 4 Peroxisomal fatty acid β -oxidation pathways. Although saturated long-chain FAs (LCFA) are preferentially degraded in mitochondria, saturated very long-chain FAs (VLCFA) and some LCFA are shortened by peroxisomal β -oxidation. Degradation of pristanic acid, the product of phytanic acid α -oxidation, and the conversion of the cholesterol-derived 27-carbon bile acid precursors DHCA and THCA (di- and tri-hydroxycholestanoic acids) to 24-carbon bile acids also require this pathway. The mechanism of entry of these substrates into peroxisomes is unknown. Four enzymatic reactions serve to shorten the substrates by either two (LCFA, VLCFA) or three (pristanic acid, DHCA, THCA) carbons. The 2-methyl group of the latter substrates is shown in brackets.

Long- and very-long-chain ACSs are associated with peroxisomes, but it has not been established whether FAs or fatty acyl-CoAs traverse the peroxisomal membrane. The basic reactions of peroxisomal β -oxidation resemble those found in mitochondria, but the peroxisomal and mitochondrial enzymes are distinct proteins (Figure 4). In fact, peroxisomes contain two sets of β -oxidation enzymes that appear to function with distinct substrates.

The first step in the oxidation of straight-chain FAs, (e.g., VLCFAs), is catalyzed by acyl-CoA oxidase (ACOX1). In humans, branched-chain acyl-CoA oxidase (ACOX2) catalyzes this initial dehydrogenation reaction for α -methyl branched-chain substrates such as pristanic acid (see FA α -Oxidation and β -Oxidation) and bile acid precursors (Figure 4). For all substrates, enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase activities are catalyzed mainly by D-bifunctional protein (HSD17B4), which contains both activities. For straight-chain substrates, peroxisomal 3-ketoacyl-CoA thiolase (ACAA1) catalyzes the final β -oxidation reaction, whereas SCPx thiolase (SCP2) catalyzes this step for pristanic acid and bile acid precursors. Peroxisomes also contain L-bifunctional protein (EHHADH), which is thought to contribute to straight-chain FA oxidation. Human deficiencies of ACOX1 and HSD17B4 are associated with significant morbidity and mortality.

The peroxisomal β -oxidation pathway also performs important biosynthetic roles. In the hepatic synthesis of bile acids from cholesterol, the aliphatic side chain, which resembles a 2-methyl-branched-chain FA, must be shortened. A single cycle of peroxisomal β -oxidation removes a three-carbon portion of the side chain (as propionyl-CoA), converting the 27-carbon bile acid precursors di- and tri-hydroxycholestanoic acids into the 24-carbon primary bile acids chenodeoxycholate and cholate, respectively. Furthermore, peroxisomal β -oxidation is required for synthesis of docosahexaenoic acid (DHA 22:6 ω 3) (see Fatty Acid Unsaturation and the Essential FAs, below).

Fatty Acid α -Oxidation and ω -Oxidation

Other important FA catabolic pathways include α -oxidation and ω -oxidation. 3-Methyl-branched FAs, for example, phytanic acid (3,7,11,15-tetramethyl-16:0; Figure 1), present in ruminant meats, fats, and dairy products consumed in the diet, cannot be degraded by β -oxidation due to the methyl group on carbon-3. Shortening the FA chain by one-carbon (α -oxidation; Figure 5) effectively shifts the position of the methyl group to carbon-2, thereby allowing subsequent degradation *via* β -oxidation. Phytanic acid is first activated to phytanoyl-CoA, and is then hydroxylated on the 2-carbon by phytanoyl-CoA 2-hydroxylase (PHYH). 2-Hydroxyphytanoyl-CoA lyase (HACL1) cleaves a one-carbon CoA derivative, formyl-CoA, yielding an aldehyde, pristanal. A splice variant of fatty aldehyde dehydrogenase (ALDH3H2) is thought to oxidize pristanal to pristanic acid (2,6,10,14-tetramethyl-15:0). Both α -oxidation and pristanic acid β -oxidation occur in peroxisomes. Deficiency of PHYH is the primary cause of Refsum disease, a peripheral neuropathy, which, if untreated, causes cerebellar ataxia, retinitis pigmentosa, and deafness.

Another mechanism for degradation of FAs that cannot undergo β -oxidation is ω -oxidation. In this process, the terminal methyl group of a FA chain is oxidized to a carboxylic acid *via* various cytochrome P450 isozymes in the endoplasmic reticulum. The resulting dicarboxylic acids are then degraded by β -oxidation from the ω -end, primarily in peroxisomes.

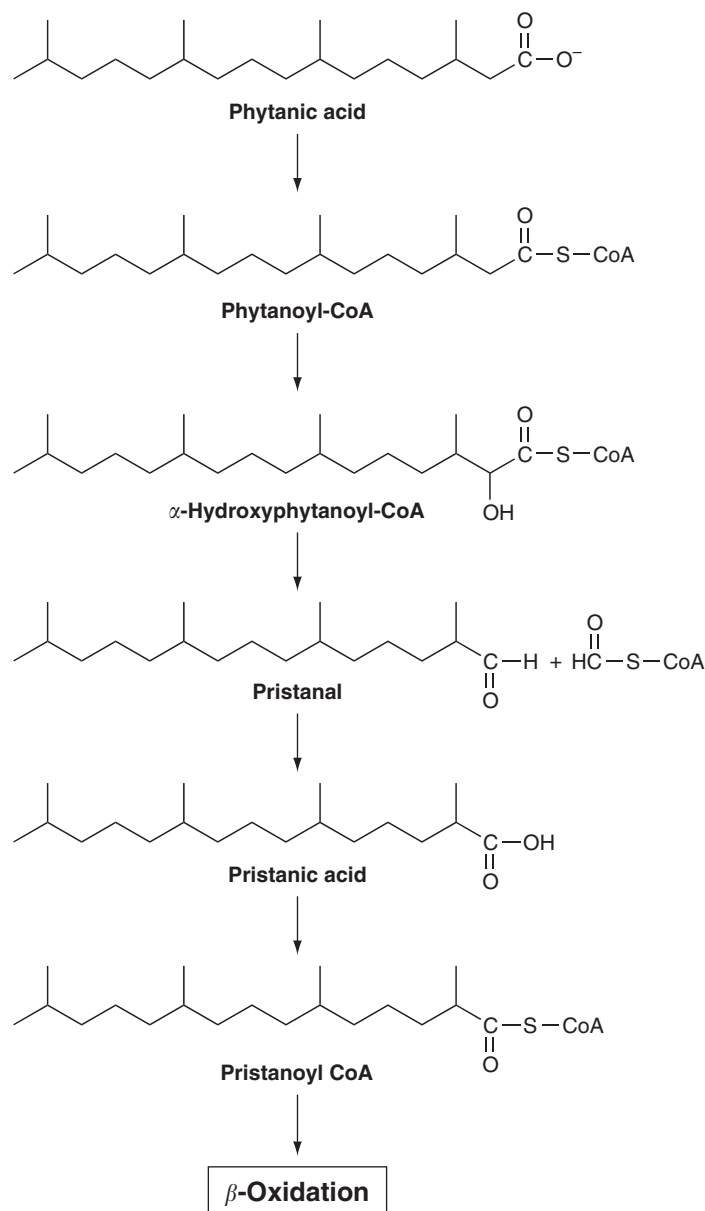


Figure 5 Peroxisomal phytanic acid α -oxidation pathway. The dietary 3-methyl-branched FA, phytanic acid, is toxic if allowed to accumulate in tissues. Its 3-methyl group prevents degradation by β -oxidation; therefore, this FA is first shortened by one-carbon. Like the substrates for peroxisomal β -oxidation, the mechanism by which phytanic acid enters peroxisomes is not known. Activated phytanic acid is hydroxylated on carbon-2. Cleavage between carbons 1 and 2 yields a one-carbon CoA compound, formyl-CoA, and an aldehyde, pristanal. After oxidation and re-activation to the CoA derivative, pristanoyl-CoA can be degraded by β -oxidation.

Fatty Acid *de novo* Synthesis

Much of our need for FAs as constituents of phospholipids and other complex lipids are met by the diet. In addition, lipogenic tissues are capable of the *de novo* synthesis of FAs (Figure 6). These tissues include liver (hepatocytes), adipose tissue, and lactating mammary gland. Much of the FAs synthesized by all three tissues are incorporated into triacylglycerol. Hepatic synthesis is primarily for export to other tissues (in very low-density lipoproteins), whereas synthesis in adipocytes and mammary gland is for local storage. In several respects, the

enzymatic reactions of FA synthesis are the converse of those in FA oxidation. However, there are key differences, which are summarized in Table 1.

The carbon utilized for FA synthesis typically derives from the products of glycolysis (Figure 6). Pyruvate enters the mitochondrion and becomes the substrate for two separate reactions. In one, pyruvate is decarboxylated *via* the pyruvate dehydrogenase complex, yielding acetyl-CoA. Lipogenic tissue mitochondria also contain pyruvate carboxylase, which converts pyruvate to the 4-carbon acid, oxaloacetate. Acetyl-CoA and oxaloacetate condense to form the 6-carbon acid, citrate.

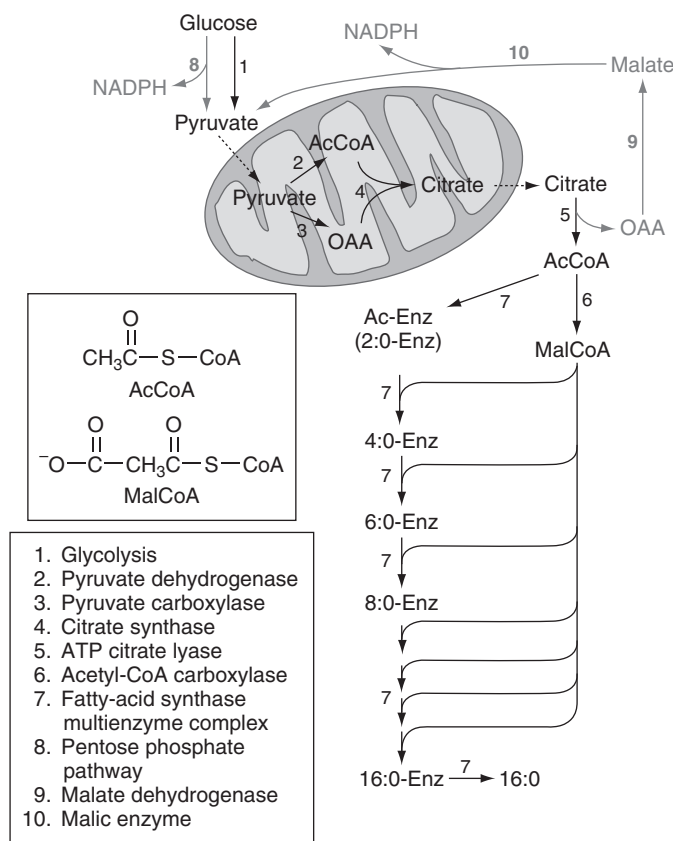


Figure 6 Fatty acid biosynthesis. Cytoplasmic acetyl-CoA (AcCoA) is the primary substrate for *de novo* FA synthesis. This 2-carbon compound most commonly derives from the glycolytic degradation of glucose, and its formation is dependent on several reactions in mitochondria. The mitochondrial enzyme, pyruvate carboxylase, is found primarily in tissues that can synthesize FAs. AcCoA is converted to malonyl-CoA (MalCoA) by acetyl-CoA carboxylase. Using AcCoA as a primer, the FA synthase multienzyme complex carries out a series of reactions that elongate the growing FA by two carbons. In this process MalCoA condenses with AcCoA, yielding an enzyme-bound 4-carbon β -ketoacid that is reduced, dehydrated, and reduced again. The product is enzyme-bound 4:0. This process is repeated six more times, after which 16:0 is released from the complex. The reductive steps require NADPH, which is derived from enzyme reactions and pathways shown in gray.

Table 1 Distinctions between fatty acid β -oxidation and fatty acid synthesis

	Fatty acid β -oxidation	Fatty acid synthesis
Tissues with active pathway	Nearly all tissues except brain, nerve, and erythrocytes	Liver, adipose, lactating mammary gland
Subcellular location	Mitochondria	Cytoplasm
Redox cofactors	NAD, FAD	NADPH
Acyl group carrier	CoA	Enzyme-bound acyl carrier protein
Stereochemistry of 3-hydroxy intermediate	L-	D-

As citrate accumulates within the mitochondrion, it is exported to the cytoplasm, where it is converted back to oxaloacetate plus acetyl-CoA in a reaction catalyzed by ATP citrate lyase (ACLY). Cytoplasmic acetyl-CoA is the fundamental building block for *de novo* synthesis of FAs.

The first enzyme unique to FA synthesis is acetyl-CoA carboxylase (ACC1), which converts the 2-carbon substrate, acetyl-CoA, into the 3-carbon product, malonyl-CoA. Citrate, in addition to being the precursor of cytoplasmic acetyl-CoA, also has a regulatory role. Citrate is an allosteric activator of acetyl-CoA carboxylase and serves as a signal that there is an ample carbon supply for FA synthesis. As mentioned, malonyl-

CoA is a potent inhibitor of CPT1. Cytoplasmic malonyl-CoA levels are high when there is significant flux through glycolysis, indicative of a high cellular energy state. Under these conditions, entry of FAs into the mitochondrion (and subsequent β -oxidation) is prevented. Interestingly, there are two isoforms of acetyl-CoA carboxylase. ACC1 is found in the above-named lipogenic tissues. ACC2 is found in many tissues that are not capable of synthesizing FAs, for example, heart. It is thought that the primary role of the second isozyme is to regulate mitochondrial FA β -oxidation by synthesizing malonyl-CoA when cellular energy needs are being met by carbohydrate metabolism.

The subsequent reactions of FA synthesis in humans are catalyzed by a multienzyme complex, fatty acid synthase (FASN). After binding of one molecule each of acetyl-CoA and malonyl-CoA to unique binding sites, a condensation reaction occurs in which CO_2 is released, and an enzyme-bound 4-carbon-3-ketoacid is formed. Subsequent reactions include reduction, dehydration, and a second reduction. The intermediates produced in these reactions are similar to those seen in β -oxidation (Figure 2), in reverse order. The product (enzyme-bound) is the saturated FA 4:0, which can then condense with another molecule of malonyl-CoA to start the process anew. After seven such cycles, the ultimate product, 16:0, is released from the complex.

The reductive steps in FA synthesis require NADPH, derived from two sources. Oxaloacetate produced by the ACLY reaction is converted to malate (*via* cytoplasmic malate dehydrogenase). Malic enzyme (ME1) then catalyzes the decarboxylation of malate to pyruvate, reducing NADP^+ to NADPH in the process. Two reactions in the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (G6PD) and 6-phosphogluconate dehydrogenase (PGD), also yield NADPH.

Fatty Acid Elongation

16:0 is the primary product synthesized by the *de novo* pathway. Although 16:0 is an important FA, there is need to synthesize longer chain-length acids. Enzymes for elongation of FA have been found in endoplasmic reticulum membranes. Reactions involved in FA elongation are very similar to those of cytoplasmic FA synthesis. The donor of the added carbon atoms is also malonyl-CoA, indicating that an active acetyl-CoA carboxylase is required for elongation. Whereas the four primary reactions of FA synthesis are found within the FASN multienzyme complex, individual proteins catalyze these reactions in FA elongation. Like FA synthesis, both reduction steps in FA elongation require NADPH.

The condensation reaction is thought to be rate-limiting, and is catalyzed by a family of very long-chain elongase enzymes (ELOVL1-7). ELOVL1, 3, 6, and 7 have been associated with elongation of saturated and monounsaturated FAs, whereas ELOVL2, 4, and 5 seem to prefer polyunsaturated FAs. The second reaction is catalyzed by 3-ketoacyl-CoA reductase (HSD17B12). No specific enzyme has been associated with the third (dehydratase) reaction. The last step is catalyzed by trans-2,3-enoyl-CoA reductase (TECR).

A minor pathway (in eukaryotes) for FA elongation is found in mitochondria. The donor of elongation units is acetyl-CoA, not malonyl-CoA, and the reduction reactions utilize NADH rather than NADPH. There is little knowledge of how FA elongation in either endoplasmic reticulum or mitochondria is regulated. However, the presence of different ELOVL isoforms suggests that each might direct its elongation product toward a specific metabolic fate.

Fatty Acid Unsaturation and the Essential FAs

Monounsaturated and polyunsaturated FAs are extraordinarily important in human health and nutrition. Thus, the ability to

insert double bonds into the carbon skeleton of an FA is a vital metabolic function. However, humans generally cannot insert a double bond closer than nine carbons from the methyl end of FAs. Thus, we are incapable of the *de novo* synthesis of two important classes of FAs, the ω 3 FAs such as docosahexaenoic acid (22:6 ω 3) and the ω 6 FAs such as arachidonic acid (20:4 ω 6). The ω 3 FAs have proven health benefits, for example, in the prevention of coronary artery disease. 22:6 ω 3 has been shown to be important for normal development of brain and retina, leading some manufacturers to include this FA into their infant formula preparations. The ω 6 FAs are important constituents of membrane lipids. 20:4 is also the precursor of prostaglandins and other bioactive eicosanoids. Because humans cannot synthesize these FAs *de novo*, we are dependent on the presence of at least some ω 3 and some ω 6 FAs in the diet. 18:2 ω 6 (linoleic acid) and 18:3 ω 3 (α -linolenic acid) are the precursors of most biologically important ω 3 and ω 6 FAs; thus, they are referred to as essential FAs.

One of the most abundant FAs in humans is 18:1 ω 9 (oleic acid), produced by inserting a *cis*-double bond in 18:0 (stearic acid). The enzyme catalyzing this reaction, stearoyl-CoA desaturase (SCD1) is called a Δ 9 desaturase because it inserts the double bond 9 carbons from the *carboxyl* carbon. Because oleic acid is so abundant, the importance of SCD1 in metabolism was initially overlooked. Oleic acid produced by SCD1 appears to be directed specifically toward triacylglycerol synthesis. SCD1 knockout mice have decreased adiposity. Furthermore, genetically obese, leptin-deficient (ob-/ob-) mice in which the SCD1 gene was also disrupted had significantly reduced body weight than did ob-/ob- mice, leading to the hypothesis that leptin regulates the synthesis of SCD1. Interestingly, dietary oleate seems to be more readily incorporated into lipids other than triacylglycerols, implying that the dietary pool and the SCD1-produced pool of this FA are metabolically distinct. Like the ω 3 FAs, dietary ingestion of monounsaturated FAs like 18:1 ω 9 have been associated with benefits to cardiovascular health. Humans and nonhuman primates also express SCD5, an isozyme found primarily in brain and pancreas, whose physiological role may be distinct from that of SCD1.

Humans are also capable of inserting *cis*-double bonds either five or six carbons from the *carboxyl* carbon of a FA (Δ 5 desaturase and Δ 6 desaturase activity, respectively). These activities, when combined with FA elongation pathways, form a powerful mechanism for synthesis of highly polyunsaturated FAs such as 20:4 ω 6 or 22:6 ω 3 from dietary essential FAs. The conversion of 18:3 ω 3 to 22:6 ω 3 also theoretically requires a Δ 4 desaturase to insert the 6th double bond (in 22:5 ω 3); however, humans lack this enzyme. It is now believed that 22:5 ω 3 is elongated to 24:5 ω 3, converted to 24:6 ω 3 by Δ 6 desaturase, and finally chain-shortened to 22:6 ω 3 by one cycle of peroxisomal β -oxidation.

Fatty Acids as Components of Complex Lipids

FAs are important building blocks for various cellular complex lipids (Figure 7). For simplicity, the pathways for incorporation of FAs into these lipids are outlined only briefly. In most cases, fatty acyl-CoA and not free FA participates in these

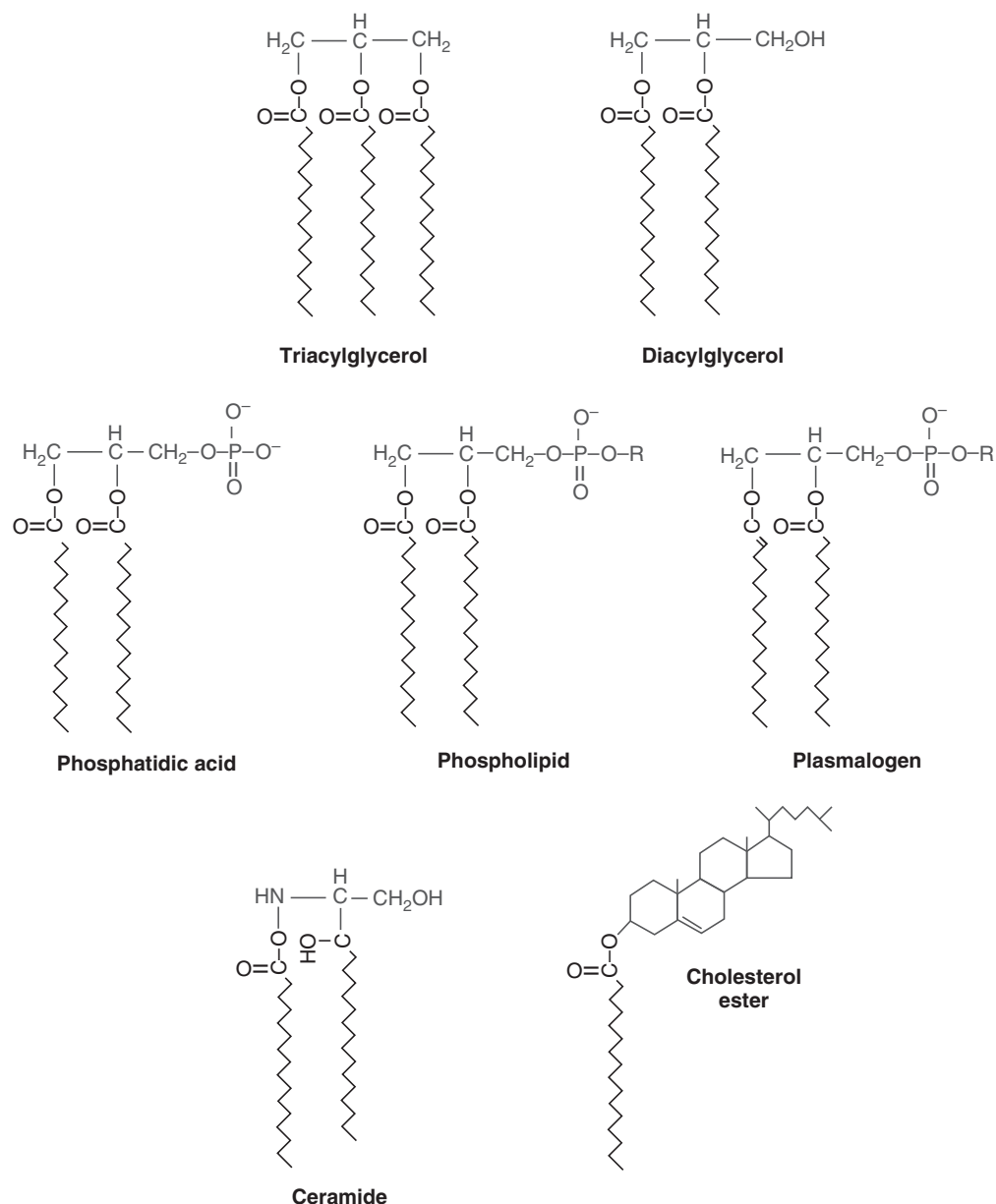


Figure 7 Fatty acids as components of complex lipids. FAs form the basis of most complex lipids. The part of the molecule derived from FAs is black, and the part derived from other sources is gray. For phospholipids and plasmalogens, R = choline, ethanolamine, inositol, serine, or similar head group.

biosynthetic reactions. Nearly all cells synthesize phospholipids, which are essential membrane constituents. Phospholipid synthesis takes place in the endoplasmic reticulum. It begins by fatty acylating the two free hydroxyl groups in α -glycerophosphate, a triose derived from glycolytic intermediates, yielding phosphatidic acid. Various head groups (e.g., choline, ethanolamine, inositol, or serine) can then be linked to the phosphate group. For synthesis of triacylglycerol, this phosphate moiety is removed, yielding diacylglycerol, and a third fatty acyl group is esterified to the free hydroxyl group. Synthesis of plasmalogens (alkyl-acyl phospholipids), which comprise approximately 20% of membrane phospholipids, requires enzymes present in both peroxisomes and

the endoplasmic reticulum. Plasmalogens are thought to be part of the cellular defense mechanism against oxidative injury.

Cholesteryl esters (ChE), in which an FA is esterified to the 3-hydroxyl group of cholesterol, are a transport and storage form of cholesterol. ChE are found in high concentrations in plasma low-density lipoproteins. Intracellular lipid droplets containing ChE are found in steroidogenic tissues and are a reservoir of cholesterol for steroid hormone synthesis. ChE most commonly contain 18:1 ω 9, which is activated to its CoA derivative before transfer to cholesterol by the enzyme acyl-CoA:cholesterol acyltransferase (**SOAT1**). ChE are also formed within lipoproteins by the transfer of a fatty acyl chain from

phosphatidylcholine to cholesterol in a reaction catalyzed by lecithin:cholesterol acyltransferase (LCAT).

Synthesis of sphingolipids, which include sphingomyelin, ceramides, cerebroside, and gangliosides, begins by the condensation of palmitoyl-CoA (16:0-CoA) with serine. The amino group of serine is then acylated by a second fatty acyl-CoA to form ceramide; the chain length of the second FA can be variable. Transfer of phosphorylcholine (from phosphatidylcholine) to the hydroxyl group of ceramide yields sphingomyelin. Alternatively, sugars (from sugar nucleotide donors) are added to produce the cerebroside, gangliosides, and related lipids.

Eicosanoid Synthesis

The FA 20:4 ω 6 (arachidonic acid) is the precursor of most eicosanoids, which include the prostaglandins, leukotrienes, and thromboxanes. Because it is an ω 6 FA, 20:4 must be derived from dietary lipids or synthesized by elongation and unsaturation of the essential FA 18:2 ω 6. Like other FAs, cellular concentrations of unesterified 20:4 are low. Conversion of 20:4 to eicosanoids begins with an agonist-induced release of the FA from the sn-2 position of membrane phospholipids *via* the action of phospholipase A2. Unlike most reactions of FAs, free 20:4 rather than its CoA derivative appears to be the substrate for eicosanoid synthesis.

Cyclooxygenases (COX1 and COX2) catalyze a complex, molecular O₂-requiring reaction that convert 20:4 to prostaglandin G2. This reaction involves carbon atoms in the middle of the acyl chain, rather than at the methyl carbon (such as occurs in ω -oxidation) or the carboxyl carbon (such as occurs in nearly all other reactions of FAs). Prostaglandin G2 can subsequently be converted to other prostaglandins or to thromboxanes. As these compounds have potent biological effects, including mediation of inflammation, COX inhibitors form an important class of anti-inflammatory drugs. Free 20:4 is also the primary substrate for the enzyme 5-lipoxygenase, which is the first step in the synthesis of leukotrienes.

Fatty Acylation of Proteins

Covalent modification of proteins is a more recently discovered role of FAs. Fatty acylation of proteins frequently serves

as a means of targeting or anchoring a protein to a membrane. Before attachment, all FAs must be activated to their CoA derivatives. Myristoylation, the addition of 14:0 to a protein, occurs at *N*-terminal glycine residues after removal of the initiator methionine. The consensus sequence for this modification is H₂N-Met-Gly-X-X-X-Ser/Thr. The reaction, catalyzed by *N*-myristoyltransferase (NMT1, NMT2), is co-translational and irreversible. *N*-myristoyl-proteins include many signal transduction-associated proteins, for example, *src* and ADP-ribosylation factors (Arfs).

Palmitoylation, the addition of 16:0 to a protein, is also commonly observed. This modification occurs post-translationally and is reversible. Most often, palmitic acid is bound to the sulfhydryl side chain of cysteine residues. Both membrane-associated proteins and integral membrane proteins can be palmitoylated; examples include ion channels, caveolin, neurotransmitter receptors, wnt, and sonic hedgehog. Palmitoylation is catalyzed by members of the large family of palmitoyltransferases (ZDHHC1-22). Three protein palmitoylthioesterases (PPT1, PPT2, and LYPLA1) that catalyze depalmitoylation of proteins have been identified. Several proteins are modified with both an *N*-terminal 14:0 and an *S*-linked 16:0 elsewhere in the protein chain. Alpha-subunits of heterotrimeric G-proteins and endothelial nitric oxide synthase are examples of myristoylated/palmitoylated proteins. In addition to palmitoylation, the signaling protein wnt is modified on a serine residue by the monounsaturated FA, palmitoleic acid (C16:1 ω 7), which is produced by the action of SCD1 on palmitoyl-CoA.

There are instances of acylation by FAs with chain length other than 14 or 16 carbons. Proteins modified by C8:0, C18:0, C18:1, and C20:4 have been reported. One nutritionally important example is the recently identified orexigenic peptide, ghrelin. The active form of this 28-amino acid peptide hormone has the medium-chain FA, C8:0, covalently esterified to the hydroxyl group of serine-3. Octanoylated ghrelin is believed to act at the level of the hypothalamus to stimulate appetite, perhaps *via* neuropeptide Y.

Vitamins and Fatty Acid Metabolism

Several of the B-vitamins are essential for normal FA metabolism (Table 2). Pantothenic acid is a constituent of CoA, and is thus required for numerous reactions of FAs. Niacin and riboflavin are necessary for the synthesis of oxidized and reduced NAD(P) and FAD, respectively. These compounds play

Table 2 Vitamins associated with fatty acid metabolism

Vitamin	Active form	Enzymes	Pathways
Pantothenic acid	CoA	Many enzymes	Most reactions involving FAs
Niacin	NAD, NADH, NADP, NADPH	Dehydrogenases; reductases	Many pathways, particularly β -oxidation and FA synthesis and elongation
Riboflavin	FAD, FADH ₂	Oxidases	β -Oxidation
Thiamine	Thiamine pyrophosphate	Pyruvate dehydrogenase complex; α -hydroxyphytanoyl-CoA lyase	FA synthesis from glucose; phytanic acid α -oxidation
Biotin	Biocytin	Acetyl-CoA carboxylase; pyruvate carboxylase	FA synthesis from glucose

essential roles in FA oxidation, synthesis, and elongation. Biotin is a constituent of acetyl-CoA carboxylase and pyruvate carboxylase, and thiamine is required for activity of the pyruvate dehydrogenase complex; all three enzymes are involved in the synthesis of FAs from glucose.

Regulation of Fatty Acid Metabolism

Regulation of FA synthesis (*see* Fatty Acid *de novo* Synthesis) and β -oxidation (*see* Mitochondrial Fatty Acid β -Oxidation) have already been discussed. More global regulatory mechanisms that deserve a brief mention include those mediated by insulin/glucagon, sterol regulatory element binding protein (SREBP)1c, and peroxisome proliferator-activated receptors (PPARs). In the fed and fasted states, control of fuel metabolism is mediated to a large extent by insulin and glucagon, respectively. Effects of glucagon are mediated *via* cAMP-dependent kinases. During fasting, increased blood glucagon levels promote release of free FAs from adipocyte triacylglycerol stores. In the liver and other tissues, glucagon promotes decreased flux through glycolysis, thereby decreasing the rate of *de novo* FA biosynthesis and increasing rates of mitochondrial β -oxidation and ketogenesis. In the fed state, insulin levels rise in response to increased blood glucose. Insulin's effects are mediated through activation of its receptor tyrosine kinase and are in general opposite to those of glucagon, stimulating glycolysis and FA synthesis while inhibiting FA degradation. Insulin and glucagon have both acute and long-term effects on FA metabolism.

SREBP1c (SREBF1) is a transcription factor thought to mediate the action of insulin in upregulating genes involved in FA synthesis such as ACC1 and FASN. PPAR γ (PPARG) is a nuclear hormone receptor that, on activation by an as yet unknown ligand, activates genes involved in adipocyte differentiation and lipid storage. It has been hypothesized that activation of SREBP1c may contribute to generation of an endogenous PPAR γ ligand.

Activation of PPAR α (PPARA) on the other hand increases rates of FA oxidation and ketogenesis. Endogenous ligands for this nuclear receptor are thought to include polyunsaturated

FAs and branched-chain FAs. Both PPARs heterodimerize with the retinoid-X-receptor (RXR), and both receptors must be ligand-bound for transcriptional activation. Several mitochondrial and microsomal, as well as peroxisomal, genes associated with FA catabolism are upregulated *via* PPAR α stimulation.

See also: Adipose Tissue: Structure, Function and Metabolism. Fats and Oils: An Overview. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids. Health Effects of Saturated Fatty Acids. Omega-3 polyunsaturated fatty acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases; Trans-Fatty Acids: Health Effects, Recommendations, and Regulations

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Glossary

C-peptide of insulin A peptide excised from the pro-insulin molecule in post-transcription processing, excreted in urine in a one-to-one ratio to insulin produced.

Energy balance The excess of energy intake over energy expenditure.

Energy flux The amount of energy flowing through the organism, approximately the same as energy expenditure.

Energy status The amount of mobilizable energy stored in organism tissues, approximately equivalent to the sum of glycogen and fat stores.

Fecundity The biological capacity to produce offspring.

Fertility The number of liveborn offspring produced.

Gonadotropin-releasing hormone (GnRH) A hypothalamic peptide that controls the reproductive hormone axis in an on-off fashion, regulated in turn by a number of amine and peptide signals, including kisspeptin.

Insulin A protein hormone secreted by the beta cells of the pancreas with major responsibility for the disposition of glucose within the organism.

Kisspeptin A family of neuropeptides capable of regulating hypothalamic release of gonadotropin-releasing hormone.

Leptin A peptide hormone produced in adipose tissue which may provide a signal of energy status to the hypothalamus via receptors on kisspeptin neurons.

Fertility and Darwinian Fitness

Reproduction is a metabolically expensive undertaking; thus fertility, the production of live-born offspring, depends critically on energy availability. A singleton, term gestation is estimated to require 325 MJ, and unsupplemented lactation may cost an additional 2.62 MJ day⁻¹. These costs are not particularly modifiable, although the strategies for meeting them are varied. Because reproduction is not necessary for survival, avoiding or postponing reproduction is possible in the face of energy constraints. Reproduction is, however, a necessary component of Darwinian fitness, the propagation of genes into subsequent generations. Thus we should expect that natural selection will have forged linkages between metabolic and reproductive physiology that help to optimize the lifetime fitness of individuals. At the most basic level, these mechanisms bias the allocation of metabolic energy toward reproductive processes at the cost of other physiological domains. A subdiscipline of evolutionary biology, life history theory, concerns itself with the formal modeling of optimal energy allocations, and interested readers may wish to consult the literature in that area. This article will focus on the empirical relationship between metabolic energy availability and human fertility, as energy availability, rather than particular nutrients or dietary composition, ultimately determines an individual's ability to reproduce.

Frisch and the Minimum Fatness Hypothesis

Rose Frisch and her colleagues deserve credit for focusing modern attention on the relationship between human

energetics and human reproduction. A series of papers in the 1970s and 80s developed an influential hypothesis, which proposed that a minimum level of body fatness was required in women for the onset and maintenance of menses. The original data to support this hypothesis were drawn from three longitudinal growth studies and the authors initially claimed that they showed that menarcheal age in girls occurred at a constant mean weight (**Figure 1**). Later, the same data were used to support a modified hypothesis, stating that a minimum (as opposed to mean) level of body fatness (as opposed to simply weight) was required (although not necessarily sufficient) for menarche (**Figure 2**). Both versions of the hypothesis were severely criticized: the first, because the data did not indicate a significant relationship between age at menarche and weight, but rather indicated that these variables were completely independent (with a correlation of zero); and the second, because the data did not support any relationship between the age at which a subject crossed the minimum fatness threshold and her age at menarche, again implying no important causal link. Nevertheless, the original versions of these hypotheses were widely cited in literature.

Other evidence accumulated at about the same time indicated that the irregularities of menstrual function observed among female athletes and sufferers from anorexia nervosa might be related to energy condition; for example, the frequency of amenorrhea increased among female runners with increasing training mileage, and the refeeding of anorexics often resulted in menstrual resumption. Other instances of either high levels of energy expenditure or excessive leanness, such as among ballet dancers or female gymnasts, were also associated with menstrual irregularities. But the relationship

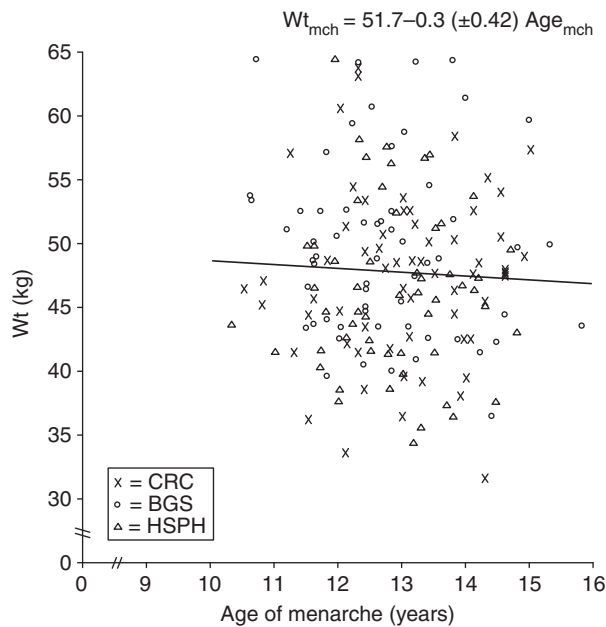


Figure 1 The original data supporting the Frisch and Revelle 'critical weight hypothesis' for menarche plotted as weight at menarche vs. age at menarche for subjects in three longitudinal growth studies (CRC, Colorado Research Center; BGS, Berkeley Guidance Study; HSPH, Stuart Growth Study, Harvard School of Public Health). The regression line is shown, but the regression itself is nonsignificant and the correlation between the variables is effectively zero. Reproduced from Frisch RE and Revelle R (1971) Height and weight at menarche and a hypothesis of menarche. *Archives of the Diseases of Childhood* 46(249): 695–701, with permission from BMJ.

of changes in menstrual function to minimum levels of body fatness was not always apparent; interruptions in athletic training due to injury, for example, often resulted in menstrual resumption before any significant weight change.

In the recent decades, research into the relationship between energetics and fertility has shifted focus from menstrual function as a proxy for fecundity to endocrine and neuroendocrine markers of fecundity, and from a focus on body fatness alone to more dynamic aspects of energetics, such as energy balance and energy flux. In addition, research has begun to focus on the physiological signaling pathways associated with the regulation of metabolizable substrates. The nature of the nutrition–fertility relationship revealed by this new research is more complex than that originally envisioned by Frisch and colleagues; nevertheless their proposal of the existence of such a relationship remains a fundamental contribution.

Energetics and Fecundity

An empirical relationship between energetics and fertility is difficult to establish. This is because human fertility, the actual production of live-born offspring, is subject to powerful behavioral regulation manifested both in the frequency and pattern of intercourse and in the use of contraceptive technology. There is some evidence that, among women attempting to conceive, both low body mass index (BMI) and

high energy expenditure are associated with longer waiting times and higher incidence of failure to conceive within a year. But more direct and extensive information exists on the relationship between energetics and the physiological determinants of fecundity, the biological potential for reproduction. Determinants of fecundity in women include hormonal and other indicators of follicular development, ovulation, luteal competence, successful implantation, and early embryonic loss. Determinants of fecundity in men include potency as well as sperm quantity and quality.

Human energetics encompasses a number of different, potentially independent variables. Most familiar, perhaps, are energy intake and energy expenditure. Methods for measuring energy intake are notoriously difficult and imprecise, although rough estimates can be made via a number of methods ranging from dietary recall to observation and portion weighing. Energy expenditure, however, can be more precisely measured over different time scales using methods such as direct and indirect calorimetry, heart rate monitoring, or doubly labeled water measurement.

Energy intake and expenditure together determine the three principal axes of human energetics: energy status, energy balance, and energy flux. Energy status refers to the stored energy available at any moment in time, and is often indexed by BMI or fat mass. Energy balance refers to the difference (positive or negative) between energy intake and energy expenditure, and is often indexed by change in weight or change in fat mass. Energy flux refers to total energy throughput and is often estimated by energy expenditure whether matched by intake or involving mobilization of stores. It is important to realize that these variables, though often correlated empirically, are in fact independent. Individuals of the same energy status can be in different states of energy balance, either gaining or losing weight. And individuals of the same energy status (for example, percent body fat) and neutral energy balance can be in different states of energy flux, either low intake and low expenditure (for example, starving) or high intake and high expenditure (for example, training for a marathon).

Energy Status and Fecundity

In women, both excessive leanness and overweight are associated with depressed indices of fecundity. Both extremes of energy status are at increased risk of ovulatory failure. Overweight women also respond poorly to ovarian stimulation during *in vitro* fertilization (IVF) treatment, requiring more gonadotropin administration and demonstrating lower estradiol responses. As a result, overweight patients have a higher rate of IVF cycle cancellation due to poor follicular growth and a higher rate of miscarriage when embryos are reintroduced, compared to normal weight women. Underweight women show no differences in response to ovarian stimulation, but have lower rates of successful pregnancy in IVF than normal weight women.

Among normal weight women differences in fecundity associated solely with differences in energy status are more difficult to demonstrate. Significant relationships between body weight or fatness in the normal range and hormonal indices of ovarian function, for example, are rarely found.

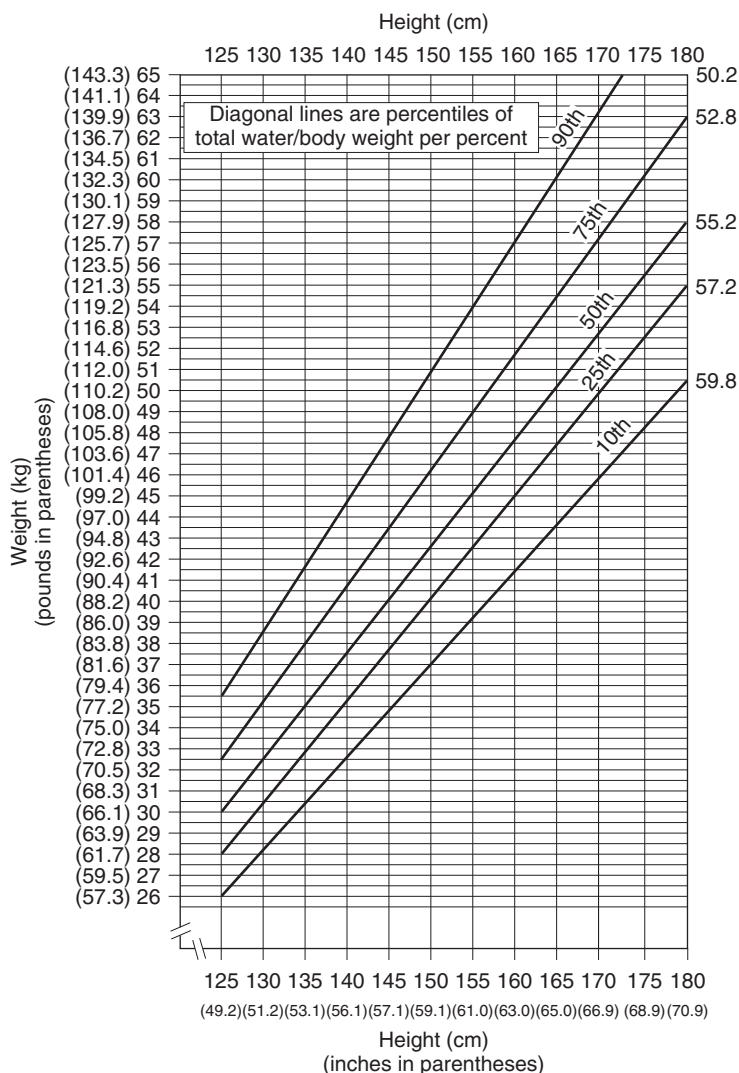


Figure 2 The same data set as in **Figure 1**, now used to support the Frisch and McArthur 'minimum necessary fat percentage hypothesis' of menarche plotted as weight at menarche vs height at menarche. The diagonal lines represent estimated percent body water, inversely related to estimated percent body fat. The lowest diagonal line represents an estimated body fat percentage of 17%, the hypothesized 'minimum necessary fat percentage' for menarche to occur. Reproduced from Frisch RE and McArthur JW (1974) Menstrual cycles: fatness as a determinant of minimum weight for height necessary for their maintenance or onset. *Science* 185(4155): 949–951, with permission from AAAS.

Some studies of men have reported an association between low BMI and both low testosterone and abnormal semen analysis. The subjects in these studies are often athletes or others engaged in routine high-energy-expenditure activities; hence it is difficult to discriminate the effects of low energy status from those of high energy flux. Studies of overweight and obese men have found negative correlations between BMI and numbers of motile sperm (**Figure 3**). Obesity is also associated with high levels of sex hormone binding globulin (SHBG) and low levels of free testosterone.

Energy Balance and Fecundity

In women, indices of ovarian function appear to respond quite sensitively to energy balance. As little as 2 kg weight

change over a month has been associated with significant changes in levels of free estradiol and progesterone. The magnitude of the reported change in estradiol levels is associated with significant changes in the probability of natural conception (**Figure 4**).

Among nonwestern populations, seasonal changes in energy balance associated with seasonal changes in energy intake and expenditure have been linked to changes in ovarian function, as well as to seasonal patterns in the frequency of conceptions and births (**Figure 5**). Periods of ongoing weight loss are associated with the lowest levels of ovarian function and the lowest conception frequencies. Both indices of fecundity reverse quickly when energy balance shifts from negative to positive, and peak indices of fecundity are attained during the period of ongoing weight gain. In fact, the highest and the lowest indices of fecundity in these populations are

associated with the periods of increasing or decreasing weight, rather than with the apex and nadir of the annual weight cycle.

In men, the relationship between energy balance and indices of fecundity does not appear to be as sensitive as in women, and may not be significant within the range of moderate changes in energy balance. Complete fasting has been associated with decreases in luteinizing hormone (LH) and testosterone over a time frame of days, and extended fasting is associated with decreases and even cessation of sperm production. Refeeding is associated with increases in gonadotropins and resumption of normal sperm production. Similar changes associated with more moderate changes in energy balance, however, have not been reported.

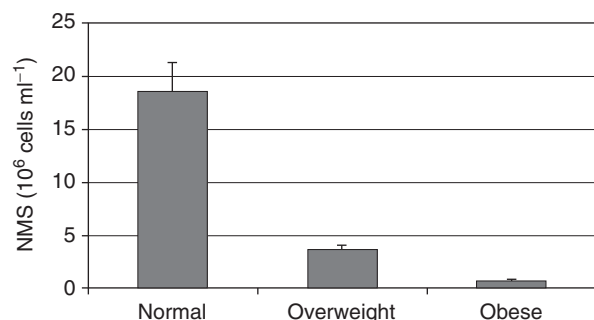


Figure 3 Mean (\pm SE) normally motile sperm cells (NMS) per ml ejaculate in 520 male subjects aged 26–45 years, grouped by BMI (normal, 20–25 kg m⁻²; overweight, 25–30 kg m⁻²; obese, > 30 kg m⁻²). Reproduced from Kort HI, Massey JB, Elsner CW (2006) Impact of body mass index values on sperm quantity and quality. *Journal of Andrology* 27: 450–452, with permission from American Society for Nutrition.

Among nonwestern populations, annual patterns of weight change of the same magnitude as those that are associated with significant changes in indices of fecundity in women have not been observed to correlate with similar indices in men, again suggesting that male reproductive physiology may be less sensitive to moderate changes in energy balance than female physiology.

Energy Flux and Fecundity

Energy flux is a difficult variable to isolate from energy status and energy balance. But women within the normal BMI range who exercise have often been found to have lower indices of ovarian function than nonexercising women, even without weight loss. And studies in rural Poland have shown that energy expenditure due to agricultural labor is negatively correlated with indices of ovarian function independently of changes in fat mass.

Extreme levels of energy flux are almost impossible to separate from extremes of energy status, especially under conditions of neutral energy balance. Extremely high levels of energy expenditure associated with athletic training are associated with lower levels of fecundity in both sexes. Men, however, often maintain sperm production at a significant level even under conditions of extreme exertion, whereas complete ovulatory failure and amenorrhea are common among women under similar conditions. Extremely low energy flux in conjunction with neutral energy balance is usually associated with extremely low energy status, and hence suppressed fecundity as noted above. High energy flux associated with high energy intake and low energy expenditure (low activity) usually results in obesity, which is itself associated with suppression of fecundity.

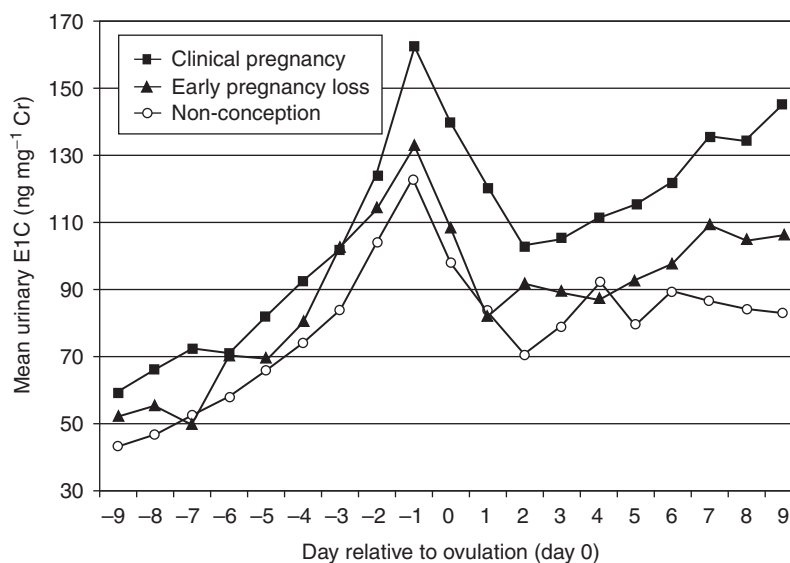


Figure 4 Daily mean (\pm SE) urinary estrone conjugate (E1C) concentration in 347 Chinese women attempting to conceive followed for up to 1 year, grouped by conception outcome (Venners, *et al.* (2006) *Human Reproduction* 21: 2272–2280). Estrogen concentrations vary between cycles in women and higher estrogen concentrations are associated with greater probability of conception. This result essentially confirms an earlier report by Lipson and Ellison based on salivary estradiol. Reproduced from Lipson SF and Ellison PT (1996) Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Human Reproduction* 11(10): 2090–2096, with permission from OUP.

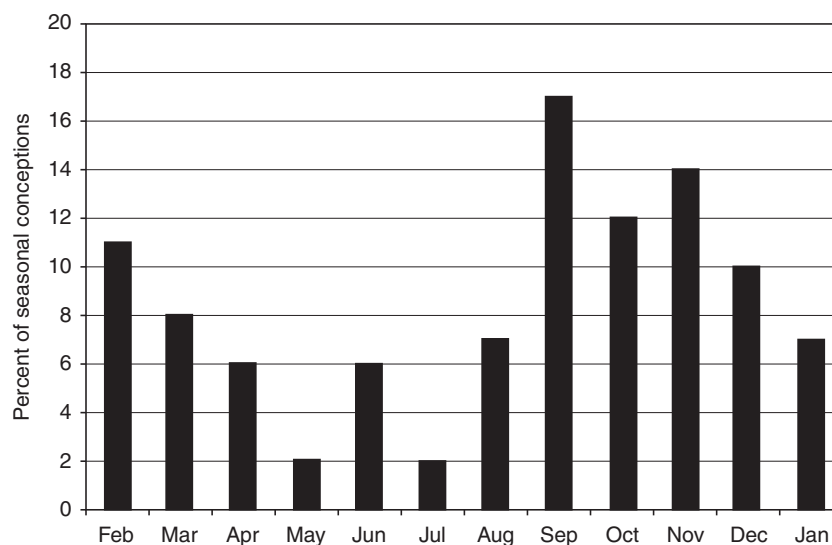


Figure 5 Seasonality in conceptions among the Lese of the Ituri Forest, Democratic Republic of the Congo, between 1980 and 1987. Harvests in this horticultural society occur between July and October. Women gain weight from July through January. The period from February through June is a period of general negative energy balance when virtually every woman in the population loses weight. Similar patterns are observed in other subsistence agricultural populations, including those in Senegal, Gambia, and Bangladesh. Reproduced from Bailey RC, Jenike MR, Ellison PT, Bentley GR, Harrigan AM, and Peacock NR (1992) The ecology of birth seasonality among agriculturalists in central Africa. *Journal of Biosocial Science* 24(3): 393–412, with permission from Cambridge.

Energetics and Pregnancy Outcomes

As noted above, the additional energy requirements imposed by a singleton, full-term gestation are estimated at 325 MJ. Strategies for meeting this requirement usually involve a mix of increased energy intake, decreased energy expenditure in activity, and changes in basal metabolism and the thermal effect of food. During the first 10 weeks of gestation, surplus energy is primarily directed towards increasing maternal fat stores and blood volume and towards placental and fetal growth. During the rest of gestation, the energy demands of fetal growth increase almost exponentially, often causing mobilization of maternal fat stores during late gestation. Maternal basal metabolic rate (BMR) normally increases during pregnancy as well, though this is more variable, as noted below.

Energy partitioning during pregnancy ordinarily places the fetus at a high metabolic priority. Constraints on maternal energy availability during pregnancy do not have a pronounced effect on offspring size or pregnancy outcome unless maternal energy status is severely compromised. Interventions based on maternal energy supplementation during pregnancy likewise have been found to have only small effects on birth weights, although the frequency of very low birth weight infants can be reduced. One exception to this generalization may be among adolescent women of low gynecological age. But even under these conditions, fetal growth may be largely buffered; energy partitioning to maternal growth is often compromised to some degree instead. Across populations, there is much greater variation in maternal fat gain and changes in BMR during pregnancy than in fetal size (Figure 6).

Remarkably, the ability of female metabolism to buffer the fetus from energy shortfalls can preserve pregnancies even under conditions of famine. During the Dutch Hunger Winter, for example, severe restrictions of caloric intake imposed by

the Nazi occupation did not result in an increased rate of spontaneous abortion of early or late gestation fetuses. It did, however, result in a shorter average gestation length and in an increase in premature live births. In late gestation, the demands of fetal brain growth begin to outstrip the placental capacity for glucose transport, leading to the initiation of labor, and this critical threshold is presumably reached sooner under conditions of acute energy shortage.

Among women in an energetically sufficient environment, excessive energy intake can lead to high infant birth weight and large fetal size, with potentially adverse consequences for pregnancy outcome. Complications of pregnancy, including preeclampsia and gestational diabetes, can occur under conditions of excessive energy intake as well.

Energetics and Lactation

Unsupplemented lactation imposes an additional energy burden on maternal physiology of some 2.6 MJ day^{-1} . Again, evidence suggests that energy partitioning to milk production is highly prioritized. This energy partitioning is partly regulated by prolactin secretion, which increases the insulin sensitivity of breast tissue; at the same time, cortisol stimulates lipolysis in somatic adipose stores, especially in the gluteofemoral region. As in pregnancy, lactating women under energy constraints maintain adequate energy partitioning to milk production by lowering allocations to BMR and activity. Energy supplementation of energetically constrained nursing mothers does not result in increased milk production or increased caloric density of milk. Rather it may moderate the reallocation of energy away from other aspects of maternal physiology. Such supplementation has been associated

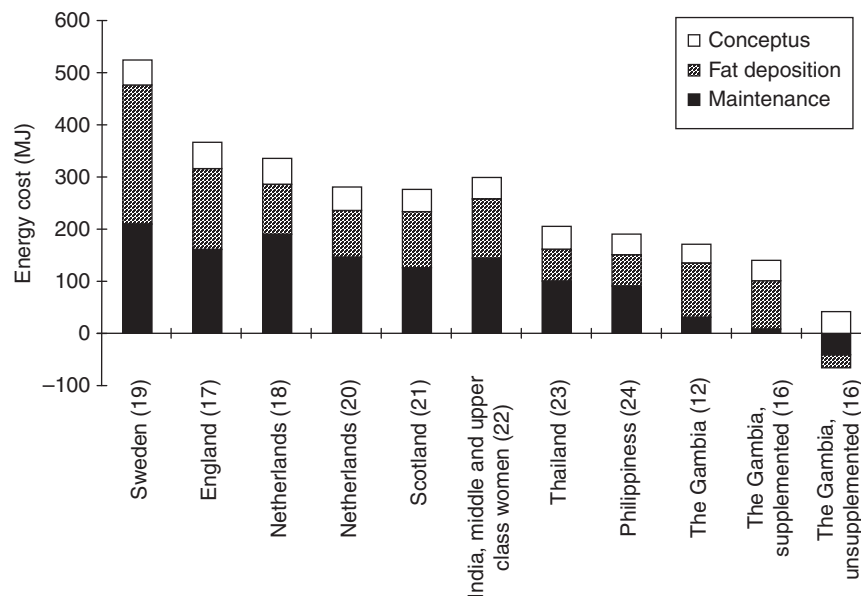


Figure 6 Total cost of pregnancy in components of fetus, maternal fat gain, and maintenance for a range of affluent and poor countries (Note: numbers in parentheses refer to bibliography entries in the original publication, not sample sizes). Reproduced from Prentice AM and Goldberg GR (2000) Energy adaptations in human pregnancy: Limits and long-term consequences. *American Journal of Clinical Nutrition* 71 (supplement 5): 1226S–1232S, with permission from American Society for Nutrition.

with more rapid reduction in postpartum prolactin levels (Figure 7), more rapid resumption of postpartum ovarian function, and a shorter interval until the next conception.

Among well-nourished women, exercise during lactation does not result in any decrease in milk production or its caloric value. Weight gain during lactation, however, can be associated with increased fat content of milk among well-nourished women.

The resumption of ovarian function postpartum may be linked to the mechanisms that regulate energy partitioning. A study of 60 lactating native women in northern Argentina revealed a pattern of increasing baseline insulin production during the 6–10 months before menstrual resumption, culminating in a transient period of relative insulin resistance during which insulin production exceeded the individual's average cycling level (Figure 8). It is postulated that the increasing trajectory of insulin production, which correlates with a trajectory of maternal weight gain, reflects increasing metabolic energy availability as the demands of milk production begin to drop. The transient period of elevated insulin immediately before menstrual resumption is probably a result of low estrogen levels. Because insulin functions as a potent gonadotropin, however, the elevated insulin levels may help to stimulate ovarian function to ovulatory levels. The resulting increase in circulating estrogens may in turn help to sensitize peripheral adipose tissue to insulin, reestablishing the normal baseline insulin levels.

Taken together, all the evidence suggests that during pregnancy and lactation, fetal growth and milk production are given a high metabolic priority and are heavily, if not completely, buffered against energy shortfalls by decreasing allocations to other maternal physiological demands. It does not appear, however, that the fetus and milk

production are similarly buffered from the effects of energy excess.

Central Regulatory Pathways

The mechanisms that link energy metabolism to fecundity probably involve both central and peripheral pathways. Signals of energy conditions to the central nervous system (CNS) may include metabolizable substrates, insulin, leptin, and potentially other adipose peptides as well.

The hypothalamus detects levels of circulating glucose via the GLUT-2 glucose transport protein. Different domains of the molecule are involved in glucose detection and glucose transport, and transgenic mice with intact transport function but blocked detection have increased appetite and energy intake. Information about circulating glucose levels appears to be communicated to the gonadotropin-releasing hormone (GnRH) secreting cells of the arcuate nucleus via orexin/hypocretin signaling with a resulting effect on pulsatile gonadotropin release.

Insulin may serve as a metabolic signal to the CNS as well. Insulin receptor (IR) is expressed in hypothalamic tissue in mice and in immortalized GnRH producing cell lines. Suppression of IR expression in the mouse hypothalamus leads to reduced GnRH pulsatility. Thus a potential exists for insulin to act centrally in the regulation of ovarian function, although the effectiveness of peripheral insulin in effecting such regulation has not been determined.

Leptin produced by peripheral adipose tissue also carries information about energy dynamics to the CNS; however the information that leptin carries is complex. Within a given sex and population, leptin levels correlate positively

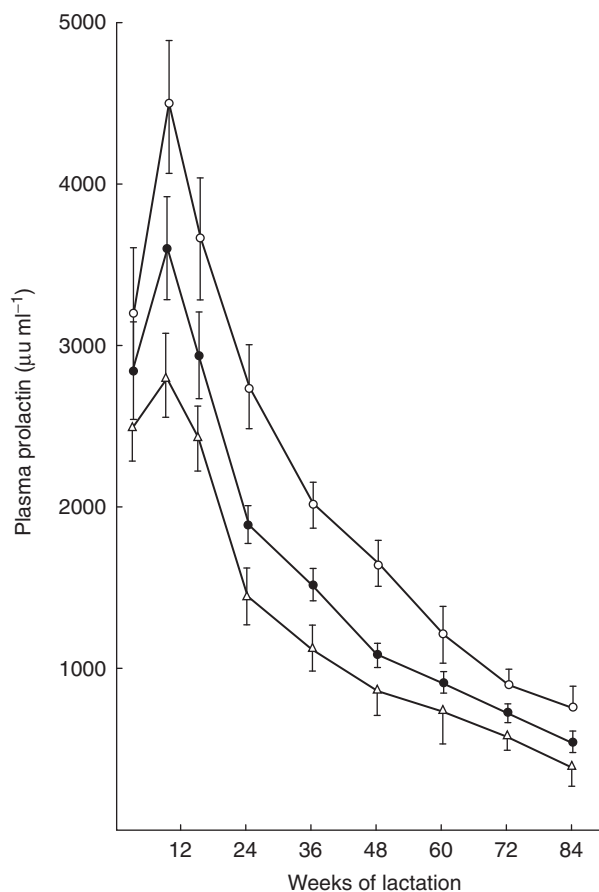


Figure 7 Mean (\pm SE) prolactin concentrations by week postpartum in lactating Gambian mothers, grouped by nutritional supplementation status. Open circles, unsupplemented; closed circles, supplemented during gestation only; open triangles, supplemented during gestation and lactation. Reproduced from Lunn PG, Austin S, Prentice AM, and Whitehead RG (1984) The effect of improved nutrition on plasma prolactin concentrations and postpartum infertility in lactating Gambian women. *American Journal of Clinical Nutrition* 39(2): 227–235, with permission from American Society for Nutrition.

with adipose tissue mass, leading to the notion that leptin signals energy status. But circulating leptin levels are also positively correlated with change in adipose mass, that is, with energy balance. There is even compelling evidence that leptin levels correlate negatively with energy expenditure independently of change in adipose mass, and thus with energy flux. Therefore, it is more helpful to think of leptin as reflecting the metabolic activity of adipose tissue, rather than simply its mass. In this context it is also important to realize that insulin is a particularly potent stimulus for leptin production. Leptin levels are more strongly correlated with insulin levels than with adiposity in some studies. Children with new-onset type I (insulin-dependent) diabetes have abnormally low leptin levels for their fat mass, but those levels quickly rise to the normal range with insulin therapy. Similarly, biliopancreatic diversion in obese subjects produces a reduction in both insulin and leptin levels and a disassociation between leptin and fat mass. Thus there is likely a significant degree of redundancy between the information

regarding energy dynamics conveyed by insulin and leptin. Leptin may have greater access to the CNS, whereas insulin, as noted in the next section, may be more important as a peripheral signal.

The fat mass–leptin relationship is strongly modified by sex in adults, presumably a consequence of the sex steroid milieu. In women, leptin levels vary across the ovarian cycle in association with estradiol levels. Within sex, the fat mass–leptin relationship varies between populations, with leptin per fat mass in many nonwestern, rural populations reported to be significantly less than in western, urbanized populations. These population differences may also reflect the effect of insulin stimulation on adipose leptin production.

The relationship of leptin to ovarian function is also complex. Based largely on analogy with animal models, negative correlations between premenarcheal leptin levels and menarcheal age in girls, and a trend toward increasing levels of leptin in girls with advancing pubertal stage, it has been hypothesized that leptin exerts permissive control on the maturation of the hypothalamic–pituitary–ovarian (HPO) axis. Experimental elevation of circulating leptin levels causes an increase in LH pulse frequency, number of dominant follicles, and circulating estradiol levels in women with amenorrhea associated with either high energy expenditure or low energy intake. These results suggest that leptin's central effects may be through modulation of pulsatile GnRH release. The mechanism of leptin action on GnRH pulsatility is unclear, however, because GnRH neurons do not express leptin receptor (LepR); some evidence suggests the pathway may be indirect, involving signaling via kisspeptin.

The pathways for conveying information about energy dynamics to the CNS are generally thought to culminate in the regulation of pulsatile GnRH secretion, which regulates pituitary gonadotropin release, in turn regulating gonadal function. Control at this level is qualitative; that is, pituitary gonadotropin release is either 'on or off' depending on whether GnRH pulsatility is within an acceptable frequency range, whereas quantitative modulation of gonadotropin release is controlled by gonadal feedback. Thus central mechanisms may well be involved in shutting down the hypothalamic–pituitary–gonadal axis in both sexes under extremes of energy availability.

Peripheral Regulatory Pathways

In addition to signals of energy dynamics that affect central regulation of reproduction, there are peripheral signals that directly affect gonadal activity. Insulin, growth hormone (GH), and insulin-like growth factor 1 (IGF-1), all stimulate steroid production pathways in the ovary, and probably the testis as well. All three are also positively correlated with energy availability. Insulin in particular bears a direct relationship to energy balance and flux. In the ovary it serves as a cogenadotropin, augmenting the response of theca and granulosa cells to gonadotropin stimulation. The potential role of insulin signaling in the postpartum resumption of ovarian function was noted above. The quantitative variation in ovarian function that has been documented to occur in

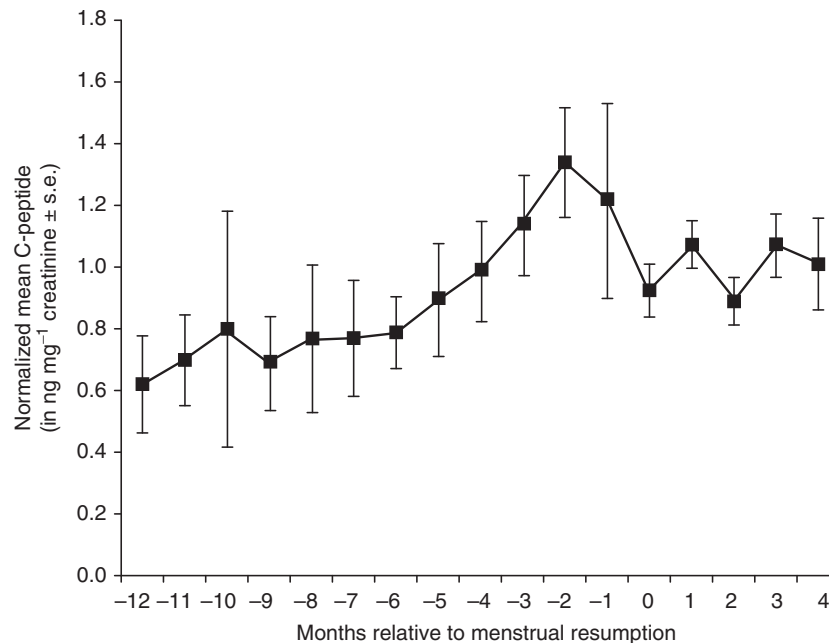


Figure 8 Mean (\pm SE) urinary C-peptide concentrations among 60 lactating Toba women in Argentina by month relative to resumption of menses. Reproduced from Vaggia CR and Ellison PT (2009) Interactions between metabolic and reproductive functions in the resumption of postpartum fecundity. *American Journal of Human Biology* 21(4): 559–566, with permission from Wiley.

association with variation in energy balance and flux may also be in response to insulin signaling.

LepR is also expressed in the ovary by both luteinized and nonluteinized granulosa cells. At the level of the ovary, however, leptin's action is primarily to inhibit ovarian steroid production, although there is also evidence of an enhancing effect on aromatase activity. It is difficult, therefore, from isolated *in vitro* studies, to fully determine the integrated effect of variation in leptin levels, acting simultaneously through central and peripheral channels, on female ovarian function. Nor is a suppressive effect on ovarian steroid production easily compatible with a hypothetical role for reduced leptin levels in down-regulating female fecundity under conditions of energy stress. Thus it may be that leptin, like GH and IGF-1, is more important as an intraovarian regulator than as a peripheral signal of energy dynamics.

The disruption of fecundity in obesity appears to be primarily a result of peripheral pathways. Obesity is associated with increased production of SHBG, increased nongonadal aromatase activity resulting in increased extragonadal estrogen production, and elevated ovarian androgen production due to inappropriate levels of insulin and IGF-1 stimulation. In men, the first two effects predominate, leading to low effective testosterone levels. In women, the last effect often predominates, generating a tendency towards hyperandrogenism, polycystic ovarian disease, and impaired fecundity.

Adaptation and Pathology in the Relationship of Energy Metabolism and Reproductive Physiology

To summarize, it appears that female fecundity responds rather sensitively to variation in energy balance and flux, male

fecundity less so. Extremes of energy status are associated with impaired fecundity in both sexes, though the pathways responsible for impairment of fecundity under conditions of low energy availability may be different from those operating under conditions of excess energy availability. Maternal energy partitioning significantly buffers both fetal growth and lactation from low energy availability, but not from energy excess.

The response of female fecundity to restricted energy availability is believed by many researchers to be adaptive, lowering the probability of commitment to a pregnancy when maternal energy availability may be constrained. A situation of negative energy balance may make further reductions in energy partitioning to maternal maintenance due to pregnancy excessively costly or impossible. High energy flux may signal higher than normal maternal energy requirements, and thus reduced capacity for energy partitioning to reproduction. At moderate levels, variation in energy balance and flux does not reduce female fecundity to zero, however. Pregnancy may still occur, though waiting times are likely to be longer. Thus situations of chronic, moderate energy constraint will still be compatible with reproduction, only with more opportunity for maternal condition to recoup between pregnancies. When energy conditions are varying, the same mechanisms will shift the probability of conception toward energetically more favorable periods. Resumption of fecundity postpartum will also be synchronized with an increase in maternal energy availability and greater opportunity for energy partitioning to a new pregnancy.

The less sensitive response of male fecundity to energy constraints is also thought to be adaptive by many researchers. Because the energy requirements of gamete production are not particularly high in men, sperm production is rarely impacted

by energy constraints. Testosterone levels respond somewhat more to restriction in energy availability, though not as quickly or as sensitively as female ovarian hormones. The most significant consequences of testosterone variation may be in determining how much energy is allocated to muscle mass and reproductive behavior, the primary regulators of male reproductive effort in most mammals.

The impairment of fecundity associated with obesity is not thought to be adaptive, but rather to be a pathological consequence of conditions that were probably uncommon in our evolutionary past. It appears that our reproductive physiology has been shaped to respond functionally to reductions in energy availability below what is optimal, but not to variation above that level. It has been argued by some, however, that the molding of our physiology by natural selection to contend with energy constraint may have generated the norms of reaction that result in pathology under conditions of energy excess. That is, by equipping us to deal with energy deficiency, natural selection may have produced the, now increasingly common, reproductive pathologies associated with energy excess.

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Contents

Physiological and Functional Effects Resistant Starch and Oligosaccharides

Physiological and Functional Effects

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Glossary

Glycemic Pertaining to sugar or glucose in the blood.

Glycemic index A quantitative ranking of the blood-glucose response to a carbohydrate food, compared to a reference food such as pure glucose.

Prebiotic A fermentable but non-digestible food component favoring the growth of beneficial bacteria in the human microbiota.

Introduction

It has long been recognized that both animal foodstuffs and human foods contain poorly digestible components that do not contribute to nutrition in the classical sense of providing essential substances or metabolic energy. With the development of scientific approaches to animal husbandry in the 19th century, the term 'crude fiber' was coined to describe the material that remained after rigorous nonenzymatic hydrolysis of feeds. During the 20th century, various strands of thought concerning the virtues of 'whole' foods, derived from plant components that had undergone only minimal processing, began to converge, leading eventually to the dietary fiber hypothesis. Put simply, this states that the nondigestible components of plant cell walls are essential for the maintenance of human health.

In the early 1970s the physician and epidemiologist Hugh Trowell recognized that the crude fiber figures available at the time for foods had little physiological significance and were of no practical value in the context of human diets. He was amongst the first to use the term dietary fiber to describe the 'remnants of plant cell walls resistant to hydrolysis (digestion) by the alimentary enzymes of man.' This definition was later refined and given the more quantitative form: The sum of lignin and the plant polysaccharides that are not digested by the endogenous secretions of the mammalian digestive tract. This definition paved the way for the development of analytical methods that could be used to define the fiber content of human foods. The use of enzymic hydrolysis to determine the 'unavailable carbohydrate' content of foods, originally developed by McCance and Lawrence, was refined by Southgate, and his technique was used for the 4th edition of the UK standard food tables, *The Composition of Foods* published in

1978. Broadly, all techniques for analysis of fiber are based on enzymic removal of the digestible elements in food, followed by either gravimetric analysis (i.e., by weight) of the residue as used in the Association of Analytical Chemists (AOAC) method, which results in the retention of some undigested starch, or chemical analysis using gas-liquid chromatography ('Englyst' method), which enables a more precise separation of starch from the structural polysaccharides of the cell wall. In the latter case, the cell wall components are defined as 'nonstarch polysaccharides' (NSPs). Whatever analytical approach is used, both 'dietary fiber' and nonstarch polysaccharides are shorthand terms for large and complex mixtures of polysaccharides. The components of such mixtures vary widely among foods and they often share few properties other than resistance to digestion in the small intestine. A summary of the main types of plant cell polysaccharides contained in the general definition of dietary fiber is given in **Table 1**.

Table 1 Major components of dietary fiber

<i>Food source</i>	<i>Polysaccharides and related substances</i>
Fruits and vegetables	Cellulose, xyloglucans, arabinogalactans, pectic substances, glycoproteins
Cereals	Cellulose, arabinoxylans, glucoarabinoxylans, β -D-glucans, lignin, and phenolic esters
Legume seeds	Cellulose, xyloglucans, galactomannans, pectic substances
Manufactured products	Gums (guar gum, gum arabic), alginates, carrageenan, modified cellulose gums (methyl cellulose, carboxymethyl cellulose)

In recent years this problem has been made more complex in some ways because of the explosion of interest in functional foods for gastrointestinal health. These often contain high levels of novel oligosaccharides, which are beta-linked saccharide polymers consisting of between three and nine monomers, or larger synthetic or purified carbohydrate polymers that behave as dietary fiber in the gut lumen. Fructose oligosaccharides, which are nondigestible in the small intestine but highly fermentable by the bacteria of the large bowel, are often added to foods as prebiotic substrates to modify the colonic microflora. Such materials may not fit the original definition of dietary fiber, but it is unrealistic in practice to exclude them from the modern concept of fiber. The need to accommodate recent developments is reflected in the 2009 definition of dietary fiber, recommended for international use by the Commission on Nutrition and Foods for Special Dietary Uses, of the Codex Alimentarius Commission, stating that: "Dietary fiber means carbohydrate polymers with ten or more monomeric units, which are not hydrolyzed by endogenous enzymes in the small intestine of human beings". This definition includes a footnote leaving the inclusion of oligosaccharides with between three and nine oligomeric units to the discretion of national governments, and so paves the way for very broad definitions of fiber that embrace commercial prebiotic products.

The widely used definition of fiber recommended by the Institute of Medicine of the National Academy of Sciences of the USA, makes a distinction between 'dietary fiber', which consists of nondigestible carbohydrates and lignin that are intrinsic and intact in plants, and what is called 'functional fiber', consisting of isolated, nondigestible carbohydrate components that have beneficial physiological effects in humans. Oligosaccharides, whether from natural or synthetic sources, are excluded from the first category but are included in the second. *Total dietary fiber* (TDF) is defined as the sum of Dietary and Functional fiber. In practice, however, no analytical technique can distinguish between dietary fiber and functional fiber when they occur as a mixture in food products. The European Food Safety Authority (EFSA) has recommended a simpler definition of dietary fiber as "all carbohydrates occurring in foods that are nondigestible in the human small intestine".

The presence in the lumen of large undigested cell wall fragments, finely dispersed particulates, or soluble polysaccharides can alter physiological processes throughout the gut. The effects of different fiber components depend on their varied physical and chemical properties during digestion, and also on their susceptibility to degradation by bacterial enzymes in the colon. The complex nature of the various substances covered by the general definition of dietary fiber means that a single analytical value for the fiber content of a food is a poor guide to its physiological effects. This article will review the main mechanisms of action of resistant polysaccharides in the alimentary tract and their implications for human health.

Sources and Types of Dietary Fiber

The main sources of dietary fiber in most Western diets are well characterized, and high-quality data are available for both food

Table 2 A Comparison of values for nonstarch polysaccharides and dietary fiber

<i>Food source</i>	<i>Nonstarch polysaccharides (Englyst method)</i>	<i>Total dietary fiber (AOAC method)</i>
White bread	2.1	2.9
Brown bread	3.5	5.0
Wholemeal bread	5.0	7.0
Green vegetables	2.7	3.3
Potatoes	1.9	2.4
Fresh fruit	1.4	1.9
Nuts	6.6	8.8

composition and dietary intakes. This is not always true for diets in developing countries, however, and this problem bedevils attempts to investigate the importance of fiber by making international comparisons of diet and disease. Another problem is that different analytical approaches give statistically significant differences for the dietary fiber content of foods. Moreover single analytical values for fiber alone do not reflect the physical and chemical properties of the different polysaccharide components. A comparison of values for NSP, and total TDF values obtained by the AOAC method, is given in **Table 2**.

In the UK approximately 47% of dietary fiber is obtained from cereal products, including bread and breakfast cereals. The level of cell wall polysaccharides in a product made from flour depends on the extraction rate, which is the proportion of the original grain present in the flour after milling. Thus a 'white' flour with an extraction rate of 70% usually contains approximately 3% NSP, whereas a 'wholemeal' flour with an extraction rate of 100% contains approximately 10% NSP. Although the terms 'soluble' and 'insoluble' fiber describe the behavior of different classes of nonstarch polysaccharides under experimental conditions *in vitro*, they do give some insight into the behavior of different components of dietary fiber during digestion, and thus partially overcome the problem of the lack of correspondence between the total analytical value for fiber and the physical properties of the measured polysaccharides. By adopting the Englyst technique for the separation and chemical analysis of nonstarch polysaccharides it is possible to specify both the soluble and insoluble fiber content of foods. Some representative values for soluble and insoluble fiber in cereal foods are given in **Table 3**, and those for fruits and vegetables, which provide a further 45% of the fiber in UK diets, are given in **Table 4**.

Fiber in the Digestive Tract

The primary function of the alimentary tract is to break down the complex organic macromolecules of which other organisms are composed into smaller molecules, which can then be selectively absorbed into the circulation by specialized mucosal epithelial cells. Food is conveyed progressively through the alimentary tract, stored at intervals, and broken down mechanically as required, by a tightly controlled system of rhythmic muscular contractions. The digestive enzymes are released into the lumen at the appropriate stages to facilitate

Table 3 Soluble and insoluble nonstarch polysaccharides in some cereal products and nuts

Food source	Nonstarch polysaccharides (g per 100 g fresh weight)		
	Total NSP	Soluble NSP	Insoluble NSP
Sliced white bread	1.5	0.9	0.6
Sliced brown bread	3.6	1.1	2.5
Wholemeal bread	4.8	1.6	3.2
Spaghetti	1.2	0.6	0.6
Rye biscuits	11.7	3.9	7.8
Cornflakes	0.9	0.4	0.5
Crunchy oat cereal	6.0	3.3	2.7
Walnuts	3.5	1.5	2.0
Hazelnuts	6.5	2.5	4.0
Peanuts	6.2	1.9	4.3
Brazil nuts	4.3	1.3	3.0

Table 4 Soluble and insoluble nonstarch polysaccharides in some vegetables and fruits

Food source	Nonstarch polysaccharides (g per 100 g fresh weight)		
	Total NSP	Soluble NSP	Insoluble NSP
Apples (Cox)	1.7	0.7	1.0
Oranges	2.1	1.4	0.7
Plums	1.8	1.2	0.6
Bananas	1.1	0.7	0.4
Potatoes	1.1	0.6	0.5
Sprouts	4.8	2.5	2.3
Peas (frozen)	5.2	1.6	3.6
Carrots	2.5	1.4	1.1
Courgettes	1.2	0.6	0.6
Runner beans	2.3	0.9	1.4
Baked beans	3.5	2.1	1.4
Tomato	1.1	0.4	0.7
Lettuce	1.2	0.6	0.6
Onion	1.7	0.9	0.8
Celery	1.3	0.6	0.7

Source: Data modified from Englyst HN, Bingham SA, Runswick SS, Collinson E, and Cummings JH (1989) Dietary fiber (non-starch polysaccharides) in fruit vegetables and nuts. *Journal of Human Nutrition and Dietetics* 1: 247–286, with permission from Wiley.

the decomposition of carbohydrates, proteins, and complex lipids. By definition, the polysaccharides that comprise dietary fiber are not digested by endogenous enzymes, though they are often fermented to a greater or lesser degree by bacterial enzymes in the large intestine.

The Mouth and Pharynx

The earliest stages of digestion begin in the mouth, where food particles are reduced in size, lubricated with saliva, and prepared for swallowing. The saliva also contains the digestive enzyme salivary amylase, which begins the hydrolysis of starch molecules. Cell wall polysaccharides are an important

determinant of food texture, and they exert an indirect effect on the degree of mechanical breakdown of plant foods before swallowing. Hard foods tend to be chewed more thoroughly than soft ones, and hence the presence of dietary fiber in unrefined foods may begin to regulate digestion at a very early stage.

The Stomach

The first delay in the transit of food through the digestive tract occurs in the stomach, where large food fragments are further degraded by rigorous muscular activity in the presence of hydrochloric acid and proteolytic enzymes. The need to disrupt and disperse intractable food particles and cell walls appears to delay the digestive process significantly. For example, the absorption of sugar from whole apples is significantly slower than from apple juice. Similarly, the rate at which the starch is digested and absorbed from cubes of cooked potato has been shown to be much slower when they are swallowed whole than when they are chewed normally. Thus, simple mechanical factors can limit the rate at which glucose from carbohydrate foods enters the circulation.

The Small Intestine

The small intestine is the main site of nutrient absorption, and it is in fact the largest of the digestive organs in terms of surface area. The semi-liquid products of gastric digestion are released periodically into the duodenum, and then propelled downstream by peristaltic movements, at approximately 1 cm per minute. The hydrolysis of proteins, triglycerides, and starch continues within the duodenum and upper jejunum, under the influence of pancreatic enzymes. The final stages of hydrolysis of dietary macromolecules occur under the influence of extracellular enzymes at the mucosal surface. The released products are absorbed into the circulation, along with water and electrolytes, via the specialized epithelial cells of the intestinal villi. Muscular activity in the small intestinal wall, together with rhythmic contractions of the villi, ensures that the partially digested chyme is well stirred. In adults, the first fermentable residues from a meal containing complex carbohydrates enter the colon approximately 4.5 h after ingestion. When a solution containing indigestible sugar is swallowed without food it reaches the colon approximately 1.5 h earlier than when the same material is added to a solid meal containing dietary fiber. The presence of solid food residues slows transit, probably by delaying gastric emptying and perhaps also by increasing the viscosity of the chyme so that it tends to resist the peristaltic flow. Soluble polysaccharides such as guar gum, pectin, and β -glucan from oats increase mouth to cecum transit time still further.

In creating the dietary fiber hypothesis, Trowell's principal interest was its role in the prevention of metabolic disorders. In particular, he believed that dietary fiber was a major factor in the prevention of diabetes mellitus, which, he argued, was probably unknown in Western Europe before the introduction of mechanized flour milling. In earlier times the near-universal consumption of unrefined carbohydrate foods

would have ensured that intact indigestible cell wall polysaccharides were present throughout the upper alimentary tract during digestion. This, according to Trowell and others, favored slow absorption of glucose, which in turn placed less strain on the ability of the pancreas to maintain glucose homeostasis. There is no doubt that Type 2 diabetes has become more common in Western countries as prosperity, and an excess of energy consumption over expenditure, has grown. It is not established that rapid absorption of glucose due to consumption of refined starches is a primary cause of diabetes, but the control of glucose assimilation is certainly a key factor in its management. Cell wall polysaccharides influence the digestion and absorption of carbohydrates in a variety of ways, and are a major determinant of the 'glycemic index', which is defined as the incremental area under the blood-glucose response curve after consumption of a standardized sample, expressed as a percentage of the response to an equivalent amount of carbohydrate consumed as glucose. This is essentially a quantitative expression of the rate of change and quantity of glucose appearing in the bloodstream after ingestion of a carbohydrate-rich food. To calculate the index, healthy volunteers are given a test meal of the experimental food containing a standardized quantity of carbohydrate, after an overnight fast. Blood samples are taken at intervals for biochemical analysis, and the change in concentration of glucose in the blood is measured and plotted over a period of time. The ratio of the area under the blood-glucose curve in response to the test meal to that produced by an equal quantity of a standard reference food is then calculated and expressed as a percentage. Individual human subjects do vary significantly in their glycemic response to food, but when glucose is used as the standard, most complex starchy foods have glycemic indices lower than 100%, and this has been shown to be a consistent property of the foods, rather than reflection of human variation. The GI values of foods have been used successfully to design diets for the management of Type 2 diabetes.

The physical resistance of plant cell walls during their passage through the gut varies considerably from one food to another. Cell walls that remain intact in the small intestine will impede the access of pancreatic amylase to starch. This is particularly true of the cells of legume seeds, which have been shown to retain much of their integrity during digestion. Legume-based foods such as lentils and chilli beans have glycemic indices that are amongst the lowest of all complex carbohydrate foods. Even when enzymes and their substrates do come into contact, the presence of cell wall polysaccharides may slow the diffusion of hydrolytic products through the partially digested matrix in the gut lumen. These effects of dietary fiber on carbohydrate metabolism emphasize once more that physiological effects cannot be predicted from simple analytical values for total fiber, because they are consequences of cellular structure, rather than the absolute quantity of cell wall polysaccharides within the food.

Many studies on postprandial glycemia have been conducted using isolated fiber supplements added to glucose test-meals or to low-fiber sources of starch. They demonstrate that, contrary to Trowell's original hypothesis, wheat bran and other insoluble cell wall materials have little effect on human glucose metabolism. However, certain soluble

polysaccharides, such as guar gum, pectin, and oat β -glucan, which form viscous solutions in the stomach and small intestine, do slow the absorption of glucose. Highly viscous food components may delay gastric emptying and inhibit the dispersion of the digesta along the small intestine, but the primary mechanism of action appears to be suppression of convective stirring in the fluid layer adjacent to the mucosal surface. The rapid uptake of monosaccharides by the epithelial cells tends to reduce the concentration of glucose in this boundary layer, so that absorption from the gut lumen becomes rate-limited by the relatively slow process of diffusion. The overall effect is to delay the assimilation of glucose and hence suppress the glycemic response to glucose or starchy foods in both healthy volunteers and in people with diabetes. A similar mechanism probably inhibits the reabsorption of cholesterol and bile salts in the distal ileum and this may account for the ability of some viscous types of soluble dietary fiber such as guar gum and β -glucan to reduce plasma cholesterol levels in humans. In one meta-analysis of randomized, controlled intervention trials it was shown that the soluble polysaccharides most commonly used in human intervention studies all modified plasma cholesterol levels to a similar though modest extent, which, in the case of oat β -glucans, amounted to a reduction of approximately 0.13 mmol l^{-1} (5.0 mg dl^{-1}) cholesterol for every 3 g of soluble fiber consumed per day.

The glycemic response to carbohydrate ingestion is the major determinant of insulin secretion, and glucose uptake also regulates the release of glucagon-like peptide 1 (GLP-1) and gastric inhibitory peptide (GIP), which are known as *incretins*. These are hormones that act to amplify the release of insulin by the pancreatic beta cell and to reduce secretion of glucagon by pancreatic alpha cells. The presence of dietary fiber in foods modulates this system. For example, the addition of viscous oat β -glucan to a test-beverage has been shown to suppress the absorption of glucose and to inhibit the release of insulin, GLP-1 and the peptide hormones cholecystokinin and PYY in humans. The importance of the viscosity of the polysaccharide is shown by the fact that these effects are abolished by hydrolysis of the β -glucan to smaller less viscous polymers. The potential significance of such endocrine effects lies in the regulatory role of PYY and other gut peptides in relation to intestinal motility and to appetite. However more research is needed to clarify their true physiological importance.

One of the main reasons for developing analytical methods to distinguish between soluble and insoluble components of dietary fiber is to provide a means of assessing the capacity of fiber-rich foods to influence carbohydrate and lipid metabolism. There is evidence that diets that provide 30–50% of their fiber in the form of soluble polysaccharides lead to lower cholesterol levels and better glycemic control than diets that contain mostly insoluble fiber. Several officially recognized sets of guidelines for patients with obesity, impaired glucose metabolism and its complications (*metabolic syndrome*) now recommend a high intake of carbohydrate foods that are rich in soluble fiber.

Some of the effects of cell wall polysaccharides in the small intestine involve specific chemical interactions with other food components. For example, there has been considerable

interest over a number of years in the possibility that the polysaccharides and complex phenolic components of cell walls contain polar groups that could interact with and bind ionized mineral species in the gastrointestinal contents, thereby reducing their availability for absorption. Intraluminal binding of heavy metals, toxins, and carcinogens might be a valuable protective mechanism, but binding of micronutrients could seriously compromise nutritional status. Interactions of this type can be shown to occur *in vitro*, and studies with animals and human ileostomists suggest that charged polysaccharides such as pectin can displace cations into the colon under experimental conditions. However, there is little objective evidence that dietary fiber *per se* has much of an adverse effect on mineral metabolism in humans. Indeed, highly fermentable polysaccharides and fructose oligosaccharides have recently been shown to promote the absorption of calcium and magnesium in both animal and human studies. The mechanism for the effect is not entirely clear, but it is probably a consequence of fermentation acidifying the luminal contents of the colon and enhancing carrier-mediated transport of minerals across the colonic mucosa.

In unprocessed legume seeds, oats, and other cereals phytate (myo-inositol hexaphosphate) is often present in close association with cell wall polysaccharides. Unlike the polysaccharides themselves, phytate does exert a potent binding effect on minerals, and has been shown to significantly reduce the availability of magnesium, zinc, and calcium for absorption in humans. Phytate levels in foods can be reduced by the activity of endogenous phytase, by hydrolysis with exogenous enzymes, or by fermentation. Dephosphorylated products may therefore be of benefit to individuals at risk of suboptimal mineral status. However, there are indications from animal and *in vitro* studies that phytate is an anticarcinogen that may contribute to the protective effects of complex fiber-rich foods. The overall significance of phytate in the diet therefore requires further assessment in human trials.

The Large Intestine

Microorganisms occur throughout the alimentary tract but in healthy individuals their numbers and diversity are maintained within strict limits by the combined effects of intraluminal conditions, rapid transit, and host immunity. The colon and rectum, however, are adapted to facilitate bacterial colonization, and the typical adult human colonic microflora has been estimated to contain approximately 400 different bacterial species. The largest single groups present are Gram-negative anaerobes of the genus *Bacteroides*, and Gram-positive organisms including bifidobacteria, eubacteria, lactobacilli, and clostridia. A large proportion of the species present cannot be cultured *in vitro* and are very poorly characterized, although this problem is being solved rapidly by the emergence of new and relatively inexpensive techniques for sequencing bacterial genomes. Most of the bacteria of the human colon utilize carbohydrate as a source of energy, although not all can degrade polysaccharides directly. It has been estimated that somewhere between 20 and 80 g of carbohydrates enter the human colon every day, about half of which is undigested

starch. Approximately 30 g of bacteria are produced for every 100 g of carbohydrate fermented.

Apart from dietary fiber, there are three major sources of unabsorbed carbohydrate for the colonic microflora. Perhaps the most important is resistant starch, which can be classified as physically inaccessible starch contained within unprocessed cell walls (RS1), resistant starch granules found in certain raw foods (RS2), retrograded amylose polymers found in foods such as potatoes and legumes that have been cooked and cooled (RS3), and chemically modified starches in manufactured foods (RS4). Nondigestible sugars, sugar alcohols, and oligosaccharides such as fructooligosaccharides and galactooligosaccharides occur only sparingly in most plant foods, but, as mentioned earlier, they are now much more common in human diets because of their use as *prebiotics* to selectively manipulate the numbers of bifidobacteria and other supposedly beneficial species in the human colon. Endogenous substrates including mucus are also important for the colonic microflora.

The beneficial effects of dietary fiber on the alimentary tract were emphasized by another of the founders of the dietary fiber hypothesis, Denis Burkitt, who based his arguments largely on the concept of fecal bulk, developed as a result of field observations in rural Africa, where cancer and other chronic bowel diseases were rare. His hypothesis was that populations consuming the traditional rural diets, rich in vegetables and cereal foods, produced bulkier, more frequent stools than persons eating the refined diets typical of industrialized societies. Chronic constipation was thought to cause straining of abdominal muscles during passage of stool, leading to prolonged high pressures within the colonic lumen and the lower abdomen. This in turn was thought to increase the risk of various diseases of muscular degeneration including varicose veins, hemorrhoids, hiatus hernia, and colonic diverticulas. Colorectal neoplasia was also thought to result from infrequent defecation, because it caused prolonged exposure of the colonic epithelial cells to mutagenic chemicals, which could initiate cancer. Epidemiological evidence continues to support a protective role of fiber against colorectal cancer, but the origins of intestinal neoplasia are now known to be far more complex than Burkitt was able to envisage.

Whatever the relationship to disease, it is certainly true that the consumption of dietary fiber is one major determinant of both fecal bulk and the frequency of defecation (bowel habit). However, the magnitude of the effect depends on the type of fiber consumed. Soluble cell wall polysaccharides such as pectin are readily fermented by the microflora, whereas lignified tissues such as wheat bran tend to remain at least partially intact in the feces. Both classes of dietary fiber can contribute to fecal bulk but by different mechanisms. The increment in stool mass caused by wheat bran depends to some extent on particle size, but in healthy Western populations it has been shown that for every 1 g of wheat bran consumed per day, the output of stool is increased by between 3 and 5 g. Other sources of dietary fiber also favor water retention. For example, isphagula, a mucilaginous material derived from *Psyllium*, is used pharmaceutically as a bulk laxative. Soluble polysaccharides such as guar and oat β -glucan are readily fermented by anaerobic bacteria, but solubility is no guarantee of fermentability, as is illustrated by modified

cellulose gums such as methylcellulose, which is highly resistant to degradation in the human gut. Fermentation reduces the mass and water-holding capacity of soluble polysaccharides considerably, but the bacterial cells derived from them do make some contribution to total fecal output. Thus, although all forms of dietary fiber are mild laxatives, the single analytical measurement of total fiber content again provides no simple predictive measure of physiological effect.

Although fermentation of fiber tends to reduce its effectiveness as a source of fecal bulk, it has other very important benefits. The absorption and metabolism of the short-chain fatty acids acetate, propionate, and butyrate derived from carbohydrate fermentation provides the route for the recovery of energy from undigested polysaccharides. Butyrate functions as the preferred source of energy for the colonic mucosal cells, whereas propionate and acetate are absorbed and metabolized systemically. Butyrate is of particular interest because of its ability to modify the expression of genes by acting as an inhibitor of histone deacetylase (HDAC) in a variety of cell types, including the epithelial cells of the colon. In *in vitro* models, butyrate causes differentiation of tumor cells, suppresses cell division, and induces programmed cell death (apoptosis). In principle, these effects might serve to suppress the development of cancer, but the true role of butyrate as a regulator of epithelial integrity in the human colon remains to be established.

The other major breakdown products of carbohydrate fermentation are hydrogen, methane, and carbon dioxide, which together comprise flatus gas. Excess gas production can cause distension and pain in some individuals, especially if they attempt to increase their fiber consumption too abruptly. In most cases, however, extreme flatus is probably caused more by fermentation of oligosaccharides such as stachyose and verbascose, which are found principally in legume seeds, rather than the cell wall polysaccharides themselves.

Conclusion

Several decades of research have confirmed that cell wall polysaccharides modify physiological mechanisms throughout the alimentary tract. Delayed absorption of glucose and lipids in the small intestine makes an important contribution to metabolic control in Type 2 diabetes, and certain types of hypercholesterolemia, respectively. Any loss of carbohydrates in the colon will lead to increased fermentative activity, and through this pathway, most of the unabsorbed energy will be recovered as short-chain fatty acids. Unfermented cell wall polysaccharides and increased bacterial mass contribute to

fecal bulk. All these established physiological effects, coupled with the possibility of using oligosaccharides as prebiotics to modify the colonic microflora, have greatly stimulated interest in nondigestible carbohydrates amongst food manufacturers and consumers in the past few years. There is little to suggest that conventional sources of fiber compromise micronutrient metabolism in otherwise healthy individuals, but the possibility of this and other adverse effects needs to be considered, as the use of novel polysaccharides as sources or analogs of dietary fiber, both for conventional products and for functional foods, continues to expand.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Carbohydrates: Requirements and Dietary Importance. Diabetes Mellitus: Dietary Management. Dietary Fiber: Physiological Effects and Health Outcomes; Role in Nutritional Management of Disease. Glycemic Index. Colon: Structure, Function, and Disorders

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Resistant Starch and Oligosaccharides

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Abbreviations

Fru	D-Fructose	NeuAc	N-Acetylneuraminic acid (or sialic acid)
Fuc	L-Fucose	NSP	Nonstarch polysaccharide
Gal	D-Galactose	RS	Resistant starch
Glc	D-Glucose	RS₁	Physically inaccessible starch
GlcNAc	N-Acetylglucosamine	RS₂	Resistant granules
HPAEC	High-performance anion exchange chromatography	RS₃	Retrograded starch
NDO	Nondigestible oligosaccharides	RS₄	Starch with nonstarch bonds
		SCFAs	Short-chain fatty acids

Glossary

Modified starch Starch with granular disorganization, polymer degradation, molecular rearrangement, oxidation, or chemical group addition produced by one or more physical, chemical, or enzymatic treatments.

Oligosaccharides Carbohydrates with 3–10 sugar units.

Resistant starch The fraction of starch that is not accessible to digestive enzymes of healthy humans.

Short-chain fatty acids Main products of carbohydrate fermentation by bacteria in the large intestine. The most abundant are acetate (2 carbons), propionate (3 carbons) and butyrate (4 carbons).

Resistant Starch

Definition

In 1992, a concerted action of European researchers defined resistant starch as 'the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals.' This concept completely changed our understanding of the action of carbohydrates in the diet because until the early 1980s, it was thought that starches were completely digested and absorbed in the human small intestine. Three important considerations are attached to this physiological definition. First, resistant starch is made up not only of high-molecular-weight polymers but also can include dextrans, small oligosaccharides, and even glucose, all derived from digested starch that escapes absorption. Second, resistant starches reach the human large intestine where they are metabolized by the complex colonic microbiota to produce a range of bioactive molecules with potential health benefits. Finally, the amount of resistant starch in a food (i.e., the amount reaching the colon) depends on the physiology of the individual and may be affected by age, by other components in the food matrix, and by disease in the gastrointestinal tract.

Classification and Dietary Sources

Food starches can be classified according to the way they are metabolized by the human small intestine into those rapidly

digested, those slowly digested, and those resistant to digestion. Similarly, resistant starch has been classified into three main types: physically inaccessible starch, resistant starch granules, and retrograded starch (**Table 1**).

Physically Inaccessible Starch (RS₁)

Type I resistant starch is physically inaccessible and is protected from the action of α -amylase, the enzyme that hydrolyzes the breakdown of starch in the human mouth and small intestine. This inaccessibility is due to the presence of plant cell walls that entrap the starch, for example, in legume seeds and partially milled and whole grains. RS₁ can also be found in highly compact processed food like pasta. The RS₁ content is affected by disruption of the food structure during processing (e.g., milling) and, to some extent, by chewing.

Resistant Granules (RS₂)

Starch granules are plant organelles where starch is produced and stored. Each plant has characteristic starch granules that differ in size, shape, amylose to amylopectin ratio, crystalline to amorphous material ratio, starch supramolecular architecture, and amylose–lipid complexes, among other features. It is believed that combinations of these factors make some granules more resistant to the attack of digestive enzymes than other granules. Type II resistant starch is found in unripe bananas, uncooked potatoes, and high-amylose starches. RS₂ disappears during cooking, especially in water, because a

Table 1 Classification of resistant starch

<i>Food source</i>	<i>Type^a</i>	<i>Content in food (g per 100 g)</i>	<i>Contribution to total RS intake</i>
Cereal products containing whole grains or grain fragments Brown breads Legumes Pastas Unripe bananas Uncooked potatoes High-amylose starches	RS ₁	1–9	Minor
Bread Cornflakes Cooked-cooled potatoes Legumes	RS ₂	17–75	Very little
Modified starches (both chemically and physically modified) Amylose–lipid complex	RS ₃ Other	1–10 Not known	Major Unknown

^aRS₁, Physically inaccessible starch; RS₂, resistant granules; RS₃, retrograded starch; RS₄, starch with nonstarch bonds.

combination of water and heat gelatinizes the starch, allowing more access to amylases.

Retrograded Starch (RS₃)

Type III resistant starch is the most abundant of the resistant starches present in food. It is formed during usual food processing by cooking and then cooling. When starch is cooked in excess water, it gelatinizes, i.e., the granular structure is disrupted, the granule swells, and amylose leaks out of the amylopectin matrix. Then when the food is cooled down, amylose (and more slowly amylopectin) recrystallizes to a new ordered and more compact structure (a process known as retrogradation), which decreases access to digestive enzymes. RS₃ production can be affected by the amylose to amylopectin ratio, amount of water and temperature during cooking, and the number of repeated cooking and cooling cycles. Retrograded starch can be found in bread, some brands of cornflakes, cooked-cooled potatoes, and legumes.

Other Sources of Resistant Starch

Amylose–lipid complex and modified starches have also been recognized as other sources of resistant starch (Table 1). Amylose–lipid complexes occur when fatty acids (12–18 carbons) are held within the helical structure of amylose. They are formed naturally during starch biosynthesis but may also be produced during cooking. Lipids may interfere with amylose retrogradation impairing the production of retrograded starch during processing. However, these complexes themselves have lower digestibility than cooked starch.

In addition to naturally resistant starch complexes, there are different types of modified starches that are manufactured by the food industry for a variety of reasons. They can be defined as native starches that have been submitted to one or more physical, chemical, or enzymatic treatments promoting granular disorganization, polymer degradation, molecular rearrangements, oxidation, or chemical group addition. According to their main physicochemical characteristics, modified starches can be classified into four main categories: pregelatinized, derivatized, cross-linked, and dextrinized

starches (Table 2). However, they are usually known as physically, chemically, or enzymatically modified starches because of the way they are produced (Table 3).

The digestibility of these modified starches is variable and depends on the type and extent of the treatment. Some authors have proposed a new category, type IV resistant starch (RS₄), to include chemically modified starches. Indeed, it has been shown that cross-linked starches have 15–19% decrease in *in vitro* digestibility compared with their native starches, and hydroxypropylated starch is only 50% digestible. However, some fractions of physically modified starches should also be considered as a category of resistant starch. Pregelatinized starches produced by drum drying and by extrusion have 3–6% and 5–11% decrease in digestibility, respectively. Part, but not all, of this reduction in digestibility is due to the formation of retrograded starch (RS₃). Moreover, stronger physical modifications, especially those produced by heating starches in a low-moisture environment, affect the starch digestibility even more. In fact, pyrodextrinization decreases starch digestibility by 55–65%. These resistant fractions are due to the occurrence of new nonstarch linkages, like (1→2) and (1→3) glycosidic bonds, in the pyrodextrinized starch that cannot be hydrolyzed by the enzymes in the gastrointestinal tract. Therefore, along with chemically modified starches, type IV resistant starch should also include physically modified starches. We propose the name ‘starch with nonstarch bonds’ for RS₄ (Table 1).

Impact of the Food Matrix

In addition to the starch properties already described, several starchy foods (for instance, cereals and legumes) have anti-nutritional factors, such as lectins, tannins, phytates, and enzyme inhibitors (both protease and amylase inhibitors). Amylase inhibitors present in raw pulses may reduce the activity of amylase in the human small intestine. However, most of these factors, especially enzyme inhibitors, are inactivated during food processing and cooking. Other components of the food matrix may also influence the digestibility of the starch such as the fat and dietary fiber content of the food that may influence transit through the stomach and small intestine and interfere with the mixing movements in the lumen.

Table 2 Classification of modified starches

Starch	Modifying agent	Physicochemical characteristic	Use in food
Pregelatinized	Extrusion Drum drying	Soluble in cold water	Cake and instant products
Derivatized	Acetyl Hydroxypropyl Phosphate	Stable at freeze–thawing cycles	Canned and frozen food
Cross-linked	Epichlorohydrin Trimetaphosphate	Stable at higher temperatures, extreme pH, and higher shear forces	Meat sauce thickeners Instant soup Weaning infant food Dressings
Dextrinized	Irradiation Heat Oxidizing agents Acid hydrolysis Amylolytic enzymes	Soluble in cold water Lower or nil viscosity	Chewing gums Jelly Syrups

Table 3 Methods of modified starch production

Treatment	Modification	Description
Physically modified	Pregelatinization Dextrinization	Starch paste is precooked and dried by extrusion or drum drying Starch polymers are hydrolyzed to smaller molecules by irradiation or by heat (pyrodextrinization)
Chemically modified	Derivatization ^a	Lateral groups are added to starch lateral chains
	Cross-linking ^a	Multifunctional groups are used to link two different starch molecules together
Enzymatically modified	Dextrinization	Starch polymers are hydrolyzed by oxidizing agents or by acid hydrolysis
	Dextrinization	Starch polymers are hydrolyzed to smaller molecules by incubation with amylases

^aDouble-derived starches are produced by combination of these two processes.

Analysis

The definition of resistant starch is based on its physiological behavior in the human small intestine. This means that resistant starch is a heterogeneous group of molecules from small oligosaccharides to large polymers with different molecular weight, degree of polymerization, and supramolecular architecture. This complexity makes it difficult to quantify accurately. All *in vitro* methods, therefore, need to be corroborated against *in vivo* models; however, *in vivo* models are also very difficult to validate.

In general, *in vitro* methods try to imitate human small intestine digestion using different sample preparation (i.e., milling, chewing, etc.), sample pretreatment (i.e., simulation of oral or stomach digestion), sample treatment (i.e., different enzyme mixtures), sample posttreatment (i.e., different resistant starch-solubilizing agents and enzyme mixtures), and incubation conditions (i.e., shaking/stirring, pH, temperature, and time) (Table 4). The choice of each of these multiple factors represents a huge analytical problem because not only does a compromise have to be made between physiological conditions and analytical handling but also the resistant starch content values must be in agreement with *in vivo* data.

However, in human *in vivo* methods, samples of digested food that reaches the end of the small intestine are taken for analysis, either from ileostomy patients (i.e., whose large intestine has been removed) or from healthy volunteers using special cannulas in the ileum. Animals can also be employed for *in vivo* experiments, such as gnotobiotic (i.e., germfree) and

pseudognotobiotic (i.e., antibiotic-treated) rats. In these cases, colonic bacterial fermentation is absent or suppressed by antibiotics and it is assumed that what reaches the end of the small intestine appears in feces. The main difficulty with these methods is that *in vivo* starch digestion may occur during the whole transit through the small intestine, which varies between individuals and the type of meals consumed. Moreover, these studies are difficult to perform in healthy volunteers, and the physiological significance of using ileostomy patients is debatable. For example, there may be some adaptation in the gut to the absence of a colon, the results may not relate to infants and children who have decreased digestive capacity, and there may be a wide range in digestion in the increasingly ageing population.

The initial *in vitro* assays were adapted from the enzymatic–gravimetric method used for dietary fiber assessment, but they could only measure RS₃. Soon, new approaches to assess other types of resistant starches were developed. The Berry method, for instance, measures both RS₃ and RS₂ using an exhaustive incubation (16 h) of milled sample with α -amylase and pullulanase, followed by centrifugation to separate the insoluble residue, which contains the resistant starch. This residue is treated with potassium hydroxide (KOH) to disperse retrograded and native starches and then hydrolyze them to glucose with amyloglucosidase. Finally, released glucose is quantified by a colorimetric assay. The Berry method has been subsequently modified by Faisant *et al.* and Goñi *et al.* by eliminating pullulanase from the enzyme mixture and adding a pretreatment with pepsin to decrease starch–protein interactions (Table 4).

Table 4 Comparison between different methods to measure resistant starch *in vitro*

Method	Sample			Treatment incubation		Types of RS measured ^a
	Preparation	Pretreatment	Treatment	Posttreatment		
Berry (1986)	Milling	None	Pancreatic α -amylase and pullulanase	KOH ^b Amyloglucosidase	Shaking for 16 h at 37 °C, pH 5.2	Sum of RS ₂ and RS ₃
Faisant, <i>et al.</i> (1995)	Same as above	Same as above	Same as above, but without pullulanase	Same as above	Same as above, but pH 6.9	Same as above
Gofri, <i>et al.</i> (1996)	Same as above	Pepsin	Same as above	Same as above	Same as above	Same as above
Englyst, <i>et al.</i> (1992)	Minced or as eaten	Same as above	Pancreatic α -amylase, amyloglucosidase, and invertase	Same as above	Shaking for 2 h at 37 °C, pH 5.2	RS ₁ , RS ₂ , RS ₃ , and total RS
Muir and O'Dea (1992)	Chewing	Salivary α -amylase then pepsin	Pancreatic α -amylase and amyloglucosidase	Thermostable α -amylase Dimethyl sulfoxide ^b Amyloglucosidase and pancreatic α -amylase	Stirring for 15 h at 37 °C, pH 5.0	Total RS
Akerberg, <i>et al.</i> (1998)	Same as above	Same as above	Same as above	KOH ^b Thermostable α -amylase	Stirring for 16 h at 40 °C, pH 5.0	Same as above
McCleary and Monaghan (2002)	Milling	None	Same as above	Amyloglucosidase KOH ^b Amyloglucosidase	Shaking for 16 h at 37 °C, pH 6.0	Sum of RS ₂ and RS ₃

^aRS, resistant starch; RS₁, physically inaccessible starch; RS₂, resistant granules; RS₃, retrograded starch.^bKOH and dimethyl sulfoxide are used as resistant starch-solubilizing agents.

Other methods have been developed to assess all types of resistant starches. Indeed, the Englyst method was developed to assess all nutritionally important starch fractions, such as rapidly digestible and slowly digestible starches, along with the three types of resistant starches initially described. In this method, resistant starch fractions are estimated altogether by difference between total and digestible starches. Sample preparation is kept to the minimum trying to mimic the way food is consumed. After a pretreatment with pepsin, the sample is incubated for more than 2 h with a mixture of amyloglucosidase, invertase, and pancreatic enzymes. Glucose released is then used to estimate the digestible starch. Next, total starch is measured as glucose released after solubilization of the nondigestible fractions with KOH, followed by amyloglucosidase hydrolysis. The Englyst method also allows evaluation of each of RS₁, RS₂, and RS₃. The main problem with this method is a low reproducibility between laboratories because of the need for careful calibration of waterbath shaking speeds, which requires skilled technical input. Two other methods of sample preparation include chewing by volunteer subjects. In the Muir method, for instance, the chewed sample is sequentially treated with pepsin and amyloglucosidase–pancreatic amylase mixture to obtain the nondigestible fraction, which is boiled with Termamyl (a thermostable α -amylase) and solubilized with dimethyl sulfoxide to yield finally glucose with another amyloglucosidase–pancreatic amylase mixture step. The Akerberg method is similar to Muir method, but it includes other steps that permit the estimation of available starch and dietary fiber along with total resistant starch (Table 4).

The most commonly used *in vitro* methods were extensively evaluated and a simplified version was proposed (McCleary method). Here, samples are treated with amyloglucosidase–pancreatic amylase mixture only and the insoluble residue, after washing with ethanol, is dispersed with KOH, followed by the amyloglucosidase step to yield glucose. This protocol has been accepted by AOAC International (AOAC method 2002.02) and the American Association of Cereal Chemists (AACC method 32-40) (Table 4). More recently, McCleary and coworkers have proposed new combined methods that measure resistant starch as part of the dietary fiber component of food along with oligosaccharides to give an overall value for ‘fiber’ as defined in the *Codex Alimentarius* definition discussed later.

Regarding the quantification of the resistant fractions in modified starches, care must be taken because some nondigestible fractions are soluble in water and can be lost during washing steps. This is particularly important with both pregelatinized starch and dextrinized by heat starch (pyrodextrin; Table 2). In addition, the presence of nonstarch linkages could impair the action of the enzyme mixtures used in the *in vitro* methods. One suitable way to look at the impact of the modification on the starch availability is to measure total starch before and after the modification.

Dietary Intake

It is very difficult to assess resistant starch intake at present because there are not enough data on the resistant starch content of foods. In addition, as the resistance of the starch to

digestion depends on the cooking method and the temperature of the food when eaten, the values gained from looking at old dietary intake data may be misleading. Despite this, an average value for resistant starch intake across Europe has been estimated as 4.1 g day⁻¹. Figures comparable with this estimation have been made in other countries, for instance, 4.3 g day⁻¹ in Venezuela and 3–8 g day⁻¹ in the United States, but in China intakes may be as high as 14.9 g day⁻¹. It is very difficult to separate the benefits of slowly, but completely, digestible starches from those that are resistant. In some groups, like small children, whose small intestinal digestive capacity is reduced, the very same food may provide more starch that is resistant to digestion than for adults.

Quantification of modified starch intake is even more difficult. First, food labels do not usually provide information about the nature of the modification used. Second, the commonly used method to estimate resistant starch (Table 4) can underestimate any nondigestible fractions that became soluble in water or formed nonstarch linkages because of the modification. At present, there are no data available about how much modified starch is eaten.

Fermentation in the Colon

The main nutritional properties of resistant starch arise from its potential fermentation in the colon. The diverse and numerous colonic microbiota ferments unabsorbed carbohydrates to short-chain fatty acids (SCFAs), mainly acetate, propionate, and butyrate, and gases (H₂, CO₂, and CH₄). Acetate is the main SCFA produced (50–70%) and is the only one to reach peripheral circulation in significant amounts, providing energy for muscle and other tissues. Propionate is the second most abundant SCFA and is mainly metabolized by the liver, where its carbons are used to produce glucose (via gluconeogenesis). Propionate has also been associated with reduced cholesterol and lipid synthesis and has been associated with increased release of satiety-related hormones. Finally, butyrate is mainly used as fuel by the colonic enterocytes but has been shown *in vitro* to have many potential anticancer actions, such as stimulating apoptosis (i.e., programmed cell death) and cancer cell differentiation (i.e., increasing expression of normal cell function), and inhibiting histone deacetylation (this protects the DNA). Resistant starch fermentation has been shown to increase the molar proportion of butyrate in the colon. Recently, *in vivo* studies have shown anticarcinogenic effects of resistant starch intake in rat, but there are still few studies to confirm these effects in human.

The main physiological effects of digestion and fermentation of resistant starch are summarized in Table 5. However, most of these effects have been observed with a resistant starch intake of approximately 20–30 g day⁻¹, which represents from five to seven times the estimated intake for most countries.

Oligosaccharides

Definition and Classification

Oligosaccharides are carbohydrate chains containing 3–10 sugar units. However, some authors also include

Table 5 Physiological effects of resistant starch intake

Energy	8–13 kJ g ⁻¹ ; cf. 17 kJ g ⁻¹ for digestible starches
Satiety	Increased satiety, may be mediated by colonic fermentation
Glycemic and insulinemic response	Depends on food, e.g., legumes (high in RS ₁) and amylase-rich starchy foods (which tend to produce RS ₃ on cooking) increase glucose tolerance, but cornflakes and cooked potatoes, both with high and similar glycemic indexes, have different resistant starch content
Lipid metabolism	Increases insulin sensitivity in humans Decreases plasma cholesterol and triacylglyceride levels in rat, but not in humans
Fermentability	Complete, although some RS ₃ are more resistant
SCFA production	Increased production, especially butyrate
CO ₂ and H ₂ production	Occurs
Colonic pH	Decreased, especially by lactate production
Bile salts	Deoxycholate, a secondary bile salt with cytotoxic activity, precipitated due to the low pH
Colon cell proliferation	Stimulated in proximal colon, but repressed in distal colon; may be mediated by butyrate
Fecal excretion	At high dose, fecal bulk increases due to an increase in bacteria mass and water retention
Transit time	Increased intestinal transit at high dose
Nitrogen metabolism	Increased bacterial nitrogen and biomass
Minerals	May increase calcium and magnesium absorption in large intestine
Disease prevention	Epidemiological studies suggest prevention against constipation and some <i>in vivo</i> studies suggest prevention against colorectal cancer, both in rat and human

carbohydrates with up to 20 residues or even disaccharides. Oligosaccharides can be made of any sugar monomers, but most research has been carried out on fructooligosaccharides (e.g., oligofructose) and galactooligosaccharides (e.g., raffinose, human milk oligosaccharides). Few oligosaccharides are hydrolyzed and absorbed in the small intestine (e.g., maltotriose), but nearly all enter the colon intact (nondigestible oligosaccharides). **Table 6** shows several examples of oligosaccharides (and disaccharides, for comparison purposes), their chemical structure, and source.

Dietary Sources and Intake

The first source of oligosaccharides in the human diet is mother's milk, which contains 5–10 g l⁻¹ unbound oligosaccharides. In human breast milk, there are more than 130 different oligosaccharides with both simple and complex structures. They are composed of galactose, fucose, sialic acid, glucose, and *N*-acetylglucosamine. Most are of low molecular weight, but a small proportion are of high molecular weight. Ninety percent of breast milk oligosaccharides are neutral; the remainder are acidic. Interestingly, the nature of these

oligosaccharide structures is determined by the mother's blood group. These oligosaccharides may have important function in the small intestine, where they can bind to the mucosa or to bacteria, interfering with pathogenic bacterial attachment and therefore acting as anti-infective agents. As they are nondigestible, they enter the colon and may act as a major source of energy for the colonic microbiota and promote the growth of typical lactic acid bacteria that are characteristic of the normal breast-fed infant, giving a prebiotic effect. Oligosaccharides (usually a mixture of galactooligosaccharides and fructooligosaccharides) have now been added to some infant formulas to mimic the actions of those in human milk. Several studies have now shown that these promote the growth of bifidobacteria in feces and make the stools more like those of breast-fed infants in terms of consistency, frequency, and pH, and may reduce the severity of eczema in affected infants.

In adults, the main dietary sources of oligosaccharides are chicory, artichokes, onions, garlic, leeks, bananas, and wheat. However, much research has been carried out on purified or synthetic oligosaccharide mixtures, mostly fructooligosaccharides derived from inulin and galactooligosaccharides. The normal dietary intake of oligosaccharides is difficult to estimate, as they are not a major dietary component. Approximately 3 g day⁻¹ has been suggested in the European diet. However, with the increasing information on the health benefits of isolated oligosaccharide sources (see later), they are being incorporated into functional foods and labeled as prebiotics.

Analysis

In general, oligosaccharides are a less heterogeneous group of compounds than resistant starches. Almost all nondigestible oligosaccharides (some fructooligosaccharides are an exception) are soluble in 80% (v/v) ethanol solution, which makes them relatively easy to isolate from insoluble components. Liquid chromatography, more specifically high-performance anion exchange chromatography (HPAEC), has been extensively employed not only to separate mixture of different oligosaccharides but also to separate, identify, and quantify individual carbohydrate moieties after appropriate hydrolysis of the oligosaccharide to its individual monomers. A more comprehensive study of the oligosaccharide structure can be achieved using more sophisticated techniques such as nuclear magnetic resonance and mass spectrometry. However, from a nutritional viewpoint, where simpler methods are needed for quality control and labeling purposes, HPAEC is usually applied to quantify the monomers (and dimers) present before and after hydrolysis of the studied oligosaccharide with appropriate enzymes and then the oligosaccharide level is worked out by difference. As with resistant starch, McCleary and coworkers have included the analysis of oligosaccharides in their recently published combined method for dietary fiber.

Fermentation in the Colon and Health Benefits

Most oligosaccharides escape digestion in the small intestine and are fermented by the colonic bacteria. They are rapidly

Table 6 Chemical structure and source of sugars and oligosaccharides

<i>Common name</i>	<i>Simplified structure^a</i>	<i>Source</i>	<i>NDO^b</i>
Sugars (disaccharides)			
Lactose	Gal β 1 \rightarrow 4Glc	Milk, milk products	No
Maltose	Glc α 1 \rightarrow 4Glc	Glucose syrups, hydrolysis of starch	No
Sucrose	Fru β 2 \rightarrow 1Glc	Table sugar	No
Cellobiose	Glc β 1 \rightarrow 4Glc	Hydrolysis of cellulose	Yes
Trehalose	Glc α 1 \rightarrow 1Glc	Mushrooms, yeast	No
Melibiose	Gal α 1 \rightarrow 6Glc	Hydrolysis of raffinose	Yes
Gentiobiose	Glc β 1 \rightarrow 6Glc β	Plant pigments, like saffron	Yes
Trisaccharides			
Maltotriose	Glc α 1 \rightarrow 4Glc α 1 \rightarrow 4Glc	Glucose syrups, hydrolysis of starch	No
Umbelliferose	Gal α 1 \rightarrow 2Glc α 1 \rightarrow 2Fru β	Plant tissues	Yes
Raffinose	Gal α 1 \rightarrow 6Glc α 1 \rightarrow 2Fru β	Legume seeds	Yes
Planteose	Gal α 1 \rightarrow 6Fru β 2 \rightarrow 1Glc	Plant tissues	Yes
Sialyl α (2-3)lactose	NeuAc α 2 \rightarrow 3Gal β 1 \rightarrow 4Glc	Human milk	Yes
Tetrasaccharides			
Stachyose	Gal α 1 \rightarrow 6Gal α 1 \rightarrow 6Glc α 1 \rightarrow 2Fru β	Legume seeds	Yes
Lychnose	Gal α 1 \rightarrow 6Glc α 1 \rightarrow 2Fru β 1 \rightarrow 1Gal	Plant tissues	Yes
Isolychnose	Gal α 1 \rightarrow 6Glc α 1 \rightarrow 2Fru β 3 \rightarrow 1Gal	Plant tissues	Yes
Sesamose	Gal α 1 \rightarrow 6Gal α 1 \rightarrow 6Fru β 2 \rightarrow 1Glc	Plant tissues	Yes
Pentasaccharides			
Verbascose	Gal α 1 \rightarrow 6Gal α 1 \rightarrow 6Gal α 1 \rightarrow 6Glc α 1 \rightarrow 2Fru β	Plant tissues	Yes
Lacto- <i>N</i> -fucopentaose I	Fuc α 1 \rightarrow 2Gal β 1 \rightarrow 3GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4Glc	Human milk	Yes
Lacto- <i>N</i> -fucopentaose II	Gal β 1 \rightarrow 3[Fuc α 1 \rightarrow 4]GlcNAc β 1 \rightarrow 3Gal β 1 \rightarrow 4Glc	Human milk	Yes
Fructans			
Oligofructose	[Fru β 2 \rightarrow 1]Fru β 2 \rightarrow 1Glc with 1–9 [Fru β 2 \rightarrow 1] residues	Hydrolysis of inulin or synthesis from Yes sucrose	
Inulin (It is a polysaccharide, not an oligosaccharide)	[Fru β 2 \rightarrow 1]Fru β 2 \rightarrow 1Glc with 10–64 [Fru β 2 \rightarrow 1] residues	Artichokes	Yes

^aFru, D-fructose; Fuc, L-fucose; Gal, D-galactose; Glc, D-glucose; GlcNAc, N-acetylglucosamine; NeuAc, N-acetylneuraminic acid (or sialic acid).

^bNDO, nondigestible oligosaccharides.

fermented resulting in a low pH and have been shown to increase the survival of the so-called probiotic organisms, i.e., lactobacilli and bifidobacteria. Probiotic bacteria have been shown to have strain-specific effects, including reduction in duration of rotavirus and other infective diarrhea and reduction in symptoms of atopic eczema. They may also have some anticarcinogenic effects, but these have not been demonstrated in human *in vivo* studies. This action of oligosaccharides to promote the growth of bifidobacteria and lactobacilli defines them as prebiotics. Some studies investigate the synergistic effects of probiotics mixed with prebiotics. These mixtures are termed synbiotics. Oligosaccharides also have similar health benefits to fermentable dietary fiber and resistant starch by increasing colonic fermentation, production of SCFA (especially butyrate), and reduction in colonic pH, and increasing magnesium and calcium absorption in the colon.

Resistant Starch, Oligosaccharides, or Just Dietary Fiber?

There has been much debate on the definition of dietary fiber over the last few decades and, in particular, whether it should include carbohydrates other than nonstarch polysaccharides.

It is increasingly recognized that oligosaccharides, resistant starch, and nonstarch polysaccharides are very similar especially in their effects on gut physiology and colonic fermentation. A comparison of their actions is summarized in [Table 7](#).

The AACC proposed a new definition of dietary fiber in 2001, which would include both oligosaccharides and resistant starch as well as associated plant substances. This definition also required complete or partial fermentation and demonstration of physiological effects such as laxation, and reduction in blood glucose or blood cholesterol. A similar approach to include beneficial physiological effects was also proposed by the Food and Nutrition Board of the US Institute of Medicine in 2002, but they divided the definition into dietary fiber (from plant-based foods) and functional fiber to include added nondigestible carbohydrates with demonstrable health benefits. The latest *Codex Alimentarius* definition of dietary fiber (published in 2009) has a similar division of types of fiber: natural components of foods, extracted components that are added to other foods, and synthetic fibers added to foods. Resistant starch was included in this definition, but the inclusion of oligosaccharides (DP 3–9) was left optional for individual country authorities. There was also a requirement for health benefits for added fibers, but they were not defined and are now open to different interpretations. This

Table 7 The physiological effects of resistant starch, oligosaccharides, and dietary fiber

Physiological effect	Resistant starch	Oligosaccharides	Dietary fiber
Energy supply	8–13 kJ g ⁻¹	8–13 kJ g ⁻¹	8–13 kJ g ⁻¹
Increased glucose tolerance	Some foods	No	Some NSP ^a
Decreased plasma cholesterol and triacylglyceride levels	No	Not known	Some NSP
Fermentability	Complete	Complete	Variable
Production of SCFA	Yes	Yes	Yes
Increased butyrate production	High	High	Variable
CO ₂ and H ₂ production	Yes	Yes	Variable
Decreased fecal pH	Yes	Yes	Some NSP
Decreased production of deoxycholate	Yes	Yes	Some NSP
Increased colonocyte proliferation	Yes	Yes	Yes
Increased fecal bulk	At high dose	No	Variable
Faster whole gut transit time	At high dose	No	Yes
Increased bacterial nitrogen and biomass	Yes	Yes	Yes
Reduced mineral absorption in small intestine	No	No	Some NSP
Increased mineral absorption in large intestine	Yes	Yes	Some NSP
Possible prevention of colorectal cancer	Yes	Not known	Yes

^aNSP, nonstarch polysaccharide.

new definition of dietary fiber has led again to some debate among fiber experts.

See also: Breast Feeding. Cancer: Carcinogenic Substances in Food; Dietary Management; Epidemiology and Associations Between Diet and Cancer; Epidemiology of Gastrointestinal Cancers Other than Colorectal Cancers; Epidemiology of Lung Cancer. **Carbohydrates:** Chemistry and Classification; Regulation of Metabolism; Requirements and Dietary Importance. **Celiac Disease.** **Cereal Grains.** **Dietary Fiber:** Physiological Effects and Health Outcomes. **Fiber:** Physiological and Functional Effects; Role in Nutritional Management of Disease. **Legumes.** **Microbiota of the Intestine:** Prebiotics; Probiotics. **Colon:** Structure, Function, and Disorders. **Whole Grains**

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FISH AND SEAFOOD

Nutritional Value

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Glossary

Cephalopods Aquatic marine molluscs with a prominent head and tentacles, such as squids, cuttlefishes, and octopuses.

Crustaceans Invertebrate animals that have a hard exoskeleton and two pairs of antennae, such as lobsters, shrimps, crabs, and barnacles.

Dietary reference intakes (DRIs) Set of four reference values related to the daily dietary intake of nutrients of healthy individuals in a particular life stage and gender group: Estimated average requirements (EAR), recommended dietary allowances (RDA), adequate intakes (AI), and tolerable upper intake levels (UL).

Elasmobranchs Aquatic vertebrate with fins and a cartilaginous skeleton, such as sharks, rays, and skates.

Molluscs (mollusks) Invertebrate animals with a soft unsegmented body, usually enclosed in a calcareous shell, such as clams, oysters, and whelks.

Surimi A fish-based food product processed to resemble the texture, color, and flavor of the meat of lobster, crab, and other seafood.

Teleosts Aquatic vertebrate with fins and a bony skeleton, such as cod, tuna, salmon, sole, and sardines.

Triacylglycerol (triglyceride) Any of a group of lipids formed from glycerol and three fatty acids, which can be saturated, monounsaturated, or polyunsaturated.

Introduction

In discussing the food uses of fishes, the term 'fish' refers to edible species of finfish, molluscs, and crustaceans coming from the marine or freshwater bodies of the world, either by capture fisheries or by aquaculture. Accordingly, 'fishery products' means any human food product in which fish is a characterizing ingredient, such as dried, salted, and smoked fish, marinated fish, canned seafood, minced fish flesh such as surimi, and miscellaneous products.

Fish have always been important in the diets of those communities living close to the sea, rivers, and lakes. The development of on-board refrigeration and freezing on fishing vessels as well as refrigerated transport, has improved both the quality and shelf-life of fish and its availability to the general consumer. The development of attractive processed products has also contributed to the widening of fish consumption. According to Food and Agriculture Organization figures, capture fisheries and aquaculture supplied the world with approximately 110 million tons of food fish in 2006, providing an apparent per capita supply of 16.7 kg. Of this total, aquaculture accounted for 47%.

Edible fish muscle contains 18–20% protein and 1–2% minerals; the percentage of lipids varies from less than 1% to more than 20% (in high-fat finfish), and fish has the added advantage of being low in saturated fat. In general, lean fish is not an important source of calories, which are mostly obtained from the staple carbohydrates in the diet. Fatty fish, however, is a significant energy source in many fish-consuming communities in both the developed and the developing worlds. Today it is recognized that fish is probably more important as a source of micronutrients,

minerals, and particularly essential fatty acids than for its energy or protein value. The essential micronutrients and minerals in fish include vitamins A and D, calcium, phosphorus, magnesium, iron, zinc, selenium, fluorine, and iodine (in marine fishes).

Several studies have demonstrated beneficial effects of a diet including two or three servings of fish per week on recognized cardiovascular risk factors, such as a reduction of plasma triacylglycerol concentrations, blood pressure, and platelet aggregation (thrombogenesis). These benefits have been attributed to the long-chain omega-3 fatty acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA), which are found primarily in fatty fish such as salmon, tuna, herring, mackerel, and sardines.

Food-borne diseases attributed to fish can result from the fish itself (i.e., toxic species, allergies) or from bacterial (i.e., *Clostridium botulinum*, *Listeria monocytogenes*, *Salmonella*, *Vibrio*, and *Staphylococcus*), viral (i.e., hepatitis, Norwalk gastroenteritis), or parasitic (i.e., *Anisakis* and related worms) contamination. Also, naturally occurring seafood toxins (i.e., scombrototoxin, ciguatera toxins, shellfish poisoning from toxic algae) can cause food-borne illnesses. In recent years, reports of contamination of fish by chemical residues have raised concerns about the healthfulness of certain fish for vulnerable groups of the population.

General Characteristics of Finfish

A very large number of species of finfish are used for food by the world's population. The carcass yield of finfish (60–70%) is similar to that of beef, pork, or poultry. The percentage of

edible tissue in the dressed carcasses of finfish (without head, skin, and viscera) is higher than that of other food animals, because fishes contain less bone, adipose tissue, and connective tissue. There are three main categories of vertebrate fish that are widely used as foods. The bony fishes (teleosts) provide two compositional categories: white fishes (or lean fishes) and fatty fish. The third category is the cartilaginous elasmobranch fishes.

White Fish

The flesh of these fishes is very low in fat and consists primarily of muscle and thin layers of connective tissue. The concentrations of most of the B vitamins are similar to those in mammalian lean meats, although fish may contain higher amounts of vitamins B₆ and B₁₂. The mineral levels are also similar, although the very fine bones that are eaten with the fish flesh can raise the calcium content; fish is also a significant source of iodine. These fishes (i.e., cod) accumulate oils only in their livers, which are a rich source of vitamin A (retinol), vitamin D, and long-chain PUFAs in their triacylglycerols.

Fatty Fish

These fishes (i.e., mackerel, herring) have fat in their flesh, which is usually much darker than that of white fishes, with similar blocks of muscle and connective tissue. The amount of fat is related to the breeding cycle of the fish, so that the fat content falls considerably after breeding. The flesh of fatty fishes is generally richer in the B vitamins than that of white fishes, and significant amounts of vitamins A and D are present. The mineral concentrations are not very different, but fatty fish is a better source of iron. The oil of these fishes is particularly rich in very-long-chain PUFA, especially those of the omega-3 (n-3) series such as EPA, and DHA. These fishes accumulate oils in their muscles, belly flap, and skin (subdermal fat).

Cartilaginous Fish

The cartilaginous fishes include the sharks and rays, whose flesh is rich in connective tissue and relatively low in fat, although they do accumulate oils in their livers. The concentrations of vitamins and minerals are very similar to those in white fish. These fishes contain urea in relatively large amounts, and so protein values based on total nitrogen are overestimated. The ammonia smell of cooked sharks and rays is not an indication that the fish is spoiled but rather is the result of enzymatic degradation of urea.

General Characteristics of Shellfish

The term 'shellfish' includes any aquatic invertebrate, such as molluscs or crustaceans, which has a shell or shell-like exoskeleton. The cephalopods have an internal shell (as in squids) or no shell (as in octopods). Owing to the presence of the tough exoskeleton, the edible portion in shellfish

(approximately 40%) is less than that in finfish, with the exception of cephalopods, whose edible yield is 70–75%. The lipid content of the edible parts of most shellfish is low, as bivalves store their energy surplus as glycogen and not as depot fat, whereas crustaceans and cephalopods store their fat in their digestive glands (hepatopancreas). In many fish-eating communities, these foods are very highly valued gastronomically.

Molluscs and Cephalopods

A wide range of molluscs are eaten by man, including bivalves (such as mussels, oysters, and scallops), gastropods (such as winkles and whelks), and cephalopods (such as squids and octopuses). The flesh is muscular with low levels of fat, although the fat is more saturated and richer in cholesterol than that of finfish. The mineral levels in shellfish are usually somewhat higher than those in finfish, and the vitamin concentrations are low. Bivalves and gastropods are often eaten whole after boiling or sometimes raw; usually, only the muscular mantles of cephalopods are eaten. In some cultures, only selected parts are eaten; for example, only the white adductor muscle of the scallop is eaten in North America. Bivalve molluscs are filter feeders and can be contaminated by toxins and pathogenic organisms from the sea water.

Crustaceans

Crustaceans include a range of species, both freshwater (such as crayfish) and marine (such as crabs, shrimps, prawns, and lobsters). These animals have a segmented body, a chitinous exoskeleton, and paired jointed limbs. The portions eaten are the muscular parts of the abdomen and the muscles of the claws of crabs and lobsters. The flesh is characteristically low in fat and high in minerals, with vitamin levels similar to those found in finfish.

Nutritional Value of Fish and Shellfish

Fish and shellfish are excellent sources of protein. A 100 g cooked serving of most types of fish and shellfish provides approximately 18–20 g of protein, or about a third of the average daily recommended protein intake. The fish protein is of high quality, containing an abundance of essential amino acids, and is very digestible by people of all ages. Seafood is also loaded with minerals such as phosphorus, magnesium, iron, zinc, and iodine in marine fish.

The caloric value of fish is related to the fat content and varies with species, size, diet, and season. Seafood is generally lower in fat and calories than beef, poultry, or pork. Most lean or low-fat species of fish, such as cod, hake, flounder, and sole, contain less than 100 kcal (418 kJ) per 100 g portion, and even fatty fish, such as mackerel, herring, and salmon, contain approximately 250 kcal (1045 kJ) or less in a 100 g serving. Most crustaceans contain less than 1% fat in the tail muscle because depot fat is stored in the hepatopancreas, which is in the head region.

Fish Lipids

In fish, depot fat is liquid at room temperature (oil) and is seldom visible to the consumer; an exception is the belly flaps of salmon steaks. Many species of finfish and almost all shellfish contain less than 2.5% total fat, and less than 20% of the total calories come from fat. Almost all fish has less than 10% total fat, and even the fattiest fish, such as herring, mackerel, and salmon, contains no more than 20% fat (Table 1). In order to obtain a good general idea of the fat contents of most finfish species, flesh color might be considered. The leanest species, such as cod and flounder, have a white or lighter color, whereas fattier fishes, such as salmon, herring, and mackerel, have a much darker color.

The triacylglycerol depot fat in edible fish muscle is subject to seasonal variation in all marine and freshwater fishes from all over the world. Fat levels tend to be higher during times of the year when fishes are feeding heavily (usually during the warmer months) and in older and healthier individual fishes. Fat levels tend to be lower during spawning or reproduction. When comparing fat contents between farmed and wild-caught food fish, it should be remembered that farmed species have a tendency to show a higher proportion of muscle fat than their wild counterparts. Also, the fatty-acid composition of farmed fish depends on the type of dietary fat used in raising the fish. Cholesterol is independent of fat content and is similar in wild and cultivated fishes.

Most protein-rich foods, including red meat and poultry as well as fish, contain cholesterol. However, almost all types of

fish and shellfish contain well under 100 mg of cholesterol per 100 g, and many of the leaner types of fish typically have 40–60 mg of cholesterol in each 100 g of edible muscle. It is known that most shellfish also contain less than 100 mg of cholesterol per 100 g. Shrimp contain somewhat higher amounts of cholesterol, over 150 mg per 100 g, and squid is the only fish product with a significantly elevated cholesterol content, which averages 300 mg per 100 g portion. Fish roe, caviar, internal organs of fishes (such as livers), the tomalley of lobsters, and the hepatopancreas of crabs can contain high amounts of cholesterol.

Omega-3 PUFA in Fish and Shellfish

The PUFA of many fish lipids are dominated by two members of the omega-3 (*n*-3) family, C20:5 *n*-3 (EPA), and C22:6 *n*-3 (DHA). They are so named because the first of several double bonds occurs three carbon atoms away from the terminal end of the carbon chain.

All fish and shellfish contain some omega-3, but the amount can vary, as their relative concentrations are species specific (Table 2). Generally, the fattier fishes contain more omega-3 fatty acids than the leaner fishes. The amount of omega-3 fatty acids in farm-raised products can also vary greatly, depending on the diet of the fishes or shellfish. Many companies now recognize this fact and provide a source of omega-3 fatty acids in their fish diets. Omega-3 fatty acids can be destroyed by heat, air, and light, so the less processing, heat, air exposure, and storage time the better for preserving omega-3 in fish. Freezing and normal cooking cause minimal omega-3 losses, whereas deep frying and conditions leading to oxidation (rancidity) can destroy some omega-3 fatty acids.

Research has shown that EPA and DHA are beneficial in protecting against cardiovascular diseases, through the

Table 1 Fat levels in marine and freshwater fish and shellfish commonly found in the marketplace

<i>Low (< 2.5% fat), less than 20% of total calories from fat</i>	<i>Medium (2.5–5% fat), between 20% and 35% of total calories from fat</i>	<i>High (> 5% fat), between 35% and 50% total calories from fat</i>
Saltwater fish		
Cod	Anchovy	Dogfish
Grouper	Bluefish	Herring ^a
Haddock	Sea bass	Mackerel ^a
Hake	Swordfish	Salmon ^a
Most flatfishes (flounder, sole, plaice)	Tuna (yellowfin)	Sardine
Pollock		Tuna (bluefin)
Shark, skate		
Snapper		
Whiting		
Most crustaceans		
Most molluscs		
Freshwater fish		
Pike	Bream	Catfish (farmed)
Perch, bass	Carp	Eel ^a
Tilapia	Trout (various)	Whitefish

^aMore than 10% fat.

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

Table 2 Selected fish and shellfish grouped by their omega-3 fatty-acid content

<i>Low-level group (< 0.5 g per 100 g)</i>	<i>Medium-level group (0.5–1 g per 100 g)</i>	<i>High-level group (> 1 g per 100 g)</i>
Finfish		
Carp	Bass	Anchovy
Catfish	Bluefish	Herring
Cod, Haddock, Pollock	Halibut	Mackerel
Grouper	Pike	Sablefish
Most flatfishes	Red Snapper	Salmon (most species)
Perch	Swordfish	Tuna (bluefin)
Snapper	Trout	Whitefish
Tilapia	Whiting	
Shellfish		
Most crustaceans	Clams	
Most molluscs	Oysters	

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

reduction in serum triacylglycerides, blood pressure and platelet aggregation (thrombogenesis). Brain, nervous tissue membranes, and the retina of the eye contain a high proportion of DHA. Limited evidence from studies of human infants suggests that learning ability and retinal function may be impaired if there is an insufficient intake of DHA in the mother's diet during pregnancy and lactation.

Fish Proteins

Both finfish and shellfish are highly valuable sources of proteins in human nutrition, supplying approximately 7.9% of the world's protein requirements and 15.3% of the total animal protein.

The protein content of fish flesh, in contrast to the fat content, is highly constant, independent of seasonal variations caused by the feeding and reproductive cycles, and shows only small differences among species. **Table 3** summarizes the approximate protein contents of the various finfish and shellfish groups. Fatty finfish and crustaceans have slightly higher than average protein concentrations. Bivalves have the lowest values if the whole body mass is considered (most of them are usually eaten whole), whereas values are roughly average if specific muscular parts alone are consumed; this is the case with the scallop, in which only the adductor muscle is usually eaten.

The essential amino acid compositions of fish and shellfish are given in **Table 4**. Fish proteins, with only slight differences among groups, possess a high nutritive value, similar to that of meat proteins and slightly lower than that of egg. It is worth pointing out the elevated supply, relative to meat, of essential amino acids such as lysine, methionine, and threonine. In addition, owing in part to the low collagen content, fish proteins are easily digestible, giving rise to a digestibility coefficient of nearly 100.

The recommended dietary allowance (RDA) of protein for human male and female adults is in the range of 45–65 g day⁻¹. In accordance with this, an intake of 100 g of fish would contribute 15–25% of the total daily protein requirement of healthy adults and 70% of that of children. A look at the dietary importance of the Mediterranean diet is convenient: one of its characteristics is the high consumption of all kinds of fish, chiefly fatty fish. In many Mediterranean countries, fish intake averages greater than 50 g day⁻¹ (edible flesh);

thus, fish protein contributes greater than 10% of the total daily protein requirements steadily over the whole year in those countries.

Nonprotein Nitrogen (NPN) Compounds in Fish

NPN compounds are found mostly in the fiber sarcoplasm and include free amino acids, peptides, amines, amine oxides, guanidine compounds, quaternary ammonium molecules, nucleotides, and urea (**Table 5**). NPN compounds account for a relatively high percentage of the total nitrogen in the muscles of some aquatic animals, 10–20% in teleosts, approximately 20% in crustaceans and molluscs, and 30–40% (and in special cases up to 50%) in elasmobranchs. In contrast, NPN compounds in land animals usually represent no more than 10% of total nitrogen.

Most marine fishes contain trimethylamine oxide (TMAO); this colorless, odorless, and flavorless compound is degraded to trimethylamine, which gives a 'fishy' odor and causes consumer rejection. This compound is not present in land animals and freshwater species (except for Nile perch and tilapia from Lake Victoria). TMAO reductase catalyzes the reaction and is found in several fish species (in the red muscle of scombroid fishes and in the white and red muscle of gadoids) and in certain microorganisms (*Enterobacteriaceae*, *Shewanella putrefaciens*).

Migratory marine species such as tuna, characterized by a high proportion of red muscle, have a high content (approximately 1%) of free histidine. The presence of free histidine is relevant in several fish species because it can be microbiologically decarboxylated to histamine, which cannot be inactivated by cooking, thus becoming a hazard to consumers. The symptoms of the resulting illness (scombroid poisoning) are itching, redness, allergic symptoms, headache, diarrhea, and peppery taste. Scombroid poisoning is most common after ingesting mahi-mahi, tuna, bluefish, mackerel, and skipjack.

Nucleotides and related compounds have a noticeable participation in flavor; moreover, some of them may be used as freshness indices. Adenosine triphosphate (ATP), adenosine diphosphate, and adenosine monophosphate decompose quickly leading to a build-up of inosine and hypoxanthine. As this corresponds well to a decline in freshness, the ratio of the quantity of inosine and hypoxanthine to the total quantity of ATP and related substances is called the *K*-value and used as a freshness index of fish meat.

Guanosine is an insoluble compound that gives fish eyes and skin their characteristic brightness. It is degraded to guanine, which does not have this property; therefore, brightness decreases until it completely disappears.

The NPN fraction contains other interesting compounds, such as small peptides. Most of them contribute to flavor; besides this, they have a powerful antioxidant activity. Betaines are a special group of compounds that contribute to the specific flavors of different aquatic organisms: homarine in lobster and glycine-betaine, butiro-betaine, and arsenic-betaine in crustaceans. Arsenic-betaine has the property of fixing arsenic into the structure, giving a useful method for studying water contamination.

Table 3 Protein content of the different groups of fish and shellfish

Fish group	g per 100 g
White finfish	16–19
Fatty finfish	18–21
Crustaceans	18–22
Bivalves	10–12
Cephalopods	16–18

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

Table 4 Content of essential amino acids in fish and shellfish (g per 100 g of protein)

Fish group	Isoleucine	Leucine	Lysine	Methionine	Phenylalanine	Threonine	Tryptophan	Valine
Finfish	5.3	8.5	9.8	2.9	4.2	4.8	1.1	5.8
Crustaceans	4.6	8.6	7.8	2.9	4.0	4.6	1.1	4.8
Molluscs	4.8	7.7	8.0	2.7	4.2	4.6	1.3	6.2

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

Table 5 Nonprotein nitrogen compounds in several commercially important fish species and mammalian muscle (mg per 100 g wet weight)

Compounds	Cod	Herring	Shark species	Lobster	Mammal
Total NPN	1200	1200	3000	5500	3500
Total free amino acids	75	300	100	3000	350
Arginine	<10	<10	<10	750	<10
Glycine	20	20	20	100–1000	<10
Glutamic acid	<10	<10	<10	270	36
Histidine	<1.0	86	<1.0	-	<10
Proline	<1.0	<1.0	<1.0	750	<1.0
Creatine	400	400	300	0	550
Betaine	0	0	150	100	-
Trimethylamine oxide	350	250	500–1000	100	0
Anserine	150	0	0	0	150
Carnosine	0	0	0	0	200
Urea	0	0	2000	-	35

NPN, nonprotein nitrogen.

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

Fish Vitamins

The vitamin content of fish and shellfish is rich and varied in composition, although somewhat variable in concentration. In fact, significant differences are neatly evident among groups, especially regarding fat-soluble vitamins. Furthermore, vitamin content shows large differences among species as a function of feeding regimes.

The approximate vitamin concentration ranges of the various finfish and shellfish groups are summarized in **Table 6**. The RDA for adults is also given, together with the percentage supplied by 100 g of fish. Of the fat-soluble vitamins, vitamin E (tocopherol) is distributed most equally, showing relatively high concentrations in all fish groups, higher than those of meat. However, only a part of the vitamin E content is available as active tocopherol on consumption of fish, because it is oxidized in protecting fatty acids from oxidation. The presence of vitamins A (retinol) and D is closely related to the fat content, and so they are almost absent in most low-fat groups. Appreciable but low concentrations of vitamin A are found in fatty finfish and bivalve molluscs, whereas vitamin D is very abundant in fatty fish. In fact, 100 g of most fatty species supply over 100% of the RDA of this vitamin.

Water-soluble vitamins are well represented in all kinds of fish, with the sole exception of vitamin C (ascorbic acid), which is almost absent in all of them. The concentrations of the rest are highly variable; however, with few exceptions, they constitute a medium-to-good source of such vitamins, comparable with, or even better than, meat. The contents of vitamins B₂ (riboflavin), B₆ (pyridoxine), niacin, biotin, and B₁₂

(cobalamin) are relatively high. Indeed, 100 g of fish can contribute up to 38%, 60%, 50%, 33%, and 100%, respectively, of the total daily requirements of those vitamins. Fatty fish also provides a higher supply of many of the water-soluble vitamins (namely pyridoxine, niacin, pantothenic acid, and cobalamin) than does white fish or shellfish. Crustaceans also possess a relatively higher content of pantothenic acid, whereas bivalve molluscs have much higher concentrations of folate and cobalamin.

A Mediterranean diet rich in fish – and especially in fatty finfish – contributes steadily over the year to an overall balanced vitamin supply. The last row of **Table 6** illustrates this; the supply of vitamins D, B₂, B₆, B₁₂, and niacin from this particular diet is more than 15% of the daily requirements; all other vitamins, except ascorbic acid, are supplied to a lesser, but significant, extent.

Fish Minerals

The approximate amounts of selected minerals contained in fish are given in **Table 7**. The first point to note is that all kinds of finfish and shellfish present a well-balanced content of most minerals, either macrominerals or trace elements, with only a few exceptions. Sodium content is low, as in other muscle and animal origin foods. However, it must be remembered that sodium is usually added to fish in most cooking practices in the form of common salt; also, surimi-based and other manufactured foods contain high amounts of added sodium.

Table 6 Vitamin content of the different groups of fish and shellfish (mg or μg per 100 g), and relation to DRIs

	A (μg)	D (μg)	E (mg)	B ₁ (mg)	B ₂ (mg)	B ₆ (mg)	Niacin (mg)	Biotin (μg)	Pantothenic acid (mg)	Folate (μg)	B ₁₂ (μg)	C (mg)
White finfish	Tr	Tr	0.3–1.0	0.02–0.2	0.05–0.5	0.15–0.5	1.0–5.0	1.0–10	0.1–0.5	5.0–15	1.0–5.0	Tr
Fatty finfish	20–60	5–20	0.2–3.0	0.01–0.1	0.1–0.5	0.2–0.8	3.0–8.0	1.0–10	0.4–1.0	5.0–15	5.0–20	Tr
Crustaceans	Tr	Tr	0.5–2.0	0.01–0.1	0.02–0.3	0.1–0.3	0.5–3.0	1.0–10	0.5–1.0	1.0–10	1.0–10	Tr
Molluscs	10–100	Tr	0.5–1.0	0.03–0.1	0.05–0.3	0.05–0.2	0.2–2.0	1.0–10	0.1–0.5	20–50	2.0–30	Tr
Cephalopods	Tr	Tr	0.2–1.0	0.02–0.1	0.05–0.5	0.3–0.1	1.0–5.0	1.0–10	0.5–1.0	10–20	1.0–5.0	Tr
RDA	700/900	5	15	1.1/1.2	1.1/1.3	1.3	14/16	30	5.0	400	2.4	75/90
% RDA per 100 g	0–11	0–100	2–20	1–20	2–38	5–60	1–50	3–33	2–20	0.3–12	40–100	0
% RDA/Md	2	50	7	5	15	25	18	5	8	2	100	0

RDA, recommended dietary allowance (women/men, 31–50 years) by Food and Nutrition Board, Institute of Medicine, National Academies of Science; Tr, trace; Md, Mediterranean diet.

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

Table 7 Selected mineral content of the different groups of fish and shellfish (mg per 100 g), and relation to DRIs

	Na	K	Ca	Mg	P	Fe	Zn	Mn	Cu	Se	Cr	Mo	I
White finfish	50–150	200–500	10–50	15–30	100–300	0.2–0.6	0.2–1.0	0.01–0.05	0.01–0.05	0.02–0.1	0.005–0.02	0.005–0.02	0.01–0.5
Fatty finfish	50–200	200–500	10–200	20–50	200–500	1.0–5.0	0.2–1.0	0.01–0.05	0.01–0.05	0.02–0.1	0.005–0.02	0.005–0.02	0.01–0.5
Crustaceans	100–500	100–500	20–200	20–200	100–700	0.2–2.0	1.0–5.0	0.02–0.2	0.1–2.0	0.05–0.1	0.005–0.02	0.01–0.05	0.01–0.2
Molluscs	50–300	100–500	50–200	20–200	100–300	0.5–10	2.0–10	0.02–0.2	0.02–10	0.05–0.1	0.005–0.02	0.01–0.2	0.05–0.5
Cephalopods	100–200	200–300	10–100	20–100	100–300	0.2–1.0	1.0–5.0	0.01–0.1	0.02–0.1	0.02–0.1	0.005–0.02	0.01–0.2	0.01–0.1
RDA	1500 ^a	4700 ^a	1000	320/420	700	18/8	8/11	1.8/2.3	0.9	0.025/0.055	0.035	0.045	0.15
% RDA per 100 g	3–33	2–10	1–20	4–50	15–100	2–50	1–90	0–10	1–100	25–100	15–60	10–100	8–100
% RDA/Md			6	5	30	18	2		2	100			100

^aAdequate intake.

RDA, recommended dietary intake (women/men, 31–50 years) by Food and Nutrition Board, Institute of Medicine, National Academies of Science; Md, Mediterranean diet.

Source: Reproduced from Ariño A, Beltran JA, and Roncalés P (2003) Dietary importance of fish and shellfish. In: Caballero B, Trugo L, and Finglas P (eds.) *Encyclopedia of Food Sciences and Nutrition*, 2nd edn., pp. 2471–2478. Oxford: Elsevier Science Ltd.

Potassium and calcium levels are also relatively low, though the latter are higher in fish than in meat; in addition, small fish bones are frequently eaten with fish flesh, thus increasing the calcium intake. Fish is a good source of magnesium and phosphorus, at least as good as meat. These elements are particularly abundant in crustaceans; fatty finfish show elevated levels of phosphorus, and bivalve molluscs have high amounts of magnesium.

Fish is a highly valuable source of most trace elements. Fatty fish provides a notable contribution to our iron supply, similar to that of meat, whereas shellfish have higher concentrations of most dietary minerals. In particular, crustaceans and bivalve molluscs supply zinc, manganese, and copper concentrations well above those of finfish. Worth mentioning is the extraordinary dietary supply of iodine in all kinds of finfish and shellfish; however, this depends on the concentration present in feed, particularly in planktonic organisms.

In summary, 100 g of fish affords low levels of sodium and medium-to-high levels of all the remaining dietary minerals. In fact, it can contribute 50–100% of the total daily requirements of magnesium, phosphorus, iron, copper, selenium, and iodine. A Mediterranean diet, rich in fatty fish and all kinds of shellfish, can lead to an overall balanced mineral supply, which may well reach greater than 20% of daily requirements of phosphorus, iron, selenium, and iodine.

Chemical Contaminants in Fish

Several chemical contaminants can find their way into fish and some of them can bioaccumulate. These compounds can be divided into three major groups:

- Toxic metals: mercury, cadmium, lead, and tin. These elements are present in water both from natural sources and as a result of human activities, such as emissions from industrial processes, biocides, and paints. These metals are taken up by marine organisms and tend to accumulate in organisms such as predatory fish which are higher up the food chain.
- Halogenated organic compounds: polychlorinated dibenzodioxins, polychlorinated dibenzofurans, polychlorinated biphenyls (PCBs), polybrominated biphenyls, and insecticides (chlorinated hydrocarbons). This is a very diverse group with a chemical stability that allows them to bioaccumulate and persist in the environment.
- Processing-related compounds: polycyclic aromatic hydrocarbons (PAHs), sulfites (used in shrimp processing), nitrosamines, and residues of drugs used in aquaculture (e.g., antibiotics or hormones).

As regards mercury in fish, methylmercury is the chemical form of most concern and can make up more than 90% of the total mercury in fish and seafood. Fish accumulate mercury as a result of its natural presence in the environment and from pollution. Large predatory fish, such as swordfish, tuna, and sharks, accumulate higher levels of methylmercury through intake over a long life-time. Large predatory species are often migratory and it is not possible to exclude fish from particular waters where background levels of mercury contamination

might be high. In addition to the setting of maximum levels, targeted consumer advice is an appropriate approach for protecting vulnerable groups of the population. In particular, the advice is aimed at women of child-bearing age and young children as methylmercury can affect the neurodevelopment of the unborn child and young children. It is recommended that pregnant women and nursing mothers should avoid certain species of fish and limit their consumption of other fish to an average of 400 g of cooked fish per week.

Organotin compounds such as tributyltin are organic substances containing the metal tin. There is evidence for marine contamination and organotin compounds bioaccumulate in organisms which is a cause for concern.

Certain fish species such as herring, salmon, and others, originating from contaminated regions of the world may contain high levels of polychlorinated dioxins and furans and PCBs, which are bioaccumulative toxic compounds. Long-term exposure may result in negative effects on the developing nervous system, as well as disruption of the endocrine system. Toxic levels in affected regions should be monitored on a regular basis, and consumers should be informed of the dietary recommendations concerning restrictions on consumption of contaminated fish, especially by identified vulnerable groups of the population.

As regards PAHs, benzo(a)pyrene can be used as a marker for the occurrence and effect of carcinogenic PAHs in food. These toxic compounds can contaminate fish during smoking processes and heating and drying processes that allow combustion products to come into direct contact with fish. In addition, environmental pollution may cause contamination with PAH in fish and fishery products.

See also: Coronary Heart Disease: Prevention. Dietary Guidelines, International Perspectives. Food Composition Data. Food Safety: Bacterial Contamination; Heavy Metals; Other Contaminants. Hyperlipidemia: Prevention and Management. Iodine: Physiology, Dietary Sources, and Requirements. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Protein: Quality and Sources. Stroke Nutritional Management. Supplementation: Dietary Supplements

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FOLIC ACID

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Introduction

In the 1930s, first Lucy Wills and then Robert Stokstad isolated a natural component of yeast (termed ‘Wills factor’ and ‘factor U’, respectively) that prevented megaloblastic anemia of pregnancy and promoted growth in chickens. In the next decade, Herschell Mitchell, Esmond Snell, and Robert Williams isolated a factor from spinach that could support the growth of the lactic acid bacteria, *Streptococcus faecalis* and *Lactobacillus casei*. They named this factor ‘folic acid’ based on *folium*, the Latin word for leaf. Shortly thereafter, Stokstad isolated the pure crystalline form of the vitamin, and it was recognized that the Wills factor, factor U, and folic acid were the same substance. Elucidation of the components and details of folic acid metabolism, its metabolic interrelationships with vitamin B₁₂ and methionine/homocysteine metabolism, its roles in pyrimidine and purine synthesis, and the determination of the molecular basis for deficiency diseases occurred through the 1950s and 1960s. In the late 1980s and 1990s, the identification of elevated plasma homocysteine (hyperhomocysteinemia) as a risk factor for vascular disease, cognitive dysfunction, and dementia renewed interest in folic acid. During this time it was also determined that periconceptional supplements of the vitamin were effective in reducing the risk of neural tube defects (NTDs). This led to wide-spread fortification of cereal and grain products with folic acid in the US and Canada beginning in the mid- to late-1990s. Today, more than 50 countries and territories have instituted folic acid fortification programs, which have been highly effective in reducing NTDs. There are, however, lingering questions regarding the safety of excess folic acid consumption.

Chemistry and Biochemical Functions

Chemical Forms

Though used generically, the term ‘folic acid’ actually refers specifically to the synthetic form of the vitamin, which is used in supplements and as a fortificant in foods. The term ‘folate’ refers generally to all forms of the vitamin. As shown in **Figure 1**, folic acid consists of a pterin moiety linked via a methylene group to a para-aminobenzoylglutamate moiety. Its metabolic activity requires reduction to the tetrahydrofolic acid (THF) derivative, addition of a chain of glutamate residues in γ -peptide linkage, and acquisition of one-carbon units.

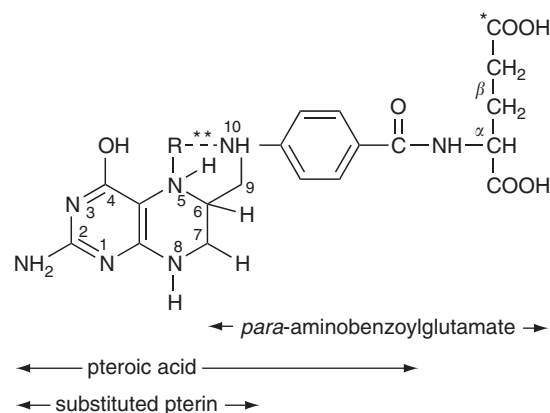


Figure 1 Structural formula of tetrahydrofolate (THF) compounds. In tetrahydrofolic acid R=H; other substituents are listed in **Table 1**. The asterisk indicates the site of attachment of extra glutamate residues; the hatched line and double asterisk indicate the N5 or N10 site of attachment of one-carbon units.

Table 1 Structure and nomenclature of folate compounds

Compound	R-group	Oxidation state
5-FormylTHF	–CHO	Formate
10-FormylTHF	–CHO	Formate
5-FormiminoTHF	–CH=NH	Formate
5,10-MethenylTHF	–CH=	Formate
5,10-MethyleneTHF	–CH ₂ –	Formaldehyde
5-MethylTHF	–CH ₃	Methanol

One-carbon units at various levels of oxidation are generated metabolically and are reactive only as moieties attached to the N5 or N10 positions of the folate molecule (**Table 1**). The range of oxidation states for folate one-carbon units extends from methanol to formate as methyl, methylene, methenyl, formyl, or formimino moieties. When one-carbon units are incorporated into folate derivatives, they may be converted from one oxidation state to another by the gain or loss of electrons.

Overview of Biochemical Functions

The biochemical function of folate substrates is to transfer and use one-carbon units in a variety of essential reactions

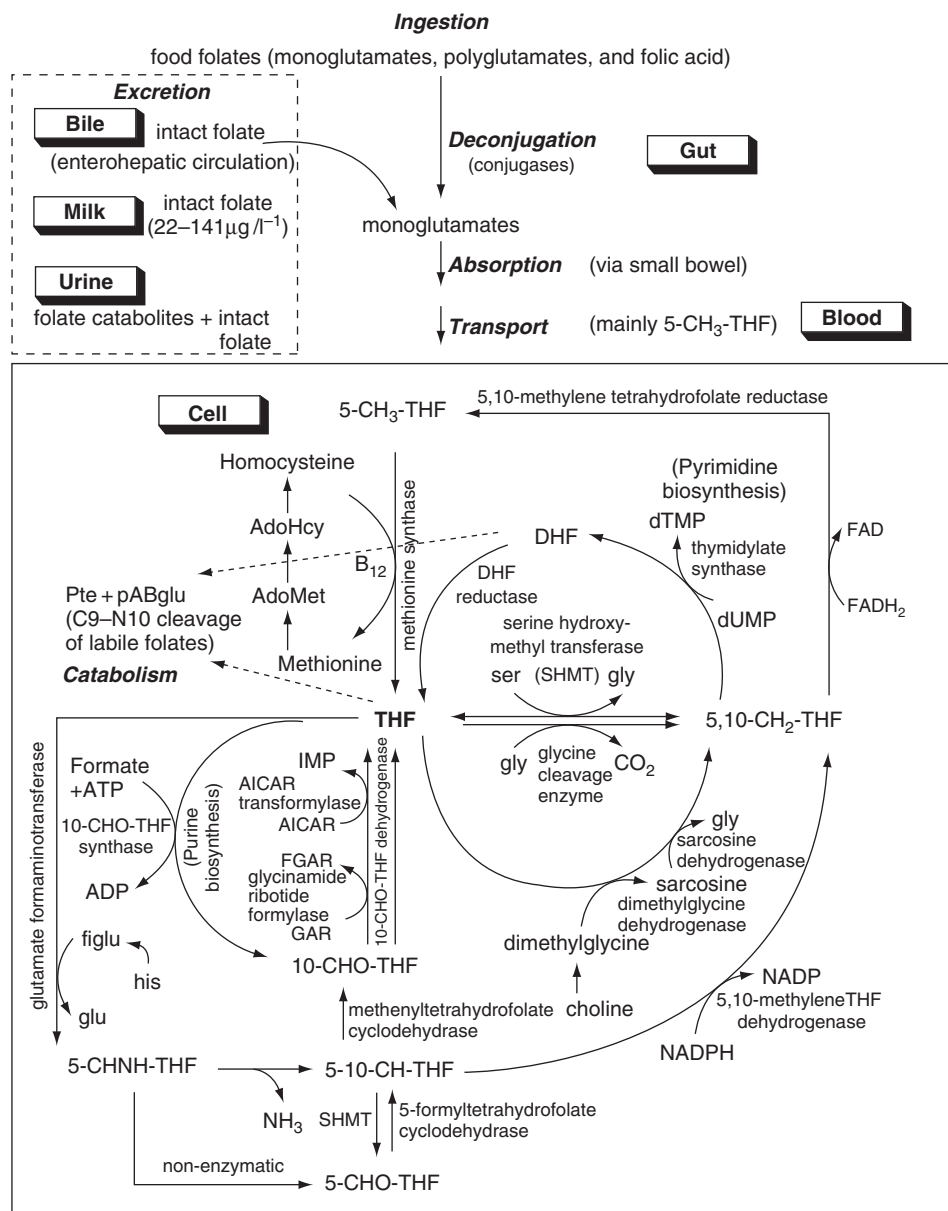


Figure 2 Physiology and metabolism of folate. GAR, glycinamide ribonucleotide; FGAR, formylglycinamide ribonucleotide; AICAR, aminoimidazolecarboxamide ribonucleotide; FIGLU, formiminoglutamic acid; IMP, inosine monophosphate.

(Figure 2), including *de novo* purine biosynthesis (formylation of glycinamide ribonucleotide and 5-amino-4-imidazole carboxamide ribonucleotide), pyrimidine nucleotide biosynthesis (methylation of deoxyuridylic acid to thymidylic acid), amino acid interconversions (interconversion of serine to glycine, catabolism of histidine to glutamic acid, and vitamin B₁₂-dependent conversion of homocysteine to methionine), and the generation and use of formate.

Many of the enzymes involved in these reactions are multifunctional and are capable of channeling substrates and one-carbon units from reaction to reaction within a protein matrix. Another feature of intracellular folate metabolism is the compartmentalization of folate coenzymes between the cytosol and the mitochondria. For instance, 5-methylTHF is

associated with the cytosolic fraction of the cell, whereas most of 10-formylTHF is located in the mitochondria. Similarly, some folate-dependent enzymes are associated with one or other compartment, though some are found in both. Metabolic products of folate-dependent reactions, such as serine and glycine, are readily transported between the two locations, but the folate coenzymes are not.

Source of One-Carbon Units

One-carbon units at the oxidation level of formate can enter directly into the folate pool as formic acid in a reaction catalyzed by 10-formylTHF synthase (EC 6.3.4.3) (**Figure 2**). Entry

at the formate level of oxidation can also take place via a catabolic product of histidine, formiminoglutamic acid. The third mode of entry at the formate level of oxidation involves the formation of 5-formylTHF from 5,10-methenylTHF by the enzyme serine hydroxymethyltransferase (SHMT) (EC 2.1.2.1). The 5-formylTHF may be rapidly converted to other forms of folate.

The enzyme SHMT is also involved in the entry of one-carbon units at the formaldehyde level of oxidation by catalyzing the transfer of the β -carbon of serine to form glycine and 5,10-methyleneTHF. Other sources of one-carbon entry at this level of oxidation include the glycine cleavage system and the choline-dependent pathway; both enzyme systems generate 5,10-methyleneTHF in the mitochondria of the cell.

Removal of One-Carbon Units

Single-carbon units are removed from folate by a number of reactions. The enzyme 10-formylTHF dehydrogenase (EC 1.5.1.6) provides a mechanism for disposing of excess one-carbon units as carbon dioxide. (Folate administration to animals enhances the conversion of ingested methanol and formate to carbon dioxide, diminishing methanol toxicity.) Additionally, single-carbon units from 10-formylTHF are used for the biosynthesis of purines (Figure 2).

The one-carbon unit of 5,10-methyleneTHF is transferred in two ways. Reversal of the SHMT reaction produces serine from glycine, but since serine is also produced from glycolysis via phosphoglycerate this reaction is unlikely to be important. However, one-carbon transfer from 5,10-methyleneTHF to deoxyuridylate to form thymidylate, a precursor of DNA, is of crucial importance to the cell. Although the source of the one-carbon unit, namely 5,10-methyleneTHF, is at the formaldehyde level of oxidation, the one-carbon unit transferred to form thymidylate appears at the methanol level of oxidation. Electrons for this reduction come from THF itself to generate dihydrofolate as a product. The dihydrofolate must in turn be reduced back to THF in order to accept further one-carbon units.

A solitary transfer of one-carbon units takes place at the methanol level of oxidation. It involves the transfer of the methyl group from 5-methylTHF to homocysteine to form methionine and THF. This reaction is catalyzed by the enzyme methionine synthase (EC 2.1.1.13) and requires vitamin B₁₂ as a cofactor. The substance 5-methylTHF is the dominant folate in the body, and it remains metabolically inactive until it is demethylated to THF, whereupon polyglutamylation takes place to allow subsequent folate-dependent reactions to proceed efficiently. Methionine is activated by reaction with ATP to form S-adenosylmethionine (SAM). SAM serves as the universal methyl donor for a variety of essential methylation reactions, including those involving deoxyribonucleic acid (DNA), ribonucleic acid (RNA), histones, proteins, phospholipids, neurotransmitters, and creatine synthesis.

Regulation of Folate Metabolism

The biochemical functions of folate can be divided into two general categories: (1) the synthesis of bases for incorporation into DNA and RNA, and (2) the metabolism of homocysteine

to form methionine and subsequently SAM. Whether folate is utilized for the former or the latter purpose is controlled by biochemical feedback mechanisms that specifically affect the conversion of 5,10-methyleneTHF to 5-methylTHF by the enzyme 5,10-methyleneTHF reductase (MTHFR) (EC 1.5.1.20). MTHFR is subject to allosteric inhibition by SAM. When intracellular SAM concentrations are low, MTHFR actively promotes the synthesis of 5-methylTHF for methionine and SAM synthesis. When SAM concentrations are high, inhibition of MTHFR by SAM reduces the synthesis of 5-methylTHF, methionine, and SAM, and promotes utilization of 5,10-methyleneTHF for thymidylate and purine synthesis. Another important inhibitor of MTHFR is dihydrofolate. Accumulation of DHF as a product of thymidylate synthesis will inhibit MTHFR, thus conserving 5,10-methyleneTHF for further thymidylate synthesis. In this way, DHF may serve as a sensor of active DNA synthesis and cellular proliferation.

Enzyme Polymorphisms

Common polymorphisms have been identified in key enzymes involved in folate metabolism. The most well studied is the C677T single nucleotide polymorphism in the MTHFR gene, which encodes for either alanine (wild-type) or valine (variant) at residue 222 within the amino acid sequence. The variant form of the enzyme is thermolabile *in vitro* and has approximately 70% lower activity than the wild-type enzyme due to reduced affinity for its substrate (5,10-methyleneTHF) and its cofactor (flavin adenine dinucleotide or FAD). Homozygosity for the variant form is found in 10–15% of Caucasians, with lower prevalence in blacks and higher in Hispanics. The lower activity of the variant MTHFR is associated with a variety of conditions, including hyperhomocysteinemia, increased risk of NTDs, and decreased risk of some cancers. Hyperhomocysteinemia and NTD risk can be attenuated by increased dietary folate intake or folic acid supplements. Prevalent polymorphisms in several other folate-metabolizing enzymes have been identified, including those within thymidylate synthase (EC 2.1.1.45), methionine synthase, methionine synthase reductase (EC 1.16.1.8), reduced folate carrier (RFC), and folylpoly- γ -glutamate carboxypeptidase (EC 3.4.17.21), among others. The effect of these polymorphisms on enzyme activity and associated pathophysiological conditions ranges from none to weak or moderate, depending on the nature of the specific polymorphism.

Nutritional Aspects

Dietary Sources

Folate is synthesized by microorganisms and higher plants, but not by mammals, for which it is an essential vitamin. The most concentrated food folate sources include liver, yeast extract, green leafy vegetables, legumes, certain fruits (e.g., orange juice and strawberries), and fortified cereal and grain products. Folate content is likely to depend on the maturity and variety of particular sources. Prolonged exposure to heat, air, or ultraviolet light is known to inactivate the vitamin; thus, food preparation and cooking can make a difference to

the amount of folate ingested. Boiling in particular results in substantial food losses. The major source of folate loss from vegetables during boiling may be leaching as opposed to folate degradation. Broccoli and spinach are particularly susceptible to loss through leaching during boiling compared with potatoes because of their larger surface areas. The retention of folate during cooking depends on the food in question as well as the method of cooking. Foliates of animal origin are stable during cooking by frying or grilling. Steaming in preference to boiling is likely to double the amount of folate consumed from green vegetables. In countries with voluntary or mandatory fortification policies, folic acid from enriched cereal grain products, including bread, cereals, pasta, flour, and rice, may constitute the largest proportion of dietary intake of the vitamin.

Bioavailability

The amount of folate that is absorbed and utilized physiologically varies among different food sources and among different chemical forms of the vitamin. Folic acid consumed as a supplement separate from food is most highly bioavailable. The bioavailability of folic acid taken with a meal or as a food fortificant is approximately 85% compared with folic acid consumed while fasting. Natural food folates are even less bioavailable at approximately 50% of the value for folic acid alone. Based on these differences in bioavailability, 'dietary folate equivalents (DFEs)' have been defined as 1 DFE = 1 μg food folate = 0.6 μg folic acid added to food = 0.5 μg folic acid taken without food.

Dietary Reference Intakes

The recommended dietary allowance (RDA) for males and nonpregnant and nonlactating females age 15 years and older is 400 μg DFE day^{-1} (Table 2). The RDA ranges from 65 to 300 μg DFE day^{-1} for ages 0–14 years. The RDAs for pregnant and lactating women are, respectively, 600 and 500 μg DFE day^{-1} , which accounts for the increased demands for folate of the growing fetus and breast-feeding infant. There is no upper tolerable limit (UL) established for food folates. However, a UL for folic acid has been set at 1000 μg day^{-1} . This is based not only on direct toxic effects of folic acid, but rather the possible masking of vitamin B₁₂ deficiency by high dose folic acid, which can correct hematological abnormalities but not the

neuropathological manifestations of B₁₂ deficiency (see the Section Folic Acid Fortification Beyond NTDs).

Absorption, Transport, and Excretion

Absorption

Food folates mainly consist of reduced polyglutamates, which are hydrolyzed to monoglutamates in the gut before absorption across the intestinal mucosa. The conjugase enzyme that hydrolyzes dietary folates, folylpoly- γ -glutamate carboxypeptidase, is found on the luminal brush border membrane in the human jejunum and has equal affinity for folate polyglutamates of various chain lengths. Uptake of folate monoglutamate into the intestinal cell is mediated by two saturable carrier-mediated processes. These include the RFC, a transporter protein that mediates the uptake of food folates, but has low affinity for folic acid, and folate binding protein (FBP), which mediates the uptake of both reduced and oxidized folates by receptor-mediated endocytosis. The luminal pH optimum for intestinal folate uptake is ~ 5.0 – 6.0 , and changes in luminal pH, as well as the presence of conjugase inhibitors, folate binders, or other food components can adversely affect the rate of hydrolysis and intestinal absorption. Such factors account for the wide variation in the bioavailability of the vitamin from foods of plant and animal origins. Some metabolism of the resultant monoglutamate, mainly to 5-methylTHF, appears to occur during the absorption process, though this may not be necessary for transport across the basolateral membrane of the intestinal mucosa into the portal circulation. The degree of metabolic conversion of dietary folic acid depends on the dose; pharmacological amounts are transported unaltered into the circulation.

Transport and Cellular Uptake

Folate circulates in the blood predominantly as 5-methylTHF. A variable proportion circulates freely or is bound either to low-affinity protein binders such as albumin, which accounts for approximately 50% of bound folate, or to a high-affinity folate binder in serum, which carries less than 5% of circulating folate. The physiological importance of serum binders is unclear, but they may control folate distribution and excretion during deficiency.

Though most folate is initially taken up by the liver following absorption, it is delivered to a wide variety of tissues in which many types of folate transporters have been described. Because these transporters have affinities for folate in the micromolar range, they would not be saturated by normal ambient concentrations of folate. Therefore, folate uptake into tissues should be responsive to any increases in serum folate levels arising from folate supplementation. An important determinant of folate uptake into cells is their mitotic activity, as would be expected given the dependence of DNA biosynthesis on folate coenzyme function. Folate accumulation is more rapid in actively dividing cells than in quiescent cells, a factor that is probably related to the induction and activity of folylpoly- γ -glutamate synthase. This enzyme catalyzes the addition of glutamate by γ -peptide linkage to the initial

Table 2 Recommended dietary allowances (RDA) for folate (US and Canada)

Category	Age	RDA ($\mu\text{g day}^{-1}$)
Infants	0–6 months	65
	6–12 months	80
	1–3 years	150
Children	4–6 years	200
	7–14 years	300
Adults	15+ years	400
Pregnancy		600
Lactation		500

glutamate moiety of the folate molecule. Although polyglutamate derivatization may be considered a storage strategy, this elongation is the most efficient coenzyme form for normal one-carbon metabolism. The activity of folylpoly- γ -glutamate synthase is highest in the liver, the folate stores of which account for half of the estimated 15–30 mg adult total body complement. Retention within the cell is facilitated by the high proportion of folate associated with proteins, and this is likely to be increased in folate deficiency.

The mobilization of liver and other stores of folate in the body is not well understood, particularly in deficiency states, though some accounts describe poor turnover rates in folate-depleted rats. Transport across cell membranes during redistribution requires deconjugation of the large negatively charged polyglutamates. Mammalian γ -glutamylhydrolases that hydrolyze glutamate moieties residue by residue and transpeptidases that can hydrolyze folylpolyglutamates directly to mono- or diglutamate forms of the vitamin have been described for a number of tissues. Thus, mammalian cells possess two types of enzymes that can play a key role in folate homeostasis and regulation of one-carbon metabolism: the folylpolyglutamate synthetase that catalyzes the synthesis of retentive and active folate, and a number of deconjugating enzymes that promote the release of folate from the cell. Polyglutamate forms released into the circulation either through cell death or by a possible exocytotic mechanism would be hydrolyzed rapidly by plasma γ -glutamyl-hydrolase to the monoglutamate form.

Excretion

Folate is concentrated in bile and enterohepatic recirculation from the intestine accounts for considerable reabsorption and reuse of folate (approximately $100 \mu\text{g day}^{-1}$). Fecal folates mostly arise through biosynthesis of the vitamin by the gut microflora, with only a small contribution from unabsorbed dietary folate. Urinary excretion of intact folates accounts for only a small fraction (1–2%) of ingested folate under normal physiological conditions. Free folates (i.e., nonprotein bound) are filtered in the glomerulus and reabsorbed in the proximal tubules, which contain a high concentration of FBP. The greater amount of excretion in urine is accounted for by products that arise from cleavage of the folate molecule at the C9–N10 bond, consisting of one or more pteridines and *p*-acetamido-benzoylglutamate. The rate of scission of the folate molecule increases during rapid-mitotic conditions such as pregnancy and growth. Scission of folate is perhaps the major mechanism of folate turnover in the body.

Deficiency and Excess

Megaloblastic Anemia

Megaloblastic anemia is characterized by larger than normal circulating red blood cells and hypersegmented neutrophils. Deficiencies of both folate and vitamin B₁₂ induce anemias that are clinically indistinguishable. The hematological effect in both cases is the result of intracellular concentrations of 5,10-methyleneTHF that are inadequate to sustain thymidylate

synthesis. In the case of folate deficiency, this is directly the result of a lack of dietary folate. In vitamin B₁₂ deficiency, this is due to what is known as the ‘methylfolate trap.’ This occurs because the conversion of 5,10-methyleneTHF to 5-methylTHF by MTHFR is an irreversible reaction. When vitamin B₁₂ is deficient, the utilization of 5-methylTHF for methionine synthesis is inhibited. Consequently, the metabolism of 5-methylTHF cannot proceed forward or backward, and thus the 5-methylTHF becomes metabolically trapped and a secondary folate deficiency occurs. Regardless of the cause of the folate deficiency, the resulting inhibition of DNA synthesis affects rapidly proliferating cells, in particular the blood cell precursors in the bone marrow. Inhibition of DNA synthesis leads to a block in cell replication, but not in cytoplasmic protein synthesis, which results ultimately in the release of megaloblastic or macrocytic red cells into the circulation. Other rapidly proliferating cells that are similarly affected by folate deficiency are the mucosal cells of the intestine. This leads to blunted intestinal villi and consequent reduced nutrient absorptive capacity.

It is important for the treatment of megaloblastic anemia to differentiate between folate and B₁₂ deficiencies as the cause in any given patient. High dose folic acid supplements correct hematological abnormalities, but not the neuropathological manifestations of B₁₂ deficiency. This occurs because folic acid is taken up into cells and is metabolized first to dihydrofolate and then THF by the enzyme, dihydrofolate reductase (EC 1.5.1.3). The THF can then be converted to 5,10-methyleneTHF and used for thymidylate synthesis. In this way, the folic acid bypasses the methylfolate trap, reverses the megaloblastic anemia, and essentially ‘masks’ the B₁₂ deficiency. The patient nonetheless remains vitamin B₁₂ deficient and is susceptible to potentially irreversible neuronal damage if the B₁₂ deficiency continues untreated.

Hyperhomocysteinemia

An important consequence of folate deficiency is the inability to remethylate homocysteine to form methionine (Figure 2). Indeed, there is an inverse correlation between the concentration of folate and that of homocysteine in the blood. Many clinical studies, beginning with the observations of children with homocystinuria presenting with vascular abnormalities and thromboembolism, have demonstrated an association between hyperhomocysteinemia and increased risk of premature atherosclerosis in the coronary, carotid, and peripheral vasculatures. Even mild hyperhomocysteinemia is recognized to be an independent risk factor for cardiovascular disease. The risk of heart disease increases proportionately in most, but not all, studies, throughout the full of range of blood homocysteine concentrations. An increase in plasma homocysteine of $5 \mu\text{mol l}^{-1}$ is associated with a combined odds ratio of 1.3 for cardiovascular disease.

Metabolically, homocysteine may be disposed of by the methionine synthase reaction (dependent on folate and vitamin B₁₂), the trans-sulfuration pathway (dependent on vitamin B₆), and the choline degradation pathway (independent of folate, B₁₂, and B₆). Deficiencies of the three vitamins are associated with hyperhomocysteinemia. Of the three vitamins, folate has been shown to be the most effective in lowering

levels of homocysteine in the blood. Evidence of the potential role of folate intake in the prevention of vascular disease has come from a significant inverse relationship between serum folate levels and fatal coronary heart disease. Although most studies have focused on the homocysteine-lowering effects of folate, other benefits have also been reported. Potential mechanisms include antioxidant actions and interactions with the enzyme endothelial nitric oxide synthase. However, it is important to note that folic acid supplements, with or without concomitant vitamin B₁₂ and B₆ supplements, though effective in lowering plasma homocysteine levels, have not proven to be particularly effective in reducing vascular disease risk.

Neurological and Cognitive Dysfunction

Folate deficiency is associated with a variety of neurological consequences, including neuropsychiatric manifestations (insomnia, irritability, fatigue, and forgetfulness), peripheral neuropathies (decreased or absent reflexes, diminished vibration sense, and restless leg syndrome), optical neuropathy, and progressive vision loss. Folate deficiency has also been associated with cognitive impairment and dementia. Both cross-sectional and longitudinal studies have verified that low folate intake or status is a risk factor for poor scores on objective measures of cognitive function and Alzheimer's disease. In addition, some, but not all studies have demonstrated a beneficial effect of folic acid supplements in preventing cognitive decline in older adults. The benefit of folic acid supplements may be limited to the period before the development or the early stages of cognitive impairment (before significant structural damage and dementia have occurred). Folate deficiency may also contribute to altered mood and depression, and may limit the efficacy of antidepressant medications.

Several potential mechanisms have been postulated to explain the effects of folate deficiency on neurological function. Most attention has focused on hyperhomocysteinemia, which in addition to cardiovascular disease is associated with atherosclerotic and thrombotic damage that acutely (i.e., stroke and infarction) or chronically limits blood flow to the brain. Homocysteine or products of its oxidative metabolism, including homocysteine sulfinic acid and homocysteic acid, may also induce excitotoxicity or oxidative stress within the brain with resultant neurodegeneration. Alternatively, hyperhomocysteinemia may be a marker of altered intracellular concentrations of SAM and SAH. Reduced SAM and increased SAH induced by folate deficiency may inhibit key methylation reactions in the central nervous system involving proteins, neurotransmitters, phospholipids, and DNA. Inhibition of these processes may in turn cause metabolic impairments and structural damage. In addition, depressive symptoms associated with folate deficiency may be the consequence of altered SAM concentrations in the brain. Oral SAM supplements have been shown to have antidepressant properties and folate deficiency may induce depressive symptoms by reducing SAM levels in the brain. This may also be relevant to cognition because depressive symptoms are a strong determinant of cognitive impairment in older adults.

Cancer

Folate has a complex relationship with cancer. Folate deficiency limits the synthesis of thymidylc acid and causes the accumulation of uridylic acid. This leads to misincorporation of uracil into DNA. Cellular repair mechanisms efficiently excise the misincorporated uracil. However, because thymidylc acid availability is reduced due to the folate deficiency, DNA strand breaks occur, which leaves DNA vulnerable to mutation. In this way, folate deficiency is a risk factor for the initiation of cancer, whereas folate sufficiency is protective. In contrast, proliferating cancer cells, like all mammalian cells, require folate for replication and proliferation. When folate is deficient, replication and proliferation are inhibited. Thus, after cancer is initiated, folate deficiency will actually retard its progression. This phenomenon, first exploited by Sidney Farber in the treatment of pediatric acute lymphoblastic leukemia, is the basis for several antifolate cancer chemotherapeutic drugs, including methotrexate (an inhibitor of dihydrofolate reductase) and 5-fluorouracil (an inhibitor of thymidylate synthase), among others.

Neural Tube and Other Birth Defects

In early vertebral development, the neural tube forms from the invagination of neural crest cells, which subsequently differentiate into the brain and spinal column. NTDs are malformations in which there is failure of the neural tube to close properly during the fourth week of embryonic life. Incomplete closure of the spinal cord results in spina bifida, whereas incomplete closure of the cranium results in anencephaly. Observations in both humans and animals in the 1960s and 1970s suggested that poor nutritional status and specifically low folate status was a cause of NTDs. In the 1980s and early 1990s, randomized control trials proved that folic acid supplements prevent the recurrence and occurrence of NTDs, which led to government-mandated fortification of cereal and grain products with folic acid in the US and Canada in 1998. Today, more than 50 countries and territories have instituted folic acid fortification programs. Assessments of the efficacy of folic acid fortification in the US, Canada, Chile, Costa Rica, and South Africa indicate that NTD incidence has decreased between 19% and 78%. The percent reduction in incidence depends on the rate of NTDs and folate status within a population before the institution of fortification. These data indicate that folic acid fortification, for its intended purpose, has been one of the most highly successful public health interventions ever devised.

International agencies have published folic acid recommendations for the prevention of NTDs. To prevent recurrence, 5 mg folic acid day⁻¹ is recommended, whereas 400 µg day⁻¹ is recommended to prevent occurrence. Because women do not usually become aware that they are pregnant until after neural tube closure has occurred in the developing fetus, it is essential that folic acid supplements be commenced before conception. For informed women and their doctors, this is typically achieved through folic acid supplements. However, for the general population, in which a high proportion of pregnancies are unplanned, fortification is the more effective strategy for prevention. The level of fortification

mandated in the US is 140 µg of folic acid per 100 g of flour, calculated to increase individual consumption of folic acid by 100 µg day⁻¹ and bring overall folate intake to the recommended 400 µg day⁻¹. Measurement of the actual folic acid in fortified foods indicates that manufacturers have added an amount closer to 200 µg folic acid per 100 g flour, presumably to provide a margin of error in achieving the mandated amount.

Folate deficiency and hyperhomocysteinemia have also been associated with other abnormal pregnancy outcomes. These include premature delivery, low birth weight, fetal growth retardation, abruptio placentae (placental infarction), preeclampsia, and congenital heart defects. Randomized controlled clinical trials are needed to determine if folic acid supplements reduce the incidence of these abnormal outcomes.

Folic Acid Fortification beyond NTDs

Because of the essential role of folate in one-carbon metabolism, other effects of folic acid fortification have been considered, including the possibility that excess intake may have negative consequences. With respect to cancer, it is postulated that increased folic acid intake at the population level could have both preventive and promoting effects. Folic acid supplements increase the grade and multiplicity of colorectal adenomas in patients who previously had colonic polyps removed. In addition, ecological studies indicate that temporary increases in the incidence of colorectal cancer occurred in the US, Canada, and Chile concurrent with the institution of folic acid fortification. In contrast, a reduction in the rate of pediatric neuroblastoma was observed after the start of fortification. With respect to breast cancer, conflicting studies indicate that risk may be decreased or increased with folic acid supplements. It is likely that these contradictory findings are explained by the timing of folic acid exposure. If exposure occurs before neoplastic initiation, folic acid will reduce the risk of cancer. However, if exposure occurs after initiation, then folic acid will promote proliferation and accelerate progression to clinical disease.

One of the positive consequences of folic acid fortification has been a significant reduction in the prevalence of hyperhomocysteinemia in the general population. Studies of whether or not this reduction in homocysteine has translated into reduced vascular disease risk are limited. No apparent reduction in coronary artery disease was observed in one study. In contrast, reduced rates of stroke were observed in the US and Canada around the time fortification was initiated, but not in England and Wales where fortification has not been initiated. These studies were ecological in design, however, and it remains to be determined if folic acid fortification reduces the risk of vascular disease of any kind.

One of the primary worries with folic acid fortification was the possible masking of vitamin B₁₂ deficiency, as described above. Recent observational studies, however, suggest that excess folic acid intake, particularly in folic acid supplement users who are also exposed to folic acid fortification, may actually exacerbate both the neurological and hematological consequences of B₁₂ deficiency. Older adults with a

combination of low serum B₁₂ and high serum folate concentrations were found to be at higher risk of cognitive impairment and anemia than adults of similar age who had both low B₁₂ and low folate. The combination of low B₁₂ and high folate was also associated with higher circulating homocysteine and methylmalonic acid and lower holotranscobalamin concentrations (metabolic indicators of functional B₁₂ deficiency) than the combination of low B₁₂ and low folate. Two hypotheses have been put forth to explain these observations. The first postulates that excess folic acid accumulation in cells causes the irreversible oxidation of intracellular vitamin B₁₂, which inactivates B₁₂ as a cofactor for homocysteine metabolism and odd-chain fatty acid metabolism. The second hypothesis proposes that dihydrofolate, which will accumulate in cells exposed to excess folic acid, inhibits both MTHFR and thymidylate synthase. Inhibition of these two reactions by dihydrofolate putatively causes inhibition of blood cell production and inhibition of homocysteine remethylation. These hypotheses currently remain untested, however, and the conclusion that excess folic acid exacerbates vitamin B₁₂ deficiency remains highly controversial.

Biomarkers and Status Assessment

The main clinical indicator of folate deficiency is megaloblastic anemia, as described above. The primary screening measurements for assessing folate status are serum and red blood cell folate concentrations. The gold-standard for folate measurement is a microbiological assay utilizing *L. casei*. Radioactive competitive binding and automated chemiluminescence assays are commonly used for research and clinical purposes, respectively. Mass spectrometry methods have also been devised. Though there is usually a high correlation between serum and red cell folate concentrations, serum folate typically reflects recent folate intake and short-term status, whereas red cell folate reflects long-term status over the 120 days life-span of the red cell. Common cut-off values used to define folate deficiency are <3 ng ml⁻¹ (<7 nmol l⁻¹) in serum and <140 ng ml⁻¹ (<305 nmol l⁻¹) in red cells. Elevated plasma homocysteine (>10 µmol l⁻¹) is another indicator of folate deficiency. However, hyperhomocysteinemia has several potential causes, including vitamin B₁₂ and vitamin B₆ deficiencies, renal disease, and hypothyroidism, and thus homocysteine is a sensitive, but not specific indicator of folate status. Another biomarker sensitive to low folate status is global DNA methylation. This is a consequence of the role of folate in the synthesis of SAM used for DNA methylation reactions.

See also: Homocysteine. Riboflavin.
Vitamin B₁₂: Physiology, Dietary Sources, and Requirements

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FOOD ALLERGIES

Diagnosis and Management

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Documenting Possible Food Allergies

The diagnosis of food allergy is made from the history, supported by investigations and by responses to avoidance of specific food triggers. Because the value of investigations is limited, it is especially important to obtain a clear history. There are a number of practical points to be made:

- *Speed of onset*: In general, the quicker the onset of the allergic reaction, the more reliable is the history. If a child develops a violent allergic reaction within a minute or two after ingesting a food, it is much easier to link the reaction to a specific food than if a reaction only occurs hours or days after eating a food.
- *Coincidences need to be excluded*: If a child becomes unwell (e.g., starts wheezing) an hour after eating a specific food, the wheezing could be caused by the food, or it could just be a coincidence. The more times that such a sequence has been observed, the more likely it is that there is a cause and effect relationship.
- *Observations need to be tested for internal consistency*: Someone may believe that he or she is allergic to a food if a symptom (e.g., urticaria) occurs on (say) three occasions after eating a specific food. It is important to find out the following:
 1. Whether the subject has had the same symptoms on other occasions when the suspect food trigger was not taken.
 2. Whether the subject has taken the suspect food on one or more other occasions without any adverse effects.

Failure to seek inconsistencies such as these is one factor that is responsible for the overdiagnosis of food allergy.

Documenting a Diagnosis of Food Allergy

If it is reported that someone is allergic to an item, it is important to probe further and find out on what basis the person has been deemed allergic. It is common to find children and adults who are believed to be allergic to a food solely on the basis of tests such as skin tests or blood tests, which are in fact almost wholly unreliable (see below). It is also common for people to believe that they are allergic to something because a

health professional said so one day, which on further enquiry turns out to be on flimsy or nonexistent grounds.

Another common problem is the misinterpretation of a sequence of events. For example, a child with an ear infection is given an antibiotic, and 3 days later gets diarrhea, so the parents come to believe the child is allergic to the antibiotic. In fact the cause of the diarrhea is far more likely to be either an underlying viral infection, or a disturbance of the gut flora. Another example is the report of a child who is believed to be allergic to sesame seeds because of the reactions occurring after eating buns coated with sesame seeds; many such children are in fact not allergic to sesame seeds but are reacting to the egg glaze that has been used as an adhesive for the seed coating. Another common example is the child with asthma who coughs and wheezes after drinking a diluted orange squash drink, with the result that it is believed that the child is reacting to the yellow-orange coloring agent tartrazine. In fact such reactions are more likely to be due to sulfite preservatives in the squash; sulfites trigger symptoms in 60% or more of children with asthma.

Practical Diagnostic Difficulties

Multiple Mechanisms

Reactions to foods are a heterogeneous group of disorders caused by a variety of different immunological and pharmacological mechanisms. In any individual case, the precise mechanism is often not known. No single type of laboratory test could possibly cover all the different types of possible mechanisms of reactions to foods. Even if one focuses on food allergy, there are a number of different possible immunological mechanisms, including IgE antibody mediated, cell mediated, and circulating immune complexes.

Inability to Predict Outcome

In many situations (e.g., atopic disease), the subject wants to know whether there will be any benefit from food avoidance (e.g., not drinking cows' milk or not eating apples). Even if there were valid tests for the diagnosis of food intolerance, the outcome of avoidance measures depends on a number of

other variables. Allergen avoidance may succeed for the following reasons:

(1) the patient was intolerant to the item; (2) coincidental improvement; (3) placebo response.

The reasons why a trial of food avoidance may fail to help can be summarized as follows:

(1) The subject is not allergic to the food. (2) The period of elimination was too short. For example, where a child has an enteropathy (damage to the small intestine) due to food allergy, it may take a week or more for improvement in symptoms to occur. (3) The food has been incompletely avoided. This may happen in a subject supposedly on a cows' milk protein-free diet who still continues to receive food that contains cows' milk proteins such as casein or whey. (4) The subject is allergic to other items, which have not been avoided. For example, a child with an allergy to cows' milk protein who fails to improve when given a soy-based milk to which they also have an allergy. (5) Coexisting or intercurrent disease, for example, gastroenteritis in a child with loose stools who is trying a cows' milk-free diet. (6) The patient's symptoms are trivial and have been exaggerated or do not exist at all and have either been imagined or made up by the parents. It is unrealistic to expect there to be a simple test that can overcome all these problems.

Diagnostic Tests

Skin Prick Tests

The principle of skin prick tests is that the skin weal and flare reaction to an allergen demonstrates the presence of mast-cell-fixed antibody, which is mainly IgE antibody. IgE antibody is produced in plasma cells, and is distributed in the circulation to all parts of the body, so that sensitization is generalized and therefore can be demonstrated by skin testing. In the presence of specific IgE antibody, mast cells in the skin release histamine, which in turn causes a visible weal and flare reaction in the skin.

The procedure involves a drop of allergen solution being placed on the skin, which is then pricked with a hypodermic needle. Two control solutions should also be used: the diluent, in order to detect false-positive reactions; a positive control (e.g., a histamine solution), to enable comparison with a positive result of an allergen solution. The skin prick test induces a response that reaches a peak in 8–9 min for histamine and 12–15 min for allergens. The size of the weal reaction (and not the larger red flare) is measured.

There are numerous problems with skin prick tests, including the following:

1. There is no agreed definition about what constitutes a positive reaction.
2. The size of the weal depends to some extent on the potency of the extract.
3. Antihistamines and tricyclic antidepressants suppress the histamine-induced weal and flare response of a skin test. The suppressive effect of antihistamines may last from a week up to several months for some of the more recently introduced nonsedating antihistamines.
4. *False-positive tests*: Skin prick test reactivity may be present in subjects with no clinical evidence of allergy or

intolerance. This is sometimes described as 'asymptomatic hypersensitivity' or 'subclinical sensitization.' Whilst many with positive skin prick tests will never develop the allergy, some subjects with positive skin prick tests do develop symptoms later. However, since the test cannot identify those who are going to develop symptoms, the skin test information is of no practical value.

5. *False-positive results*: Skin prick test reactivity may persist after clinical evidence of intolerance has subsided. For example, in a study of children with egg allergy, it was noted that five out of 11 who grew out of egg allergy had persistently positive skin prick tests after the allergy had disappeared.
6. *False-negative tests*: Skin prick tests are negative in some subjects with genuine food allergies.
7. Skin prick tests mainly detect IgE antibody. However, many adverse reactions to food are not IgE mediated, in which case skin prick tests can be expected to be negative. Taking cows' milk protein intolerance as an example, patients with quick reactions often have positive skin prick tests to cows' milk protein, but those with delayed reactions usually have negative skin prick tests.
8. False-negative results are a problem in infants and toddlers, when the weal size is much smaller than later in life.
9. There is a poor correlation between the results of provocation tests (e.g., double-blind food challenges) and skin prick tests. For example, in one study of 31 children with a strongly positive (weal > 3 mm in diameter) skin prick test to peanut, only 16 (56%) had symptoms when peanuts were administered.
10. Commercial food extracts (sometimes heat treated) and fresh or frozen raw extracts may give different results (more positives with raw foods), reflecting the fact that some patients are allergic to certain foods only when taken in a raw state. In others the reverse is the case.

Skin prick tests are mainly used in research studies. The results of skin tests cannot be taken alone, and standard textbooks on allergy acknowledge that "the proper interpretation of results requires a thorough knowledge of the history and physical findings." The problems in clinical practice are, for example, whether or not a subject with atopic disease (eczema, asthma, or hay fever) or symptoms suggestive of food intolerance will benefit from attempts to avoid certain foods or food additives. However, skin prick test results are unreliable predictors of response to such measures.

Skin test results are known to be misleading in cases of inhalant allergy (e.g., allergy to dust mites or grass pollen) but skin prick tests for food allergy are especially unreliable because of the large number of false-positive and false-negative reactions.

Intradermal Testing

Intradermal testing comprises the intradermal injection of 0.01–0.05 ml of an allergen extract. It can cause fatal generalized allergic reaction (anaphylaxis), and is only performed if a preliminary skin prick test is negative. Intradermal tests are more sensitive than skin prick testing, and hence also produce even more false-positive reactions, making the interpretation

of the results of intradermal testing even more difficult than that for skin prick testing. The difficulty in interpretation of the results, the pain of intradermal injections, and the risk of anaphylaxis mean that intradermal testing has no place in the routine investigation of food allergy.

Skin Application of Food before Food Challenges

There is one situation where direct application of food to the skin may be of practical value, and that is before a food challenge in a child in whom one fears an anaphylactic reaction. An example might be a 6-month-old infant with a history of a severe allergic reaction to egg. If the parents wish to see if the child has outgrown the allergy without directly administering egg and risking a violent reaction, a simple approach is to rub some raw egg white into the skin and observe the skin for a few minutes. If the skin application of egg in this way causes an urticarial reaction, then a gradual diminution and disappearance of this response during the succeeding months and years can probably be taken to indicate the development of tolerance, and a continuing brisk response to skin contact would constitute a deterrent to an oral challenge. However, this is only an approximate guide, and there are a number of possible reasons why such testing may give false-positive (e.g., using a raw food when the food is usually eaten cooked, such as egg or potato) or false-negative (e.g., the child is receiving antihistamine drug) results.

Tests for Circulating IgE Antibodies: The Radioallergosorbent (RAST) Test

The RAST test is the best known of a number of laboratory procedures for the detection and measurement of circulating IgE antibody. Unfortunately, the clinical interpretation of RAST test results is subject to most of the same pitfalls as that for skin prick testing. Additional problems with RAST tests are the cost, and the fact that a very high level of total circulating IgE (e.g., in children with severe atopic eczema) may cause a false-positive result. Depending on the criteria used for positivity, there is a fair degree of correlation between the RAST test and skin prick test results.

Provocation Tests

A provocation test may be useful to confirm a history of allergy. An example might be a child who developed wheezing and urticaria minutes after eating a rusk that contained, as its main ingredients, wheat and cows' milk protein. To determine which component, if any, caused the reaction, oral challenges with individual components can be conducted.

However, the results of provocation tests cannot prove that improvement in a disease has been caused by food avoidance. For example, a child with atopic eczema is put on a diet avoiding many foods, and the eczema improves. This improvement could be a coincidence, a placebo effect, or due to the diet. Just because the child is shown to react to a single food does not prove that avoidance of that food was the cause of the improvement.

Open and Blind Challenges

Where the subject and the observer knows the identity of the administered material at the time of the challenge, the procedure is said to be an 'open' challenge. In a 'single-blind challenge' the observer but not the patient or family know the identity of the test material. To avoid bias on the part of the observer, a double-blind challenge is required. A 'double blind' challenge involves exposing the subject to a challenge substance, which is either the item under investigation or an indistinguishable inactive (placebo) substance. Neither the subject nor the observer knows the identity of the administered material at the time of the challenge or during the subsequent period of observation.

The Purpose of Provocation Tests

The aim of a food challenge is to study the consequences of food or food additive ingestion. Provocation tests are helpful in the following ways:

(1) to confirm a history (parents' observations of alleged food allergy are notoriously unreliable, as are adults' beliefs about their own allergies); (2) to confirm the diagnosis, for example, of cows' milk protein allergy in infancy, where the diagnostic criteria include improvement on elimination diet and relapse on reintroduction; (3) to see if a subject has grown out of a food intolerance; (4) as a research procedure. The food challenge should replicate normal food consumption in terms of dose, route, and state of food. It should also be performed in such a way that the history can be verified. Thus, for example, there is no point solely looking for an immediate reaction if the parents report a delayed reaction.

Open food challenges are the simplest approach, but open food challenges run the risk of bias influencing the parents' (or doctors') observations. Often this is unimportant. But in some cases belief in food intolerance may be disproportionate, and where this is suspected there is no substitute for a double-blind placebo-controlled challenge. An open challenge may be an open invitation to the overdiagnosis of food intolerance. For example, in the UK parents widely believe that there is an association between food additives and bad behavior, but in one series, double-blind challenges with tartrazine and benzoic acid were negative in all cases in a study of 24 children with a clear parental description of adverse reaction.

The double-blind placebo-controlled challenge is regarded as the state-of-the-art technique to confirm or refute histories of adverse reactions to foods. The ability to unravel food-related problems is said to be limited only by the imagination of the physician and a clever dietitian. In fact, the technique is subject to a number of potential limitations, not all of which can be overcome.

Effect of Dose

In some cases of food intolerance, minute quantities of food (e.g., traces of cows' milk protein) are sufficient to provoke florid and immediate symptoms. In other cases, much larger quantities of food are required to provoke a response. Hill *et al.* demonstrated that whereas 8–10 g of cows' milk powder (corresponding to 60–70 ml of milk) was adequate to provoke an adverse reaction in some patients with cows' milk protein allergy, others (with late onset symptoms and particularly

atopic eczema) required up to 10 times this volume of milk daily for more than 48 h before symptoms developed.

Concealing Large Doses is Difficult

Standard capsules that contain up to 500 mg of food are suitable for validation of immediate reactions to tiny quantities of food, but concealing much larger quantities of certain foods (especially those with a strong smell, flavor, or color) can be very difficult.

Route of Administration

Reactions to food occurring within the mouth are likely to be missed if the challenge by-passes the oral route, e.g., administration of foods in a capsule or via a nasogastric tube. In practice, patients whose symptoms are exclusively confined to the mouth are unusual, and where there is a history of purely oral reactions an alternative challenge procedure can be employed. In subjects who are intolerant to sulfites, it is well recognized that the administration of sulfites in capsules or directly into the stomach via a nasogastric tube usually fails to provoke an adverse reaction, whereas the oral administration of solution will succeed in doing so.

Problems with Capsules

Capsules are unsuitable for use in children who cannot swallow large capsules, and this is a major limitation as most cases of suspected food allergy are in infants and toddlers. Furthermore, it is unsatisfactory to allow patients or parents to break open capsules and mix the contents with food or drink, as the color (e.g., tartrazine) or smell (e.g., fish) will be difficult or impossible to conceal and the challenge will no longer be blind.

Anaphylactic Shock Danger

There is a danger of producing anaphylactic shock, even if it had not occurred on previous exposure to the food. For example, in Goldman's classic study of cows' milk protein intolerance, anaphylactic shock had been noted before cows' milk challenge in five out of 89 children, but another three developed anaphylactic shock as a new symptom after cows' milk challenge. In a study of 80 children with atopic eczema treated with elimination diets, anaphylactic shock occurred in four out of 1862 food challenges. The risk appears to be greatest for those who have received elemental diets.

Effect of Disease Activity

A food challenge performed during a quiescent phase of the disease (e.g., urticaria, eczema, or asthma) may fail to provoke an adverse reaction.

Additive Effect of Triggers

Although some patients react repeatedly to challenges with single foods, it is possible (but unproven) that some patients only react adversely when multiple allergens are given together. There certainly are some subjects who only react in the presence of a nonfood trigger, such as exercise or taking aspirin.

Special Types of Provocation Testing

Other than giving a suspect food by mouth, and asking the subject to swallow it, there are some alternative approaches, which are outlined below.

Oral Mucosal Challenge

A small portion of food is applied to the mucosa inside the mouth, and one looks for reactions such as swelling of the lips, and tingling or irritation of the mouth or tongue, possibly followed by other more generalized symptoms such as urticaria, asthma, vomiting, abdominal pain, or anaphylactic shock. Patients with food intolerance commonly make use of these oral symptoms, spitting out and avoiding further consumption of a food that provokes the symptom.

Gastric Mucosal Challenge

In this procedure, an allergen is applied directly to the gastric mucosa via an endoscope, and the mucosa is then observed for signs of a reaction. In addition, it is possible to take biopsies of the gastric mucosa to study the histological changes and measure the tissue concentration of mediators of inflammation such as histamine.

Rectal Challenges

The standard test to confirm a diagnosis of celiac disease is the jejunal biopsy, in which a small portion of jejunal mucosa is obtained with the aid of a special capsule that is swallowed, and which passes into the small intestine. When in the correct location, the capsule is triggered and withdrawn; it contains a portion of intestinal mucosa, which can then be examined under the microscope. Alternatively, gluten can be instilled into the rectum, in order to look for a reaction that would signify celiac disease. This procedure requires multiple biopsies from the rectum, and it is uncertain whether the results are reliable.

Management

Dietary Elimination

The management of food allergy consists largely of elimination from the diet of the trigger food or foods. Elimination diets are used either for the diagnosis or the treatment of food intolerance, or for both. A diet may be associated with an improvement in symptoms because of intolerance to the food, a placebo effect, or the improvement may have been a coincidence. The degree of avoidance that is necessary to prevent symptoms is highly variable. Some patients are intolerant to minute traces of food, but others may be able to tolerate varying amounts. Strict avoidance and prevention of symptoms are the aims in certain instances, but in many cases it is unknown whether allowing small amounts of a food trigger could lead to either enhanced sensitivity or to the reverse, increasing tolerance. The duration required for dietary avoidance varies. For example, intolerance to food additives may last only a few years, whereas intolerance to peanuts is usually lifelong. Although food allergy is common in children, most have grown out of the problem by the age of 5 years; an important exception is those with nut allergy.

Malnutrition

Malnutrition is a major risk of unsupervised diets.

Calcium

Cows' milk is an important source of calcium, and avoidance of cows' milk and its products carries the risk of an inadequate intake of calcium. Unfortunately, it is far from clear what constitutes an adequate intake for various different age groups.

Protein and Energy

Milk, eggs, fish, meat, wheat, and their respective manufactured food products are important sources of protein and energy. Avoidance of these without the provision of alternative sources of protein and energy runs the risk of an inadequate intake, and growth failure, serious malnutrition, and weight loss are well documented sequelae of unsupervised and inappropriate dietary elimination.

Iodine

Cows' milk and dairy products are important sources of dietary iodine. Exclusion of cows' milk products and a number of other items from the diet, coupled with the consumption of large amounts of soy milk, which has been reported to cause hypothyroidism by increasing fecal loss of thyroxine, have resulted in hypothyroidism and growth failure due to dietary iodine deficiency.

High-Risk Factors

The risk of malnutrition from an elimination diet is particularly high in the following situations:

- (1) The diet is not supervised by a dietitian.
- (2) There is chronic disease before diagnosis, or concurrent chronic disease such as severe atopic eczema. The subject's nutrient requirements may be increased.
- (3) Malabsorption or enteropathy increases the risk of malabsorption of nutrients.
- (4) The subject is avoiding sunlight. The risk of vitamin D deficiency may compound the effects of a low calcium intake.
- (5) The subject is already on a diet that excludes multiple foods, e.g., vegan or macrobiotic diet.

The Role of the Dietitian

The dietitian has three roles in the management of elimination diets. One is to ensure that the resulting diet is nutritionally adequate, and to prevent potential deficiency states by recommending (in an infant) appropriate amounts of infant milk formula, and (in older children or adults) supplements of calcium, vitamins, and so on. Another role is to advise how to avoid specific foods, particularly those contained in manufactured foods. Third, the dietitian makes suggestions as to how to make the diet practical and palatable, and suggests recipes for use with a limited range of foods (e.g., how to make biscuits with potato flour).

Cows' Milk Protein Avoidance

Any form of cows' milk, whether fresh, skimmed, condensed, or evaporated, needs to be avoided. Also forbidden are milk

products that contain casein, whey, and nonfat milk solids. Where milk substitutes are required, the choice lies between formulas based on soy protein, casein hydrolysate, or whey hydrolysate. Soya formulas are cheaper, but unsuitable for those who are also intolerant to soya.

Butter, margarine, cream, cheese, ice cream, and yogurt all need to be avoided. Fats that can be used instead include margarines made from pure vegetable fat (e.g., Tomor) and lard. Caution is required with baby foods, as a large number of manufactured products, e.g., rusks, contain milk protein. A common trap is the so-called 'vegetarian' cheese, often wrongly believed to be safe for subjects with cows' milk allergy. In fact, it differs from ordinary cheese only in the use of nonanimal rennet and is unsuitable for people with cows' milk allergy. Meat, game, and poultry are all allowed, but sausages and pies should be avoided unless it is known that they are milk free. Intolerance to cows' milk protein is not a reason to avoid beef. Eggs are allowed, but not custard or scrambled egg, which may contain milk. Fish is permitted, unless it is cooked in batter (which unless otherwise stated should be assumed to contain milk) or milk. Lemon curd, chocolate spread, chocolate (unless stated to be milk-free), toffee, fudge, caramels, and butterscotch are all unsuitable. All ordinary cereals (e.g., oats) are allowed, but caution is required with manufactured breakfast cereals, some of which contain milk powder.

It is essential to check the list of ingredients on the label of any manufactured foods. There is a special problem with unwrapped foods, because there is no label of ingredients. Examples include bread, sausages, or confectionery.

Egg Avoidance

Eggs (both the white and the yolk) and all products that contain egg or albumen must be avoided. As well as hen's eggs, eggs of other birds such as geese, turkeys, and quails must be avoided. Eggs are widely used to make cakes and are sometimes used in the manufacture of bread. Egg wash or glaze is commonly brushed on to the surface of rolls, buns, or baps, and also bread, cakes, and pastry used in puddings (e.g., apple pie). Sweets can be a hazard because they are usually sold without information about ingredients, and egg is included in several products.

Mayonnaise normally contains egg; custard usually does not, with the exception of egg custard and egg custard tarts. Eggs are an essential ingredient of souffles and certain sauces, such as Bearnaise or Hollandaise sauce.

Egg allergy is not a reason to avoid eating chicken.

Soy Avoidance

The major difficulty is mass-produced bread, because in the UK soy is often included as an ingredient in flour. Soy is also found in manufactured products that contain hydrolyzed or textured vegetable protein, and minced beef, which unless described as 'pure beef' has been known to include quantities of soy protein.

Wheat-Free and Gluten-Free

These terms cause confusion; they are not interchangeable. Subjects who are allergic to wheat cannot tolerate foods that

contain any type of wheat. Subjects with celiac disease can tolerate all wheat proteins other than the gluten fraction.

Peanut Avoidance

Peanut is also known as groundnut or arachis, so these three names need to be sought on labels of manufactured foods as well as some pharmaceutical products. The difficulty comes with 'vegetable oil,' which may include peanut oil; only by writing to the manufacturer of individual products can the composition of the vegetable oil be determined. It is not known to what extent subjects with peanut allergy should avoid peanut oil. Most peanut oil used in food manufacture is highly refined, and contains only very minute quantities of peanut protein. In a number of small-scale studies, subjects with peanut allergy were found not to react when given highly refined peanut oil. However, it remains possible that such oil contains traces of protein sufficient to result in enhanced reactivity, such that when the subject does ingest peanut accidentally the reaction is worse than previously. On this basis, subjects with peanut allergy should really be advised to avoid peanut oil.

Drug Treatment in the Management of Food Allergy

At present, drug treatment has little part to play in the management of food allergies. There are two exceptions. First, there are a very small number of cases in which the reaction to a food is exclusively gastrointestinal, and in whom the reaction can be blocked by taking the drug sodium cromoglycate by mouth 20 min before the trigger food is swallowed. Second, there are a small number of individuals who develop the life-threatening reaction, of anaphylactic shock when exposed to a trigger food. There are three ways in which anaphylactic shock may prove fatal. First, rapid swelling of the soft tissues in the pharynx may completely obstruct the airway; the treatment is to bypass the obstruction, either by passing an endotracheal tube, or by performing a tracheostomy. Another mechanism is severe shock, with a profound drop in blood pressure; the life-saving treatment is to restore the circulating volume with intravenous fluids and to give oxygen. The third mechanism is severe bronchoconstriction (asthma); here, the life-saving treatment is with bronchodilator drugs and artificial ventilation. If patients with life-threatening anaphylactic shock are to be saved, they must be given urgent (within minutes) medical attention. For individuals who have already experienced a life-threatening allergic reaction to a food, it is a common practice to provide them with a syringe preloaded with adrenaline (epinephrine), with the aim that this should be administered while waiting for medical help. Unfortunately, self-administered adrenaline is not without its hazards (e.g., inadvertent intravenous administration causing fatal cardiac arrest), and there is no proof that it is life saving; indeed, there are many cases in which the subject died despite the use of epinephrine. Nevertheless, it is the best one can do when faced with someone who is experiencing a life-threatening allergic reaction to a food. The need for urgent medical help cannot be overemphasized.

There is little evidence that antihistamine drugs are of any value. It would be reasonable to take a nonsedating fast-acting antihistamine such as terfenadine if experiencing an allergic reaction to a food, but it is questionable whether it will have much effect.

A number of new approaches to the treatment of IgE-mediated food allergy are being examined. In a double-blind placebo-controlled study of monthly injections of a preparation of anti-IgE antibodies, treated patients with peanut allergy required significantly greater amounts of peanut protein to elicit allergic symptoms compared with control subjects. Another anti-IgE preparation has been used in the treatment of asthma but has not been evaluated in peanut allergy. Theoretically, anti-IgE antibody treatment should be protective against multiple food allergens, although it would have to be administered indefinitely. Other experimental approaches include a concoction of traditional Chinese herbs, injection of heat-killed *Escherichia coli* containing mutated recombinant peanut proteins Ara h 1 to Ara h 3, the use of immunostimulatory sequences, and the use of chimeric protein that could form complexes with allergen-specific IgE bound to mast cells and basophils.

Desensitization

In theory it ought to be possible to desensitize subjects with food allergy by giving injections of gradually increasing quantities of an appropriate extract of the food trigger. In practice, such treatment is not available. One at present insurmountable difficulty is that desensitization (also known as hyposensitization) treatment carries a small risk of death from the treatment itself. A subject has a series of injections without any major problem, but then without warning drops dead from anaphylaxis after the next injection. There is some data to show that desensitization performed in this way can work, but such subjects would probably require maintenance injections on a permanent basis, and the very subjects most at risk of fatal anaphylaxis from accidental injection are quite probably also the ones most at risk from fatal anaphylaxis resulting from desensitization treatment.

See also: Celiac Disease. Eggs. Food Intolerance. Lactose Intolerance. Malnutrition: Secondary, Diagnosis and Management

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FOOD CHOICE

Behavioral Aspects

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Introduction

Food choice decisions are not the same as intake volume decisions. The former determine what we eat (soup or salad); the latter determine how much we eat (half of the bowl or all of it). Large amounts of money, time, and intelligence have been invested into understanding the physiological mechanisms that influence food choice. Much less has been invested in understanding how and why our environment influences food consumption volume. Yet environmental factors (such as package size, plate shape, lighting, variety, or the presence of others) increase our food consumption volume far more than we realize.

This is one of the puzzles of food consumption research. Although people can acknowledge that environmental factors influence others, they wrongly believe they are unaffected. Perhaps they are influenced at a basic level of which they are not aware or do not monitor. Understanding these drivers of consumption volume has immediate implications for research, policy, and personal interventions. There are three objectives of this chapter: (1) explain why environmental factors may unknowingly influence consumption; (2) identify two resulting myths that may lead to misspecified models or misguided policy recommendations; and (3) offer implications to move research, policy, and personal dietary efforts forward with more certainty and focus.

Why do We Overeat?

Although there are many environmental factors which influence consumption, consumption norms and consumption monitoring are themes that tie these factors together. Many seemingly unrelated environmental factors consistently influence eating behavior because they alter our perceived consumption norm for a situation, or because they interfere with our monitoring of how much we have consumed.

Consumption Norms Offer Suggestible Benchmarks

For many individuals, determining how many ounces of cola to drink or how many pieces of pizza to eat for lunch is a low-involvement behavior that can be based on how much one normally buys or consumes. Yet consumption can also be unknowingly influenced by environmental cues – a benchmark or reference point – that may subtly suggest a

consumption norm that is appropriate, typical, reasonable, and normal.

For instance, the number of items in an assortment or the eating behavior of a dinner companion may serve as a benchmark that a person uses to gage how many items should be eaten or how much should be drunk. Similarly, large packages, plates, serving bowls, serving spoons, and even pantries have all been shown to increase how much a person serves and consumes by 15–45%. The consumption norms suggested by these large sizes have been shown to influence experts – leading professional bartenders to overpour alcohol and nutritional science professors to overserve themselves ice cream. Moreover, the tendency to be biased by these cues may be even as powerful – within limits – as the taste of the food itself. When moviegoers in a Philadelphia suburb were given either medium- or large-size containers of stale, 14-day-old popcorn, they still ate 38% more despite its poor taste.

All of these cues perceptually suggest that a larger amount of food is normal, appropriate, typical, and reasonable to consume. Most individuals dutifully follow these implicit suggestions. The use of consumption norms, as with normative benchmarks in other situations, may be relatively automatic and may often occur outside of conscious awareness.

Consumption Monitoring and Calorie Estimation is Highly Inaccurate

Eating is multidimensional and difficult to monitor. This can cause people to focus more on food choice than on their consumption volume of the chosen food, and it can lead to unmonitored and unintended results. The biggest danger of not monitoring intake is that a person eats more calories than they would otherwise want. Studies involving calorie estimation have shown that the general process of estimating how many calories one has consumed is tremendously influenced by the external environment.

In general, all people underestimate their calorie consumption by a predictable amount. Mathematical modeling shows that the magnitude of the underestimation bias increases as the actual size of the meal increases.

In addition to this basic tendency to underestimate one's calories as a function of the size of a meal, people are also biased by the 'health halos' that accompany labels. A series of studies where foods were falsely labeled as being 'low fat' led consumers to overconsume these foods relative to control foods. Even when taking into account the average (11%)

reduction in the calorie content of low-fat offerings, these people ate 34% more calories than the control group. A similar result was found with regard to how much a person ordered and ate from restaurants they perceived as healthier versus less healthy (e.g., Subway vs McDonalds). That is, although consumers visiting Subway ate 11% fewer calories than when at McDonalds, they estimated they had eaten 37% fewer.

Food Psychology and Eating Behavior

Psychologists have experimented extensively with food consumption decisions. Here we summarize the most important principles of this literature. More thorough reviews of this literature can be found in Just in 2007 and Wansink in 2004. This summary will provide a background for the policy discussion in the following section.

Individuals tend to view goods in terms of a moral structure. Some goods are viewed as being virtuous, and others as sinful or extravagant. This may be of particular importance in food consumption where this moral structure may be reinforced by public information campaigns, and food or diet advertising. It has been shown that individuals are willing to pay much less to acquire an item than they are willing to accept to part with the item once it is given to them. This endowment effect appears to interact with the type of item. For instance, it has been found that utilitarian (or virtuous) goods are salient when choosing among goods to acquire, while hedonic (or sinful) goods are salient when deciding which must be given up. In terms of diet, this suggests that individuals are much more willing to add good foods to their diet than they are willing to give up bad foods, making it difficult to reduce overall caloric intake.

Epstein describes the choice between 'wants' and 'shoulds' as a battle between rational thought and emotion. He proposes the Cognitive-Experiential Self-Theory (CEST) to model this battle. This model supposes that the individual evaluates each stimulus using two separate processes: (1) an experiential system is used to make rapid evaluations based on affect and (2) a cognitive process is used to make deliberative evaluations based on rational thinking. Epstein shows that which process dominates depends primarily on the availability of processing resources. These resources can include time, distractions, or the volume of decisions that need to be processed, among others. Impulsive behavior can result from the presence of stress, time constraints, the presentation of food choices, or the sheer number of choices available. Others have found that individuals are much more likely to choose cake than fruit salad when given a simple cognitive task to perform than when no task is given.

Surprisingly, preferences and taste appear to have less to do with the amount people eat than environmental factors. It has been suggested that the external factors having the greatest impact on consumption volume are the eating environment (atmosphere, effort, social facilitation, and distractions) and the food environment (salience, structure, size, stockpiling, and shape). The eating environment refers to the attributes of the areas surrounding the individual as she/he eats. The food environment refers to the presentation of the food itself.

Importantly, most of these factors affect consumption volume without the individual being aware of the effect.

CEST also plays a role in determining the quantity of food consumed. As individuals become distracted, they have less ability to monitor the amount of food they have consumed. This generally leads to overconsumption in distracting eating environments. Distractions that are known to increase consumption include conversation, reading, watching television, listening to music, or watching sporting events. For example, social gatherings tend to extend the duration of meals, leading to greater consumption; the larger the gathering, the greater the consumption. The presence of others can affect consumption not only through distraction but also through the setting of social norms. When eating in groups, individuals tend to try to eat amounts similar to their peers. Additionally, the convenience with which food can be obtained can have a disproportionate impact on consumption quantity. It has been found that significantly more ice cream is purchased when the cooler door is left open than when shut.

The food environment can affect consumption through several separate mechanisms. First, the simple viewing of food can lead to unplanned consumption. This happens because viewing the food acts as a reminder of a pleasurable experience, and because viewing and smelling food actually induces the release of dopamine, stimulating hunger. Simply asking an individual to describe the last time they ate soup more than doubles the amount of soup consumed on average in the next 2 weeks. Similarly, having large quantities of a food on hand increases the consumption of that food significantly regardless of replacement cost.

Cosmetic differences in the food can also have a large impact. Offering a greater variety of foods (or perceived variety) increases consumption. Additionally, packaging can impact consumption by introducing simple consumption monitoring mechanisms. For example, individually wrapping items can dramatically reduce consumption.

People tend to eat more when they are presented with larger packages or portions of food. Doubling portion sizes increases consumption anywhere from 18% to 25% for meal related foods and up to 45% for snack foods. Moreover, larger package sizes lead individuals to severely underestimate their consumption after the fact. Astonishingly, individuals will eat more when given larger portions even if the food is reported to be repulsive by the subjects. A general result is that individuals tend to focus on consumption volume when determining a stopping point rather than specific levels of nutrients or calories.

Three Myths of Mindless Eating

The unique context of eating may challenge the assumptions that researchers and public policy officials have about consumers and rational decision-making. When faced with food, people respond differently than when faced with, for example, a car purchase. This can lead researchers and public policy officials to make assumptions about mindful eating that take on an untested yet near mythical surety in research and policy realms.

Mindless Eating Myth 1: People know How Much they Want to Eat

In one study, 62 Master of Business Administration students were presented with a 90-min class session that used lectures, videos, demonstrations, and group activities to underscore that if they were presented with a gallon serving bowl of Chex Mix, they would serve and eat more than if they were instead presented with two half-gallon serving bowls. At the end of this session, these were informed, intelligent consumers. Six weeks later, these same students were invited to an apparently unrelated Super Bowl party where they were presented either gallon-size serving bowls of Chex Mix or twice as many half-gallon bowls. Those presented with the gallon bowls served 53% more and ate 59% more. When asked if they believed the size of the serving bowls influenced their behavior, they denied it influenced them.

Similarly, consider the studies showing that Philadelphia bartenders poured 28–32% more into short, wide tumblers than tall, narrow high-ball glasses. Immediately after they poured and after pointing out their bias, the bartenders were asked to pour again. Although they were a bit more accurate, they still poured 21% more into the wider glasses than the taller ones.

Even when shown that larger packages bias consumption by at least 20%, many people in lab and field studies wrongly maintain that they were unaffected. The same is true with other studies examining low-involvement behaviors. Although people readily acknowledge that these environmental factors influence other people, they deny the influence on themselves.

In relating this to consumption, it is well supported that the size of a package can increase consumption, as can the size of portion servings in kitchens and in restaurants.

The impact of packages and portions on consumption is sizable. When packages double in size, this has generally translated into a 18%–25% increase in consumption for many meal-related foods (such as spaghetti) and a 30%–45% increase in many snack-related foods. Such predictable increases in consumption occur even when the energy density of the food is altered, thus indicating that something is driving people to consume these foods past the point of satiation. In effect, the volume of food eaten tends to be a better indicator of how ‘full’ one considers oneself than does the calorie density of the food.

Mindless Eating Myth 2: People Know When they are Full

One objection to studies that show that people overserve themselves in response to environmental cues is to argue that people may get tricked into overserving themselves, but they would not overeat. This presupposes that a person is more responsive to their internal cues of satiation (such as hunger or taste) than to external cues.

Sociologically, this may not be as true for Americans as for others. One study asked a matched set of 150 Parisians and Chicagoans when they knew they were through eating dinner. The Parisians said they knew they were through eating dinner when they ‘were no longer hungry’ or when the ‘food no longer tasted good’ – both of these are internal cues of satiation. In contrast, the Chicagoans said they knew they were

through eating dinner when their ‘plate was empty’ or when the TV show they were watching ‘was over’ – external cues of satiation. Regardless of their culture, overweight people used external cues more than internal cues.

This physiological view toward satiety was further challenged in a study that suggested that people stop eating when their dish is empty. A soup bowl was designed to automatically refill itself. Those who were given these bowls ate an average of 73% more than those sitting across from them with a regular bowl. After 15 min, the study was stopped and those with refillable soup bowls were asked to rate their satiety. Following this, when asked if they were full, a common response was, “How can I be full, I still have half a bowl left?” A similar study involving the continuous removal of chicken wing bones at an all you can eat restaurant by waitresses showed a similar result. Those whose chicken wings had been bussed ate 34% more but did not believe it.

People may believe they know when they are full, but studies in the field suggest they eat more with their eyes than with their stomach. Indeed, we may think we know when we are full, but that is our fallibility.

Mindless Eating Myth 3: Changing Prices Changes Food Choice

Several studies have suggested that poor diets may be a simple result of price differences. Although real food prices have declined, it is hard to reconcile the notion that simple price fluctuations have caused the obesity problem with the commonly held belief that the elasticity of demand for food is very small. For example, using a simple utility model of food consumption, the effects of the US dairy program on nutrient intakes has been estimated. The dairy program has the overall effect of raising prices on fresh milk, whereas lowering prices on processed milk products. In accordance with the simple utility model, it was supposed that this would lead consumers away from fresh milk, to fattier and less-healthy processed milk products. However, an empirical analysis, employing data covering US consumption from 1949 to 1994 suggests that demand for milk products is very price inelastic. Thus even though price changes were large, changes in nutrient intake for all nutrients associated with dairy products changed by less than 1%. The impacts of food price changes on consumers appear to have been more financial than nutritional.

It should not be terribly surprising that price has little to do with consumption. Most individuals live in multiperson homes. In a typical family, one individual may do most of the shopping, whereas all family members eat. Most individuals have no opportunity to observe changes in prices when making consumption decisions. Those that do may have little memory of small changes in prices by the time the food is consumed. It may require substantial changes in food prices before casual eating behaviors are affected.

Another analysis reported the effects of price and availability of fast food and other food sources on obesity levels. Employing the Behavioral Risk Factor Surveillance System for years 1984 through 1999, they estimate that, at best, raising

the prices on fast food by 50% should result in a loss of 5 lbs for an average height and weight male (slightly more for an obese individual). Although the relationship between food prices and obesity is significant, it is also very small in magnitude. However, availability of fast food appears to play a much larger role in obesity. They find a much stronger relationship between the number of fast-food establishments and obesity. This suggests that individuals may not be so deliberative when considering fast food, rather reacting to impulses when presented with an opportunity (e.g., while driving by a restaurant when one happens to have extra time).

Some models of food consumption have lead many to explore the possibility of taxing fatty or sugary foods and subsidizing more healthy fare. If consumption behavior, especially for the most fatty and sugary foods, is unresponsive to price, such policies will fail to improve diets substantially. Additionally, such a tax could have the unintended effect of transferring wealth away from those who have the least wealth to begin with.

One important line of economic research examines the impact of media and government health information on diet. There is some disagreement between the economics and marketing literatures regarding the impact of health information on consumption. The economics literature has guessed that health information is a significant determinant of consumption, and, thus, when new health information arrives, behavior incorporates this new information. In studies designed to determine the impact of health information and schooling on obesity, it has been found that those with a knowledge of the link between diet and disease are much less likely to be obese. Econometric models were also found to be extremely sensitive to the inclusion of diet knowledge as an independent variable.

Many applied economists have attempted to analyze the impact of health information on consumers' perception by utilizing different health resources and health information sources in the United States and European countries. Diverse conclusions were reached. For example, researchers focusing on US studies find that health information is a significant and large factor in consumption, but EU data show that this factor is negligible. Meanwhile, the marketing literature has concluded that health information plays little to no role in food consumption decisions, far outweighed by concerns of price, taste, and ease of preparation. Some of this disparity might be due to the different types of information examined in the two literatures. Although economists tend to look for the aggregate effect of any health information, marketing scientists have examined more specifically the effect of specific pieces of positive health information on individual consumption. Certainly some consumers are affected by the information but in very different ways. By eliminating the restricted structure imposed by economists, the marketing studies consistently show little impact of health information. Indeed, it has been found that information connecting eggs and cholesterol has no long-term impact on egg demand when economic models allow information to decay over time. Impacts of health information on average decayed entirely after 1 month. In summary, there is substantial reason to doubt the effectiveness of traditional policies in changing long-term eating behavior.

The Future of Mindless Eating

Food consumption volume is not the same as food choice. The mechanisms behind each of these are very different. Although impressive resources have been invested into understanding food choice, it is now becoming increasingly important to better understand what drives food consumption volume. Given the unknowing impact that environmental factors have on consumption, consumer welfare will advance if these discoveries help them personally and effectively alter their environment without them having to continually monitor how much they eat.

Although the potential to use food psychology in food assistance policy exists, several challenges must first be overcome. Very little is known about how food behaviors interact with prices and other traditional mechanisms. Thus, although initial evidence suggests the usefulness of behavioral policies, their true effectiveness is a mystery. More work must be done to measure the effects of behavioral mechanisms on the functioning of behaviors, such as those involving food-assistance programs. The goals of this research should be to: calibrate the effects of offering behavior-targeted options; determine the cost effectiveness of such options; and to evaluate the trade offs in costs, benefits, health, social stigma, and membership for traditional policies offering similar behavioral changes.

It is difficult to argue the importance of policies targeting willpower, underestimation of quantities, or decisions made in haste. However, there is some precedent in the banning of certain money-making schemes (such as pyramid marketing or certain investment vehicles) or the regulation of walkaway periods for many contracts. More research into the relationship between behavioral-based marketing and the consequences they may have on unsuspecting consumers may highlight the need for such policies in the food industry. Clearly, food assistance must strike a delicate balance between the nutritional minimums of the participants, participant behavior, and the motivations of the food marketers; which may be perverse. The mechanisms are not in place to strike this balance. Rather, traditional mechanisms used for food assistance may increase the ability of food marketers to leverage behavioral anomalies.

For consumers, in general, current policy on food marketing concerns primarily the truthfulness of the health claims made on packaging. Ironically, truthful claims may often mislead consumers into thinking items that have 'less fat' are necessarily better than their normal fat counterparts – even if sugar or other items have been added to compensate. Despite the evidence that smaller packages can lead to healthier portion sizes, marketers are currently forbidden from advertising such a fact.

The current prevalence of overweight and obesity in the US has prompted many policy discussions. If much of eating behavior is determined by reflexive behaviors and decisions made with few cognitive resources, it is unlikely that policies designed to appeal to highly rational and cognitive thought will have much of a positive effect.

What can be done? The environment can work for people or against people. On one hand, it can unknowingly entice and contribute to our overconsumption of food. On the other

hand, a personally altered environment can help people more effortlessly control their consumption and lose weight in a way that does not necessitate the discipline of dieting or relinquishing self-governance to another. For some, this might involve repackaging food into single-serving containers, storing tempting foods in less convenient locations, and preplating one's food before beginning a meal. For others, simply using narrow glasses and smaller plates might be all that is required to make their environment less conducive to overeating.

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FOOD COMPOSITION DATA

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Glossary

Food composition The level of nutrients and other components in specific foods.

Imputed nutrient composition An estimate of the nutrient level in a food based on an analyzed nutrient level for a similar food.

INFOODS International Network of Food Data Systems at the Food and Agriculture Organization.

LanguaL An automated system for describing, capturing, and retrieving data about food.

Supplement composition The level of nutrients and other components in specific dietary supplements.

Tagnames Standardized names for nutrients, as proposed by INFOODS.

Overview – Why Compile Food Composition Tables?

Food composition data are an integral component of evaluating and planning nutrient intakes. Without information on the nutrient content of foods, it is not possible to convert dietary intake data, based on foods consumed, into nutrient intake data. The science of developing accurate food composition data has advanced substantially with the advent of sophisticated laboratory equipment and methods for food analyses, as well as increasingly powerful computers that are utilized to compile and store the results. The International Network of Food Data Systems (INFOODS) at the Food and Agriculture Organization (FAO) has provided guidelines and training to help countries improve their food composition tables. However, comprehensive analyses of the many nutrients and other bioactive components of foods remain both challenging and expensive, and given the enormous variety of foods consumed around the world, food composition tables may be incomplete. Often, the intake calculations that are based on these tables must be regarded as estimates of true nutrient intakes. Nonetheless, for many research and public health purposes, nutrient intake estimates are essential, and can lead to actions that improve the health of both individuals and populations.

Procedures for Compiling Food Composition Data

Nutrients to Include

As the number of recognized biologically active components of foods increases, compilers of food composition tables are faced with an ever-expanding list of possible nutrients and other components to be included. Some of these are given in **Table 1**. The current version of the US Department of Agriculture's Standard Reference Database (release 23) contains up to 146 components for each of more than 7500 foods. Because a wide variety of analytic methods are available for

determining and reporting nutrient levels in foods, it is useful to have a common convention for naming the nutrients. Many compilers are using standard nutrient names, called tagnames, that have been proposed by INFOODS.

Nutrient values in a food composition table normally reflect the level in 100 g of the food item. Thus, the intake of a nutrient from a specific food can be calculated if the amount consumed is recorded in gram weights (e.g., if 100 g of whole milk has 119 mg of calcium, and a person drank a cup of milk weighing 244 g, then the intake of calcium from the cup of milk would be 291 mg). Many food composition tables also contain the weight of typical portions of each food item, and thus the nutrient profile for these portions can readily be calculated.

A related issue is whether to show nutrient profiles per 100 g of the food as consumed, or 100 g of the food as purchased. Because some parts of a food may be discarded as inedible, the nutrients per 100 g as purchased will be lower for these foods. For example, a banana skin is approximately one-third of the weight of a banana. If 100 g of a banana without peel has an energy content of approximately 90 kcal, then the energy content of 100 g of banana with peel is only 60 kcal. Composition tables may simply carry a variable for the average percent of the food that is edible, but it is obviously important to match the method used to measure the food intake (with or without inedible portions) with the way the composition of the food is given in the table.

Foods to Include

There are also a constantly expanding number of foods available in most regions of the world, due to changing agricultural practices, increases in imported foods, and new commercial product formulations. Including all these foods in a single composition database has not been attempted, and instead, regions and countries have focused on compiling food composition tables that are specific for their populations.

Table 1 Nutrients and other food components that are often included in food composition tables

<i>Nutrients/food components</i>	<i>Typical units (usually per 100 g)</i>	<i>Related components that may be present in a table</i>
<i>Macronutrients</i>		
Energy	kcal or kJ	
Protein	g	Individual amino acids; nitrogen
Fat	g	
Carbohydrate	g	May be calculated by difference (100 minus grams of the other macronutrients)
Alcohol	g	
Water	g	
Ash	g	
<i>Carbohydrates and fiber</i>		
Sugars	g	Individual monosaccharides and disaccharides
Starch	g	
Dietary fiber	g	May be divided into soluble and insoluble fiber
Nonstarch polysaccharides	g	
Lignin	g	
Glycemic load	g	Glycemic index
<i>Fats</i>		
Saturated fatty acids	g	Individual fatty acids
Monounsaturated fatty acids	g	Individual fatty acids
Polyunsaturated fatty acids	g	Individual fatty acids
Omega-3 fatty acids	g	
Omega-6 fatty acids	g	
Trans fatty acids	g	
Conjugated linoleic acid	mg	
Cholesterol	mg	
<i>Minerals</i>		
Calcium	mg	
Phosphorus	mg	
Magnesium	mg	
Iron	mg	Heme iron, nonheme iron
Zinc	mg	
Sodium	mg	
Potassium	mg	
Selenium	µg	
Copper	µg	
Chromium	µg	
Molybdenum	µg	
Manganese	mg	
Fluoride	mg	
Iodine	µg	
<i>Vitamins</i>		
Vitamin A	IU, µg RE, µg RAE	
Carotenoids	µg	Individual carotenoids
Retinol	µg	
Vitamin E	mg α-tocopherol	mg α-tocopherol equivalents, synthetic α-tocopherol
Tocopherols	mg	Individual tocopherols
Vitamin C	mg	
Vitamin D	µg, IU	
Thiamin	mg	
Riboflavin	mg	
Niacin	mg	Niacin equivalents
Folate	µg, µg dietary folate equivalents	Synthetic folic acid
Vitamin B ₆	mg, µg	
Vitamin B ₁₂	µg	
Pantothenic acid	mg	
Biotin	µg	
Vitamin K	µg	
<i>Other food components</i>		
Isoflavonoids	mg	Individual isoflavonoids
Flavonoids	mg	Individual flavonoids

Several types of foods are usually found in such composition tables.

Basic agricultural commodities are considered essential in most tables. These would include both plant and animal foods that are typically consumed by the population of interest. Frequently, composite values are given in composition tables, and reflect an average of multiple samples collected from different regions of the country. For example, nutrient profiles of oranges in the USA are an average of different species of oranges grown primarily in California and Florida; the average is weighted to reflect the production of different types of oranges. Composition tables may contain both cooked and raw values for a food item, which can be helpful if a food is consumed both ways (e.g., tomatoes). Because nutrients may be lost during cooking, and also because the water and fat contents may change, it is important to have nutrient values that correspond to the form of the food that is actually consumed. In addition to cooked and uncooked forms, basic foods may also be available in processed forms, such as canned, frozen, or dried. Many of these processing procedures can alter the nutrients in foods, and thus it is sometimes desirable to have composition data for the differently processed forms of the food.

In addition to basic foods and ingredients, food composition tables usually also contain values for mixed dishes. Some of these mixtures may reflect common recipes that are used at home, and others may represent commercially available foods, either in food stores or in restaurants. Because recipes may vary greatly, it is particularly useful if the software that accesses the composition table allows the user to alter the recipe ingredients.

Food Descriptors to Use

It is a challenging task to clearly and completely describe the foods that are in the food composition table. Food names in most tables are devised by the compilers, using common names plus appropriate descriptors (e.g., cooked, raw, canned). Ideally, the food descriptors should fully define the food item, to ensure there is no ambiguity about the scientific name, the part of the plant or animal that is consumed, and

any cooking or processing that has been applied. Several schemes for describing foods have been proposed, including guidelines from INFOODS, and the LanguaL system that is being used by several European countries.

Sources of Composition Data

Food composition data come from a variety of sources (Table 2). Those based on laboratory analyses of foods are considered the gold standard for composition tables. Appropriate methods are often specified by the Association of Official Analytical Chemists (AOAC). Accurate analytic procedures also should incorporate quality control methods, including the proper use of internal standards, and the analysis of duplicate samples to determine inter-sample variability.

The scheme that is used to obtain and prepare the food samples for analysis is also important. Ideally, the sample collection scheme would match the foods that are reported by the population of interest. For example, if the purpose of the analysis is to determine the nutrient content of a specific person's diet, then the analyses should be performed for a composite of the foods actually consumed, or in the case of feeding studies, for a composite of the foods to be fed. Because such analyses are usually not feasible, more general composition data are often used. Most food composition tables are intended for use across a broad population, and thus the sampling scheme should reflect the types of foods typically consumed. Often, this is an expensive and challenging task, particularly for national and regional tables. Once the sampling plan is devised, it is also necessary to decide on the protocol for storing and preparing the samples for analysis. Considerable nutrient losses can occur if samples are handled improperly, as many nutrients are labile to heat, light, and exposure to oxygen. Methods of indicating the quality of analytic data for foods have been proposed, including attaching a confidence code to each data point, so that users can decide if the composition data are appropriate for their purposes.

Analytic data are published in various forms. Many countries or regions publish tables, either in print or electronic form. For example, large electronic tables (often called food

Table 2 Sources of data for food composition tables

Sources	Comments on accuracy
<i>Analytic values</i>	
By the table compilers	Generally the most accurate type of data if sampling and analyses are appropriate
From published literature	May not be correct if the food items differ on important characteristics
From the food industry	Values from food labels may be underestimates for nutrients added to foods
From another composition table	May not be correct if the food items differ on important characteristics
<i>Imputed values</i>	
Based on a similar food	The accuracy of this process depends on how closely the foods can be matched
Assumed zero	Can be very accurate for some nutrients (fiber in animal products; vitamin B ₁₂ in plant products)
<i>Calculated values</i>	
From another form of the same food	Usually requires assumptions about changes such as losses due to cooking
From a recipe	Typical recipe ingredients and proportions may be difficult to collect
From a product formulation	Useful method for obtaining nutrients' values that are not on the product label

composition databases or databanks) are compiled by the US Department of Agriculture, and by the Food Standards Agency in the UK. Other sources of analytic data include journal articles and books. A particularly useful journal for food composition values is the *Journal of Food Composition and Analysis*, edited by the INFOODS Secretariat.

However, analytic data may not be available for all foods and nutrients of interest, and time and cost constraints may prohibit chemical analyses of these foods. In some cases, these values are left blank, and such missing values are assumed to be the same as zero values by most programs that calculate nutrient intakes. Because an appropriately estimated value for a nutrient is usually superior to a value of zero, several methods are used to derive such estimations. A frequent approach is to obtain data from the food composition table of another region or country. If the foods are of the same genus and species, then the nutrient profiles should be similar. Although variations can occur due to different cultivars within a species, as well as different conditions during growing, storage, and processing, such borrowed composition values are considered preferable to a missing value. Another approach to estimate nutrient profiles is to impute a value from a similar food that has analytic data. If the known nutrients are similar for two foods (e.g., the macronutrient profiles), and the type of food is similar (e.g., dark-green vegetables), then the missing value may be replaced with an imputed value from the similar food. Sometimes calculations are performed to adjust for differences between the foods. Values for a cooked food can be imputed from a raw food by applying factors for nutrient losses during cooking, and adjusting for differences in moisture (and sometimes also in fat) content.

Another common method of obtaining composition data is to calculate the values from the ingredients in a mixture. For home-prepared foods, such calculations involve determining the proportions of each ingredient (a recipe) and any changes in moisture content during preparation (the yield). There are many challenges in determining recipes that are appropriate for a large group of individuals, but it is equally challenging to try to collect appropriate samples of these mixtures for chemical analysis. For some mixtures, multiple recipes, and thus multiple entries on the food composition table, may be needed (e.g., home-prepared beef stew, commercially canned beef stew, and beef stew from a restaurant).

Composition data may also be obtained from the nutritional labels on commercial food products, if they are available. Most countries now require a list of ingredients on the label, and if the proportions of each can be estimated, then a recipe can be devised. It is more useful, however, if the label gives information on the nutrient profile, for at least some of the main nutrients. These values can be incorporated directly into the composition table, and also are useful in estimating the proportions of each ingredient (e.g., the amount of wheat flour might be estimated from the carbohydrate content). Because even the most comprehensive nutrition labels seldom give values for all nutrients of interest for the users of food composition tables, recipes will be needed to estimate values for nutrients not shown on the label. Caution should be used with label values for nutrient-fortified products. Good

manufacturing practice dictates that the label underestimate the levels of any nutrient, particularly vitamins, that may degrade with time. This ensures that nutrient levels are at least as high as those stated on the label, even after a substantial time on the shelf. Thus, it is always preferable to obtain average nutrient values directly from the product manufacturer, if possible.

Compilations of Composition Data for Dietary Supplements

As the use of dietary supplements increases worldwide, there is an increasing need to quantify intakes of nutrients and botanical products from these sources. Compiling nutrient profiles of such products into tables can be very time consuming, as the number of products continues to grow, and formulations of existing products often change over time. Furthermore, average analytic data are seldom available from the supplement manufacturers, and thus database compilers must rely on whatever information is available from the product label. In many countries, a label showing the amount of each nutrient in the product is required.

Uses of Food Composition Data

Evaluate or Plan Nutrient Intakes

Uses of food composition data are varied, and the method of compiling the data may need to be tailored to the application of interest. Perhaps the most common use of composition data is to estimate intakes of individuals. Dietitians and other health professionals may wish to evaluate the quality of a person's current diet, or to plan for changes in a diet to meet specific nutrient goals. For example, a person with elevated serum cholesterol may be counseled to reduce saturated fat and cholesterol intakes, and given specific menus of diets low in these nutrients. To compile these menus, a nutritionist would require access to composition data for saturated fat and cholesterol in a variety of commonly consumed foods. Although the composition data are often averages across many samples of a food, this level of precision is usually acceptable for counseling applications, where long-term compliance with dietary recommendations is being examined.

Similarly, researchers often evaluate or plan diets for individuals as part of nutrition studies. However, for these applications, the required level of precision of the data may be higher. In a feeding study, it may be crucial that the composition of the menus be tightly controlled, and thus average values across many samples are not appropriate. Indeed, it may be necessary to conduct laboratory analyses of the diets that are used in feeding studies, rather than rely on more general composition data.

Food composition data are also used to plan and evaluate intakes of population groups, as in dietary surveys, or in choosing menus for institutions such as schools and hospitals. When intakes are to be evaluated and averaged across a large number of people, the use of aggregated food composition data is appropriate, and would lead to less error in the estimates than relying on only a small number of samples.

Some users of food composition data may wish to evaluate the nutrient content of foods as purchased at stores or markets. For example, food consumption data may be evaluated for households, rather than for individuals, and these data are usually recorded as foods that are purchased for the household. In this case, the composition data must also be given per quantity of food as purchased, before any inedible portions are removed, and before cooking. Likewise, nutrition education for families may focus on making shopping lists of nutritious foods for the household, and composition data for foods as purchased will be helpful.

Food composition data may be used at an even more aggregated level in estimating food use for a region or country. For example, the US Department of Agriculture estimates the nutrient content of the US food supply annually, in order to track trends. These data, often called disappearance data, assign nutrient composition values to the major commodities that are produced (minus any exports) or imported for use as food (e.g., flour, sugar, butter). The amount of each commodity that is available for consumption is multiplied by the corresponding nutrient composition to give an estimate of nutrient consumption per capita.

Estimate Nutrient Profiles for Product Labels

The food industry also uses food composition data to justify health claims for their products (e.g., to indicate that a food product is low in fat), and in many countries, to obtain information for printing nutrient information on the product labels. Nutrition labels are often required, and are considered an important consumer guide to select healthy diets. Large manufacturers of processed foods usually obtain laboratory analyses of the nutrients in their products, but smaller companies may rely on calculating nutrient profiles from the product's ingredients. Restaurant chains are also increasingly likely to provide nutrient composition data for the items on their menus.

Evaluate or Plan Food Intakes

Yet another use of food composition data is to examine intakes from food groups – at the level of the individual, the population group, or the nation. Such analyses are facilitated if each of the food items in a food composition table is assigned to a food group, using a predetermined food grouping scheme. Once foods are categorized into groups, it is possible to examine intakes from each group (as grams per day) as well as nutrient intakes from each group (e.g., dietary fiber from grains). A further refinement of the food group assignments includes an indication of the number of servings that each food contains (usually per 100 g of the food). Thus, 100 g of orange juice contains approximately one-half of a serving of fruit (assuming three-fourth cup, or 188 g, of juice is considered a serving). Using such a scheme, it is possible to calculate the number of servings consumed from each food group in a day, and compare these intakes to dietary guidance for a country. MyPyramid is used for such guidance in the USA, and the US Department of Agriculture has developed a MyPyramid Equivalents Database that may be used to calculate intakes of 32 food groups (Table 3).

Table 3 Food groups on the MyPyramid Equivalents Database 2.0, 2003–04

Grain group	Total grain Whole grain Nonwhole/refined grain
Vegetable group	Total vegetables Dark-green vegetables Orange vegetables White potatoes Other starchy vegetables Tomatoes Other vegetables
Fruit group	Total fruits Citrus fruits, melons, and berries Other fruits
Milk group	Total milk Milk Yogurt Cheese
Meat and beans group	Meat, poultry, fish Meat (beef, pork, veal, lamb, and game) Organ meats (meat, poultry) Frankfurters, sausage, and luncheon meats (made from meat or poultry) Poultry (chicken, turkey, other) Fish and shellfish high in <i>n</i> -3 fatty acids Fish and shellfish low in <i>n</i> -3 fatty acids
Oils	Eggs Cooked dry beans and peas Soybean products (tofu, meat analogs) Nuts and seeds
Extras	Discretionary oil Discretionary solid fat Added sugars Alcoholic beverages

Potential Limitations of Food Composition Data

Poor Analytic Procedures

Accurate chemical analysis of the nutrient content of foods is a challenging process, and may yield inaccurate results for a variety of reasons. For some nutrients (and other food components of interest), accurate procedures may not be available. For example, the usual procedures for analyzing the folate content of foods are known to underestimate the actual levels, and thus estimates of folate intakes are likely to be low. Both the extraction procedures and the enzyme digestion treatments may be less than optimal for food folate, and although more recent procedures solve some of these problems, folate values on most food composition tables are probably underestimated. Dietary fiber in foods provides another example of possibly incorrect methods. Many older food composition tables contain a variable named fiber, but the values are for crude fiber. Crude fiber is measured using procedures that destroy some of the physiologically important fibers, and thus it is an underestimate of the true dietary fiber content. More recent methods measure either total dietary fiber (defined as all fibers that are not digested in the human gut, including lignin) or nonstarch polysaccharides (which excludes lignin).

Inaccurate analyses may also occur when access to the best laboratory equipment is not available, either because the costs are too high or because the technical expertise on its usage is not available. For many of the antioxidant compounds such as carotenoids and tocopherols, quantification by mass spectrometry (MS) yields the most sensitive detection limits, although analysis using high-performance liquid chromatography (HPLC) is adequate in most cases. However, because the equipment, maintenance, and reagents are often too expensive, laboratories (particularly in developing countries) may use older methods, such as spectrophotometry combined with open column chromatography. Nutrient values derived using such methods are less accurate than those resulting from HPLC and MS methods.

Users of food composition tables should ask when and how analytic values were obtained. Likewise, compilers of these tables should clearly document the analytic procedures used to obtain all values and ensure that such information is readily available to users.

Inappropriate Sampling Procedures

The way foods are sampled and collected can also impact the quality of the composition data. Many nutrient values vary substantially across multiple samples of the same food. Nutrient composition can be affected not only by the species and cultivar of a plant, but also by the growing conditions, time of harvest, and length of storage. Because it is seldom feasible to match all these factors with the diets to be analyzed, composite values, based on the average of multiple samples, are usually given in food composition tables.

Inappropriate Nutrient Forms and Expressions

An important limitation for some food composition tables is the method of expressing the activity of the nutrient. The estimation of nutrient activity is a large and expanding field of research, and includes studies of both the absorption of the nutrient, and its bioavailability for metabolic processes. For example, the iron bioavailability has been debated extensively, and many algorithms for calculations have been proposed. Virtually all of them require separating the iron that is found as heme iron in animal products from nonheme sources of iron. If these two variables are not carried on the composition table, it will not be possible to calculate the iron bioavailability for specific intakes.

Vitamin A also illustrates the complexity of properly expressing the physiologically meaningful form of a nutrient. Until 1967, the vitamin A value of foods was expressed in international units (IUs), which was equivalent to 0.3 μg of retinol and 0.6 μg of β -carotene. This form of expression is still used in many composition tables, and also on nutrition labels for both foods and dietary supplements. A more relevant unit of activity, microgram (μg) of retinol equivalents (REs) was adopted in 1967, and has been used to set recommended nutrient intake levels. A lower relative provitamin A activity of carotenoids was assumed, and thus, it is not possible to directly convert IUs into REs, unless both the retinol and the carotenoid levels of a food are given. Recently, the estimated

provitamin activity of carotenoids has been further reduced, and a newer unit proposed: microgram (μg) of retinol activity equivalents (RAEs). Again, it is not possible to convert between REs and RAEs (or between IUs and RAEs), unless the retinol and carotenoid components of a food are available. Increasingly, food composition tables carry separate variables for the specific forms of nutrients like vitamin A and iron, but this is not the case for many older tables. Such disaggregation has an obvious advantage, as it allows for recalculation of nutrient activity when there is a scientific consensus that new availability factors are needed. Tables that cannot be easily updated to reflect new information will lag behind the current knowledge, and thus will have increasingly limited usefulness.

Lack of Internal Consistency and Integrity

Compiling food composition tables involves recording nutrient profiles for many foods and nutrients, and errors can easily occur during this process. Quality control is important in this field, just as it is in the development of any product. Developers of the most accurate composition tables always include procedures that ensure the numbers are correct. In addition to having several people review any new data before they are added to the table, several automated types of integrity checks are possible. For example, the energy value of a food item should approximate the value calculated from the main components (4 kcal g^{-1} times the grams of protein and carbohydrate, plus 9 kcal g^{-1} times the grams of fat, plus 7 kcal g^{-1} times the grams of alcohol), and any deviations should be investigated. Likewise, the sum of all the macronutrients (water, protein, fat, carbohydrate, alcohol, and ash) should be approximately 100 g if the nutrient profiles are given per 100 g of the food. Such quality control procedures should be an integral part of the compilation of food composition data.

Conferences on Food Composition Issues

Most of the issues discussed in this article have been addressed at conferences specifically convened to present advances in food composition data. In the USA, the National Nutrient Databank Conference has been held annually since 1976. In addition, the International Food Data Conference has been held biannually since 1993. The proceedings from several recent conferences have been published in the *Journal of Food Composition and Analysis*.

See also: Dietary Intake Measurement: Methodology. Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Supplementation: Dietary Supplements

Further Reading

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FOOD CULTURE

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Glossary

Complementary and alternative medicine (CAM) A group of diverse medical and health care systems, practices, and products not presently considered to be part of traditional medicine. Examples include homeopathy and macrobiotic or fruitarian diets.

Culture Integrated pattern of human knowledge, belief, and behavior that depends on the capacity for learning and transmitting it to succeeding generations.

Folklore Traditional customs and tales that are preserved and shared widely among people of a culture.

Food folklore Traditional beliefs, legends, and customs about food that have been transferred from one generation to the next by word of mouth.

Foodways Eating habits and culinary practices of people, regions, or historical periods.

Introduction and Definitions

Food is an important part of culture as well as essential for biological well being. In addition to study by the biological and physical sciences, the societal and cultural aspects of food and nutrition are also matters for scholarship, and these are addressed by social scientists. Some definitions set the context for this large field. Culture is the integrated pattern of human knowledge, belief, and behavior that depends on the capacity for learning and transmitting knowledge to succeeding generations. Culture also refers to the customary beliefs, social forms, and material traits of a social, racial, or religious group, and the characteristic features of everyday existence as a way of life that are shared by people in a particular place or time. Foodways are eating habits and culinary practices of people, regions, or historical periods. Many cultural forces are involved in causing individuals to choose or refuse certain foods, and thus they are of interest to clinicians.

Folklore is traditional customs, tales, and the like that are preserved and shared widely among the people of a different culture. It includes legends, oral history, proverbs, popular beliefs, and customs that are traditions of a culture or group. Food folklore consists of traditional beliefs, legends, and customs about food that have been transferred from one generation to the next by word of mouth. For thousands of years, folklore espousing food's nutritional and medicinal benefits has influenced dietary practices. Food folklore is often considered to be similar to mythology because both are beliefs that do not correspond with the dominant belief structures in the society. In many instances such folklore has little impact on health related behaviors or nutrition and it can be ignored by health professionals. Complementary and alternative medicine (CAM) is a group of diverse medical and health care systems, practices, and products that are not presently considered to be part of

conventional medicine. They include many beliefs and customs related to healing and health in food lore.

Because food folklore and CAM coexist with formalized education based and institutionalized systems of thinking about food and nutrition sciences, occasionally clashes emerge between the two belief systems about food and eating practices that are guiding health related behaviors emerge, and the folklore may actually be harmful to health. When that happens, clinicians must deal with the discrepancy and encourage patients to adopt healthful practices. But the first problem they face is knowing what is going on. Patients do not always know that they should tell providers about their use of CAM or food folklore that has an impact on their medical care. Others are afraid to tell health professionals for fear that they will be berated by the health care provider for unscientific beliefs. Recently it has become clear to many health professionals that rather than ignoring or condemning such food folklore a more fruitful approach is to engage in a dialog with patients. This has proven useful in discussing and dealing with beliefs about complementary and alternative medicine in the National Center for Complementary and Alternative Medicine's (NCCAM) 'Time to Talk' program (<http://nccam.nih.gov/timetotalk/forpatients.htm#jump3>). The notion is that if patients tell their health care providers about their CAM use they can more effectively manage their own health. And when providers ask their patients about CAM use, they can ensure that patients are fully informed and that they as providers can help patients make informed health care decisions.

History of Food in Culture

Food is essential to culture because it sustains life by providing essential nutrients. But it also has a larger role in culture, such

as influencing food choice. Key cultural factors that influence food choices include geography, environment, biology, physiology, the senses, technology, politics, and economics, among others. Food also plays a very important role in the social structure of society, and in psychological attitudes toward eating and health.

Food plays a symbolic role in both religious ceremonies and cultural traditions. For example, rice has been associated with fertility in many cultures for millennia and in many countries it continues to be thrown on newly married couples today. Similarly, bread has been regarded as a symbol of divinity and has played an important role in many religious services and observances.

Curative properties have also been ascribed to many foods for thousands of years. In ancient Rome, cabbage was considered the perfect medicinal plant and was prescribed frequently for a wide range of ailments including warts, deafness, and drunkenness. Apples, herbs, garlic, honey, milk, peppers, and many other foods were also highly regarded in ancient cultures for their therapeutic qualities. The prescription of foods as medicines was not necessarily based on scientific evidence but instead was often based on early medical theories or magic. The ancient Greeks believed that the body was composed of four humors: blood (hot and moist), phlegm (cold and moist), yellow bile (hot and dry), and black bile (cold and dry). Health was thought to result from a balance of the humors, and illness from an imbalance. To counteract imbalances and restore health, physicians often prescribed specific foods, based on their perceived degree of heat and moisture. For example, fever, a hot and dry condition was attributed to an excess of yellow bile, and cool and moist foods, such as cucumbers, were prescribed to treat it. In contrast, edema, a cool and moist condition, was treated with foods that were viewed as warm and dry. The hot, cold, moist, and dry properties of food were also regarded as important in other ancient societies, including China, where achieving a balance between the opposing forces of yin (cold/moist) and yang (hot/dry) has guided traditional Chinese medical practice for centuries and continues to be popular today.

Table 1 Food names related to food folklore

Herb (botanical name)	Folklore
Blackeye root (<i>Tamus communis</i>)	Heals bruises, removes discoloration
Bloodroot (<i>Sanguinaria canadensis</i>)	Cures blood disorders and heart disease
Birthwort (<i>Aristolochia longa</i>)	Alleviates complications associated with childbirth
Eyebright (<i>Euphrasia officinalis</i>)	Cures disorders of the eyes
Ginseng (<i>Panax quinquefolium</i>)	General human panacea
Heartsease (<i>Viola tricolor</i>)	Relieves heart ailments
Liverwort (<i>Anemone hepatica</i>)	Relieves liver disorders
Lungwort (<i>Sticta pulmonaria</i>)	Cures pulmonary diseases
Maidenhair fern (<i>Asplenium trichomanes</i>)	Prevents balding, promotes hair growth
Snakeroot (<i>Aristolochia serpentaria</i>)	Antidote for snake bites
Spleenwort (<i>Asplenium</i>)	Remedy for disorders of the spleen

The Doctrine of Signatures, based on the notion that 'like cures like', was popular in the nineteenth century. Therapies were chosen on the basis of similarities of color, aroma, shape, and other characteristics. For example, beet juice, which is deep red, was thought to be an effective cure for blood diseases, whereas yellow plants were believed to alleviate jaundice and other liver ailments. The pungent odors of onions and garlic were thought to ward off disease, stimulate strength and bravery, arouse the libido, and banish evil spirits. Walnuts resemble the brain and so were eaten to improve intellect. The ginseng root, with its resemblance to the human torso, was used by the Chinese as a panacea.

The common names of many herbs and botanicals reflect folklore about their curative properties, as shown in [Table 1](#). For example, the word ginseng is derived from the root words *gin*, meaning man, and *sing*, meaning essence.

Food Folklore and Food Culture Today

Although some food folk beliefs continue to be passed down from generation to generation, others have been discarded over the years, and new ones have been introduced. Today, food folklore is spread not only by word of mouth from person to person, but also to large numbers of people simultaneously *via* the mass media, the Internet, Twitter, etc. The growing popularity of CAMs, dietary supplements, organic products, and functional foods has led to the development of new food folklore and increased the popularity of some traditional notions. Several examples of commonly held food folk beliefs of both the past and present are provided in [Table 2](#).

Food and nutrition-science concepts that have developed over the past two centuries are newcomers to human thinking about the relationships between food and health. Although some food beliefs, such as the association of carrots with eyesight, have some scientific basis, many others remain unsupported by, or in opposition to, recent scientific findings. Pseudoscience, rather than sound evidence, provides the basis for much of today's food folklore. It is important for food, nutrition, and biomedical professionals to be knowledgeable about current food folk beliefs, because these ideas influence popular views about diet–health relationships. To identify beliefs that are of major health significance, first to consider is the prevalence of the belief, the likelihood the belief will be followed, and the seriousness of the effect that acting on the belief is likely to engender. Then it is useful to consider both the strength of the belief in folklore and the strength of the scientific evidence surrounding it, as shown in [Table 3](#). When folkloric belief and scientific evidence are both strong and in agreement with each other, so that the behavior is in line with scientific research, the folklore is unlikely to pose a major health threat, and may even be beneficial, for example, 'eat for two'. Similarly, when food folklore and the scientific evidence surrounding it are both weak, few practical problems exist. However, when scientific findings refute, or fail to support, popular food folk beliefs, and outcomes of following beliefs are serious, individual or public health may be threatened. For example, despite folklore that ephedra promotes rapid weight loss, evidence-based reviews suggested that it was harmful. Some hypotheses or theories, such as cold fusion, lack both theory and evidence to

Table 2 Food folklore: Current and historic beliefs

<i>Food</i>	<i>Folklore</i>
<i>Fruits</i>	
Apple	Preventive. 'An apple a day keeps the doctor away'; prevents caries and tooth decay
Blueberries	Cure for kidney and urinary-tract ailments; improves vision and memory
Cherries	Cherry gum dissolved in wine relieves a cough; ensures continued fertility
Citrus fruits	Prevent scurvy; cause low blood pressure; cure the common cold
Cranberries	Prevent scurvy; prevent or cure urinary-tract infections
Currant	Relieves sore throat
Fig	Relieves toothache; mild laxative
Grapefruit	Should be avoided completely when taking medication; burns calories, dissolves fat, aids in weight loss; is 'good for you'
Raspberry	Raspberry leaf tea promotes labor contractions and aids in childbirth
<i>Vegetables</i>	
Beets	Cure for anemia; helps build iron-rich blood
Carrots	Good eyesight
Celery	Promotes weight loss
Garlic	Stimulates digestion; inhibits germs; cleanses the blood and intestines; lowers cholesterol and blood pressure
Lettuce	Induces sterility
Onions	Cooked onions cure the common cold; good for the heart
Peppers	Cures headaches
Potatoes	Cure for impotence, scurvy, and soothe and soften the skin; but are fattening
Spinach	Builds strong muscles
<i>Grains</i>	
Bread	Cures disease and protects against evil; is a fattening food; brown bread has more fiber than white bread
Flaxseed	Cure for constipation; prevents cancer; lowers cholesterol
Oats	Oatmeal and oat bran prevent heart disease
<i>Dairy</i>	
Milk	Prevents scurvy; heals ulcers; causes constipation; unpasteurized milk is more nutritious than pasteurized; a glass of milk before bed causes drowsiness; mothers who drink a lot of milk have colicky babies; milk and other dairy products are fattening and should be avoided on a low-fat diet; the calcium in milk and other foods causes kidney stones, raw milk is superior to pasteurized milk healthwise
Yoghurt	Prevents vaginal yeast infections; cures vaginitis, constipation, and diarrhea; yoghurt applied topically heals a sunburn
<i>Meat</i>	
Beef	Beef and other red foods cause high blood pressure; extra protein from beef makes muscles stronger
Chicken soup	Cure for the common cold
Eggs	Raw eggs help build muscle; brown eggs are healthier than white eggs; people with high cholesterol should not eat eggs
Legumes	Beans are a natural laxative
Seafood	Fish is a brain food; is good for the heart and prevents heart attacks; pregnant women should avoid eating fish; oysters increase sexual potency
<i>Fat, sweets, and alcohol</i>	
Olive oil	Protects against breast cancer
Cod liver oil	Relieves rheumatism, aching muscles, and stiff joints; prevents rickets
Sugar	Causes hyperactivity; eating too much causes diabetes and heart disease
Honey	Is natural and will not raise blood-sugar levels; a mix of honey and water is a good cure for colic
Chocolate	Causes acne; prevents heart disease
Salt	A no-salt diet protects against high blood pressure; sea salt is healthier than table salt; salt tablets prevent muscle cramps
Alcohol	Helps to warm the body in cold weather; acts as a sleep aid if consumed before bedtime; red wine is good for the heart; a nip of brandy cures a cold; drinking alcohol with raw oysters makes them safe and free of food-borne infection

support causal inference. Often, though, it is that the scientific evidence regarding food folklore is weak, unproven or undetermined, indicating that more research needs to be done. It is impossible to test all possible food folklore beliefs for their effects on health. Usually formal reviews or experiments are reserved for beliefs that are likely to have major impacts on the public health. Examples are, beliefs that circulation of foods cause cancer, use of raw instead of processed milk is healthier that vitamin deficiency causes HIV-AIDS, etc.

Food, Culture, Folklore, and Evidence-Based Nutrition

Totality of the Evidence

Nutrition science strives to be evidence-based, relying on the totality of the scientific evidence for making conclusions. This has given rise to the term evidence-based nutrition. Food folklore cannot be taken as fact without evidence to support it.

Table 3 Folklore: Separating fact from fiction

		Scientific evidence	
		Strong	Weak
Folklore	Strong	Fact ^a	Fiction/ undetermined ^b
	Weak	Emerging science ^a	Fiction ^a

^aUnlikely to be a major threat.^bPotential threat to public health.**Table 4** Ranking the quality of the evidence

Highest quality	Randomized double-blind placebo-controlled trials
	Observational studies Prospective studies Retrospective studies
	Uncontrolled clinical trials
	Patient reports/Case studies
Lowest quality	Non-human trials <i>In vitro</i> studies <i>In vivo</i> studies

Single studies are also usually inadequate for demonstrating cause–effect relationships, and no single study alone is enough to prove that something is fact or folklore. It is important to consider the totality of evidence and the type and quality of the available research. When many different types of evidence are all supportive of a relationship, the weaknesses of individual studies are mitigated and causal inference is strengthened.

Ideally, the best way to conduct a scientific evaluation of a question is to perform an evidence-based expert review of many randomized double-blind placebo-controlled clinical trials, meta-analyses, and other studies. An evidence-based review often entails the use of statistical techniques to re-analyze the results of many small studies, as well as expert judgment. If all systematic evidence-based reviews of randomized trials produce comparable conclusions, the scientific evidence is good that the folklore belief is justified. Comprehensive reviews of observational studies are also useful although causal inference is weaker. These use a number of grading systems now that help to rank the results of different kinds of studies in assessing cause and effect relationships.

Comprehensive evidence-based reviews are especially important for far-reaching questions that have implications for large populations. They are also necessary for questions

regarding important issues, including life and death, and those that involve very large costs or imply large reimbursements. However, because such reviews are significantly time consuming and require much expertise and money, they can be done only for a few very important questions. For example, the National Institutes of Health (NIH) has sponsored such reviews of obesity treatment, the American Institutes of Cancer Research has done reviews to substantiate their population-based dietary recommendations, and the health effects of many other dietary interventions have been the subject of reviews by the Cochrane Collaborative, the Agency for Health Care Research on Quality (AHRQ) of the US Department of Health and Human Services, and the American Dietetic Association (ADA) Evidence Analysis Library. The 2010 Dietary Guidelines for Americans used several evidence reviews to examine some questions that relate to folklore. Unfortunately, for much food folklore, such studies have never been done.

Type of Evidence

Although the combined historical experience of various cultures on the optimal relationship between food, diets, and health is helpful, by itself it is insufficient to determine whether the relationships are valid or not. In determining the validity of food folklore or CAM, not only the totality of evidence but also the type and quality of available evidence are important. As shown in **Table 4**, the strength of the association between eating a food (cause) and a health outcome (effect) can be ranked according to the type of evidence presented. The best evidence comes from studies that have the most control over the claim or treatment being evaluated and eliminate other factors that may suggest an effect was present, when really it was not. Although randomized double-blind placebo-controlled clinical trials are considered the ‘gold standard’ for determining diet–health relationships, such studies are rarely available for many nutrition questions. Lesser levels of evidence must usually be used. Also, randomized, double-blind clinical trials are too small to assess all adverse effects that are important to monitor – observational studies are better at doing this, as was the case in demonstrating the adverse effect of ephedra for weight loss in large amounts and the lack of major adverse effects of olestra on bowel function.

Randomized Double-Blind Placebo-Controlled Trials

When several randomized double-blind placebo-controlled trials show a relationship between a specific food and a health effect, the evidence of a cause–effect relationship is considered to be very good. These studies exert rigorous control over the claim or treatment being evaluated and over the people who are subjected to it (by randomization) and the assumptions of both the experimenters and the study participants (by placebos and blinding). Multiple studies of this type, with an expert review of all other types of data, are considered to be the ‘gold standard’ for establishing cause–effect relationships. Other types of evidence and studies are lower in the hierarchy, because they are not as definitive in identifying true cause and effect.

Although single randomized trials are somewhat less definitive, they are still valuable, because they also permit control over the treatment being evaluated. Often, however, these studies are not large enough, or the study sample is not representative, so the results cannot be generalized to the population of interest. Other factors that may weaken these studies are not counting dropouts, lacking or unconvincing placebos, and inappropriate events or biomarkers serving as surrogate end points.

Observational Studies

Human studies that involve observation rather than direct intervention provide evidence that is satisfactory but less conclusive than randomized, double-blind clinical trials. These studies are designed to test a relationship between an exposure of interest (folk belief) and a health outcome. Observational studies include both cohort studies (prospective) and case-control studies (retrospective). In a prospective study, a group exposed to the treatment of interest and an unexposed group are followed forward in time. The health outcomes in both groups are observed and evaluated after controlling for confounding factors with the use of statistics. In contrast, retrospective studies compare individuals who have already developed an outcome of interest (case) against those who have not (control). Factors contributing to the development of the outcome are then determined by looking backward in time. Because observational studies cannot be precisely controlled, it is more difficult to establish cause and effect. However, when confounding factors can be adequately controlled for, these studies provide suitable evidence to support diet-health relationships. They are useful for discovering adverse events or beneficial effects that are attributable to the treatment.

Uncontrolled Clinical Trials

Clinical studies in which everyone is treated, in which only those who ask for the treatment are treated, or in which some are treated based on unsubstantiated clinical convictions are suspect. In such studies, no randomization occurs and neither the researcher nor the participant is blinded to the treatment. Therefore, it cannot be determined whether the treatment is actually the cause of the observed results or whether biased convictions of either or both experimenters and study participants are falsely contributing to the results. Better evidence is needed before it can be stated with assurance that the folklore based on such observations is true.

Patient Reports, Case Studies, and Folklore

Even weaker human evidence of cause and effect comes from single medical case reports and anecdotal evidence. These types of evidence are also biased because those who experience success from the treatment are much more likely to report their stories than those who do not.

Animal Studies and Laboratory Experiments

Nonhuman studies involving living animals (*in vivo* studies) or tissue cultures (*in vitro* studies) are useful in providing information on the possible mechanisms of action, biological plausibility, dose response, and action of a treatment. However, their ability to predict outcomes in humans is poor.

Therefore, these studies are unconvincing by themselves of effects in humans and should be used only to support other types of evidence.

Guide for Evaluating Food Folklore Collaboratively with Patients in Clinical Situations, and a Practical Example

For summarizing and evaluating food folklore involving diet-health relationships, health professionals must not only evaluate the evidence but also need to use their clinical judgment and communications skills to relate their findings to clients or patients. How can food folklore be evaluated in discussions with laypeople and in counseling situations? The strategies are similar to those employed in research and in more formal evidence-based reviews, but contextual realities require tailoring of the approach. One method of evaluation and resolution called the 6R method is provided, as shown in Table 5, and an actual clinical example follows.

The Problem

One example of currently popular food folklore is the notion that people on medication should not ever drink grapefruit juice. Although there is scientific evidence that grapefruit juice interacts with certain medications, making them incompatible, the facts do not suggest either that grapefruit must be eliminated from the diet of those taking medications or that all drugs exhibit these interactions. The process of reviewing this food folklore with the patient to arrive at this conclusion is outlined in detail below.

In the late 1980s, in a study examining the interaction between alcohol and felodipine (a calcium antagonist used to lower blood pressure), it was accidentally discovered that grapefruit juice, which was being used as a placebo, dramatically altered the drug's metabolism. The drug was a common one, the juice dose – approximately 6 oz (180 ml) – was within the range many people drink, and the effects were large (similar to a doubling of the drug dose). Therefore, the finding was of potential clinical importance. Since then, more than 200 scientific papers have been published in peer-reviewed journals on the issue of drug interactions with grapefruit, confirming the original observations. By the mid-1990s, the finding had received a great deal of media coverage and the notion that grapefruit juice was dangerous for those on prescription drugs had become a subject of food folklore. This particular bit of folklore is an example of a strongly held belief for which there is some scientific evidence. Under the circumstances, how should clinicians advise patients?

Table 5 Steps to evaluating food folklore in clinical situations using the 6R's

1. Report
2. Review
3. Recall
4. Relate
5. Recommend
6. Revise

Steps for Evaluation and Resolution

Report

In counseling, it is important for the clinician to relate to the patient and establish two-way communication to learn about the folk belief. It may be useful to determine the strength of the individual's conviction about this belief as well as its source and whether it is likely to jeopardize the patient's treatment. When the health professional actively listens, it is more likely that the patient will listen, understand, accept, and follow recommendations.

Review

In clinical situations, it is also important for health professionals to review all the evidence surrounding the patient's food belief. A vital piece of information to consider is safety. Although many prescription drugs have side-effects, they are taken under the supervision of a physician, who can monitor adverse effects and take steps to control them. Because folk remedies and alternative medicines are often self-administered, such safeguards are lacking. If there is evidence that the implementation of food folklore in self-medication is likely to be hazardous to the patient's health, it must be discouraged. The implementation of some folk alternative medicines has little or no adverse effects, and it can be disregarded.

Specifics are important, such as the drug in question. For example, when the patient's prescription drug is one that is metabolized by cytochrome 3A (CYP 3A), a dramatic effect can occur if grapefruit juice or other forms of the fruit are consumed. Grapefruit juice enhances the effects of these drugs over time by decreasing their oral clearance. However, the effects of the interaction depend on the nature of the drug (for some drugs there is little or no effect) and the size of the interaction. Interaction occurs only if the drug is metabolized by CYP 3A, if it normally undergoes presystemic extraction with CYP 3A, and if it is given orally. The interactions vary. For example, with the statins – drugs commonly used to lower serum cholesterol – they are strong for simvastatin and lovastatin, moderate for atorvastatin and cerivastatin, and low for fuvastatin and pravastatin. Similarly, sedatives, hypnotics, and other drugs vary as to whether they induce interactions or not. Thus, for some drugs, grapefruit juice is contraindicated whereas for others it is not.

Recall

The CYP P450 superfamily consists of many enzymes. They are labeled as follows: CYP (family 1, 2, 3, etc.) (subfamily A, B, C, etc.) (isoform 1, 2, 3, 4, etc.). There is individual variability in the expression of these enzymes, which is probably in part genetic.

CYP 3A is an enzyme that is involved in the metabolism of many drugs. It is present in the liver and gut mucosa and is induced or inhibited by drugs and other chemicals. Under certain conditions, components of foods can also affect it. The CYP 3A enzyme in the gut mucosa (enteric CYP 3A) is affected by grapefruit juice, but CYP 3A in the liver is not. The phytochemicals that are thought to have these effects are furanocoumarin derivatives in the juice, which reversibly and irreversibly inhibit the CYP 3A in the gut mucosa. When grapefruit juice and certain drugs are taken together orally, this

leads to effects on their presystemic extraction (first-pass metabolism). In consequence, presystemic extraction of the drug is reduced, and, because of this, more of the drug reaches the circulation over time. With this increased systemic exposure to the drug, there is an increased drug effect. The duration of the juice's effect depends on the dose and on the time it takes for the enzyme to regenerate. The liver CYP 3A is not affected by grapefruit juice (although it may be affected by some drugs that also affect gut CYP 3A). Thus, the grapefruit–drug interaction does not happen with drugs administered intravenously, because they bypass the gut.

Approximately one-fifth of American households consume grapefruit juice, and it is considered a good food since it has the American Heart Association 'heart check' and the American Cancer Society endorsement. Many older people take their medications and juice together at breakfast. Many people who are elderly take medications, and some of the drugs may pose problems if taken with grapefruit juice. Therefore, this folklore is highly relevant from the clinical standpoint.

For the grapefruit interaction to take place, the patient must be taking a drug that affects the gut CYP 3A and the patient must express a significant amount of CYP 3A. People differ in these respects, and there may be racial as well as other genetic differences that are not yet clear.

Relate

The clinician scientist must build on his or her own knowledge and that available from expert reviews or sources and place it in the clinical context. Common sense is needed to fit the information to the patient's realities. The facts, which are that some but not most drugs do not interact with grapefruit, must be related to the folklore and the patient's actual condition. Many grapefruit–drug interactions are modest and not clinically important. Fortunately, for every therapeutic class of drugs there is an option that is not affected by the grapefruit interaction, and that can be prescribed to avoid the problem.

Recommend

In responding and making recommendations, considerations include their importance, feasibility, and effectiveness for the patient. The information is then individualized to fit the patient or questioner's problem and needs. This is the time to particularize for the patient and lead him or her to the next level of understanding. With many drugs there is no interaction, and the patient can be told to drink the juice if he or she wishes but to alert the physician if adverse reactions occur. It may be possible to change the medication if a major interaction exists, or, for modest interactions, it may be enough to avoid taking drugs and grapefruit juice at the same time and to avoid consuming large quantities (four or more glasses) of juice. The patient should be praised for asking about possible food–drug interactions and told that these reactions sometimes occur, but do not usually exist. Is the information relevant – that is, if the patient is on a statin, is it the type that is involved in interactions? Does the patient want to drink grapefruit juice? If not, the issue is moot.

Revise

Fortunately, in counseling, although a relatively rapid response is usually required, there is an ongoing relationship with the individual that permits follow-up after additional sources have been consulted. For example on this question, additional information is available at <http://www.powernetdesign.com/grapefruit> and at www.foodmedinteractions.com. The clinician can follow-up and revisit the issue later if necessary when more information becomes available or when additional questions arise.

For example, a patient might ask whether, since the drug effect is enhanced with the consumption of grapefruit juice, he or she could save money by taking more grapefruit juice and continuing with lovastatin. The response to this legitimate question is no, not because it is theoretically impossible but because it is difficult to titer the drug, dose; individual differences exist, and reactions are unpredictable, so such a strategy is not recommended.

Conclusions

Food culture, folklore, and CAM are alive and well and likely to continue. For summarizing and evaluating food folklore involving diet–health relationships, health professionals need to not only evaluate the evidence but also use their clinical judgment and communications skills to relate findings to patients. The 6R's (report, review, recall, relate, respond, recommend, and revise if necessary) provide a guide for evaluating food folklore with patients in clinical situations. Many of the other aspects of food and culture that are not discussed here are matters dealt with in the symposia and journals of the Association for the Study of Food and Society, the Society for Anthropology of Food and Nutrition, and the Agriculture, Food and Human Values Society.

See also: Drug–Nutrient Interactions. Functional Foods: Health Effects and Clinical Applications

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FOOD FORTIFICATION

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Programs

Technological Aspects

Programs

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Glossary

Effectiveness Extent to which an intervention attains its expected impact, under real conditions.

Evaluation Assessment, as systematic and objective as possible, of a planned, ongoing, or completed program that covers its need, design, implementation, impact, efficiency, and sustainability, so as to incorporate lessons learned into the decision-making process and inform policy.

Fortified foods Foods (for example, staples and condiments) that have had micronutrients added.

Logic model Visual representation of the of the core components of the program that map the relationships

between program resources, activities that will take place, and outputs and outcomes that may result in the short, medium, and long term.

Mass food fortification program Organized, planned, and usually ongoing effort designed to deliver fortified foods widely consumed by the general population.

Monitoring (implementation evaluation) Ongoing process of collecting, analyzing, interpreting, and reporting indicators, to compare how well a program is being executed according to predefined criteria.

The findings and conclusions in this report are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

Mass Food Fortification: Programs

The main goal of a mass food fortification program is to help correct inadequate intake of micronutrients to prevent or reduce the severity and prevalence of micronutrient deficiencies, without exposing the population to the risks from excessive intake.

Fortification is a cost-effective approach to improve the vitamin and mineral intake of the overall population, including women of reproductive age (WRA) and children. Although many foods (such as salt wheat flour, edible oils, and margarine) have been fortified for years in some countries, this approach has not yet been scaled up in many lower-income countries.

As of March 2011 there were 60 countries with legislation or decrees that mandate fortification of one or more types of wheat flour with either iron or folic acid (**Map 1**). The flour produced in these countries, plus the flour that is fortified voluntarily, represents 30% of the world's wheat flour that is produced in large roller mills. One hundred and twenty five

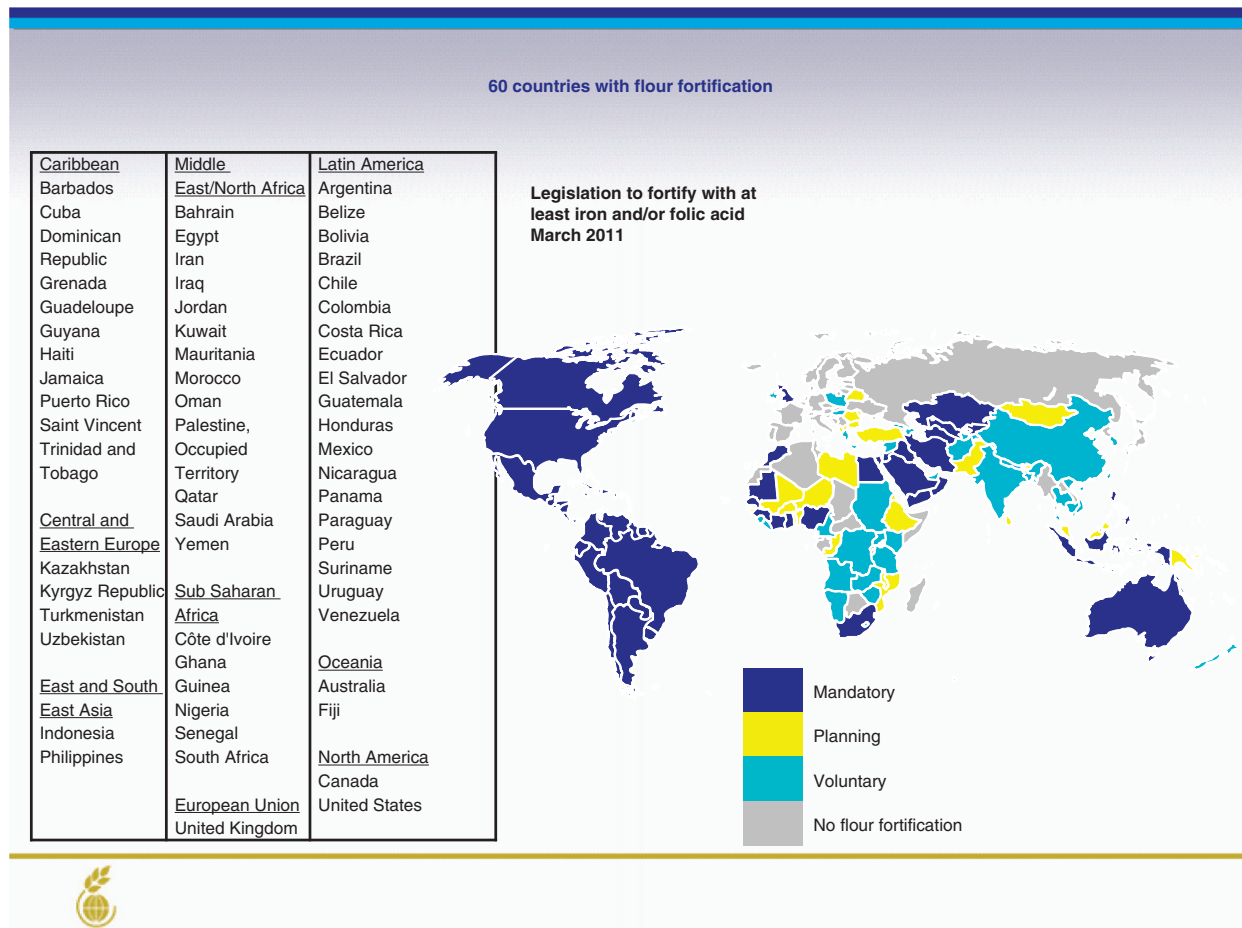
countries are now implementing and reporting on salt iodization programs, which translates into 72% of all households in developing countries now consuming adequately iodized salt. These are examples of how the need for these fortification programs is being expanded and of the massive coverage they can have.

How to Determine the Need for Fortification Programs?

The need for a fortification program is based on evidence or assumption that micronutrient deficiency exists in the population and that fortification will produce a health benefit for this population. Other micronutrient interventions already being implemented in the country should be considered in the assessment of this need.

Micronutrient Status of the Population

The nutritional status of micronutrients in a population can be assessed by various biomarkers related to the micronutrients' metabolism. This information can be supplemented with clinical indicators related to the deficiencies. For example, vitamin A deficiency can be measured by the prevalence of low serum retinol, but can also be assessed by the prevalence of night blindness. Iron deficiency can be measured by



Map 1 Wheat flour fortification status, countries fortifying with at least iron and/or folic acid. Reproduced with permission from Flour Fortification Initiative (FFI). Flour fortification status as of March 2011. <http://www.sph.emory.edu/wheatflour/globalmap.php> (accessed 26 March 2011).

the prevalence of low serum ferritin levels (adjusted for inflammation) but can also be approximated by the prevalence of anemia. Folic acid deficiency can be measured by the prevalence of low folate levels in the serum or red blood cell but can also be assessed by the prevalence of neural tube defects.

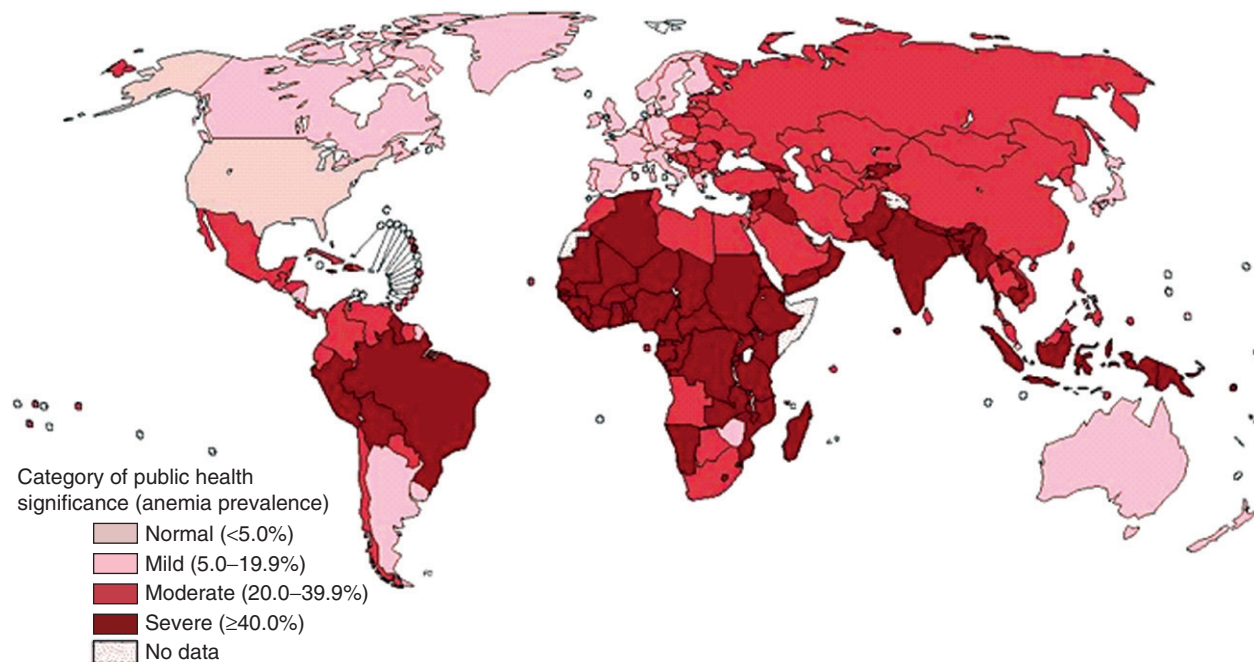
World Health Organization (WHO) maintains a Vitamin and Mineral Nutrition Information System to monitor the micronutrient status of populations and produces estimates of the major deficiencies affecting the world. For example, anemia is a public health problem worldwide, and vitamin A deficiency in preschool children is quite widespread as well (Maps 2 and 3). The high prevalence of anemia and vitamin A deficiency is usually accompanied by other deficiencies like zinc, vitamin B₁₂, and riboflavin (B₂) because these micronutrients all come from animal source foods. The nutritional gaps or biomarkers associated with these deficiencies are the primary source of information when considering the need for a fortification program. The major micronutrient deficiencies in different populations around the world are iron, vitamin A, iodine, folate, zinc, and vitamin B₁₂.

National health surveys carried out every 4–6 years by several countries in the world provide data on anemia, and

national micronutrient surveys provide data on iron, vitamin A, and iodine status. In recent years these surveys have started to incorporate measurements of folate, zinc, and vitamin D. However, most countries do not have an updated micronutrient status report on its population. **Table 1** shows the prevalence of anemia in several countries in recent years, and it can be seen that the situation has either remained stagnant or worsened in these countries. WHO suggests that prevalence of anemia between 20% and 39.9% indicates a moderate public health problem and prevalence $\geq 40\%$ a severe problem. Changes in trends from moderate to severe may suggest the need to consider fortification programs as an option to improve the anemia status of nonpregnant women.

Micronutrient Intakes

The adequacy of the diet is a second source of information to assess the need for a fortification program. The usual intake of micronutrients in the population compared with their requirements indicates the adequacy of the diet in each country and shows if there is a need to correct the intake through fortification. For example, in the chapter entitled 'Food



Map 2 Anemia as a public health problem by country: Preschool-age children. Reproduced with permission from De Benoist B, McLean E, Egli I, and Cogswell M (2008) Worldwide prevalence of anemia 1993–2005. WHO Global Database on Anemia. Geneva: World Health Organization. http://whqlibdoc.who.int/publications/2008/9789241596657_eng.pdf (accessed 26 March 2011).

Fortification: Technological Aspects' it was mentioned that 69% of children 24–59 months and 47% of WRA showed an inadequate intake of vitamin A in Kampala, Uganda in 2008, clearly indicating the need to improve the diet. Precise measures of daily household consumption of specific foods by categories of individuals (men, women, and children) are ideal; however, very few countries (approximately 5%) in the world have the latest information on usual intake of micronutrients.

When the usual intake of individuals is not available, one option is to approximate it using household consumption data. For example, the mean purchases per adult equivalent unit per day can be estimated from national household income and expenditure surveys that most countries run at regular intervals. With the average consumption of these foods, the likely deficit in micronutrient intake or the contribution of fortification to the intake can be estimated. For example, in Guatemala it was estimated that wheat flour fortification contributed 2.3 mg day^{-1} of iron and 90 mg day^{-1} of folic acid per adult equivalent. Assuming 5% bioavailability, wheat flour fortification provided 6% of the estimated average requirement (EAR) iron for WRA and 33% of the EAR of folic acid. This analytical approach can also be used to identify potential food vehicles in the design of a fortification program.

How to Decide on the Types and Levels of Fortificants?

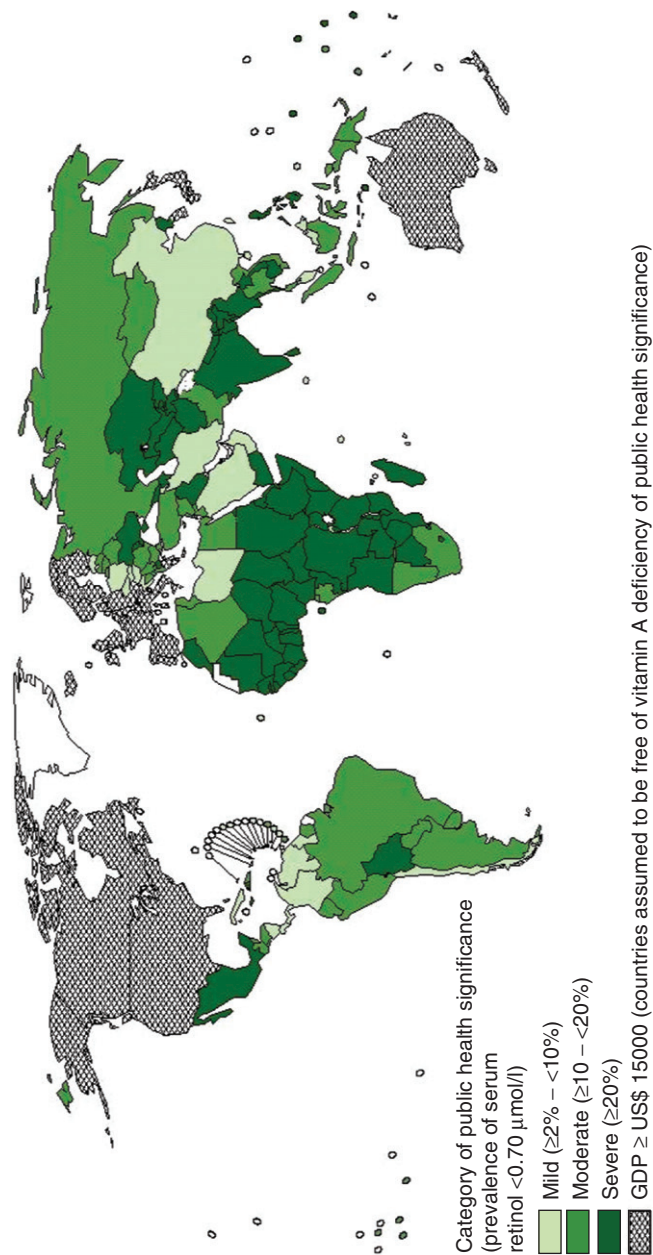
The potential sensory and physical effects of added micronutrients to food vehicles (food/s to be fortified), and the cost implications of the fortificant premix are addressed in the next chapter. In addition to these factors, consumption of the food vehicle(s), the potential exposure to excess levels of vitamins and minerals due to high consumption of fortified foods, and

the efficacy and effectiveness of fortification are the other three factors that help determine the levels and types of compounds in fortification in order to provide maximum benefits and minimum risks for the population.

However, in the past, in countries where mass fortification has been in place for many years, fortification levels were set up combining experience with technological and cost limitations. **Table 2** shows these levels for staples and condiments worldwide. Minimum monitoring and evaluation of these levels has been done.

Consumption of Food Vehicle(s) and Potential Exposure to Excess Intake

It is necessary for each country to estimate, with the best available information, the consumption of the food vehicle to be fortified. This information is used to assess if the group at high risk of deficiency of a specific nutrient will consume enough of the fortified food to improve their nutrient intake significantly, and simultaneously if the group who has the highest prefortification intake will be at risk of excessive intakes after fortification. As discussed previously (see the Section Micronutrient Intakes) the usual individual intake data provide the best estimates of fortified food consumption. If they are not available then household income and expenditure data can be used as good proxies. For example in Guatemala where there are no data on usual individual intake, using the World Bank supported household income and expenditure survey, it was estimated that the median amount of fortified wheat flour consumed was 50 g/day per adult equivalent. If household consumption data are not available, the food balance sheets produced by Food and Agriculture



Map 3 Prevalence of vitamin A deficiency among preschool-age children by country. Reproduced with permission from WHO. Global prevalence of vitamin A deficiency in populations at risk 1995–2005. WHO global database on vitamin A deficiency. Geneva, World Health Organization, 2009. http://whqlibdoc.who.int/publications/2009/9789241598019_eng.pdf (accessed 26 March 2011).

Table 1 Trends in national anemia prevalence in nonpregnant women in selected countries

Country	Survey year	Prevalence (%)
Uganda	2000	30.3
	2007	43.1
Zimbabwe	1999	34.3
	2006	37.3
Egypt	2000	26.7
	2005	38.8
Bangladesh	2001	29.0
	2004	43.0
India	1998	51.9
	2006	53.2
Philippines	1998	32.5
	2003	42.1
Mexico	1990	14.0
	2006	15.5
Peru	2000	31.6
	2004	38.2

Source: Reproduced with permission from ACC/SCN. Sixth Report on the World Nutrition Situation. UN Standing Committee on Nutrition, Geneva, 2010. <http://www.unscn.org/files/Publications/RWNS6/html/index.html> (accessed 26 March 2011).

Organization (FAO) can be used as a last resort to estimate fortified food consumption; these sheets provide the average per capita availability of common foods (g day^{-1}) for almost every country in the world. The limitation with this approach is the lack of information about the distribution of the availability for the whole population and by population subgroups within each country.

There is also a need to ensure that the various population groups will not be exposed to high intakes of vitamins and minerals that could pose health risks due to potential overconsumption of fortified foods. One approach used to evaluate this factor is the nutrient density (nutrient/1000 kcal) approach. This method considers the effect of different fortification levels on intakes of age and gender groups with different recommended nutrient intakes (RNI) and different energy requirements (ERs). The ratio of these two (RNI/ERs) varies across age and gender groups, and can be used to identify the group with the highest recommended intake per 1000 kcal. For example if flour is fortified to a level that will satisfy the recommendation for this group, then that level of fortification is likely to satisfy the recommendations for all other groups. Similarly, the nutrient density approach can be used to identify the age and gender group with the lowest ratio of the tolerable upper intake level (UL) to ER. This is the group that would be most vulnerable to exceeding the UL at a given level of fortification. **Table 3** shows the results of the application of this approach in the case of wheat flour fortification for zinc, vitamin A, folate, iron, and B_{12} . For example, if the daily average consumption of flour is 57 g day^{-1} , the folic acid fortification level could be 4.0 mg kg^{-1} in the flour. This level will avoid 100% UL for the group with the highest risk of potential adverse effects due to excessive intake.

Efficacy and Effectiveness of Fortification

If a fortificant is going to be added to a food vehicle, first it is necessary to know if it will improve the intake and

micronutrient status of different population groups, under controlled conditions (efficacy). After the fortificant has been shown to work then its performance under real conditions (uncontrolled) needs to be assessed to ascertain its impact (effectiveness). In general, efficacy is evaluated based on systematic reviews of randomized controlled trials (for example, Cochrane reviews) and meta-analysis of the independent studies included in the systematic reviews. Rating of the quality of resultant evidence is done using the system Grading of Recommendations Assessment, Development, and Evaluation (GRADE). The effectiveness should be rated similarly; however there are very few studies available on the measurement of the impact of fortification programs. The Campbell Collaboration has a database containing reviews of effectiveness of social and educational policies and practices, in the future it will be useful to register the effectiveness of food fortification programs as well.

When the recommendations for the fortification of wheat flour were developed, an effort was made to summarize the available evidence for different fortificants that potentially could be used. For example, in the case of iron, efficacy studies with foods fortified with NaFeEDTA, ferrous sulfate, ferrous fumarate, electrolytic iron, hydrogen-reduced iron, and ferric pyrophosphate were reviewed to determine the minimum daily amounts of additional iron that have been shown to significantly improve iron status in children, adolescents, and WRA. If consumption of low extraction ($\leq 0.8\%$ ash) wheat flour is $150\text{--}300 \text{ g day}^{-1}$, the addition to flour of 20 mg kg^{-1} of NaFeEDTA or 30 mg kg^{-1} of ferrous sulfate or ferrous fumarate may improve iron status. Seventy-eight country programs were also reviewed and it was found that the majority used low-bioavailability, atomized, reduced or hydrogen-reduced iron, and only nine national programs were likely to have a significant positive impact on iron status when assuming fortified flour coverage of 80% or more. This is due to the levels and compounds being used. Approaches similar to those described for iron were used to assess the efficacy and effectiveness of folic acid, B_{12} , vitamin A, and zinc.

Global Guidelines

WHO is the international public health agency that produces global guidelines for health interventions. In the WHO/FAO 'Guidelines on food fortification with micronutrients' the characteristics of fortificants for iron, vitamin A, iodine, zinc, folate, B vitamins, vitamins C and D, calcium, selenium, and fluoride are summarized for some food vehicles; however, unanswered questions remain regarding the types and levels of fortificants to be used in fortification. Nevertheless, programs should move forward guided by global recommendations based on evidence, benefits and harms, values and preferences, and costs and resource implications.

In 2009 WHO issued recommendations on wheat and maize flour fortification based on scientific reviews prepared for a Flour Fortification Initiative (FFI) technical workshop. Various organizations actively engaged in the prevention and control of vitamin and mineral deficiencies along with relevant stakeholders, met and discussed specific practical

Table 2 Examples of levels of micronutrients currently added to staples and condiments worldwide (mg kg⁻¹)

Nutrient	Milk	Evaporated milk	Powdered milk	Margarine	Vegetable oil	Sugar	Wheat flour	Pasta	Com mass flour	Pre-cooked maize flour	Maize flour	Maize meal	Soy/fish sauce	Salt
Vitamin A	0.7–1.0	2–3	4.5–7.5	5–15	5–15	5–15	1–5	–	–	2.8	–	1–2	–	–
Vitamin D	0.01	0.01	0.05–0.06	0.02–0.15	–	–	0.014	–	–	–	–	–	–	–
Vitamin E	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Vitamin C	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Thiamine (vitamin B ₁)	–	–	–	–	–	–	1.5–7.0	8–10	1–6	3.1	2.4	2–3	–	–
Riboflavin (vitamin B ₂)	–	–	–	16	–	–	1–5	3–5	1–5	2.5	–	1.7–2.5	–	–
Niacin (vitamin B ₃)	–	–	–	180	–	–	15–55	35–57	25–50	51	1.6	19–30	–	–
Vitamin B ₆	–	–	–	20	–	–	2.5	–	–	–	–	2–3	–	–
Folic acid	–	–	–	2	–	–	0.5–3.0	–	0.5–3.0	–	–	0.4–0.5	–	–
Vitamin B ₁₂	–	–	–	–	–	–	0.01 ^a	–	–	–	–	–	–	–
Iron ^b	–	–	–	–	–	–	–	–	–	–	–	–	–	–
NaFeEDTA	–	–	–	–	–	–	–	–	–	–	–	–	250	500 ^c
Ferrous	–	–	–	–	–	–	–	–	22	–	–	–	–	–
Bisglycinate	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Ferrous sulfate or fumarate	–	–	–	–	–	–	30–45	30	30	30 +	–	–	–	–
Electrolytic iron	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Zinc (oxide)	–	–	–	–	–	–	45–60	25–35	30–60	20 +	–	9–14	–	–
Calcium	–	–	–	–	–	–	15–30	–	15–30	–	–	–	–	–
Iodine	–	–	–	–	–	–	2100–3900	–	–	–	–	–	–	–
	–	–	–	–	–	–	–	–	–	–	–	–	–	15–60

^aAs recommended at a recent PAHO/WHO meeting (339).^bUsually foods are fortified with only one iron compound, but in the case of pre-cooked maize flour trials are currently underway to assess the viability of using more than one iron fortificant.^cAs encapsulated ferrous sulfate, but to date this has only been used only in experimental trials.

NaFeEDTA, sodium iron ethylenediaminetetraacetic acid.

Source: Reproduced with permission from Allen L, de Benoist B, Dary O, and Hurrell R (eds.) (2006) *Guidelines on Food Fortification with Micronutrients*. Geneva: World Health Organization, 341 pp. <http://www.who.int/nutrition/publications/micronutrients/9241594012/en/index.html> (accessed 27 May 2011).

recommendations to guide flour fortification efforts being implemented in many countries by the public, private, and civic sectors. **Table 4** summarizes the levels and compounds of fortificants recommended for flour fortification with iron, folic acid, B₁₂, vitamin A, and zinc. The recommendations in this table considered the intake of the flour, efficacy and effectiveness of fortificants, potential excessive intakes of these micronutrients, potential sensory and physical effects of fortificants, and costs of the fortification premix. As with any other global guideline, the final selection of the type and quantity of vitamins and minerals to add to flour, either as a voluntary standard or a mandatory requirement lies with national decision makers in each country.

How to Monitor and Evaluate Food Fortification Programs?

Monitoring of food fortification programs is required to compare how well these interventions are being implemented so managers can put into action timely remedial measures when needed. Impact evaluation of these programs is required

Table 3 Fortification levels to avoid 100% UL for the group with the highest risk of adverse effects due to potential excessive intake

Flour (g day ⁻¹)	57	114	229	400
Micronutrient mg kg ⁻¹ in flour				
Zinc	111.4	55.7	27.9	15.9
Vitamin A	9.0	4.5	2.2	1.3
Folic acid	4.0	2.0	1.0	0.6
Iron	247.3	123.7	61.8	35.3
Vitamin B ₁₂	0.040	0.020	0.010	0.006

Source: Reproduced with permission from Flour Fortification Initiative (FFI). Second Technical Workshop on Wheat Flour Fortification: Food Consumption Work Group. Stone Mountain, GA, 30 March to 3 April 2008.

Table 4 Average levels of nutrients to consider adding to fortified flour based on extraction, fortificant compound, and estimated per capita flour availability

Nutrient	Flour extraction rate	Compound	Level of nutrient to be added in parts per million (ppm) by estimated average per capita wheat flour availability (g day ⁻¹) ^a			
			< 75 ^b g day ⁻¹	75–149 g day ⁻¹	150–300 g day ⁻¹	> 300 g day ⁻¹
Iron	Low	NaFeEDTA	40	40	20	15
		Ferrous sulfate	60	60	30	20
		Ferrous fumarate	60	60	30	20
		Electrolytic iron	NR ^c	NR ^c	60	40
	High	NaFeEDTA	40	40	20	15
Folic acid	Low or high	Folic acid	5.0	2.6	1.3	1.0
Vitamin B ₁₂	Low or high	Cyancobalamin	0.04	0.02	0.01	0.008
Vitamin A	Low or high	Vitamin A palmitate	5.9	3.0	1.5	1.0
Zinc ^d	Low	Zinc oxide	95	55	40	30
	High	Zinc oxide	100	100	80	70

^aThese estimated levels consider only wheat flour as the main fortification vehicle in a public health program. If other mass-fortification programs with other food vehicles are implemented effectively, these suggested fortification levels may need to be adjusted downward as needed.

^bEstimated per capita consumption of < 75 g day⁻¹ does not allow for addition of sufficient level of fortificant to cover micronutrients needs for women of childbearing age. Fortification of additional food vehicles and other interventions should also be considered.

^cNR: not recommended because very high levels of electrolytic iron could negatively affect sensory properties of fortified flour.

^dThese amounts of zinc fortification assume 5 mg zinc intake and no additional phytate intake from other dietary sources.

Source: Reproduced with permission from WHO (2009) Recommendations on wheat and maize flour fortification. *Meeting Report: Interim Consensus Statement*. Geneva: World Health Organization. http://www.who.int/nutrition/publications/micronutrients/wheat_maize_fort.pdf (accessed 26 March 2011).

to document if they have produced the expected outcomes and achieved their purpose in the population.

The WHO/FAO in their 'Guidelines on food fortification with micronutrients' present a generic monitoring and evaluation (M&E) system for food fortification programs (**Figure 1**), which has a regulatory monitoring component to track the supply of adequately fortified foods, and a household/individual M&E component to assess the provision, coverage, utilization, and impact of fortification in the target population.

The Centers for Disease Control and Prevention framework for program evaluation in public health is a useful guideline for the design and implementation of a monitoring and evaluation (M&E) system. It includes six steps: engage stakeholders, describe the program, focus the M&E design, gather credible information, justify conclusions, and ensure use of M&E results.

Step 1: Engage Stakeholders

Stakeholders are people or organizations that are invested in food fortification programs, and have a stake in what will be done with the M&E results. For example, in the regulatory monitoring component mentioned above, the food industry, the premix vendors, and the ministries of health and commerce are key stakeholders. The M&E managers need to identify and involve key stakeholders through the next five steps.

Step 2: Describe the Program

A complete program description explains all the components and intended outcomes of a program and thus helps focus the M&E on the most important questions to be considered. A comprehensive program description includes the following

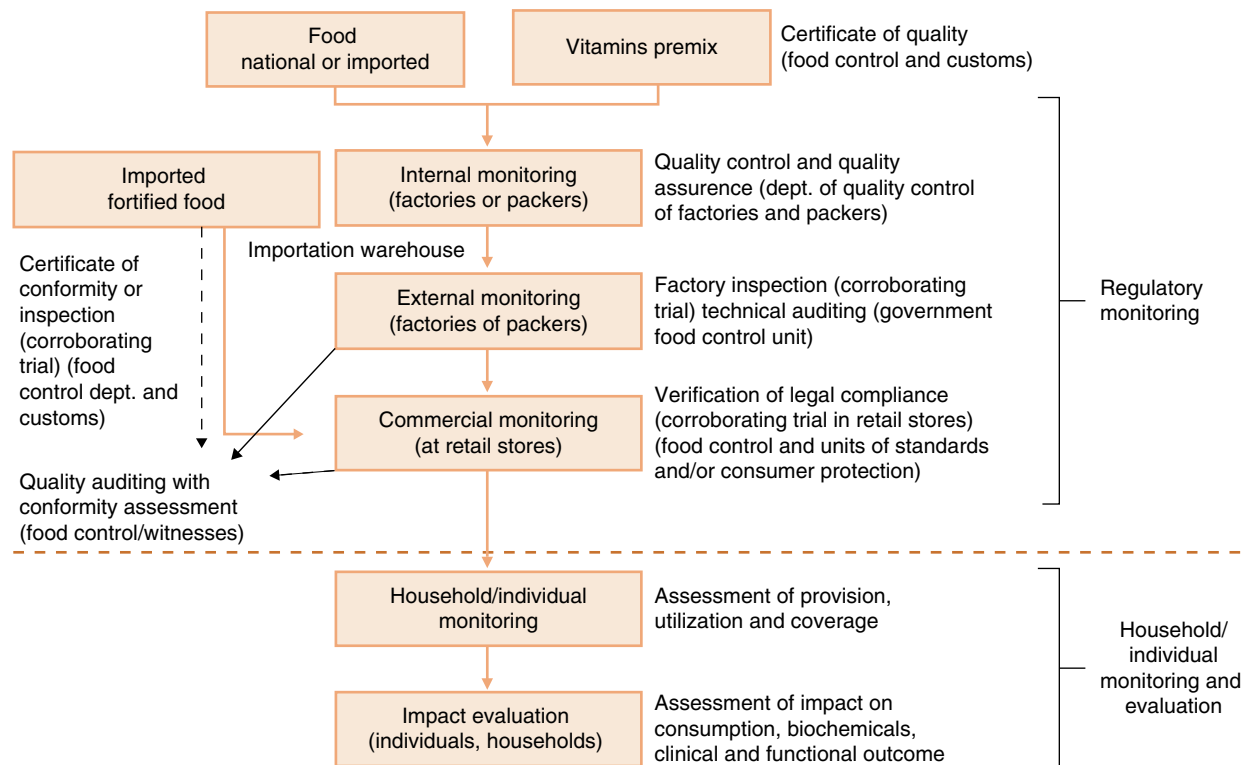


Figure 1 A monitoring and evaluation system for food fortification programs. Reproduced with permission from Allen L, de Benoist B, Dary O, and Hurrell R (eds.) (2006) *Guidelines on Food Fortification with Micronutrients*. Geneva: World Health Organization, 341 pp. <http://www.who.int/nutrition/publications/micronutrients/9241594012/en/index.html> (accessed 26 March 2011).

program components: needs, inputs, activities, outputs, targets, and outcomes. It also includes a discussion of the stage of development of the program and the context of where it is operating. Participation by stakeholders in describing the program ensures a consensual understanding of the program components among all involved.

Logic models are graphic descriptions of the relationship between a program's activities and its expected outputs and outcomes. Logic models portray expected outputs and outcomes of a program's activities, rather than reality, at any point in time. That is, of all activities that could have been undertaken to address this problem, these activities were chosen because, if implemented as intended, they should produce outputs that lead to the expected outcomes.

An adaptation of the proposed WHO/Centers for Disease Control and Prevention (CDC) generic logic model for micronutrient interventions for a flour fortification program designed to improve iron and folate status in WRA is presented in **Figure 2**. For example, under the column of Access and Coverage, one of the expected outputs is an increase in the purchase of fortified flour and byproducts by households, which in turn will contribute to the demand of these food vehicles by non pregnant WRA (Knowledge and Appropriate Use outputs column). These two outputs lead into the appropriate use of the fortified flour, which will increase the iron and folate intake in WRA.

A more complete approach to describing a program is the logical framework ('logframe'), which follows the same principles as the logic model but in greater detail. The logframe is

summarized in a four (goal, purpose, outputs, and activities) by four (narrative, indicators, sources and assumptions, and risks) matrix.

Step 3: Focus the M&E System

The stage of implementation of the food fortification program guides the focus of the M&E system. In early stages of implementation the most important questions are related to the execution of activities, for example, in the flour fortification case some questions could be as follows: Are the legislation and standards for flour fortification in place? Is fortified flour being produced? Have the retailers increased the stock and sales of fortified flour? Is quality assurance/quality control done correctly at the mills? Are the mass media materials being distributed as planned? (See Production and Supply, Delivery, Quality, and Behavior Change Communication under the Activities column in **Figure 2**.) As time progresses, the interest moves to outputs: Is fortified flour accessible to WRA? Is fortified flour of acceptable quality? Do WRA purchase fortified flour? Is fortified flour being consumed by WRA in appropriate amounts /frequency? For established programs with appropriate coverage and a minimum predetermined implementation period of time, one of the key questions could be as follows: Have the iron and folate status of the WRA improved? Thus, the focus can go from monitoring implementation to impact evaluation. The stakeholder's input in focusing the M&E system ensures that the key questions of most importance will be included according to the program

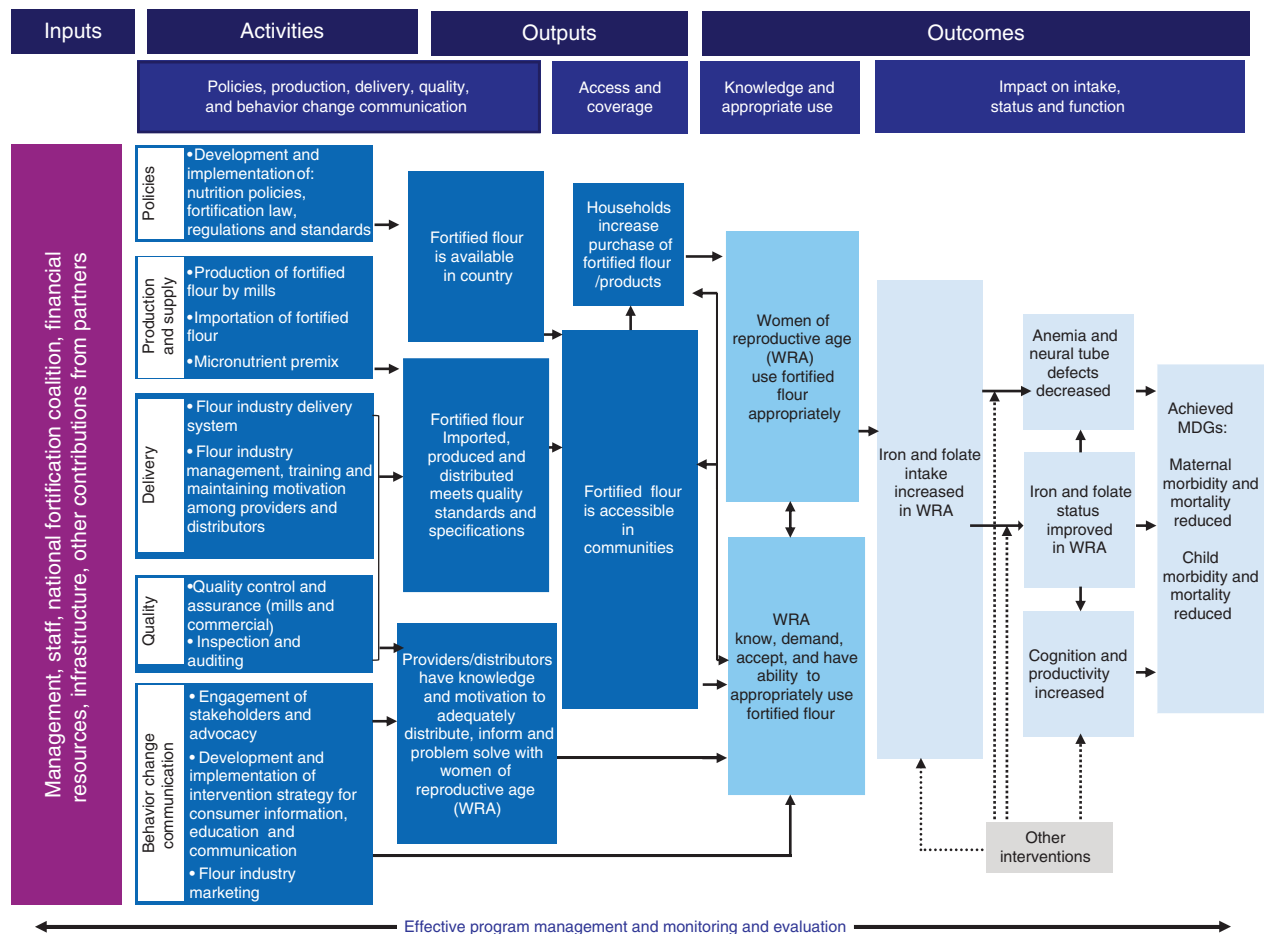


Figure 2 Proposed Logic model for a national wheat flour fortification program. Adapted from De-Regil LM, Peña-Rosas JP, Flores-Ayala R, and Jefferds ME (2011) WHO/CDC logic model for micronutrient interventions in public health. *Experimental Biology Meetings*. Washington, DC, with permission from WHO/CDC.

stage of implementation. These questions define the objectives and users of the M&E system. For example, if the objective is to ensure that implementation is proceeding as planned, then the main user is the manager of the fortification program.

Step 4: Gather Credible Evidence

For each M&E question identified in the previous step, at least one indicator needs to be defined along with its benchmark or expected change. As seen in the logic model, the indicators put the components of the flour fortification program into operation. For example, for the output 'increase in the purchase of fortified flour and byproducts by households' one indicator could be percentage of households with fortified bread and corresponding benchmark of 75%. For the improved iron and folate status in WRA outcome, one indicator could be prevalence of iron deficiency anemia in nonpregnant WRA, and its expected decrease by 10% after 1 year of adequate program implementation with coverage above 80%.

For each indicator the following questions need to be answered:

- For whom will the indicator be collected (stakeholders)?
- How will the indicator be collected (data collection sources)?

- How often will the indicator be collected (frequency)?
- Who will report the indicator?
- Who does what based on the information collected?

Sources from which to gather information on the indicators could be existing data (secondary data collection) or new data (primary data collection). Depending on the M&E questions and indicators, some secondary data sources may be appropriate data collection sources. The most frequent primary data collection methods are as follows: cross-sectional surveys, lot quality-assurance sampling, sentinel surveillance, focus groups, observation, and document review. Choosing the best method from the previous options needs to take into account both the context in which the question is asked and the content of the question. As an example, for the iron and folate status outcome, the before and after cross-sectional survey is the method of choice.

Step 5: Justify Conclusions

The data collected need to be analyzed, synthesized, and interpreted to make conclusions about the program performance and significance. These claims are justified by comparing the evidence against the established program benchmarks, expected

changes, stakeholder values and other information available. Conclusions will lead to the recommendation of actions to be taken related to program performance, significance, and sustainability. A clear and understandable report needs to be written after each round of data collection of indicators.

For example in 2003 South Africa launched its national food fortification program, which mandated that any wheat flour bread or maize meal needed to be fortified with eight micronutrients including vitamin A, folic acid, iron, and zinc. In 2007 the impact evaluation of the fortification program showed a significant decline in birth defects with reductions in spina bifida and anencephaly by 41.6% and 10.9%, respectively. The impacts found in South Africa are consistent with decreases observed in other countries that have fortified foods as well.

Step 6: Ensure Use of M&E Results and Share Lessons Learned

M&E results can be used to demonstrate the effectiveness of the program, identify ways to improve it, modify program planning, demonstrate accountability, and justify funding. Right after the M&E report is available the process of communicating it to relevant audiences in a timely, unbiased, and consistent manner needs to be started. The audiences have been identified in Steps 1 and 3 (responses to the question: Who does what based on the information collected?). For the M&E process to be effective, feedback is necessary throughout all stages in the program cycle.

Because stakeholders have been involved throughout the M&E process they are more likely to take up the results and follow up by taking action. However, the manager of the program is ultimately responsible for using the results of the M&E system for the benefit of the program and the target population.

Further key points that merit attention are as follows:

- Developing a monitoring system is necessary and needs to be considered for all fortification programs.
- To the extent possible, fortification programs should use and build on the monitoring systems that are already in place.
- It is important to convince stakeholders of the importance of impact evaluation when a national program is established for the first time and then the monitoring system will indicate future needs. In the case of food fortification there is an urgent need to document the effectiveness of iron-fortified flour, vitamin A-fortified edible oil, and iron-fortified soy sauce fortification programs.
- It is important to design an impact evaluation before starting implementation of the program, as these considerations may determine how the actual evaluation can be carried out. For example one best practice for impact evaluation is to control for pre- and postprogram differences in participants, therefore baseline data will be needed before the food fortification program starts.

Further Reading

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Relevant Websites

A2Z Project: <http://www.a2zproject.org/>

A2Z is the latest USAID Project in micronutrients (2005–11), and the website contains publications, reports, manuals, presentations and similar material about micronutrients, including food fortification. The site also presents similar documents from the predecessor project MOST (1999–2005).

Flour Fortification Initiative: <http://www.sph.emory.edu/wheatflour/>

The Flour Fortification Initiative (FFI) is a public–private network of organizations dedicated to promote the use of wheat flour fortification worldwide to improve status of several micronutrients. Website contains documents, information, and tools associated to this area.

Micronutrient Initiative: <http://www.micronutrient.org/English/View.asp?x=699>

The Micronutrient Initiative (MI) is an international technical NGO based in Ottawa, Canada, with offices in Asia and Africa, and programs in several developing countries; its focus is to improve micronutrient status in vulnerable populations, and food fortification has been one of the main interventions.

CDC/IMMPaCt <http://www.cdc.gov/impact/index.html>

The site of the International Micronutrient Malnutrition Prevention and Control (IMMPaCt) Program of the CDC (USA), covers a wide range of information about micronutrient biology and interventions, including food fortification.

WHO/Micronutrients unit: <http://www.who.int/nutrition/topics/micronutrients/en/>

The site of the Macronutrients unit of WHO covers a wide range of information about vitamins and minerals science and interventions, including food fortification.

Global Alliance for Improved Nutrition: <http://www.gainhealth.org/>

This site contains news, reports, resources, and announcements about nutrition interventions, mainly implemented under public–private partnerships, including food fortification.

Technological Aspects

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Glossary

Codex Alimentarius Inter-country organization of the United Nations system, led by FAO and WHO, with the purpose of proposing general principles, standard models, practices, and guidelines, aimed to protect the health of consumers and ensure fair trade associated with foods. The Codex recommendations are recognized by the World Trade Organization as international reference points in those matters.

Estimated Average Requirement (EAR) The average daily nutrient intake level estimated to meet the needs of 50% of the healthy individuals in a particular age- and sex-specific population group.

Fortificants The chemical forms that are the sources of micronutrients in food fortification.

Premix A blend of fortificants with antioxidants, anticaking, excipient, and other substances to provide the

adequate physical and chemical properties that are required to produce homogeneous and reasonably stable fortified products.

Recommended Daily Allowance (RDA) In this chapter, RDA is assumed to have the same meaning as RNI. The RDAs are set by the Institute of Medicine for the United States and Canada, as the EAR plus two standard deviations (i.e., covering 97.5% of the population).

Recommended Nutrient Intake (RNI) The daily intake set by FAO/WHO that meets the nutrient requirements of almost all apparently healthy individuals in an age- and sex-specific population group.

Tolerable Upper Intake Levels (ULs) The highest average daily nutrient intake level unlikely to pose a risk of adverse health effects to almost all (97.5%) apparently healthy individuals in an age- and sex-specific population group.

What is Food Fortification?

The Codex Alimentarius defines food fortification or enrichment as the addition of micronutrients to foods, whether or not they are normally contained in the food, for the purposes of preventing or correcting a demonstrated deficiency. The Codex also mentions that the amount of micronutrients to add should be sufficient to correct or prevent the deficiency when the food is consumed in normal amounts by the population at risk, but not likely to result in excessive intakes by individuals with a high intake of the fortified food. As stated, these recommendations are applicable to single foods.

The WHO/FAO proposed a more appropriate definition in the Guidelines on Food Fortification with Micronutrients, which focuses on the diet rather than on single foods. A single food may contribute toward improving the nutritional quality of the food supply but may not necessarily be sufficient as the only solution to prevent a micronutrient deficiency. This is the concept adopted in this chapter.

In food technology, food fortification and food enrichment have different meanings: fortification is reserved for the addition of micronutrients to a food that does not contain those compounds naturally, whereas enrichment is applicable when the natural contents of some micronutrients normally available in the food are intentionally increased. Two related terms are frequently used: restoration, when micronutrients are added to recover the original levels in a food that has partially or totally lost them during processing, for example, adding vitamin A and D to defatted milk to reproduce the content of those vitamins in whole milk, and nutritional equivalence, when the content of micronutrients of a manufactured food is modified to imitate the content of a natural food that is intended to be replaced, as, for example, adding

vitamin A and D to margarine to achieve their natural contents in butter.

Micronutrients are vitamins and minerals that are required by humans in very small amounts; most of them cannot be synthesized by the human body and therefore they should be obtained directly from the diet. The chemical sources of micronutrients used in food fortification are called fortificants. Thus, for example, ferrous sulfate, ferrous fumarate, and NaFeEDTA are fortificants used to increase the content of iron in foods. Fortificants are generally added to foods as part of premixes, which constitute the main ingredients in the fortification process.

How does Food Fortification Work?

Many articles in the scientific literature intend to demonstrate that consumption of fortified foods has positive health outcomes without emphasizing the fact that those outcomes are directly associated with the quality and amount of the micronutrients that are added to the foods rather than to only the consumption of the fortified foods. For example, vitamin A can be added to several food vehicles such as oil, sugar, wheat flour, milk, and even bouillon cubes, but the biological impact will be directly related to the amount of vitamin A that is being delivered to the consumer rather than just its presence in the food vehicle. The purpose of using a food vehicle is the ability to reach the largest number of individuals of a vulnerable population in an efficient and low-cost manner. Food fortification takes advantage of an existing delivery system – and the manufacturing structure of the food industry and its trade partners – to supply the needed micronutrients in sufficient amounts to populations with inadequate diets. In other

words, food fortification favors coverage, but the impact depends on the proportion of individuals who fully meet their nutritional needs through the additional amounts of micronutrients supplied by the fortified foods, and that can be estimated as described in eqn [1]:

Additional micronutrient intake:

$$\begin{aligned} &\text{Micronutrient intake } (\mu\text{g d}^{-1}) \\ &= \text{micronutrient in food } (\text{mg kg}^{-1}) \\ &\quad \times \text{consumption of food } (\text{g d}^{-1}) \end{aligned} \quad [1]$$

The magnitude of the biological impact depends on the extent to which the nutritional gap is corrected. Therefore, the impact varies from population to population, and hence the same fortification formula does not necessarily replicate results from one community to another, even under similar patterns of consumption of the fortified food, because the magnitude of the nutrient intake gap may differ.

Although impact depends on the micronutrient and not on the fortified food itself, certain characteristics of the food vehicle might have some influence. For instance, some compounds that are present in the food vehicles can react negatively with the fortificants, thus decreasing the quantities of micronutrients that are available to the consumer. Some compounds, such as phytates, can reduce the absorption of iron, zinc, and other minerals. However, sufficient intake and high bioavailability do not necessarily lead to good biological impact if the metabolic conditions of the individuals are suboptimal. For example, absorption of synthetic β -carotene in a zinc-deficient individual might be good but bioconversion to vitamin A may be impaired. Equation [2] illustrates how increased micronutrient intakes may lead to biological impact (bioefficacy).

Factors that modulate the impact of additional micronutrient intakes:

$$\begin{aligned} &\text{Bioefficacy} = \text{Additional micronutrient intake } (\mu\text{g d}^{-1}) \\ &\quad \times \% \text{ bioavailability} \times \% \text{ bioconversion} \end{aligned} \quad [2]$$

In summary, food fortification increases the supply of micronutrients in the diet by using products manufactured by the food industry, but the biological impact depends on the extent to which individual nutritional gaps are corrected, which, in turn, is affected by three main factors: (1) micronutrient quantity (additional intake); (2) interactions of the fortificants with other substances present in the diet (bioavailability and stability); and (3) general nutritional status of each individual (bioconversion). In any case, the micronutrient content of the fortified food is an important element for estimating bioefficacy.

Which Factors Limit the Amount of Micronutrients in Fortified Foods?

In the past, it was assumed that the design of a fortification formulation should be based on an estimation of the nutritional gap divided by the average amount of the food vehicle that is consumed by the target population. In theory, this

calculation would estimate the necessary micronutrient addition through fortification to fill the nutrient gap. However, experience has shown that this is not necessarily the case, as many factors limit the amount of micronutrients that can be incorporated into food vehicles.

Assuring Safety for Almost Everyone

Both the Codex and the WHO/FAO statements about food fortification point out that fortification should be designed in such a way that it should prevent almost all individuals in the population from having excessive micronutrient intakes. The total micronutrient intake for most individuals of the population should not exceed the recommended tolerable upper intake levels (ULs). Young children and adult males have lower and higher UL values, respectively. Thus, for instance, the safety of fortification in packaged foods with fixed serving sizes (e.g., breakfast cereals) should be checked against the UL values for young children. However, for fortified staples, the UL values of both adolescents/adult males and young children may limit the fortification levels, because of the large food intakes of the former and the low UL values of the latter. It is important to point out that the calculations of total intake should be carried out for the overall supply (regular diet plus all the fortified foods) and not separately for each fortified food.

Technological Compatibility

In general, fortificants should not change the stability, color, odor, or flavor of the food vehicles. Negative interactions between fortificant and vehicles impede the adoption of high micronutrient contents. For example, vitamin B-2 and β -carotene, when added to food matrices with white color (refined flours and rice), cause yellowish-orange colors. Using a change of color as a distinctive feature of the fortified product may be an advantage instead of a limitation when this feature is intended for identifying a fortified product. Some iron salts with good water solubility, such as ferrous sulfate and ferrous bisglycinate, favor rancidity, whereas others such as NaFeEDTA might produce discoloration. Technological incompatibility greatly reduces the impact of iron fortification because the fortificants with higher bioavailability cannot be added at high contents. In this case, the selection of the fortificant rests on a decision that combines estimation of bioavailability and the highest feasible fortification level. For example, for maize flour, it is possible to use 40 mg iron kg^{-1} from ferrous fumarate but only 15 mg iron kg^{-1} from NaFeEDTA. If one assumes that the bioavailabilities are 5% and 15%, respectively, any of these two iron fortificants would have a similar biological impact, and the compound with the lowest cost would be preferred. In this example, the use of NaFeEDTA would be more attractive only if the technological compatibility allows the iron content to be higher than 15 mg iron kg^{-1} .

Physical Segregation

Dry food matrices may also suffer segregation (separation) of the fortificant particle from the food particles, due to differences in sizes and densities. This factor affects mainly food

matrices with large particle sizes such as sugar and coarse salt. In the case of sugar fortified with vitamin A, the vitamin-containing beadlet is attached to the surface of the sugar crystal by means of a layer of vegetable oil. However, during storage and transportation the friction among sugar crystals may separate some of the vitamin A beadlets and therefore reduce the content of vitamin A. Figure 1 illustrates how the vitamin A beadlets are fragile when attached to the surface of the sugar crystals. Recently, novel sugar premixes with vitamin A embedded in sugar-containing particles have been introduced for overcoming this limitation.

Relative Cost

The cost of adding fortificants to foods mainly depends on the price of the premix and the fortification levels. To design programs that are congruent with the usual production and trade practices, the increment in the price due to fortification should not be too high. Fortificants with high costs can only be added up to certain amounts. Table 1 illustrates why the addition of iodine to salt is much easier to implement than the addition of vitamin A or iron. For supplying 100% of the estimated average requirement (EAR) to an adult male consuming 10 g d^{-1} of salt, the fortification costs for iodine, vitamin A, and iron are



Figure 1 Microphotography of vitamin A beadlets (fortificant) attached to sugar crystals (food matrix) by means of a thin layer of vegetable oil. Reproduced with permission from DSM.

US \$0.49, US \$36.61, and US \$149.04, respectively. These costs represent 0.25%, 18.3%, and 74.5% of the retail salt price, respectively (assuming US \$0.20/kg). In the case of food staples, viable programs have cost increases lower than 5%. Consequently, the addition of vitamin A and iron to salt should be substantially reduced for a program to be feasible, and the contribution of salt to intakes of these micronutrients would be proportionally lower; therefore, more than one fortified food or micronutrient intervention may be necessary.

Packaged-processed fortified foods may accept larger price increases due to fortification because the price of these products is set on the basis of factors other than the cost of the food ingredients.

Dilution Factor

The amount of the fortificant required to reach the target micronutrient content in a given food is rarely examined when food fortification programs are being considered. Table 1 shows the dilution factors for iodine, vitamin A, and iron at fortification levels that are required to provide 100% of the EAR; these are 1:60 000, 1:1750, and 1:85 (the dilution factors are reduced further when the fortificants are incorporated into premixes), respectively. This means that in the case of iron, even though the cost may not be restrictive, the low dilution factor requires the use of relatively large amounts of the fortificant; in the case of salt, it would be approximately 12 kg per metric ton. Other products derived from salt, such as bouillon cubes and powdered soups, are likely to face the same type of limitation for fortification with iron if biologically important levels are desired.

The Special Case of Rice Fortification

In developing countries, rice is washed before consumption and some micronutrients may be washed out in the process. Therefore, rice fortification requires the use of either artificial fortified kernels or coated grains. The main constraint for rice fortification is the dilution factor, because it should be as large as possible to reduce the cost of producing the fortified kernels. In practice, dilution factors of 1:200 (i.e., 5 kg of fortified kernels per metric ton of fortified rice) are being used. Experiments have been carried out with dilution factors of 1:50 (i.e., 20 kg of

Table 1 Conditions for fortification of salt with different micronutrients

Nutrient	Fortificant	EAR ^a of adult males (mg)	Nutrient content ^b (mg kg ⁻¹)	US\$/metric ton ^c	% Price ^d	Dilution factor ^e
Iodine	Potassium iodate	0.100	10	0.49	0.25	1:60 000
Vitamin A	Dry 250 000 IU Vitamin A	0.429	42.9	36.61	18.3	1:1750
Iron	Micronized ferric pyrophosphate	30.0	3000	149.04	74.5	1:85

^aEstimated average requirement (EAR), and assuming a diet with an iron bioavailability of 5% and 3.5% for micronized ferric pyrophosphate.

^bEstimated for supplying 100% of the EAR of adult males with a salt intake of 10 g d^{-1} , and without losses of the micronutrients.

^cBased on prices of 2010.

^dAssuming that the salt price is US \$0.20 kg⁻¹.

^eConsiders that the fortificants are not 100% micronutrient. In this case, iodine in potassium iodate is 59%; vitamin A in a dry beadlet form is 7.5%; and iron in micronized ferric pyrophosphate is 25%.

fortified kernels per metric ton of fortified rice) to improve the homogeneity of the fortified product, but this increases the cost 3 to 4 times beyond that of the 1:200 dilution, hence making the large-scale use of a 1:50 dilution prohibitive.

In rice fortification, half or more of the cost is associated with the process of manufacturing the fortified kernels. Therefore, it is preferable to incorporate most micronutrients that are low in the diet and not only one or two. For other fortification cases, 90% or more of the total cost of fortification is attributable to the cost of the fortificants.

Assessing the Contribution of Food Fortification to Nutrient Intake (Selecting the Alternatives with the Highest Potential for Effectiveness)

A conclusion of this section is that, in practice, fortification levels are usually neither defined by the nutritional gap nor by the expected consumption of the fortified vehicle but by many other factors such as safety and technological and economic conditions. Indeed, feasible micronutrient contents for each food matrix fall within relatively narrow ranges of values. However, it is always important to select the most suitable level for each population based on an assessment of the desired or the expected reduction in the proportion of the population whose intakes are below their EAR. It is important to explain here that if EAR values are used as cut-off points, the population average intake will be larger than the recommended nutrient intake (RNI). The use of RNI as the reference cut-off point is not appropriate for population interventions, because it will unnecessarily increase the intakes of the entire population and may place some individuals at risk of excessive intakes, as shown in **Figure 2**.

Example of Estimating the Impact of Vitamin A Fortification in Uganda

Table 2 illustrates the estimation of the potential impact of vitamin A fortification in Uganda, where vitamin A

inadequacy is a serious public health problem both for children 24–59 months old and for women of reproductive age. The severity of the problem increases from the rural South West to urban Kampala, and from the latter to the rural North, where almost everyone has a vitamin A intake below the EAR. The most promising vehicles to deliver vitamin A are oil and sugar, the former in the whole country and the latter with a potentially larger impact in Kampala. The best combination would be oil and sugar, as inadequacy would be practically eliminated in Kampala and greatly reduced in the rural South West and North. However, despite double fortification, vitamin A supplementation would still be needed in the North. The addition of vitamin A to maize flour does not seem to be potentially effective in the country, because maize flour is consumed in relatively low amounts (the main sources of energy in Uganda are plantains and starchy roots). The addition of vitamin A to wheat flour is unnecessary if both oil and sugar are fortified but, in the absence of sugar fortification, it might further reduce the prevalence of low vitamin A intakes in Kampala beyond the impact of fortified oil.

Types of Food Fortification

Three main types of food fortification were identified by the WHO/FAO based on application and scope: mass-, target-, and market-driven. **Figure 3** illustrates their complementary contribution to meet the nutrient gaps in a safe manner. In a population, the combined supply of micronutrients should cover the largest number of individuals and at the same time avoid excessive intakes. To achieve this aim, all the fortification programs should be regulated based on studies of intake. Panel (b) of **Figure 3** shows that uncontrolled market-driven fortification may jeopardize the good impact of mass fortification by supplying excessive amounts of micronutrients. Thus, all types of fortification should have standards that consider the micronutrient supply from both the regular diet and the fortified food and other micronutrient-supplying interventions.

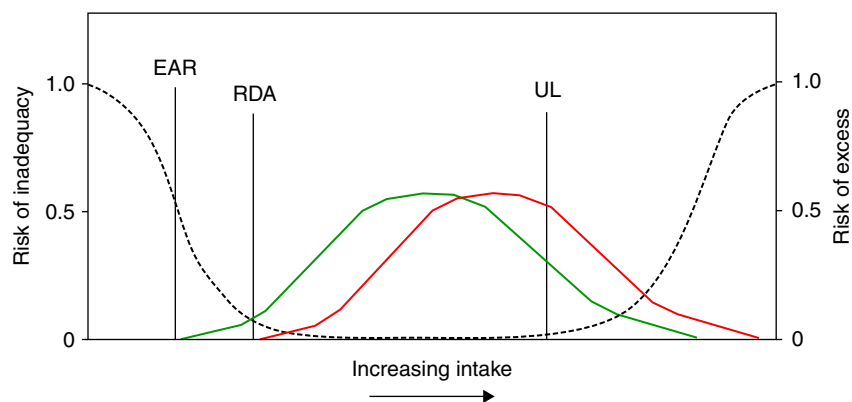


Figure 2 Risk distribution of micronutrient inadequacies and excesses in function of the micronutrient intakes, and intake distribution profiles – if symmetrical – using the EAR and RDA (RNI) values as cut-off points. Intersection areas of the intake profiles below the risk curves of inadequacy and excess represent the proportion of the population at risk. Modified from the *Institute of Medicine (1998) Dietary Reference Intakes for Thiamin, Riboflavin, Niacin, Vitamin B₆, Folate, Vitamin B₁₂, Pantothenic acid, Biotin, and Choline*, p. 464. Washington, DC: National Academy Press.

Table 2 The predicted prevalences (%) of inadequate intake of vitamin A for children 24–59 months and women of reproductive age (WRA) in the absence and in the presence of fortification, in Kampala, South-West (S-W), and North Uganda, 2008

Region	Age					
	Children 24–59 months			WRA		
	Kampala	S-W	North	Kampala	S-W	North
Diet alone	69	52	99	47	30	98
Plus oil ^a	20	28	56	6	17	45
Plus wheat flour	48	46	99	30	29	98
Plus w. flour and oil	10	25	54	3	17	45
Plus sugar	6	31	90	2	19	87
Plus oil and sugar	0	16	43	0	13	33
Plus wheat flour and oil & sugar	0	14	42	0	13	33
Plus maize flour	64	49	99	40	28	97
Plus wheat flour and oil and sugar and maize flour	0	13	39	0	12	31

^aThis is a closer approximation to the current situation in Uganda, because more than 85% of vegetable oil is already fortified with vitamin A. The assumed vitamin A contents in the fortified food at households were oil: 24.5 mg kg⁻¹; sugar: 7 mg kg⁻¹; wheat flour: 2.4 mg kg⁻¹; and maize flour: 0.8 mg kg⁻¹.

Source: http://www.a2zproject.org/pdf/Uganda_Food_Consumption_Survey_Final_08152011.pdf

Mass Fortification

This is defined as fortification targeted to the general population. In mass fortification, serving sizes of the fortified food are determined by the consumers and vary considerably among countries, regions, age groups, and socio-economic strata. This variation in food intakes limits the amount of micronutrients that can be added because of the need to avoid excessive intakes by individuals with high food intakes. Mass fortification uses staples and condiments, which are products extensively and widely consumed. This type of fortification is usually mandatory and instigated by governments, and the standards are usually made compulsory through specific regulations. However, under certain circumstances, the standards may be voluntary, as is the case in Uganda with oil fortification. In this country, three oil industries supply more than 90% of the national oil demand, and they have all adopted fortification. However, in other countries, it may be necessary to make oil fortification compulsory when the food industry is not willing to start fortification unless it is mandatory.

Targeted Fortification

This describes the fortification strategy designed for specific groups of the population, such as infants and young children (complementary foods), school-feeding, and public health or social programs implemented for benefiting vulnerable groups. The fortified foods are generally given in specific daily serving sizes and, because other fortified products are rarely available, the contents of micronutrients are large in order to meet the daily nutrient requirements using a few products. Furthermore, because the products are given free or subsidized and some of them are pre-cooked and processed, higher contents of micronutrients can be used because retaining the characteristics of the unfortified foods is not too restrictive.

Market-Driven Fortification

This type of fortification occurs when the food industry decides to introduce an additional perceived value to their

products to attract the interest of the consumers toward foods with a better nutritional value. These products are usually packaged, labeled, and branded. The application of the fortification standards is voluntary – the industry is free to fortify or not – but once fortification is claimed on the products, following the specified standards should be made compulsory. Very few countries in the world have introduced standards for this type of fortification, although this is changing.

Type of Fortification is Independent of the Food

The same food can support any of the three types of fortification. For example, rice could be mass-fortified if the country decides that all rice distributed to the population should be fortified as it is the case in Costa Rica. Fortified rice may be used for target fortification if it is produced and distributed through specific social programs, such as it is currently in the Philippines, where there is a compulsory regulation for rice fortification, but it is now mainly applied to the rice handled and delivered by the National Food Authority to poor sectors of the population. Finally, rice could also be used for market-driven fortification as in China, where industries launched fortified rice aimed at high-income groups.

Home Fortification or Fortification at the Point of Consumption

Nowadays, the terms home fortification and fortification at the point of consumption are being increasingly used to describe the addition of micronutrient powders (which are in fact micronutrient supplements) to meals just before consumption. The final result is an increment in the micronutrient density of the meal. The specific serving size of the micronutrient powders allows formulating them with amounts close to the RNI values, because the serving size is fixed. Nevertheless, countries should prepare standards for

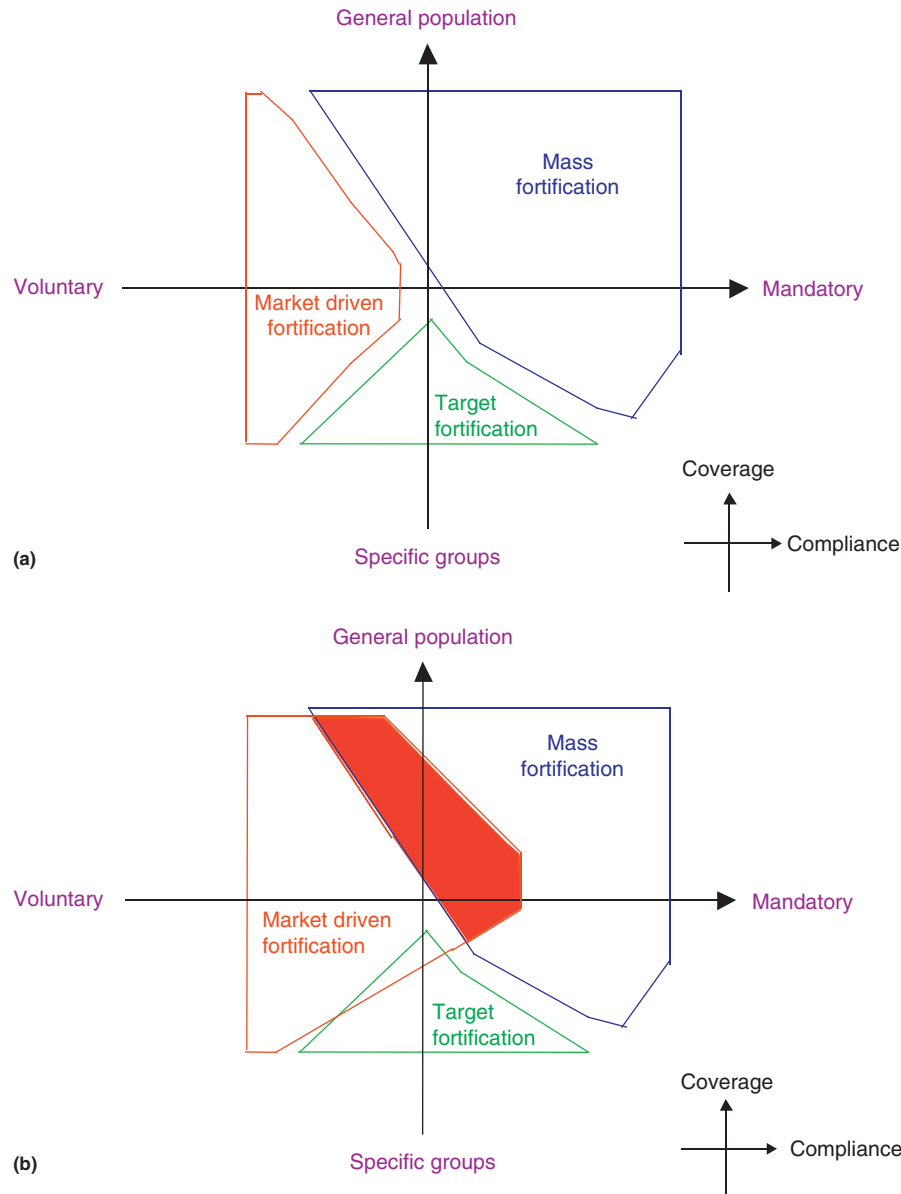


Figure 3 Panel (a) illustrates the complementary contribution of the three types of food fortification to fulfill the nutritional gap (total square) of a population, and position associated with extension of coverage (from specific groups to the general population) and type of compliance of the standards (from voluntary to mandatory). Panel (b) shows a situation when market-driven fortification is not regulated. Modified from Allen L, de Benoist B, Dary O, and Hurrell R (eds.) (2006) *Guidelines on Food Fortification with Micronutrients*, p. 341. Geneva: World Health Organization, <http://www.who.int/nutrition/publications/micronutrients/9241594012/en/index.html> [accessed on 13 March 2011].

these products to ensure safety when other interventions are in place.

Although the term home fortification was coined after the recent introduction of such micronutrient powders, it is interesting that the definition is also applicable to iodized salt, which has been in use since the beginning of the 20th century. Salt is not a food (i.e., it does not provide energy), iodized salt is incorporated into meals during preparation or before consumption, and the daily amount of iodine supplied is large enough to satisfy the requirements for this mineral for almost all individuals. These characteristics are not found in other fortified foods. In summary, iodized salt works as a type of home fortification.

What Else is Needed to Implement a Fortification Program?

Regardless of the type of fortification, the technical and economic feasibility of the fortified product, the accessibility to the vulnerable groups (coverage and utilization of the fortified product), and the expected biological impact of the fortified food (fulfilling the nutritional gap through the additional micronutrient intake), the success of a food fortification program also requires that it is programmatically controllable. This implies the existence of practical, reliable, and efficient mechanisms to enforce the standards and regulations.

Enforcement is based on the usual practices of food control, which have two components: internal supervision and external monitoring. Internal supervision refers to quality assurance (actions during production that are needed for complying with standards and other technical specifications) and quality control (chemical and physical analyses of samples of fortified food to check compliance of the standard and specifications) by the food industry. External monitoring includes auditing (review of the industry quality assurance procedures and their documentation) and inspection (confirmation that the product complies with standards and technical specification through sampling at the production places, distribution centers and retail outlets, and importation sites) by governmental authorities responsible for food control.

Quality control and inspection require that the standards and regulations provide reference values to check that the fortified product complies with the criteria of presentation, labeling, and packaging; toxicological and microbiological safety; expected technical properties; and micronutrient content. The latter is based on target values, which are the average contents of a micronutrient in the fortified food, after addition to its intrinsic content in the unfortified food. A tolerable range of heterogeneity should be estimated for each micronutrient and food matrix to specify the variation that will be allowed. In the past, and still currently in many countries, it was preferred to use a minimum value rather than an average in combination with a tolerable range of variation (heterogeneity). The minimum content was adopted with the assumption that it would not only simplify the sampling process (because most samples should comply with the minimum content) but that it would also pressure the industries to narrow the variability of the process and ensure the minimum required content. However, the use of the minimum content has not worked properly because many industries see it as the target value, without realizing that under some circumstances the variability may be very large due to the combination of many factors: the physical nature of the fortified product (dispersion is greater in solids than in liquids); the particle size (dispersion is greater in coarse than in fine products); the

form of addition of the fortificant (dispersion is greater when the fortificant is added in dry rather than in a liquid or a spray form); the size of the sample used for chemical analysis (dispersion is higher when small amounts of the fortified product are used); the performance of the analytical assay (dispersion is greater when the resolution and precision of the assay are low); and the efficiency of the mixing process. Under the current policy of the minimum content, many samples are

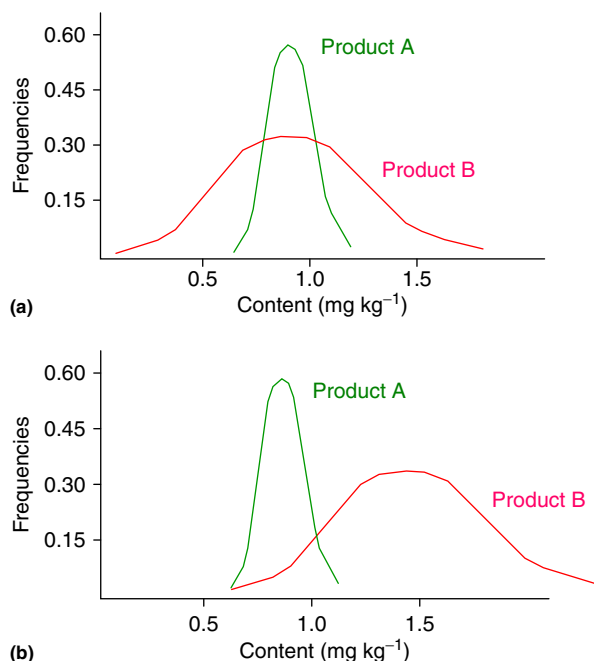


Figure 4 Panel (a) shows the average and dispersion of micronutrient content of two combinations of fortificant and food vehicle with distinct grades of variation. Panel (b) illustrates the distribution of micronutrient contents if the same minimum value is applicable to the two cases. For complying with the minimum content, product B should be formulated with twice the amount of the fortificant as product A.

Table 3 Iodine content in coarse (Guatemala) and refined (Costa Rica) salt

Parameter	Coarse (Guatemala)		Refined (Costa Rica)	
	Single samples	Composite samples ^a	Single samples	Composite samples
<i>N</i>	43	8	35	7
Mean (mg kg ⁻¹)	26.1	26.5	36.5	36.5
S.D. (mg kg ⁻¹)	11.8	4.4	5.4	2.0
S.E. (mg kg ⁻¹)	1.8	1.6	0.9	0.7
C.V. (%)	45.2%	16.6%	14.8%	5.4%
Samples < 5 mg kg ⁻¹	4%	0%	0%	0%
<i>Tolerable ranges of heterogeneity for single results</i>				
80% compliance (P-10 to P-90) ^b	11.0–41.2	20.8–32.1	29.6–43.4	33.9–39.1
90% compliance (P-5 to P-95)	6.7–45.5	19.2–33.7	27.6–45.4	33.2–39.8
99% compliance (P-0.5 to P-99.5)	0.0–56.5	15.1–37.8	22.6–50.4	31.3–41.7

^aComposite samples prepared by mixing five single samples.

^bP-X denotes the percentile X in mg iodine per kg salt.

Source: Consumer Protection Association (LIDECON for its acronym in Spanish), Guatemala, 2009; and National Reference Center of Oral health, INCIENSA, Costa Rica, 2009. Results are from the same brand of salt for each country.

Table 4 Prediction of vitamin A contents at households based on the added levels at factories and estimated stability – Example of Uganda 2008

Food	Added level (mg kg ⁻¹)	Stability from factory to homes (%)	Predicted micronutrient at homes (mg kg ⁻¹)
Vegetable oil	35.0	70	24.5
Sugar	10.0	70	7.0
Wheat flour	3.0	80	2.4
Maize flour	1.0	80	0.8

usually found below that value, which tends to cause conflicts between the government and industry. Large amounts of fortificants may be needed to comply with a minimum; **Figure 4** illustrates this situation.

If the coefficient of variation of an acceptable fortification process is larger than 30%, it would be necessary to prepare composite samples (a blend of single samples from the same batch or brand) in order to reduce variation. If this is not done, the valid values of the tolerable range of heterogeneity would be so large that it would make no sense. **Table 3** shows the situation for iodized salt in Guatemala and Costa Rica. In Guatemala, coarse salt is iodized using very simple mixing by shoveling. Although the program provides sufficient iodine to the population, the variability in single samples is so high that the use of composite samples is required; otherwise, up to 4% of salt samples with very low iodine levels (<5 mg kg⁻¹) should be accepted. In Costa Rica, the variation of the iodine content is very narrow; thus, both single and composite samples could be used for enforcement, although the use of the latter would reduce the amount and cost of the analytical work.

Finally, a food fortification program should be evaluated for its performance and effectiveness at the household and the consumer level. The change in biomarker values associated with the added micronutrients should be evaluated and linked to the proportion of the population that moves from inadequate to adequate intakes. For this purpose, the intake due to fortified foods should be estimated before and during the existence of the fortification programs, taking samples from foods served at home. A prediction of the possible levels can be made beforehand using stability data from other countries. **Table 4** shows the calculations made in Uganda for estimating the micronutrient content of foods consumed by households, and then evaluating the impact of fortification as presented in **Table 2**.

See also: Bioavailability. Food Fortification: Programs. Iodine: Deficiency Disorders and Prevention Programs. Iron: Physiology, Dietary Sources, and Requirements. Supplementation: Dietary Supplements; Programmatic Issues. Vitamin A: Deficiency and Interventions. Zinc: Deficiency Disorders and Prevention Programs

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Relevant Websites

- <http://www.a2zproject.org/>
A2Z Project: A2Z is the latest USAID Project in micronutrients (2005–2011), and the website contains publications, reports, manuals, presentations, and similar material about micronutrients, including food fortification. The site also presents similar documents from the predecessor project MOST (1999–2005).
- <http://www.cdc.gov/impact/index.html>
CDC/IMMPaCt: The site of the International Micronutrient Malnutrition Prevention and Control (IMMPaCt) Program of the CDC (USA) covers a wide range of information about micronutrient biology and interventions, including food fortification.
- http://www.codexalimentarius.net/web/index_en.jsp
Codex Alimentarius: This site contains guidelines, general principles, manuals, standard models, and similar materials for food safety and trade, including recommendations for food fortification, health and nutrient claims, nutritional panel, and labeling.
- <http://www.sph.emory.edu/wheatflour/>
Flour Fortification Initiative: The Flour Fortification Initiative (FFI) is a public–private network of organizations dedicated to promote the use of wheat flour fortification worldwide to improve the status of several micronutrients. The website contains documents, information, and tools associated with this area.
- <http://www.gainhealth.org/>
Global Alliance for Improved Nutrition: This site contains news, reports,

resources, and announcements about nutrition interventions, mainly implemented under public–private partnerships, including food fortification.

<http://www.ilsa.org/Pages/HomePage.aspx>

ILSI: The International Life Science Institute (ILSI) is a nonprofit organization focused on nutrition and health, food safety, risk assessment, and the environment in support of public health policies and programs, through the collaboration among academia, government, and industry. The website contains several publications and resources useful for food fortification.

<http://www.micronutrient.org/English/View.asp?x=699>

Micronutrient Initiative: The Micronutrient Initiative (MI) is an international

technical NGO based in Ottawa, Canada, with offices in Asia and Africa, and programs in several developing countries; its focus is to improve micronutrient status in vulnerable populations, and food fortification has been one of the main interventions.

<http://www.sustaintech.org/>

SUSTAIN: SUSTAIN (Sharing U.S. Technology for Improve Nutrition) is a nonprofit organization based in the USA whose objective is to transfer food technologies that are operationally feasible and cost-effective from industrial countries to developing regions by means of voluntary experts and researchers. Most of the publications and reports refer to food fortification.

FOOD INTOLERANCE

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Definition of Food Intolerance

Food intolerance can be defined as a reproducible adverse reaction to a specific food or food ingredient, and it is not psychologically based. Although this appears straightforward, a key limitation of this definition is the lack of consistent definition on what constitutes an 'adverse reaction.' The phrase has a strong subjective component, from the part of both the patient as well as the physician. The problem is compounded by the wide variation in tolerance to the different 'reactions' associated with eating in the general population.

Any Food taken in Excess may be Harmful

The definition above does not take into account the dosage. Large quantities of certain foods may result in disease in certain individuals, although such disorders are not usually included in the category of food intolerance. Any food, however harmless, can be harmful if taken in excess. Notable examples of this are:

1. Apples, pears, and honey are rich sources of fructose – a sugar which in early childhood is not well absorbed if taken in large quantities. Thus, if a child takes a quantity of fructose in excess of that which can be absorbed in the gastrointestinal tract, the result will be loose stools (diarrhea) due to the osmotic effect of unabsorbed fructose. It should be noted that whereas this applies to normal children, there are, in addition, a few children who are especially poor at handling ingested fructose, and in these children even small quantities of fructose-containing foods will cause florid diarrhea.
2. Chicken liver is a rich source of vitamin A. There are reported cases of infants who were fed large quantities of chicken liver, and who developed raised intracranial pressure as a consequence of vitamin A toxicity.
3. In those who are genetically predisposed, ingestion of an excess of purine-rich foods contributes to hyperuricemia, leading to gout, a disorder which is not usually regarded as a form of food intolerance.

Principal Mechanisms and Pathophysiology of Food Intolerance

Food Allergy

The term 'allergy' implies a definite immunological mechanism. This could be antibody mediated, cell mediated, or due

to circulating immune complexes. The clinical features of an allergic reaction include urticaria (nettle rash), angioedema, rhinitis (sneezing, nasal discharge, blocked nose), worsening of preexisting atopic eczema, asthma (wheezing, coughing, tightness of the chest, shortness of breath), vomiting, abdominal pain, diarrhea, and anaphylactic shock.

Enzyme Defects

Inborn errors of metabolism may affect the digestion and absorption of carbohydrate, fat, or protein. In some subjects the enzyme defect is primarily gastrointestinal, causing defects in digestion or absorption. An example is lactase deficiency (see below). In other subjects, the enzyme defect is systemic. An example is the rare disorder of hereditary fructose intolerance, described below.

Lactase Deficiency

An example of an enzyme defect causing food intolerance is lactase deficiency. In this condition, which is primarily a disorder that affects infants and young children, there is a reduced or absent concentration of the enzyme lactase in the small intestinal mucosa. Affected individuals are unable to break down ingested lactose, the main sugar found in milk, and which if unabsorbed passes into the large intestine, where there are two consequences. One is an osmotic diarrhea. The other is that some of the unabsorbed lactose is broken down by intestinal bacteria, accompanied by the production of gas (hydrogen) leading to abdominal distension and flatus and the production of organic acids that cause perianal soreness or excoriation. The elimination in exhaled air of hydrogen produced in the colon from unabsorbed carbohydrate can be used as a simple yet reliable test for malabsorption (breath hydrogen test). Most commonly used to assess lactose malabsorption, the test provides quantitative estimates of the amount of carbohydrate that was not absorbed in the small intestine and reached the colon. Because hydrogen is produced by colonic bacteria, the test requires a normal gut flora and is therefore affected by antibiotic use, diarrhea, or other large intestine disorders.

The management of lactose intolerance is to avoid foods that contain lactose (mainly cows' milk and its products). For infants it is worth noting that the soya-based infant formulas are lactose free. In theory, an alternative is to add microbial β -galactosidase to cows' milk, which can produce a lactose-free

milk, with the inconvenience that it has a sweeter flavor and requires a 24-h incubation period at 4 °C.

In infants and young children, lactase deficiency is usually a transient problem occurring after an episode of gastroenteritis, but it is commonly a feature of any disease that causes damage to the intestinal mucosa (e.g., celiac disease). Levels of lactase tend to fall during mid- to later childhood, and in a number of populations (e.g., African, Mexican, and Greenland Eskimo) a high proportion of adults have very little lactase activity. This adult deficiency is believed to have a genetic basis. Man is the only animal apart from the domestic cat that drinks milk after weaning, and deficiency of lactase in adults could in certain populations be considered the normal state.

Hereditary Fructose Intolerance

In this condition, which has autosomal recessive inheritance, there is deficiency of the liver enzyme fructose 1,6-bisphosphate aldolase. As a result, fructose-1-phosphate accumulates in liver cells, and acts as a competitive inhibitor for phosphorylase. The resulting transient inhibition of the conversion of glycogen to glucose leads to severe hypoglycemia (low blood glucose concentration). Affected infants are symptom free as long as their diet is limited to human milk. If they receive milk formulas or any food that contains fructose they develop attacks of hypoglycemia, shock, coma, and convulsions. There may be jaundice, an enlarged liver, and sometimes progressive liver disease. The treatment requires the complete elimination of fructose from the diet, which may be difficult as fructose is a widely used food additive and a sweetener. A trivial but interesting feature of the condition in survivors is a notable reduction in dental caries, a beneficial result from the need to avoid many types of confectionery.

Pharmacological Mechanisms

Caffeine

A good example of a pharmacological agent found in food with the ability to cause adverse reactions is caffeine. The stimulant effect, which may be welcome at times but unwelcome at others, of 60 mg caffeine in a cup of tea or 100 mg caffeine in a cup of coffee are well recognized. What is less well recognized is that heavy coffee or tea drinkers can suffer a number of other side effects of caffeine, which stimulates gastric secretion and can cause heartburn, nausea, vomiting, diarrhea, and intestinal colic. Also common are irregular heartbeats, episodes of rapid pulse, sweating, tremor, anxiety, and sleeplessness. Caffeine also has a diuretic effect.

Sodium Nitrite

Another pharmacological effect occurs when unusually large quantities of sodium nitrite are ingested. Sodium nitrite is an antioxidant used as an antibacterial agent, and in quantities of 20 mg or more it can cause dilatation of blood vessels causing flushing and headache, and urticaria.

Tyramine, Histamine, and Other Vasoactive Amines

A further example of a pharmacological mechanism is the adverse effect of various vasoactive amines such as tyramine,

serotonin, tryptamine, phenylethylamine, and histamine, which are found in a range of foods such as tuna, pickled herring, sardines, anchovy fillets, bananas, cheese, yeast extracts (such as Marmite), chocolate, wine, spinach, tomato, and sausages. There appear to be three main mechanisms in operation:

1. An abnormally high intake of vasoactive amines, such as histamine or tyramine, either because of a high content in food or because of synthesis of these chemicals in the gut by bacteria.
2. An abnormal effect whereby drugs or chemicals in food interfere with the enzymes that break down vasoactive amines.
3. An abnormal release from mast cells of histamine and other mediators of inflammation, triggered by eating certain foods such as strawberries, shellfish, and alcohol.

Vasoactive amines are the normal constituents of many foods. They arise mainly from the decarboxylation of amino acids, but they may also develop during normal food cooking and during the storage of food. **Table 1** shows the histamine level of various sausages. The term 'semidry' when applied to sausages (**Table 1**) means sausages that are fermented for varying periods. During this sausage-ripening process, the histamine concentration increases, depending on the length of the ripening process. It is estimated that 70–1000 mg of histamine ingested in a single meal is necessary for the onset of toxicity, depending on individual sensitivity. Thus, 130 g of the pepperoni sample that contained 55.0 mg histamine per 100 g (see **Table 1**) would be necessary to cause symptoms in the most sensitive individuals.

The largest amounts of histamine and tyramine are found in fermented foods such as cheese, alcoholic drinks, sausage, sauerkraut, and tinned fish. Badly stored food (see below) such as mackerel and tuna can also contain large amounts of histamine.

The effects of large doses of tyramine, histamine, and other vasoactive amines are extremely variable. Histamine causes

Table 1 Histamine levels in sausages

Type of sausage	Histamine level (mg/100 g)	
	Mean	Range
<i>Cooked sausages^a</i>		
Bologna	0.55	0.19–0.84
Cooked salami	0.83	0.47–5.86
Kosher salami	0.50	0.33–0.97
<i>Semidry sausages^a</i>		
Thuringer cervelat	2.35	1.03–3.63
Thuringer	1.19	0.31–2.56
<i>Dry sausages^a</i>		
Italian dry salami	2.14–24.5 ^b	0.42–36.4 ^b
Pepperoni	1.03–38.1 ^b	0.72–55.0 ^b
Chorizo	2.29	0.60–8.08

^aThe sausages were obtained from retail markets in the San Francisco Bay area.

^bDepending on the brand tested.

Source: Reproduced from Taylor SL, Leatherwood M, and Lieber ER (1978) A survey of histamine levels in sausages. *Journal of Food Protection* 41: 634–637, with permission from International Association For Food Protection

flushing (by dilatation of blood vessels), constriction of smooth muscle in the intestine and the bronchi, increased heart rate, headache, fall in blood pressure, and asthma. Tyramine causes constriction of blood vessels, and it stimulates the release of noradrenaline from nerve endings. It can also cause the release of histamine and prostaglandins from mast cells. Dietary tyramine is known to induce hypertension and headache in patients who are taking monoamine oxidase inhibitor drugs. This effect has been shown to be due to inhibition, by these drugs, of intestinal and hepatic metabolism of tyramine, so that the amine accumulates.

The variable effect of histamine taken by mouth is in part due to the varying degree of inactivation in the gastrointestinal tract. Histamine is inactivated by mucoproteins that are produced in the gastrointestinal tract mucosa, but this inactivation can be blocked by other amines such as cadaverine and putrescine, which also bind strongly to mucoproteins. Thus, when food that contains cadaverine and putrescine is ingested, more histamine can be absorbed. In fact, most of the histamine that is absorbed is degraded as it is transported across the mucosa by the intestinal enzyme diamine oxidase. Cadaverine and putrescine also have a high affinity for diamine oxidase and can also interfere with the inactivation of histamine by this enzyme. Another barrier to the absorption of histamine is provided by the liver enzyme methyl transferase.

Thus, the effect of histamine and other vasoactive amines on an individual will depend on a number of factors, which include:

1. The amount of vasoactive amine that is present in food.
2. The amount of histamine released (as a result of an allergic process).
3. The permeability of the gastrointestinal tract, including inactivation by mucus and by mechanisms in the gut mucosa.
4. Interference with the synthesis or release of enzymes involved in amine breakdown (e.g., liver damage causing reduced activity of methyl transferase).

Tyramine and Migraine

There has been interest in a possible relationship between dietary tyramine and migraine. One hypothesis is that some patients with migraine have defective metabolism of ingested tyramine in the intestinal wall, which leads to increased absorption, apparently explaining why foods that contain tyramine can provoke attacks in susceptible individuals. However, there is no evidence that the activity of monoamine oxidase, the main tyramine-metabolizing enzyme, is lower in patients with food-induced migraine than in other individuals prone to migraine, although levels of monoamine oxidase in platelets are generally lower in patients with migraine.

Set against these theoretical arguments, in fact, most attempts to induce migraine by tyramine challenge in children and adults have been unsuccessful. Furthermore, a controlled study of exclusion of dietary vasoactive amines in children with migraine failed to demonstrate benefit. In the latter study, patients were randomly allocated to either a high-fiber diet low in dietary amines or a high-fiber diet alone. Although there was no significant difference in the results for the two groups, both

groups showed a highly significant decrease in the number of headaches, emphasizing the need for a control diet in studies designed to show that dietary manipulation improves disease.

Of the foods reported to be common triggers of attacks of migraine, only cheese is rich in tyramine. Chocolate is low in this and other vasoactive amines, and red wine usually contains no more tyramine than white wine. Alcoholic drinks, particularly red wine, are commonly reported to provoke attacks of migraine. Whether these attacks are due to the alcohol itself or some other compound is a matter of debate. The major chemical difference between red and white wines is the former's high concentration of phenolic flavonoids such as anthocyanins and catechins, which as well as having direct effects on blood vessels may also inhibit the enzyme phenolsulfotransferase. Patients with food-induced migraine were shown to have significantly lower levels of platelet phenolsulfotransferase activity, and it has been hypothesized (but not proven) that low activity of this enzyme could lead to an accumulation of phenolic or monoamine substrates, which in turn might directly or indirectly provoke attacks of migraine.

Regardless of the possible mechanism, there are a number of subjects with migraine who are made worse by specific dietary triggers such as cheese or wine, for whatever reason, and avoidance of specific food triggers in susceptible subjects may prove helpful in reducing the frequency of attacks.

11 β -Hydroxysteroid Dehydrogenase and Liquorice

Liquorice contains an enzyme that inhibits 11 β -hydroxysteroid dehydrogenase, resulting in sodium and water retention, hypertension, hypokalemia, and suppression of the renin-aldosterone system.

Irritant Mechanisms

Certain foods have a direct irritant effect on the mucous membranes of the mouth or gut, such as the irritant effect of coffee or curry. In certain individuals, food intolerance only occurs in the presence of a coexisting medical disorder. For example, the ingestion of spicy food, coffee, or orange juice provoke esophageal pain in some patients with reflux esophagitis. This effect is unconnected to the temperature or acidity of the food, or to any effect on the lower esophageal sphincter. The treatment in susceptible individuals is to avoid the trigger food item.

Specific Drug-Food Combinations

One example of drug-induced food intolerance is potentiation of the pressor effects of tyramine-containing foods (e.g., cheese, yeast extracts, and fermented soya bean products) by monoamine oxidase inhibitor drugs. Another is the effect of taking alcohol in patients with alcohol dependence during treatment with disulfiram (Antabuse). The reaction, which can occur within 10 min of alcohol and may last for several hours, consists of flushing and nausea.

Toxic Mechanisms

Nature has endowed plants with the capacity to synthesize substances that are toxic, and thus serve to protect them from

predators whether they be fungi, insects, animals, or humans. Thus, many plant foods contain naturally occurring toxins. On a worldwide scale, reactions to naturally occurring toxins may outnumber allergic reactions, although it is currently fashionable to pay more attention to the latter.

Protease Inhibitors

Soya beans were originally introduced into the US as a source of oil, the extracted meal being used as a by-product that could provide animals with a source of protein. However, it was recognized that it was necessary to subject soya beans to heat treatment if they were to support the growth of animals. It was later found that the substance responsible for growth inhibition in raw soya beans was a protease (trypsin) inhibitor, and it is now known that protease inhibitors are widely distributed throughout the plant kingdom, particularly in legumes, and to a lesser extent in cereal grains and tubers. In addition to inhibition of growth, one of the most characteristic responses of most animals to trypsin inhibitor is enlargement of the pancreas. The depression of growth is believed to result from endogenous loss of protein (i.e., loss into the gastrointestinal tract) due to hypersecretion by the pancreas. Soya bean products that have been adequately heat treated to inactivate trypsin inhibitor are safe for consumption.

Lectins

There is a protein present in most legumes and cereals that has the property of being able to agglutinate the red blood cells of various species of animals: The so-called phytohemagglutinins or lectins. Some of these lectins, such as ricin from the castor bean, are extremely toxic. Others, such as those in the soya bean, are nontoxic. Lectins appear to be responsible for the fact that many other legumes, unless properly cooked, not only fail to support the growth of animals but can lead to death. Lectins are found in many food items commonly consumed in the human diet including tomatoes, bean sprouts, raw vegetables, fruits, spices, dry cereals, and nuts, and it is not known whether these are harmful in any way. However, it is well recognized that inadequate cooking of red kidney beans can cause severe gastrointestinal upset, with vomiting and diarrhea. It is for this reason that it is recommended that raw red kidney beans should be cooked by initially boiling hard for 10 min.

Lathyragens

Lathyrism is a paralytic disease that is associated with the consumption of chickling pea or vetch, *Lathyrus sativus*. The causative factor is believed to be an amino acid derivative, β -N-oxalyl-, -diaminopropionic acid; this is a metabolic antagonist of glutamic acid, a substance that is involved in the transmission of nerve impulses in the brain.

Mimosine

Mimosine is an amino acid that comprises 1–4% of the dry weight of the legume *Leucaena leucocephala*, and consumption of its leaves, pods, and seeds leads to hair loss in animals. Mimosine is also a goitrogen (see below).

Djenkolic Acid

In parts of Sumatra the djenkol bean is a popular food item. The bean is a seed of the leguminous tree, *Pithecolobium lobatum*, and resembles the horse chestnut in size and color. Consumption of this seed leads to kidney failure that is accompanied by blood and needle-like clusters in the urine, which have been identified as containing the amino acid djenkolic acid.

Goitrogens

Substances capable of producing goiter are present in plants belonging to the cabbage family, including cabbage, turnip, broccoli, cauliflower, brussel sprouts, kale, rape seed, and mustard seed. Cows' milk is a vector for the transmission of goitrogens from animals fed kale and turnips, and may have been responsible for endemic goiter in countries such as Australia and Finland.

Cyanogens

A number of plants are potentially toxic because they contain glycosides from which hydrogen cyanide may be released by enzymatic hydrolysis. The most common plants eaten by humans, in order of their potential cyanide content, are: Lima beans (*Phaseolus lunatus*), sorghum, cassava, linseed meal, black-eyed pea (*Vigna sinensis*), garden pea (*Pisum sativum*), kidney bean (*Phaseolus vulgaris*), Bengal gram (*Cicer arietinum*), and red gram (*Cajanus cajan*s).

Vicine and Convicine

These are β -glucosides that are present in broad beans (*Vicia faba*). When consumed by individuals with deficiency of the enzyme glucose-6-phosphate dehydrogenase, these substances precipitate the condition of favism, which is characterized by anemia caused by hemolysis of red blood cells. The enzyme deficiency is a genetic disorder that is confined largely to inhabitants of countries surrounding the Mediterranean basin (Italy, Sicily, Lebanon, Israel, and North Africa) although individuals of the same ethnic background residing in other countries may also suffer from favism.

Cycasin

Cycad seeds or nuts are obtained from *Cycad circinalis*, a palm-like tree that grows throughout the tropics and subtropics. The seeds, unless thoroughly washed, are extremely toxic, causing poisoning in humans and tumors in experimental animals. The toxic ingredient methyl-azoxymethanol, the aglycone of cycasin, is released on hydrolysis of cycasin by intestinal bacteria.

Pyrrolizidine Derivatives

Pyrrolizidine alkaloids are found in a wide variety of plant species. The toxic ingredient belongs to a class of compounds that are derivatives of pyrrolizidine. Large numbers of people have been poisoned through consumption of cereal and grain crops contaminated with pyrrolizidine-containing plants. It is also possible that milk from cows grazing on pastures that contain such plants could act as a vector for the transmission of pyrrolizidine to humans. In one part of western USA one such plant, the tansy ragwort (*Senecio jacobea*) is readily consumed by cows and goats, and the milk from such animals has

been shown to contain significant amounts of a pyrrolizidine derivative, jacoline.

Lupin Alkaloid

Milk from animals that have eaten plants from the lupin family, notably *Lupinus latifolius*, may contain quinolizidine alkaloids such as anagryne. There is strong evidence that these alkaloids are teratogenic in animals, causing severe bony deformities, and there is some evidence that similar defects may occur in the offspring of human mothers who drink alkaloid-containing milk in pregnancy.

Other Examples

There are numerous other examples of toxic substances present in foodstuffs. These include solanidine in potatoes, cyanide in tapioca, mycotoxins in mushrooms and cereal grains, and phototoxic furocoumarins in angelica, parsley, dill, and celeriac, which in sufficient quantities can give rise to a wide variety of toxic reactions (Tables 2 and 3).

Food Storage

Chemical changes in food during storage can produce substances that cause food intolerance. An example is intolerance to ripe or stored tomatoes in subjects who can safely eat green tomatoes, where ripening of the fruit produces a new active glycoprotein. Some adverse reactions resulting from food storage come into the category of toxic reactions, such as the rise in levels of histamine and tyramine in certain foods during storage as a result of bacterial decarboxylation. An example of this is the production of histamine in badly stored mackerel and other fish: Scombroid fish poisoning. Contamination of food by antigens such as storage mites or microbial spores

may give rise to adverse effects, particularly asthma and eczema. Contamination of food by microorganisms may result in adverse effects. For example, celery, parsnip, and parsley may become infected with the fungus *Sclerotinia sclerotiorum* ('pink rot'), resulting in the production of the photosensitizing chemicals psoralen, 5-methoxypsoralen, and 8-methoxypsoralen.

Practical Applications

Food arouses not only the appetite but also the emotions. The passion for food that is natural (i.e., free from extraneous ingredients) is not new; in 1857, a survey of adulterants in food showed that childrens' sweets were commonly colored by red lead (lead oxide), lead chromate, mercuric sulfide, and copper arsenite. By the late 1850s, 'pure and unadulterated' had become the stock advertising slogan of those anxious to cash in on the then newly awakened fears of the public. The current scale of the use of additives in food comes as a surprise to most people, and it is understandable that many should find these substances vaguely menacing. Nonetheless, the current phobia of food additives and food processing, and the obsession for the so-called natural or health food arises largely out of misinformation and ignorance. Obsession with the so-called natural or health food ignores the wide range of naturally occurring toxins in foods. The concept of health food is wholly misleading. For example, a survey of 'crunchy' peanut butter showed that 11 out of 59 samples from health food producers contained over $100 \mu\text{g kg}^{-1}$ of aflatoxins, over 10 times the proposed maximum permitted level for total aflatoxins. Only one of the 26 samples from other producers contained aflatoxins in excess of $10 \mu\text{g kg}^{-1}$, and none contained more than $50 \mu\text{g kg}^{-1}$.

Table 2 Examples of toxic constituents of plant foodstuffs and their role in plant physiology

Toxic constituent	Type of food containing toxic constituent	Physiological role of toxic constituent	Role in plant defense: mechanism of toxic constituent
Protease inhibitors	Legumes, cereals, potatoes, pineapple	?Prevents degradation of storage protein during seed maturation	Part of defense against invading microbes following mechanical damage to leaves
Hemagglutinins	Legumes, cereals, potatoes	(a) Attach glycoprotein enzymes (b) Role in embryonic development/differentiation (c) Role in sugar transport or store (d) ?Involved in root nodule nitrogen-fixing bacteria symbiosis	(a) Counteract soil bacteria (b) Antifungal (c) Protect against seed predators
Glucosinolates	Radish, horseradish, turnip, cabbage, rape seed	?Disease & insect resistance role	
Cyanogens	Almonds, cassava, corn, peas, butter beans, bamboo shoots		
Saponins	Alfalfa, French beans, soya beans		

Source: Adapted from Leiner IE (ed.) (1980) *Toxic Constituents of Plant Foodstuffs*, 2nd edn. New York: Academic Press.

Table 3 Examples of foodborne toxins or toxin-producing organisms, excluding plant foodstuffs

Pathogen or toxin	Principal symptoms	Common food source
<i>Bacillus cereus</i>	(a) Diarrhea	Proteinaceous food, vegetables, sauces, puddings
<i>Bacillus subtilis</i>	(b) Vomiting Vomiting, diarrhea Flushing, sweating	Fried rice Meat and pastry Meat/seafood with rice
<i>Bacillus licheniformis</i>	Diarrhea	Cooked meat and vegetables
<i>Clostridium botulinum</i>	Neuroparalytic disease (botulism)	Meat, fish, vegetables, hazelnut conserve
<i>Clostridium perfringens</i>	Diarrhea, abdominal pain	Meat, poultry
<i>Salmonella enteridis</i>	Diarrhea, abdominal pain, fever, vomiting	Poultry, eggs
<i>Staphylococcus aureus</i>	Vomiting, abdominal pain, diarrhea	Numerous but specially cooked high-protein foods
Verotoxin-producing <i>Escherichia coli</i>	Hemorrhagic colitis	Ground beef
<i>Listeria monocytogenes</i>	Listeriosis	Unpasteurized cheese, undercooked meat
Dioxins and dibenzofurans	Adverse effects uncertain when consumed in quantities found in food	Fish
Cantharidin	Sensitivity to urethra and genitalia; priapism	Frogs that have Meloidae (blister beetles)
Methyl mercury	Brain damage	Fish, bread
Toxic alkaloid (saxitoxin) in dinoflagellates and plankton	Diverse neurological disorders (paralytic shellfish poisoning)	Clams, oysters, scallops, and mussels
Brevetoxins	Paresthesia, abdominal pain, diarrhea, transient blindness, paralysis, death (neurotoxic shellfish poisoning)	Clams, oysters, scallops, and mussels
Ciguatera toxin	Diverse gastrointestinal and neurological disorders	Fish (especially reef predators)
Tetrodotoxin	Diverse gastrointestinal and neurological disorders	Puffer fish, certain newts
Domoic acid	Vomiting, diarrhea, hyperexcitation, seizures, memory loss (amnesic shellfish poisoning)	Mussels
Okadaic acid, dinophys toxins, yessotoxin, pectenotoxins	Diarrhea, vomiting, abdominal pain (diarrhetic shellfish poisoning)	Mussels, scallops
Scombrotoxin (usually histamine)	Headache, palpitations, gastrointestinal disturbance	Mackerel, tuna, and related species
Tetramine (red whelk poisoning)	Diplopia, dizziness, leg pains	Whelks
Grayanotoxins (in honey from areas of Turkey where <i>Rhododendrons</i> are grown)	Hypotension, bradycardia, vomiting, sweating	Honey
Unknown (? in algae) (turtle flesh poisoning)	Cardiorespiratory failure, death	Turtles

See also: Caffeine. Food Allergies: Diagnosis and Management. Food Safety: Mycotoxins – Occurrence and Toxic Effects. Fructose: Absorption and Metabolism. Lactose Intolerance. Vitamin A: Deficiency and Interventions

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FOOD SAFETY

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Glossary

Anaerobic Without oxygen.

Antigens Markers on the surface of microbes such as bacteria that allow antibodies to recognize them.

Cholinergic nerves Nerve cells that employ acetylcholine as their neurotransmitter.

Farinaceous Consisting or made of starch.

Hazard analysis critical control point system A management system in which food safety is addressed through the analysis and control of biological, chemical, and physical hazards from raw material production, procurement, and handling, to manufacturing, distribution, and consumption of finished product.

Hemolytic uremic syndrome Formation of clots in small blood vessels of kidneys which can lead to renal failure.

Meningitis A serious inflammation of the thin membranous covering of the brain and spinal cord.

Oviduct A passage through which ova leave the maternal body or pass to an organ communicating with the exterior of the body.

Septicemia Systemic disease associated with the presence of pathogenic microbes or their toxins in the blood.

Serotype A group of closely related microorganisms distinguished by a characteristic set of antigens.

The burden of gastroenteritis (GE) in the world, in terms of both morbidity and mortality, is extensive. In the developing world (e.g., Southeast Asia), diarrhea vies with acute respiratory tract infection as the leading cause of death in childhood. Even in the more developed world, GE caused by infectious microbes is a significant cause of illness and time lost from work, and death does occur, especially among highly sensitive human populations with weak immune response. The more advanced foodborne disease surveillance systems become, the more food-associated GE illnesses they uncover.

Not all GE is caused by contaminated food. Some GE is caused by poor hygiene resulting in direct or indirect transmission of infectious microbes without a food vehicle. Nevertheless, contaminated food is a major vehicle of infectious GE throughout the world. The general definition of foodborne illness (FI) is an acute GE caused by consumption of food contaminated with foodborne pathogens or their preformed toxin. Typhoid fever and brucellosis, however, are not usually considered FIs, whereas botulism is, even though it

causes paralysis and not GE, as is listeriosis, which principally causes septicemia and meningitis.

Norovirus is the most common known cause of FI, followed by bacterial FI. Many types of bacterial FI, such as salmonellosis and enterohemorrhagic *Escherichia coli* (*E. coli*) O157:H7 infections, are more common in the summer than winter months.

Bacteria produce their effects on the intestinal tract either by direct invasion of the mucosa or by the production of toxin. Some of the toxins are produced in food outside the intestinal tract; others are formed or released in the intestine. Some invasive bacteria also produce a toxin in the intestine. This article provides an overview of the bacterial causes of FI.

Bacterial Toxins

There are three main forms of bacterial toxins that cause FI: (1) enterotoxin, which produces excess fluid secretion into the

gut (e.g., cholera and staphylococcal toxins); (2) cytotoxin, which causes inflammation and mucosal damage (e.g., *Shigella* and enterohemorrhagic *E. coli*); and (3) neurotoxin, which affects the nervous system (e.g., botulinum toxin).

Some *E. coli* strains invade and produce toxin(s) and are addressed under Invasive Bacteria. Red kidney bean toxin, scombrototoxin and other fish toxins, and heavy metal poisoning are addressed elsewhere in this book.

Staphylococcal Food Poisoning

Background

Staphylococcal food poisoning (SFP) is one of the causes of bacterial FI that is commonly attributed to a food handler. Humans frequently carry *Staphylococcus aureus* either in an infected site or asymptotically. Infected sites include wounds and abscesses, which may be the source of large numbers of staphylococci. Asymptomatic sites include the throat, nostrils, fingernails, or hair. In general, coagulase-positive staphylococci (*S. aureus*), and only certain types, produce enterotoxin. Some coagulase-negative *S. aureus* strains may also produce toxin, but rarely. Because the organism is also carried by many animals, outbreaks attributable to inadequate food handling, which often involves temperature abuse of a precontaminated food can also occur.

Survival and Growth

Staphylococci are killed by normal cooking temperatures. Any staphylococci that survive because of inadequate heating or, more frequently, by postcooking contamination from a food handler will, if it is an enterotoxigenic strain and given the right conditions of warmth, moisture, pH, and time, produce toxin. Growth of staphylococci and production of toxin are greatest at approximately 20–37 °C, but growth can occur between 8 and 48 °C. This toxin is heat stable, being tolerant to boiling for 1 h. Canning under pressure at 121 °C for 30 min is sufficient to destroy the toxin. The toxin is also resistant to gamma irradiation.

Many foods have been associated with SFP. *S. aureus* can grow in foods with high salt or sugar content, and salted meat products such as ham have been a common vehicle of SFP, as have desserts, especially those containing cream. Other foods implicated in SFP in addition to meats include other high-protein foods, salads, canned mushrooms, cream, cheese, salami, and eggs. *S. aureus* can also contaminate milk by mastitic cows infected with *S. aureus*.

Characteristic Sequence of Events

A whole leg of ham is prepared for consumption and cooked. It is then sliced warm by a chef who has no skin lesions but is a nasal-carrier of *S. aureus*. The slices are stacked on a tray. The tray is covered and left to cool for several hours before being refrigerated. Staphylococci from the nose of the chef are conveyed to the warm ham slices. Because of the large surface area, the large bulk of covered overlapping slices of meat require several hours to cool, during which staphylococci grow and produce toxin. Refrigerating, freezing, or reheating the meat will not destroy toxin. Holding cooked food, subsequently contaminated with *S. aureus* by a food handler, at

ambient temperature for several hours is the major contributing factor to outbreaks of SFP.

Clinical Features

Staphylococcal toxin is an enterotoxin and a receptor in the gut appears to be necessary. There may also be a neurotoxic effect that acts on the vomiting center in the brain. With SFP, onset of symptoms is often dramatic. Vomiting is the most prominent symptom, generally occurring between 2 and 4 h, but may range from 30 min to 8 h, after eating. Nausea, abdominal cramps, and diarrhea are also common. Generally, as with most toxins, the higher the toxin concentration (or the greater the amount ingested), the shorter the incubation period and the more severe the symptoms. Individual susceptibility is also a determining factor in severity. The illness usually completes its course within a day or two, but deaths have occurred, sometimes as a result of acute hypotension (another well-known but rare effect of the toxin).

Diagnosis

Many people carry in their throat or nostrils staphylococci asymptotically, hence in order to identify a food handler as the source of an SFP outbreak, it is important to confirm that the type of *S. aureus* causing an outbreak of FI is the same in the carrier and in those affected; merely showing that a food handler carries staphylococci is insufficient. The organism can be grown from, or enterotoxin can be detected in, implicated foods. Generally, *S. aureus* must grow to populations of $> 10^6$ colony-forming units per gram to produce sufficient amounts of enterotoxin to produce the illness. *S. aureus* can also be isolated from vomit or stool of patients and from the hands, nose, abscess, or an infected wound of the food handler. Phage typing of strains, with detection and typing of enterotoxin, can also be performed. Enterotoxins A–K (but not F) are recognized, although A is the most common. With the advent of polymerase chain reaction detection technology, enterotoxin types other than A are being associated with illness more frequently. As with all FI, the absence of laboratory-supporting evidence does not necessarily mean that the diagnosis is wrong or the implicated food innocent.

Bacillus cereus

Background

Bacillus cereus is widely distributed in the environment and can occur in food. It is found in rice and other natural foods, such as herbs and spices, cream, and dry foods.

Survival and Growth

Unlike *S. aureus*, *B. cereus* is a sporeforming bacterium that can survive prolonged boiling. It causes two fairly distinct types of food poisoning, emetic and diarrheic. The diarrheal toxin is heat labile and, like *Clostridium perfringens* food poisoning, this toxin is released in the gut. The foods commonly associated with diarrheagenic *B. cereus* FI are 'proteinaceous' and, like *C. perfringens* FI, associated with meats, stews, desserts, and sauces. The emetic type is 'farinaceous', associated mainly with cooked rice, and produces an illness similar to SFP. Different serotypes of *B. cereus* cause these two different forms of

FI, and the toxins are also different. Some strains can grow at refrigeration temperature in milk and other foods.

Clinical Features and Characteristic Sequence of Events

The emetic type of *B. cereus* FI is caused by preformed toxin (cereulide) in food, usually rice that has cooled slowly. This typically happens when a large amount of rice, as occurs in some Chinese restaurants, is allowed to cool at room temperature for many hours, often overnight. The center of the mass will remain warm for a long enough period for the spores to germinate and form toxin. The toxin is heat stable and will not be inactivated by the quick frying rice typically received in a restaurant. The incubation period is usually short (1–6 h), and the symptoms, predominantly vomiting, tend to be milder than those of SFP, which it otherwise resembles.

The diarrheal form of *B. cereus* FI is similar to that caused by *C. perfringens*. The toxin, unlike the emetic type, is an enterotoxin released in the intestine and is heat labile. The predominant symptoms are watery diarrhea and abdominal cramps. The incubation period, is also longer (6–15 h), and persists for approximately 24 h. A wide variety of foods, including meat, vegetables, and dairy products, have been associated with this type of *B. cereus* FI.

Diagnosis

The mere presence of *B. cereus* in a food is insufficient evidence to confirm the food as the vehicle of FI because *B. cereus* is a normal contaminant of many natural foods. The diagnosis is confirmed by finding *B. cereus* in high concentrations [10^6 – 10^8 g⁻¹, minimum 10^5] in cooked rice, or other foods for the diarrheic type, and obtaining it from the stool or vomit of those who are ill. Alternatively, the same serotype of *B. cereus* should be present in the implicated food and patient specimens. Detection of the emetic toxin in the food may also be sufficient.

Clostridium botulinum

Background

Clostridium botulinum is an anaerobic sporeforming bacterium widely distributed in soil and mud. Botulinum toxin is the most lethal substance known to man, with an LD₅₀ of 0.000 03 µg kg⁻¹ body weight. In one incident, an adult was paralyzed for more than 6 months after eating less than two teaspoonfuls of a contaminated rice salad. For comparison, tetanus toxin from *Clostridium tetani* and ricin from the castor bean (the next most toxic substances) have LD₅₀ values of 0.000 1 and 0.02 µg kg⁻¹, respectively. The seven toxin types, A–G, affect the nervous systems of vertebrate animals, birds, and man. Birds in aquatic environments are especially susceptible to mass die-offs caused by botulism. Invertebrates are not susceptible but can harbor the bacteria and toxins in their bodies. Botulinum toxin types A, B, and E most frequently affect man. Type E is typically acquired from fish. Type C is the primary toxin causing botulism in birds, although types D and E are also important.

Survival and Growth

C. botulinum is an anaerobe, hence special conditions (no oxygen) must be present for it to produce toxin. Although the organism is widespread in the environment, botulism in humans is uncommon. First, the spores must be present, which, depending on the type of food, occurs because they are widely distributed in soil and aquatic environments. Second, the spores must survive cooking, which often occurs because they can survive heating at 100 °C for 2 h. Third, they must germinate and grow in anaerobic conditions. Although accidents have occurred, and occasionally still occur, especially with home canned foods and occasionally with preserved meat (the term botulism is derived from *botulus*, the Latin term for sausage) and preserved rotting or fermenting food, botulism is usually rare among humans. Commercial canning, except for the occasional process deviation, destroys spores by the heating processes used. The vegetative forms of *C. botulinum* are as susceptible to heat as most other vegetative bacteria, and the toxin can be destroyed by boiling for 5 min. The pH of food is also important: the lower the pH, the less resistant the spores are to heat, and a low pH (<4.6) prevents vegetative cells from growing and producing toxin. Hence, bottled vegetables pickled in vinegar tend to be safe. High concentrations of salt also affect the viability and toxin-forming properties of *C. botulinum*.

Clinical Features

The incubation period of botulism is 18–36 h (range, 4 h–8 days). The toxin destroys the cholinergic nerves in the motor end plates (MEPs) of cells. These are the junctions of the nerves within muscle, preventing the release of acetylcholine from the cholinergic nerves in the MEP and paralyzing the muscle. Once this has happened, antitoxin, which is used to treat patients with botulism is ineffective. The combination of nausea, vomiting, or diarrhea followed by symmetrical descending paralysis of cranial and autonomic nerves is almost diagnostic. The characteristic neurological symptoms are blurred vision, dry mouth, difficulty in swallowing, dysarthria, diplopia, and descending paralysis. Recovery occurs when new MEPs form. The fatality rate was once high, but with respirators patients are often kept alive artificially until new nerve terminals have formed new MEPs, which may take several months.

Infant Botulism

Some babies, usually younger than 6 months of age, acquire a form of botulism that is caused by ingested *C. botulinum* spores colonizing the baby's intestine and multiplying, and subsequently forming toxin. The initial symptom is typically constipation, leading to poor appetite, irritability, neck paralysis, and generalized weakness. Honey is a primary vehicle of infant botulism.

Diagnosis

The diagnosis is made by demonstration of botulinum toxin in food, stool, or serum. Growing the organism from food is suggestive but not diagnostic, whereas fecal isolates are uncommon except in affected individuals.

Clostridium perfringens

Background

Food poisoning caused by *C. perfringens* is also toxin mediated. It is similar to the diarrheal form of *B. cereus* FI in that toxin is formed in the intestine after ingestion of the bacteria. Like other clostridia, it is anaerobic (but can grow in the presence of low concentrations of oxygen), Gram-positive, and sporeforming. There are five types, classified A–E according to the enterotoxin formed; type A is the one that causes FI. Some strains, but not generally those that cause FI, can cause gas gangrene. *Clostridium perfringens* is primarily found in soil and is transmitted to animals and man by ingestion of vegetables and other plants. It is thus commonly found in the intestine of man and animals. When animals are eviscerated, the organism contaminates the inside of the carcass. Flies can transmit the organism to food.

Clostridium perfringens FI is frequently reported in some countries such as the UK; however, it is rarely fatal except for those who are debilitated or immunocompromised.

Survival and Growth

Clostridium perfringens does not typically multiply on the surface of raw meat. It grows optimally at a warm temperature of approximately 43 °C (range, 10–54 °C) and where there is little to no oxygen in the interior of a cooked dish. The cooking process can remove oxygen and thereby facilitate germination and subsequent growth of the organism. Vegetative cells are not resistant to heat, but spores of the FI strains of *C. perfringens* can survive boiling conditions. If cooling is slow, vegetative cells form and can grow rapidly. After ingestion, toxin is formed from multiplying cells in the intestine, although both toxin and vegetative cells appear to be necessary to produce symptoms.

Clinical Features and Characteristic Sequence of Events

A casserole is prepared containing, among other ingredients, meat pieces. It is cooked for 1 or 2 h until ready. However, it may not be consumed immediately, and because of its bulk and the lack of refrigeration facilities it is left unrefrigerated overnight in a warm kitchen. It is warmed the next day before serving. Symptoms of diarrhea with intense abdominal pain typically begin 8–24 h after exposure. The illness may last for up to 24 h, and there are no sequelae, except in those who are already debilitated, in which less severe symptoms can last for 1–2 weeks.

Diagnosis

The organism can be cultured from the stools of affected people and should be compared by molecular subtyping and for toxin production with isolates from food. Enterotoxin detection in stools is important confirmatory evidence. The organism has to be detected in high numbers ($> 10^5 \text{ g}^{-1}$) in food to be significant.

Vibrio cholerae

Background

Cholera originated in India and spread to Asia in 1817–1823, the first pandemic. The second pandemic reached Europe in

1826–1837, and subsequent to this there were five additional pandemics. The most recent began inexplicably in 1961 with a mild strain, the El Tor biotype, which had been endemic in Indonesia since 1937. More recently, cholera has become endemic in areas of South America. *Vibrio cholerae* O139 is a relatively new strain that emerged in the Indian subcontinent in 1992.

Cholera was responsible for the introduction of sanitation and the development of ‘public health’. Although not a common cause of FI or GE in developed countries, the vibrios, especially *V. cholerae*, still cause large, mainly waterborne outbreaks in the developing world. It is the only gastrointestinal infection that is internationally notifiable. Because large numbers of organisms are required for infection, person-to-person transmission is uncommon.

Survival and Growth

The bacteria are aquatic and prefer briny waters. They can be found in many warm plankton-rich coastal waters, including the Mediterranean, Gulf of Mexico, and those of Southeast Asia and South America. Bivalved mollusks concentrate them, and other fish and shellfish can also be contaminated. Inadequate cooking and unrefrigerated storage will allow *V. cholerae* to survive and grow to sufficient cell numbers to cause FI. Vibrios are generally associated with moist, slightly salty foods. The El Tor strain is more likely to produce asymptomatic infections, persist longer in the environment, multiply more rapidly in food, and produce less immunity than the classical type. The organism causes illness by producing an enterotoxin in the intestine.

Clinical Features

Cholera, in its most severe form, is characterized by an acute outpouring of watery diarrhea (rice water stools) and vomiting resulting in death within 24 h by acute loss of fluid and electrolytes. However, the clinical syndrome can also be mild. *V. cholerae* is not invasive, and if the loss of fluid and salts can be counterbalanced by infusion of equal amounts of fluid supplemented by electrolytes, the patient will survive. Patients with an absence of acid in the stomach, and those with blood group O, are especially prone to severe symptoms. The incubation period is typically 1–3 days (range, 12 h–5 days).

Characteristic Sequence of Events

Seafood or water contaminated with human sewage is by far the most common vehicle of infection. Vibrios can grow prolifically in cooked rice and other grains contaminated by food handlers, and salad vegetables can be contaminated by water.

Diagnosis

V. cholerae is usually isolated from the stool using special media. It can also be distinguished by light microscopy, and specific antisera will halt motility of the organisms. Agglutination tests with antiserum will distinguish O1 from O139 and other serovars. The bacterium can also be isolated from the environment using enrichment media. Toxin production or the presence of the toxin gene can, and should, also be demonstrated.

Invasive Bacteria

Salmonella Infections

Background

Salmonellae are among the most common known causes of bacterial FI in developed (and possibly less well-developed) countries of the world.

There are more than 2500 serotypes of *Salmonella*. They are typed according to their somatic [O] or flagellar [H phases 1 and 2] antigens according to the Kauffman–White scheme and are sometimes named after a geographical location. Further typing or molecular subtyping such as by pulsed-field gel electrophoresis (PFGE) can be done to distinguish strains of the more common serotypes. All serotypes are considered pathogenic for humans, although most have not been detected in infected humans. Salmonellae are Gram-negative bacilli that do not form spores but can survive for remarkably long periods (months or years) in dried foods such as nonfat dried milk.

Salmonellae are frequently carried in the intestinal tract and excreted in the feces of a variety of animals, hence environmental contamination often occurs through contact with manure. *Salmonella*-contaminated protein-based feeds processed in bulk for livestock and poultry have caused widespread infection in animals and subsequently in humans. In the UK, for example, fishmeal imported from Peru and fed to poultry caused a large outbreak of *Salmonella* Agona infection in humans that lasted for many years, through the late 1960s and early 1970s. Since then, there have been outbreaks of *S. Hadar* infection in turkeys. More recently infections in poultry and hens' eggs by *S. Enteritidis* of various phage types have caused salmonellosis outbreaks in many countries. In eggs, transmission of *S. Enteritidis* is mainly 'vertical' (i.e., through oviducts to the interior of eggs laid by infected hens). Before this, salmonellae largely gained entry to the interior of eggs through the shell. If egg shells were removed in bulk, contamination of just one or two shells would be enough to contaminate the entire batch of eggs and then salmonellae would grow under favorable temperature conditions.

Other important sources of *Salmonella* contamination include sewage, manure, polluted water, and direct fecal contamination of foodstuffs. Hence, many fresh, unprocessed foods are bought already contaminated. Examples include raw meat and poultry, unpasteurized milk and eggs, legume and vegetable sprouts, and fresh produce. Many multistate outbreaks of salmonellosis in the United States have been traced to tomatoes, cantaloupes, bagged spinach, and fresh-cut lettuce. Cross-contamination in a kitchen or restaurant from raw meat or poultry has also been responsible for numerous outbreaks. Direct contamination of a food by a food handler can also occur. Although cases of human carriers with prolonged fecal shedding of *Salmonella* spp. occur, they are infrequent.

Survival and Growth

Although salmonellae do not form spores, and are fairly easily destroyed by heat (71 °C in a moist food), they can survive for a remarkably long period of time (years) in a dry environment. An outbreak of *S. Virchow* and *S. Saintpaul* infection associated with green lentils (mung beans) imported from

Queensland occurred in several countries of Europe. The lentils were used to produce bean sprouts, which were grown overnight in a warm waterbath. Drying of salmonellae makes them more resistant to heat. The presence of moisture is important when using heat to kill salmonellae.

Salmonellae grow best at 37 °C, with the danger zone at 30–45 °C. Generally, growth does not occur below 7 °C and above 46 °C. Antibiotic-resistant strains are becoming an increasing concern, especially those strains that multiply are resistant to antibiotics used for human therapy.

The infective dose of salmonellae in humans can be quite low, e.g., 1 cell g⁻¹, depending on a variety of factors, including the strain of *Salmonella*, type of food vehicle, and immune status of the person. Fatty foods such as chocolate, cheese, salami, peanut butter, and mayonnaise generally can confer illness with much smaller doses, and patients with immunosuppression, low acid levels in their stomach (achlorhydria), as well as the elderly and debilitated may also be particularly vulnerable. Salmonellae can be transmitted nosocomially, especially in geriatric or psychogeriatric wards of hospitals, and in such outbreaks food may not be the source.

Characteristic Sequence of Events

Examples of scenarios of outbreaks of salmonellosis are the following.

A chicken dish is undercooked and then left in a warm environment for many hours before consumption. Alternatively, the chicken may be thoroughly cooked but is then placed in an unwashed container or on a plate with juices from the uncooked chicken, or cut with a knife that was used for raw chicken, and then held at room temperature for a few hours. The contaminated utensil may also be used on another food such as lettuce, thus contaminating it with salmonellae.

A *Salmonella*-contaminated dried herb or spice, such as black pepper, is added to a casserole after cooking and some cooling but while still warm, and the food is then held at room temperature.

Raw egg is added to a product without cooking, such as with ice cream, egg nog, or mousse, or only lightly cooked. When the light cooking involves several hundred eggs, one contaminated egg and holding at a warm room temperature are enough to create a highly infective dose. In one outbreak, 800 eggs were used and left at room temperature for several hours before being lightly cooked to make a hollandaise sauce. More than 100 guests at a wedding at which it was served became ill.

Clinical Features

The incubation period of salmonellosis is generally 12–36 h, but can range from 6 to 72 h. Clinical features of salmonellosis can range from mild to severe. Enteric fever (typhoid) is usually caused by *S. Typhi* or *S. Paratyphi* A. Salmonellae can cause severe diarrhea with fever and abdominal pain. Additional, less common symptoms include chills, headache, vomiting, and nausea. Symptoms usually resolve within 4 to 7 days. Some *Salmonella* spp. such as *S. Cholerae-suis*, may cause multiple abscesses, and people with sickle cell disease may develop bone abscesses caused by a variety of *Salmonella* spp. Septicemia (blood poisoning), meningitis, Reiter's Syndrome,

and some localized infections are also occasional complications of salmonellosis. Patients with AIDS and other immunosuppressive conditions are particularly vulnerable to severe complications, which can lead to death.

Salmonellae and Hens' Eggs

In the late 1980s, *S. Enteritidis* rapidly became the most common cause of human salmonellosis in the United Kingdom. Previously, *S. Typhimurium* had been the most frequently reported *Salmonella* species associated with human illnesses. Between 1984 and 1987, the number of human *S. Enteritidis* infections increased by approximately 50% per year. In 1988, the number more than doubled and by 1993 it was virtually 10 times that diagnosed in 1984. By 1993, *S. Enteritidis* accounted for approximately five times the number of *S. Typhimurium* infections. Most of this was due to consumption of undercooked contaminated eggs, although some cases were also attributed to chicken. Many European countries and the USA experienced similar trends.

Diagnosis

The diagnosis of a salmonellosis is usually made by isolating salmonellae from stool or food. Some salmonellae, such as *S. Typhimurium* and *S. Enteritidis*, are so common that further differentiation is necessary for epidemiologic purposes. Further characterization of the *Salmonella* serovar can be determined by molecular subtyping (PFGE) or phage typing.

Campylobacter Infections

Background

Campylobacter jejuni was determined to be a cause of human GE in the mid-1970s, and is now recognized as the most common bacterial cause of GE and FI in many developed countries. In less developed countries, asymptomatic *Campylobacter* infection is more common. *Campylobacter jejuni* is the most common species causing diarrhea, but *C. coli* is also common in some areas.

Campylobacter spp. are carried in the intestinal tract of many animals and birds, including cattle and horses, household pets, and chickens. Rates of contamination of chicken carcasses vary from >75% in the United Kingdom to <30% in Sweden and Norway. Some of these differences may be due to the isolation or detection method used. The estimated annual incidence of human campylobacter enteritis in the USA is ca. 2 million cases.

Survival and Growth

The reason for the late recognition of campylobacters as causes of human GE is the fastidious growth conditions required to culture and isolate them. They grow best in an O₂ concentration of 5% (but not well, if at all, anaerobically or in the presence of 21% O₂) in a special medium and at a temperature of 42 °C. They are sensitive to heat, being destroyed readily by cooking, and do not survive for long (probably a few hours only) when dried on the surfaces of foods or kitchen utensils. They nevertheless are highly successful in causing infection, probably because of their ubiquity in food-producing animals and birds and the relatively small dose needed for infection

(possibly no more than a few thousand organisms may be enough).

Characteristic Sequence of Events and Clinical Features

Although campylobacters undoubtedly cause FI, the vehicle of infection in most instances is unknown. It is probable that many cases are caused by direct contact with animals, birds, the environment in which fecal contamination occurs (both domestic and outside), meat carcasses, and possibly other people. Foodborne outbreaks have been traced to untreated water and unpasteurized milk, and also milk from bottles whose tops have been pecked by birds. Undercooked poultry is a major risk factor, and meat prepared at barbecues, which includes pork, veal, and beef as well as chicken, has also been implicated as a vehicle of infection. Eating grapes was determined in one study to be a risk factor, and salads and fresh vegetables have also been implicated as vehicles, but it is possible that some of these foods were contaminated by another source or directly by a food handler.

Other risk factors include travel to foreign countries; handling and cooking of food, especially raw meat; contact with animals and pets (especially those with diarrhea); and visiting an animal farm.

The incubation period is generally 2–5 days. Symptoms are mostly associated with the lower GI tract hence vomiting is uncommon, with abdominal pain and diarrhea being the main symptoms. An accompanying fever, abdominal pain, and headache are usual, and the diarrhea can be bloody. The illness may last a few days, and the antibiotic ciprofloxacin is the treatment of choice for severe or prolonged illnesses.

Septicemia or other localized infections are rare complications. One of the well-known complications of campylobacter infection is Guillain-Barré syndrome, in which a symmetrical paralysis affects the body some weeks after the infection. Recovery is usually spontaneous but may take several months. In the acute phases of the illness, respiratory support may be needed. Reactive arthritis is also a complication, although infrequent, of campylobacter infections.

Diagnosis

The organism can be cultured from stools, rectal swabs, and food. Special media and an atmosphere of 3 to 5% O₂ are needed for culturing *Campylobacter*.

Escherichia coli

Background

Escherichia coli are a remarkable group of bacteria causing a wide range of infections, including gastroenteritis, meningitis, septicemia, and urinary tract infections. Many strains are nonpathogenic. Those that cause GE have a wide range of pathogenic mechanisms and are divided into various fairly distinct groups: enteropathogenic (EPEC), enterohemorrhagic (EHEC), enteroinvasive (EIEC), and enterotoxigenic (ETEC) are the main ones, although some groups – diffusely adherent (DAEC) and enteroaggregative (EAEC) – have been described. EHEC strains, which include *E. coli* O157:H7, produce one or two primary Shiga toxins and possess specific adherence factors. However, because the O157 strains are much more

common than the other EHEC strains that cause FI, EHEC strains are classified as O157 and non-O157. Shiga toxin is produced by other bacteria also, including *S. dysenteriae* type 1. Only these EHEC Shiga toxin-producing strains are considered in detail here because they are commonly foodborne and can cause serious illness and death.

EPEC is not identified routinely by stool culture methods commonly used. Infections with EPEC strains are common in developing countries worldwide at all ages. These strains are most commonly known to cause travellers' diarrhea, but can also be spread by food. This toxigenic group includes strains that produce heat-labile and heat-stable enterotoxins. Heat-labile enterotoxin is closely related to cholera toxin and causes profuse watery diarrhea. EPEC strains largely cause infections in neonates and infants, which tend to spread from person-to-person, and are not commonly associated with FI. EIEC and the two newer strains are rare. EIEC outbreaks related to food have been occasionally described, including one associated with French cheese exported to the United States.

In the United States, *E. coli* O157:H7 is estimated to cause 96 000 cases and 31 deaths annually, with 68% of cases being foodborne. Swimming in contaminated water can also transmit the infection. *Escherichia coli* O157:H7 was first recognized as a cause of FI in 1982. Some strains with the Shiga toxin gene produce a toxin that causes the hemolytic-uremic syndrome (HUS). Many serovars of *E. coli* that can produce Shiga toxin have not been associated with human illness.

Escherichia coli, including *E. coli* O157:H7, is an asymptomatic inhabitant of the intestines of many ruminants, including cattle, sheep, and goats. Contamination can occur directly from the intestinal content to carcass to meat or via the feces of these animals to raw vegetables and other foods.

Survival and Growth

E. coli O157:H7 can survive for days to weeks on contaminated meat and vegetables. The organism is more resistant to acid than *Salmonella*. *E. coli* O157:H7 can survive for 21 days in cider at a pH of 3.7–3.9 at 4 °C, with only approximately a 5% kill-off. It can grow successfully over several weeks in manure slurries. The infectious dose is thought to be very small (less than 100 cells), so person-to-person transmission may occur. Like most vegetative bacteria, it is destroyed by heat (71 °C in moist foods).

Characteristic Sequence of Events

In a town in North Cumbria, England, 61 patients had diarrhea, many with blood, over 3 weeks. A total of 114 people were infected, ranging in age from 3 months to 85 years. Investigations implicated a farm supplying pasteurized milk. Nine days before the first case, a problem had occurred in the heat-exchanger plates of the pasteurization unit. No tests were undertaken after new plates were fitted, and temperature monitoring was inadequate. The unit was one that a few months before had been the subject of a food hazard warning. *Escherichia coli* O157 was isolated from 66 environmental and animal feces samples on the farm but not from the milk or the pasteurization plant.

In an outbreak in the United States, more than 700 became ill after eating inadequately heated hamburgers from a

restaurant chain. More than 30 cases developed HUS and four died.

Undercooked hamburgers and ground beef are a common vehicle of *E. coli* O157:H7 infection. The process of grinding beef can spread the bacteria from the surface of the meat to the inside. Other vehicles of infection include raw milk, unchlorinated water, apple juice, unwashed fruits and vegetables including alfalfa sprouts and bagged spinach and lettuce, or swimming in unchlorinated pools.

Clinical Features

The infectious dose for *E. coli* O157:H7 is thought to be fewer than 100 bacteria. The typical incubation period is 2–5 days (ranging from 2 to 8 days). Symptoms are mostly associated with the lower GI tract. Severe bloody diarrheal and abdominal cramps are the most common symptoms, but nonbloody diarrhea also occurs. Fever is unusual. The illness may last a few days, and may progress to hemolytic-uremic syndrome (HUS) which is characterized by hemolytic anemia and renal failure, and occurs in approximately 5% of reported *E. coli* O157:H7 cases, most frequently in young children and the elderly.

Diagnosis

The usual method of diagnosis is to isolate the bacteria from stools or food. However, because most of the *E. coli* in the intestine is part of the normal flora and nonpathogenic, it is necessary to demonstrate virulence by further tests or assigning it to a serotype, which normally requires more sophisticated techniques in specialized laboratories. Serotyping is performed on the somatic cell wall antigens (O antigen) and the flagellar antigen (H). On the basis of serotyping the O and H antigens and detecting known virulence factors or virulence genes such as toxins, the organisms can be classified as EHEC, EPEC, ETEC, etc. Molecular-based tests are increasingly being used. Toxins can be tested for using immunoassays or molecular tests based on gene sequences. Serology tests of blood from infected humans are also used, but they are not reliable indicators of recent infection.

Other Organisms

Shigella Species

Humans and primates are the known reservoirs of *Shigella*, hence this bacterium is often spread by person-to-person, especially among kindergarten and primary school children. Affected patients may excrete shigellae for weeks. Many large outbreaks of shigellosis are associated with consumption of food contaminated by sewage-polluted water or food handlers. In 1995, an extensive *S. sonnei* outbreak associated with lettuce imported from Spain affected people in many countries in northern Europe. In another outbreak associated with shrimp consumption, infection was transmitted by a food handler who mixed the shrimp by hand with mayonnaise and tomato sauce. The incubation period is typically 2–4 days (ranging from 12 h to 7 days) and although large-volume bloody or mucoid diarrhea and high-grade fever are the usual symptoms, it is characteristically accompanied by tenesmus – a feeling of wanting to defecate without being able to do so.

Listeria monocytogenes

Listeriosis, caused by the bacterium *Listeria monocytogenes*, is an unpleasant and rare infection that typically affects the more vulnerable, such as fetuses, infants, pregnant women, the elderly, and the immunocompromised. It causes septicemia and meningitis, which is unusual for a FI bacterium. Fatality rates for invasive disease are high, as many as one in four. GI symptoms may be absent or mild. Also unusual, this pathogen can grow (albeit slowly) at normal refrigeration temperatures (0–4 °C). It is also very resistant in the environment, both to cold and to heat, so that it can survive for long periods of time (months to years), especially at refrigeration temperature. The incubation period for invasive listeriosis tends to be long (up to 3 weeks), but for GI symptoms very short periods of 1 day have been recorded. Deli meats (especially poultry-based), certain soft cheeses, and pâtés have been associated with large outbreaks of listeriosis.

Yersinia enterocolitica

Like *L. monocytogenes*, *Y. enterocolitica* can grow at refrigeration temperature (4 °C). It is often missed in the laboratory because it requires special media and generally grows best at 25–30 °C. Many strains are nonpathogenic, although those of serotype O 3, 8, or 9 are most commonly associated with illness. The bacterium is largely associated with swine, especially the oral-nasal cavities. Outbreaks have been associated with raw milk and dairy products, as well as undercooked pork and tofu. In one incident, a caregiver who handled swine intestines passed the infection on to some infants. These infections appear to be more common in Scandinavia than elsewhere. The incubation period is typically 4–7 days, and symptoms of diarrhea often bloody, abdominal pain, and fever, may last from 1 to 3 weeks or longer. In older children and adults severe right-sided abdominal pain may be confused with appendicitis. Infants and young children are most often affected. Clinical features are characteristically fever and profuse watery diarrhea, but may mimic acute appendicitis, resulting in an unnecessary operation. Occasionally, in vulnerable patients, septicemia may occur.

Vibrio parahaemolyticus

Like *V. cholerae*, *V. parahaemolyticus* is an aquatic bacterium that thrives in shallow coastal waters. Deep-sea fish do not tend to harbor the organism and usually become contaminated in fish markets. Precooked frozen shrimp may be contaminated and transmit FI if served without further cooking, as in a seafood cocktail. *Vibrio parahaemolyticus* FI is associated with raw, undercooked, or cross-contaminated seafood and is especially common in Japan and probably other countries in which seafood is a staple of the diet. Cross-contamination from raw to cooked seafood is a common mode of transmission. The incidence of *V. parahaemolyticus* FI has increased in many Asian countries and the United States since 1996, and this is thought to be caused by a pandemic clone. Diarrhea, abdominal pain, and nausea are the predominant symptoms. The diarrhea can be severe, with blood or mucus in the stool. Vomiting is a less common feature, but fever can occur. The incubation period

ranges from 4 h to 4 days, but most cases occur between 12 and 24 h. Death is uncommon.

The diagnosis is made by culture of the bacterium from feces or food. *Vibrio parahaemolyticus* can be easily isolated from most aquatic environments, but such strains are predominantly Kanagawa negative. Only the Kanagawa-positive strains (i.e., those producing a thermostable hemolysin that can be confirmed in a laboratory) cause GE, and it is thought that they multiply selectively in the human intestine. The infectious dose is reportedly 10^5 to 10^7 cells.

Vibrio vulnificus

Like with the other vibrios, infection with this bacterium is acquired from seafood, largely from consumption of contaminated raw oysters. It can cause a fulminating septicemia by ingress through a skin lesion in the food handler and in people with chronic liver disease through consumption of raw seafood. Gastroenteritis can also occur.

Brucellosis

Although not usually considered as a FI, brucellosis deserves mention because it is associated with food consumption. *Brucella melitensis*, in particular, is largely foodborne, and cheese, milk, and other dairy products made from unpasteurized milk are primary vehicles. Occasionally, contaminated meats may be responsible. *Brucella abortus* is associated with cattle and bovine products, *B. melitensis* with goats, and *B. suis* with swine. Brucellosis is a serious and prolonged systemic illness, with fever, night sweats, headache, aches and pains, and, sometimes, profound depression. Many developed countries have eradicated *Brucella* from livestock.

Streptococcal Pharyngitis

Notwithstanding the definition of FI as causing GE, streptococcal sore throat with fever has been well documented to spread via foods. Usually, a food handler has a *Streptococcus* group A infection in his or her throat, which may be asymptomatic, and transfers this to a food that is then left in a warm environment for several hours before consumption. Foods that have been implicated in illnesses include cheese, milk, eggs, and meat. The incubation period is 24–48 h. To confirm the vehicle, typing of strains is important, as is sound epidemiologic evidence, because many people carry these streptococci in their throats.

Prevention of Bacterial Food Poisoning

With the increasing trend toward manufacturing of foods in large quantities for distribution not only nationally but also internationally, the potential for large outbreaks of foodborne disease is considerable. Outbreaks of salmonellosis and *E. coli* O157:H7 FI associated with cheese, salami, chocolate, peanut butter, beef jerky, dried infant formula, ground beef, and even raw cookie dough have all been documented. In one outbreak of *E. coli* O157:H7 infection, 34 lots of 281 000 lb of beef

patties were manufactured in one plant, and 7 of 21 lots tested were contaminated. The introduction of hazard analysis and critical control point (HACCP) systems in food manufacturing processes has been a significant advance in the production of safer food and the prevention of FI. Microbiological criteria now exist for ready-to-eat foods. The establishment of PulseNet in the USA and similar outbreak-identification surveillance systems elsewhere is an important tool in the early detection of foodborne outbreaks and the curtailment of their effects. This is a surveillance system that accumulates and shares molecular subtyping ('fingerprinting') information on microbes causing FI. For example, most outbreaks of FI recently detected in the USA are widespread in scope, with cases occurring in many states. The most common problems in the preparation of food are inadequate cooking, leaving prepared food too long at too high a temperature, and allowing cross-contamination from raw to cooked food.

Salads and other vegetables or fruits eaten raw may be contaminated, and outbreaks have been associated with lettuce (*S. sonnei*, *Salmonella*, and *E. coli* O157:H7), spinach (*E. coli* O157:H7), cantaloupes (*Salmonella* Poona), tomatoes (*Salmonella* Newport), peppers (*Salmonella* Saintpaul), raspberries and strawberries (*Cyclospora cayentanensis* and hepatitis A), alfalfa sprouts (*S. Enteritidis*), and radish sprouts grown hydroponically (*E. coli* O157:H7). Some of these foods were contaminated at the source by polluted water or sewage, others during harvesting or processing by infected food handlers (norovirus and hepatitis A), and others by food handlers during preparation. It is difficult to avoid or prevent such infections in the kitchen short of cooking everything, and more stringent codes for hygiene at the growing farms and processing plants are required.

In the kitchen, it is important to keep raw, such as beef, and ready-to-eat foods entirely separate. Salads and fruit are in the ready-to-eat category. Raw meats, especially, should be handled with separate utensils, surfaces, and cutting boards than for cooked food, unless the utensils and cutting boards are washed thoroughly in very hot water and detergent or a dishwasher and then left to dry. Otherwise, many FI microbes can transfer from raw to cooked food and grow.

Cooking food, especially meat, thoroughly will kill vegetative microbes, including salmonellae, although bacterial spores can survive. If the cooling down period is too long – normally approximately 2 h is considered the limit before refrigeration is necessary – *C. perfringens* or *B. cereus* that have survived as spores will grow. So will salmonellae and many other FI bacteria if the food was inadequately cooked. Cooking will not normally destroy preformed toxins of *S. aureus*. Infected food handlers may also cause outbreaks of FI by contaminating food during preparation. Generally, *B. cereus* and *C. perfringens* originate in the food. Infected food handlers whose hands have been contaminated with their feces are the usual source of *Shigella*, hepatitis A, or norovirus in outbreaks of FI.

Moist food should be held either hot (above approximately 60 °C) or cold (below 8 °C, preferably 4 °C). Cooling food, even freezing it, will not destroy foodborne bacterial pathogens. Undercooked chicken that has been refrigerated will still need thorough cooking before it is safe to eat. When in large amounts, frozen meat or poultry should be thawed before cooking. Large frozen turkeys may need several days in a refrigerator to thaw fully. The inside of the meat is the last to thaw and the last to cook.

Grinding meat will disperse organisms through it. Hence, hamburgers and sausages need thorough cooking.

Drinking raw milk is hazardous: A large variety of bacterial pathogens, from *E. coli* O157:H7 to *Salmonella*, can be spread in this way. Hens' eggs have been the vehicle of many cases of salmonellosis FI (mainly *S. Enteritidis*), especially since the 1980s throughout much of Europe and the United States. The rate of contamination is typically low, such as 1 per 20 000 eggs, but the number of cases has been large because of the popularity of eggs as a food and because it is common practice to eat them less than fully cooked, not only on their own but also in other dishes such as sauces and mousse. Screening and vaccination of flocks in recent years have reduced the risk of contamination. Irradiation of food is effective in mitigating foodborne pathogen contamination, but is not popular with the public.

Education of food handlers may be straightforward, but, especially in countries in which food handlers have low status and pay, compliance is more difficult. Education of the general public has been slow but has progressed, e.g., most people now realize the importance of thawing poultry and meat thoroughly before cooking, and the large outbreaks of salmonellosis FI that used to occur at Christmas time in England and Wales caused by inadequately defrosted and cooked turkeys are now uncommon.

See also: Eggs. Fish and Seafood: Nutritional Value. Food Safety: Mycotoxins – Occurrence and Toxic Effects. Meat, Poultry and Meat Products: Nutritional Value

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Heavy Metals

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Glossary

Ataxia Lack of coordination of muscle movements.

Choreoathetosis Involuntary leg and arm movements.

Encephalopathy Disorder or disease of the brain.

Paresthesia Sensation of prickling, tingling, or numbness of skin.

Lead

How Does Lead Contaminate Food?

Although lead is primarily known as an environmental contaminant that is ingested in paint chips by young children in urban slums, or from contaminated soil or inhaled in the form of house dust or automobile exhaust, it may also enter the food and water supply. Ways in which this can occur include fuel exhaust emissions from automobiles that may contaminate crops and be retained by them, especially green leafy vegetables. Animals used for food may graze on contaminated crops and thus may also be a potential source of lead. Moreover, lead from soldered water pipes may contaminate tap water used for drink or for food production.

Permissible Intakes

In the United States, the maximum quantity of lead in the water supply that is permitted by the Environmental Protection Agency is 15 mg l^{-1} (15 ppb or $0.07 \text{ } \mu\text{mol l}^{-1}$). The Food and Drug Administration (FDA) advisory panel recommends that no more than $100 \text{ } \mu\text{g}$ ($50 \text{ } \mu\text{mol}$) of lead per day should be ingested from food products.

Dietary Lead: Absorption and Consequences

People with certain macro- and micronutrient deficiencies are prone to experience increased absorption of lead from the diet. Thus, depletion of iron, calcium, and zinc may promote lead absorption through the gastrointestinal tract. Whereas adults may normally absorb approximately 15% of their lead intake, pregnant women and children may absorb up to 3.5 times that amount; the explanation for this difference is not clear.

The effects of the entry of lead into the circulation depend on its concentration. Thus, the inhibition of an enzyme active in hemoglobin synthesis, δ -amino levulinic acid dehydratase (ALAD), occurs at blood lead concentrations of $5\text{--}10 \text{ } \mu\text{g per 100 ml}$ ($0.25\text{--}0.5 \text{ } \mu\text{mol l}^{-1}$). Another enzyme active in heme biosynthesis, erythrocyte ferrochelatase, is inhibited at a blood lead level of $15 \text{ } \mu\text{g per 100 ml}$ ($0.75 \text{ } \mu\text{mol l}^{-1}$). Reduction of the renal enzyme 25-hydroxyvitamin D-1- α hydroxylase, which converts circulating 25-hydroxyvitamin D to its biologically active steroid hormone, $1\alpha,25\text{-dihydroxyvitamin D}$

($1,25(\text{OH})_2\text{D}$) or calcitriol, is observed at a blood lead concentration of $25 \text{ } \mu\text{g per 100 ml}$ ($1.25 \text{ } \mu\text{mol l}^{-1}$). Behavioral changes and learning problems may begin to occur at blood levels previously thought to be normal, $10\text{--}15 \text{ } \mu\text{g/100 ml}$ ($0.5\text{--}0.75 \text{ } \mu\text{mol l}^{-1}$).

Manifestations of Lead Toxicity

Perhaps due to their higher absorption of lead from the diet, children appear to be more susceptible to the toxic effects of lead. These involve the nervous system, including cognitive dysfunction; the liver; the composition of circulating blood; kidney function; the vitamin D endocrine system and bone (Table 1); and gene function, possibly with resultant teratogenic effects. Chronic exposure results in high blood pressure, stroke, and end-stage kidney disease in adults.

Full-blown lead encephalopathy, including delirium, truncal ataxia, hyperirritability, altered vision, lethargy, vomiting, and coma, is not common. Although peripheral nerve damage and paralysis may still be reported in adults, the most common toxicity observed is learning disability and an associated high-frequency hearing loss occurring in children with blood lead levels previously assumed to be safe. At low blood levels of lead (less than $10 \text{ } \mu\text{g/100 ml}$), children may lose intelligence quotient (IQ) points, possibly due to the interference of lead in normal calcium signaling in neurons and possibly by blocking the recently reported learning-induced activation of calcium/phospholipid-dependent protein kinase C in the hippocampus. The physicochemical basis of these changes derives largely from small animal data. Rats exposed to lead from birth develop mitochondrial dysfunction, neuronal swelling, and necrosis in both the cerebellum and the cerebrum. Exposure on day 10 of life elicited only the cerebellar pathology, and lead exposure after $3\frac{1}{2}$ weeks of life failed to produce any of these changes. In combination with manganese, lead has also produced peroxidative damage to rat brains and has been shown to inhibit nitric oxide synthase in the brains of mice. Additionally, an increase in blood arachidonic acid and in the ratio of arachidonic to linoleic acid following lead exposure in several species, including humans, may provide evidence in support of a peroxidative mechanism of damage to neural tissue following lead exposure. Lead has also produced necrosis of retinal photoreceptor cells and swelling of the endothelial lining of retinal blood vessels in rats. Lead may also damage

Table 1 Heavy metal toxicities in different tissues

<i>Tissue</i>	<i>Heavy metal</i>	<i>Dietary source(s)</i>	<i>Toxicity</i>
Neurological	Lead	Green, leafy vegetables, canned food with lead solder, water	Learning disability, ataxia, encephalopathy, irritability
	Mercury	Seafood, agricultural crop contamination	Psychomotor retardation, paralysis, microcephaly, convulsions, choreoathetotic movements
Bone	Bismuth	Medications	Paresthesias, tremors, ataxia, reduced short-term memory
	Lead	See above	Reduced conversion of vitamin D to active form, reduced osteoclast function
	Mercury	See above	Reduced bone formation and bone density
Bone marrow	Cadmium	Seafood, plant roots in contaminated soil	High bone turnover, secondary hyperparathyroidism
	Lead	See above	Decreased hemoglobin synthesis, decreased erythrocyte survival
	Mercury	See above	Increased hemolysis, alteration of T helper and T suppressor lymphocytes
	Cadmium	See above	Reduced erythrocyte count
	Nickel	Vegetables, especially legumes, spinach and nuts	Decreased helper T cells and increased suppressor T cells
Gastrointestinal	Lead	See above	Decreased binding of L-tryptophan to hepatocellular nuclei
	Mercury	See above	Anorexia, fetal hepatic cell damage
	Cadmium	See above	Abdominal pain, vomiting, diarrhea
Renal	Lead	See above	Proximal tubular dysfunction: glycosuria, aminoaciduria, hyperphosphaturia, decreased renal conversion of 25-hydroxyvitamin D to 1,25-dihydroxyvitamin D, the biologically active form
	Mercury	See above	Renal tubular dysfunction, proteinuria, autoimmune damage
	Cadmium	See above	Proteinuria, glycosuria

the auditory nerves in rats, and it may be partially responsible for the high-frequency hearing loss observed in humans. Finally, organic lead compounds may also disturb brain microtubular assembly.

Liver

Although there are no outwardly recognizable manifestations of lead toxicity to the liver, studies in rats indicate that amino acid binding to hepatocyte nuclei may be altered by lead. Thus, liver function may be subtly or subclinically affected and further studies are needed to elucidate this possibility.

Blood Composition

The major consequences of lead toxicity to the blood are microcytic anemia and decreased erythrocyte survival. The anemia is largely due to the inhibition of ALAD and erythrocyte ferrochelatase, which are critical to heme biosynthesis. Although the pathogenesis of the decreased red blood cell survival is not clear in humans, animal data indicate that the pentose phosphate shunt and glucose-6-phosphate dehydrogenase (G6PD) are inhibited by lead, suggesting that increased hemolysis may also contribute to the reduction in erythrocyte survival.

Kidney Function

Studies from the US National Institute of Occupational Safety and Health have reported that lead exposure reduced glutathione S-transferase expression in the kidneys of rabbits, indicating increased susceptibility to peroxidative damage. Renal proximal tubular dysfunction is described with lead

intoxication and can result in glycosuria, aminoaciduria, and hyperphosphaturia as well as a reduced natriuretic response to volume expansion. This latter effect of lead exposure may possibly offer an explanation of how lead accumulation may contribute to hypertension.

Vitamin D Endocrine System and Bone

As previously mentioned, lead can contribute to the reduced conversion of 25-hydroxyvitamin D to 1,25(OH)₂D. The extent to which this action may contribute to vitamin D deficiency is not known, but there is at least the potential for lower circulating levels of 1,25(OH)₂D to play a role in reduced intestinal calcium absorption. This in turn may result in further lead absorption. Vitamin D and lead levels both increase in children's plasma in summer months although whether the higher levels of vitamin D cause more lead absorption is not certain. Additionally, lead accumulating in bone has been reported to cause osteoclasts to develop pyknotic nuclei and manifest inclusion bodies, possibly lead, in the nucleus and cytoplasm. Although it has yet to be proven, these findings suggest a reduction in the resorptive function of osteoclasts. This may be a protective mechanism by the body to prevent the liberation of lead stored in bone, but at the same time lead may prevent the uptake by bone of additional calcium.

Genetic/Teratogenic Effects

Lead has been reported to alter gene transcription by the reduction of DNA binding to zinc finger proteins. This interruption of transcription has the potential to produce

Table 2 Recommended management of toxic symptoms caused by heavy metal contaminants in food

Element	Agent	Comments
Lead	Dimercaptosuccinic acid	Blood lead levels greater than $25 \mu\text{g}$ ($1.2 \mu\text{mol}$) l^{-1} treatment of children with blood levels exceeding $10 \mu\text{g}$ ($0.5 \mu\text{mol}$) l^{-1} advocated due to learning problems
Mercury	Dimercaptosuccinic acid	Dimercaprol and D-penicillamine have also been used, but dimercaprol is complicated by increased amount of mercury in brain
Cadmium	Diethyldithiocarbamate	Also used: dimercaprol, D-penicillamine, and dicalcium disodium EDTA
Nickel	Insufficient studies for recommended agent	Parenteral administration of diethyldithiocarbamate for acute toxicity may be helpful but unproven
Bismuth	Insufficient studies for recommended agent	Dimercaprol has been used anecdotally and reversed the symptoms of myoclonic encephalopathy; many choose to stop bismuth-containing drugs with a gradual resolution of symptoms

congenital anomalies in animals or humans. Studies have reported that lead crossing the placenta has produced urogenital, vertebral, and rectal malformations in the fetuses of rats, hamsters, and chicks.

Management of Lead Toxicity

Chelation therapy with dimercaprol succinic acid has been recommended for anyone with a blood lead level higher than $25 \mu\text{g l}^{-1}$ ($1.2 \mu\text{mol l}^{-1}$), as shown in Table 2. Today children with elevated blood levels typically have concentrations $<30 \mu\text{g l}^{-1}$ and few exceed $50 \mu\text{g l}^{-1}$. Chelation therapy should be used with caution and under the guidance of an expert in lead chemotherapy. For example, local experts are available through centers for disease control and prevention lead poisoning branch.

Mercury

How Does Mercury Contaminate Food?

The primary portal of mercury contamination of food is via its industrial release into water, either fresh or salt water, and its conversion to methyl mercury by methanogenic bacteria. As the marine life takes up the methyl mercury, it works its way into the food chain and is ultimately consumed by humans. This is the scenario that occurred following the release of inorganic mercury from an acetaldehyde plant into Minimata Bay in Japan in 1956 and 1965 and is responsible for the so-called Minimata disease. Furthermore, acid rain has increased the amount of mercury available to be taken up by the tissues of edible sea life and can enhance the toxicity of certain fish. An unfortunate consequence of seafood contamination with methyl mercury is the contamination of fish meal used to feed poultry, resulting in mercury accumulation in the poultry as well as in the eggs. Additionally, mercury-containing pesticides can contaminate agricultural products.

Permissible Intakes

Limits of mercury intake set by the UN Food and Agriculture Organization (FAO) and the World Health Organization (WHO) are 0.3 mg per person per week, of which not more

than 0.2 mg should be methyl mercury. Furthermore, FAO and WHO have set limits of mercury contamination of foods as not to exceed 50 parts per billion wet weight ($50 \mu\text{g l}^{-1}$). Hair mercury content is used as a marker of methyl mercury burden.

Dietary Mercury: Absorption and Consequences

Although the precise mechanism of mercury absorption and transport has not been clarified, one possibility is its use of molecular mimicry. Studies of methyl mercury show that it binds to reduced sulfhydryl groups, including those in the amino acid cysteine and glutathione. Methyl mercury-L-cysteine is similar in conformation to the amino acid methionine and may be taken up by the methionine transport system in the intestine. A Swedish study reported a direct correlation between the amount of seafood consumed by pregnant mothers and the concentration of methyl mercury in their umbilical cord blood. Although fetal tissue mercury concentration is generally lower than the maternal concentration, the exception to this is liver. According to a Japanese study, mercury is stored in the fetal liver, bound to metallothionein. With development, the amount of metallothionein decreases and the mercury in liver is redistributed primarily to brain and kidney. In studies of offspring of animals exposed to mercury vapors, behavioral changes have been detected. Pregnant women are advised to consume no more than $1.6 \mu\text{g}$ per kg body weight per week, in order to protect the developing fetus. Swordfish and shark are the foods most important to avoid during pregnancy, to minimize risk of high lead intake.

Manifestations of Mercury Toxicity

With regard to toxicity, mercury affects the skin, kidneys, nervous system, and marrow, with consequent effects on the blood cells, immune system, and bone formation.

Skin

Mercury produces a symptoms complex called acrodynia. Its main features are redness of the lips and pharynx, a strawberry tongue, tooth loss, skin desquamation and pink or red fingertips, palms, and soles. The eyes are also affected, and photophobia and conjunctivitis are seen. In addition, there is

enlargement of the cervical lymph nodes, loss of appetite, joint pain, and, occasionally, vascular thromboses, possibly by the induction of platelet aggregation, which has been shown in *in vitro* experiments. There is also a neurological component to this symptom complex: irritability, weakness of the proximal muscles, hypotonia, depressed reflexes, apathy, and withdrawal.

Kidneys

Mercury has been hypothesized to stimulate T lymphocytes to produce a glomerular ant basement membrane antibody, which produces sufficient damage to lead to the proteinuria observed with mercury toxicity (Table 1). The basis for this theory derives from studies in rats in which mercuric chloride injection produced these antibodies, both as IgG and IgM. There was also an observed increase in CD8⁺ (suppressor) T cells in the glomeruli. In addition, the rats developed proximal tubular necrosis. However, it is not clear that this theory is correct because methyl mercury can induce apoptosis, or programmed cell death, of the T lymphocytes, possibly by damaging mitochondria and inducing oxidative stress.

Nervous System

In the large epidemics of methyl mercury ingestion reported in both Japan and Iraq, infants were reported to have psychomotor retardation, flaccid paralysis, microcephaly, ataxia, choreoathetotic motions of the hands, tonic seizures, and narrowing of the visual fields (Table 1). Studies of neonatal rats injected with methyl mercuric chloride reported postural and movement changes during the 4th week of life. These were associated with degeneration of cortical interneurons, which produce γ -aminobutyric acid (GABA) as a neurotransmitter. In the caudate nucleus and putamen, these GABAergic and somatostatin immunoreactive interneurons manifested the abnormalities. Pregnant rats given methyl mercury by intraperitoneal injection demonstrated rapid (within 2 h) effects on their fetuses, including mitochondrial degeneration of cerebral capillary endothelial cells, which led to hemorrhage. In turn, the bleeding disrupted normal neuronal migration.

In addition, methyl mercury may disrupt neuronal microtubular assembly and, perhaps by molecular mimicry (as described previously), may bind to the sulfhydryl groups of glutathione, causing peroxidative injury to the neurons. Following intracerebral injection in the rat, methyl mercuric chloride distributes in the Purkinje and Golgi cells of the cerebellum as well as in three different layers of cerebral cortical cells – III, IV, and VI. Mercury exposure in humans can result in deficits in attention and concentration, especially under pressure of time deadlines. One report suggests that this may be due to mercury damage to the posterior cingulate cortex, where these functions are regulated. Finally, *in vitro* studies of rat cerebellar granular cells suggested that incubation with methyl mercury caused an increased, although delayed, phosphorylation of certain proteins. The 12- to 24-h time course from mercury exposure to phosphorylation was believed to be consistent with the alteration of gene expression by mercury. Thus, the effects of mercury on the nervous system are multiple.

Bone Marrow: Immune Cells, Blood Cells, and Bone Formation

A toxic effect of mercury on bone marrow would explain the abnormalities in red cell production, immune cell production, and bone formation (Table 1); all of the cells involved arise from stem cells found in the marrow and are presumably affected by mercury.

With regard to the immune cells, mercury induces an autoimmune response manifested by an increase in CD4⁺ (helper) and CD8⁺ (suppressor) T lymphocytes and in B lymphocytes in peripheral lymphoid tissue. This may explain in part the autoimmune nephropathy as well as the enlarged lymph nodes of acrodynia, previously described. Additionally, mercury may impair integrin signaling pathways in neutrophils, which may give rise to neutrophil dysfunction.

Hemolysis of red blood cells resulting from mercury exposure may be at least in part due to peroxidative damage in as much as studies on workers chronically exposed to mercury vapors demonstrate a reduction in erythrocyte enzyme activity of glutathione peroxidase and superoxide dismutase, as well as in G6PD.

Finally, although the effects of mercury exposure on bone have not been studied in humans, experiments in mice indicate that the administration of an antimetallothionein antibody and mercury results in decreased biochemical markers of bone formation and decreased bone mineral density. The mechanism for this is unknown, but mercury interference with differentiation of osteogenic precursor cells is postulated.

Genetic/Teratogenic Effects

The uptake and redistribution of mercury by fetal hepatic tissue have been previously discussed. Abnormalities described with *in utero* exposure to mercury during epidemics in Japan and Iraq have included low birth weight, malformation of the brain (both cerebrum and cerebellum), an abnormal migratory pattern of neurons, mental retardation, and failure to achieve developmental milestones. This remains a problem today for pregnant women who consume seafood. The FDA recommends that intake of large predator fish, such as swordfish and shark, be limited because they contain large amounts of mercury. Even tuna is considered to contain more mercury than most other seafood.

Management

Chelation with dimercaptosuccinic acid is recommended (Table 2).

Cadmium

How Does Cadmium Contaminate Food?

Cadmium enters the food chain in much the same way that lead and mercury do – by means of industrial contamination. Cadmium is often used as a covering of other metals or in the manufacture of batteries and semiconductors; it readily transforms into a gas as the metal ores are smelted. The cadmium then condenses to form cadmium oxide, which deposits in soil and water near the source. Cadmium

accumulates in lower marine life, such as plankton, molluscs, and shellfish, and continues through the food chain as these organisms are consumed. However, contamination of the human food supply is limited by this route because cadmium is toxic to fish and fish embryos. In contrast to seafood, vegetables are affected differently because cadmium is taken up by the leaves and roots of plants, so those near industrial sources may be very high in cadmium.

Permissible Intakes

A 1991 study of adults with 'itai itai' disease as a result of consuming rice contaminated with cadmium in the Kakehashi River Basin of Ishikara, Japan, correlated cadmium intake with renal tubular dysfunction and established a maximum allowable intake of $110 \mu\text{g day}^{-1}$. Canadian studies have estimated daily intake in study populations to be approximately half that, and the French have estimated cadmium exposure in the diet as being only 3 or $4 \mu\text{g day}^{-1}$. The provisional tolerable weekly intake established by FAO/WHO is $7 \mu\text{g}$ per kg body weight per week, a slightly more conservative estimate than the Japanese study but still in general agreement with it.

Dietary Cadmium: Absorption and Consequences

Fortunately, only 2–8% of dietary cadmium is absorbed and significant cadmium ingestion is accompanied by vomiting. Therefore, the gastrointestinal route is not as significant as inhalation of dust particles as a source of significant exposure. Toxic manifestations of cadmium ingestion include renal dysfunction, osteoporosis and bone pain, abdominal pain, vomiting and diarrhea, anemia, and bone marrow involvement (Table 1).

The mechanisms for cadmium's effects on the gastrointestinal tract are not certain. Whether these toxicities stem from an irritative effect of the metal or whether there is cellular damage has not been resolved in animal or *in vitro* studies. One possibility is that *in vitro* studies of neural tissue suggest that cadmium blocks adrenergic and cholinergic synapses. Therefore, it is possible that cadmium interferes with autonomic nervous system influence on gastrointestinal motility. Renal tubular dysfunction is manifest in patients with itai itai disease including glycosuria and proteinuria, and excessive excretion of α - and β -microglobulin. Approximately 50–75% of cadmium accumulation in the body occurs in the liver and kidneys. Urinary cadmium excretion of $200 \mu\text{g}$ ($1.78 \mu\text{mol g}^{-1}$) of renal cortical tissue has been associated with tubular dysfunction. In the kidney, cadmium is bound to metallothionein. When the amount of intracellular cadmium accumulation exceeds metallothionein binding capacity, this is the point at which renal toxicity is hypothesized to occur.

Bone Marrow and Bone

In short-term accumulation of cadmium in the marrow, there is a proliferation of cells in the myeloid/monocyte category. However, with longer-term burden, marrow hypoplasia is reported, including decreased production of erythropoietin. Although a reduction in marrow cells may indicate that the osteogenic precursors in the marrow may also be reduced

(Table 1), this is not borne out by studies both in humans and in rats. In these cases, biochemical markers of bone formation (osteocalcin) and resorption (deoxypyridinoline) are both increased, indicating a high turnover state. In rats, circulating parathyroid hormone levels are also elevated, suggesting that the high turnover is due to secondary hyperparathyroidism and subsequent inability of the bone matrix to mature and bind calcium and phosphate. Parenteral administration of 1,25-dihydroxyvitamin D has been reported to decrease circulating parathyroid hormone in the rat and to reduce bone turnover. Moreover, other animal studies report that cadmium interferes with hydroxyapatite nucleation and growth, thus making it difficult for bone matrix to bind to calcium.

Management

Chelation therapy is recommended using calcium, disodium ethylenediaminetetraacetic acid (EDTA), dimercaprol, D-penicillamine, or diethyldithiocarbamate (Table 2).

Nickel and Bismuth

Dietary Contamination

Nickel and bismuth are not considered to be common dietary contaminants. Nickel is mainly inhaled as a dust by workers, whereas bismuth is mainly ingested in bismuth-containing medications such as Pepto-Bismol. Vegetables contain more nickel than other foods, and high levels of nickel can be found in legumes, spinach, lettuce, and nuts. Baking powder and cocoa powder may also contain excess nickel, possibly by leaching during the manufacturing process. Soft drinking water and acid-containing beverages can dissolve nickel from pipes and containers. Daily nickel ingestion can be as high as 1 mg (0.017 mmol) but averages between 200 and $300 \mu\text{g}$ (3.4 and $5.1 \mu\text{mol}$).

Permissible Intakes

The maximum permissible intake of nickel is not known. Bismuth intake is related to whole blood bismuth levels. If these levels exceed $100 \mu\text{g l}^{-1}$, bismuth-containing medication should be discontinued.

Toxicity

Nickel ingestion by women resulted in an increase in interleukin-5 levels 4 h after ingestion and a decrease in CD4^+ and an increase in CD8^+ lymphocytes 24 h following the nickel intake. Thus, alterations in the immune response may be associated with excessive nickel ingestion, consistent with reports of tumor production in animals and humans by inhalation of nickel-containing dust or powders. The mechanism for nickel-associated toxicity is purported to be oxidative. For bismuth, neurotoxicity, including irritability, numbness and tingling of the extremities, insomnia, poor concentration, impairment of short-term memory, tremors, dementia masquerading as Alzheimer's disease, and abnormal electroencephalograms, has been reported. Discontinuation of the bismuth may result in

restoration of normal neurological function. Production of these symptoms in animals was associated with a brain bismuth concentration of $8 \mu\text{g g}^{-1}$ brain tissue; a brain bismuth concentration of $4 \mu\text{g g}^{-1}$ brain tissue was not associated with these neurotoxic manifestations. However, hydrocephalus was reported. At $1 \mu\text{g bismuth g}^{-1}$ brain tissue, no neurotoxic features were observed in animals. Nephropathy, osteoarthropathy, and thrombocytopenia have also been reported with bismuth toxicity.

Management

Insufficient controlled clinical trials have been performed to make clear-cut recommendations for pharmacotherapy for toxicity from either nickel or bismuth. Diethyl dithiocarbamate chelation therapy when promptly administered intravenously has been reported to be effective in acute nickel carbonyl poisoning. In addition, there have been anecdotal case reports of the reversal of myoclonic encephalopathy caused by bismuth with use of dimercaprol. However, no recommendations can be given at the present time.

See also: Ascorbic Acid (Vitamin C): Deficiency States. Food Safety: Other Contaminants. Vitamin D: Physiology, Dietary Sources, and Requirements

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Mycotoxins – Occurrence and Toxic Effects

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Glossary

Aflatoxins Potent hepatotoxins and carcinogens produced by *Aspergillus* species.

Fumonisin Group of mycotoxins having significant neurotoxicity. Possible contributors to esophageal cancer in humans.

Mycotoxins Class of metabolites produced by various fungal species having a spectrum of toxicological activities.

Ochratoxins Produced by some of the *Aspergillus* strains. One of the most potent renal toxins and carcinogens in a wide number of animal species.

Trichothecenes Mycotoxins from the *Fusarium* strains of molds.

Introduction

Mycotoxins are toxic fungal metabolites of enormous chemical diversity that contaminate the human food supply. These compounds induce an array of toxicologic effects when consumed in sufficient quantities. The three major genera of mycotoxin-producing fungi are *Aspergillus*, *Fusarium*, and *Penicillium*. Fungal growth and mycotoxin production can occur both before and after crop harvest. Mold potentiating conditions such as the unsuitability of the plant hybrid to the local environment, drought, insect herbivores, or mechanical harvesting can enhance mycotoxin production in field conditions; whereas high temperatures, moisture, and pests enhance mycotoxin accumulation in stored food. The major crops affected in the world are corn, peanuts, cotton, small cereal grains, and tree nuts.

Following the discovery of the carcinogenic aflatoxins (AFs) 50 years ago, the search for mycotoxins has led to the identification of more than 100 toxigenic fungi and more than 300 mycotoxins worldwide. Most of these toxins have not been linked to any toxic syndromes in animals or people, but some, such as AFs, certain trichothecenes, fumonisins, and ochratoxins have been implicated in highly lethal episodic outbreaks of poisoning in exposed animals or human populations. Mycotoxins with carcinogenic potency in experimental animal models include AFs, sterigmatocystin, ochratoxin A, fumonisins, and patulin. Of these agents, 'naturally occurring mixes of aflatoxins' has been classified as a Group 1 human carcinogen by the International Agency for Research on Cancer (IARC). The objective of this article is to describe the occurrence, biological effects, mechanisms of action and, where available, epidemiological associations of dietary exposure to major mycotoxins with human disease outcomes.

Aflatoxins

Chemistry and Occurrence

The AFs were discovered as the causative agent of turkey X disease in the UK, which resulted in the death of thousands of turkey poults, ducklings, and chicks fed contaminated peanut

meal. Chemically, the AFs are a highly substituted coumarin moiety containing a fused dihydrofuran moiety. Four major AFs designated B₁, B₂, G₁, and G₂ are produced by *A. flavus* and *A. parasiticus*. AFB₁ and AFB₂ were named because of their strong blue fluorescence under ultraviolet light, whereas AFG₁ and AFG₂ fluoresce greenish-yellow.

Commodities that are frequently found to contain AFs are peanuts, various tree nuts, cottonseed, and corn. Human exposure can occur from consumption of AFs from these crops and the products derived from them, as well as from tissues, eggs, and milk (AFM₁) from animals that consume contaminated feeds. Within a given geographical area, the levels or final concentrations of AFs in the crop can vary from less than 1 ppb to greater than 12 000 ppb. Obvious contamination of a commodity with *Aspergilli* does not necessarily indicate the presence of AFs, and the appearance of a sound, uninfected commodity does not preclude the existence of significant quantities of AFs.

Widespread concern regarding the toxic effects of AFs and possible transfer of residues from animal tissues and milk to humans has led to regulatory actions governing the interstate as well as global transport and consumption of AF-contaminated food and feed commodities. The US Food and Drug Administration (FDA) has set action levels of AF in commodities. For feeding mature nonlactating animals, the action levels are 100–300 ppb total AF (AFB₁ + AFB₂ + AFG₁ + AFG₂); for commodities destined for human consumption, dairy and immature animal consumption, pet food, and interstate commerce, 20 ppb total AF; and for milk at 0.5 ppb AFM₁.

Toxic and Carcinogenic Effects

AFs may be lethal when consumed in large doses; sublethal doses produce chronic toxicity, and low levels of chronic exposure can result in cancer, primarily liver cancer, in many animal species. AFB₁, the most potent and most commonly occurring AF, is acutely toxic to all species of animals, birds, and fishes tested. Chronic aflatoxicosis is characterized by bile duct proliferation, periportal fibrosis, icterus, and cirrhosis of liver. Prolonged exposure to low levels of AFB₁ leads to hepatoma, cholangiocarcinoma, or hepatocellular carcinoma

(HCC) and other tumors. Some cases of acute aflatoxicosis in humans have been reported in the literature, especially in subpopulations of developing countries. AFB₁ is a potent liver carcinogen in many animal species, including rodents, non-human primates, and fish.

Metabolism

Metabolism plays a critical role in the biological activity and disposition of AF. To cause DNA damage, AFB₁ undergoes an initial two-electron oxidation by the cytochrome P450-family members CYP1A2 and CYP3A4, yielding aflatoxin-B1-8,9-oxide. This epoxide reacts with the N7 atom of guanine to form a promutagenic DNA adduct (aflatoxin-N7-guanine). The aflatoxin-DNA adduct is unstable and undergoes depurination, leading to its urinary excretion. Aflatoxin-B1-8,9-oxide is also a substrate for several isoforms of human glutathione S-transferases (GSTs), which yield a stable, nontoxic, polar product that is excreted in bile. The aflatoxin-glutathione product also undergoes sequential metabolism in the liver and kidneys to be excreted as a mercapturic acid (aflatoxin-N-acetylcysteine) in the urine. Aflatoxin B1 also undergoes extensive oxidation, which is catalyzed by cytochrome P450s. In addition to formation of the 8,9-oxide, oxidation by CYP1A2 yields a stable metabolite, aflatoxin M₁, excreted in milk and urine. Aflatoxin M₁ is less carcinogenic and mutagenic than aflatoxin B1, but equally toxic. Collectively, these end products of AF biotransformation are biomarkers of exposure to AF and risk of HCC.

AF and Human Cancer

HCC is the fifth leading cause of cancer mortality worldwide and accounts for nearly 70% of all cancer deaths in some parts of Africa and Asia. Owing to lack of symptoms in early stages and rapid growth rates of tumors, most HCCs are discovered in very advanced stages. The 5-year mortality rate for individuals diagnosed with HCC exceeds 95%. In the People's Republic of China, HCC accounts for at least 250 000 deaths per year, with an incidence rate in some counties approaching 100 cases per 100 000 per year. Moreover, in high-risk regions of the world, the median age of onset of HCC is decades earlier than in the US.

Nested case-control studies conducted in Shanghai and Taiwan utilized AF specific biomarkers to establish a significant association between AF and HCC. They showed that the risk of HCC increased dramatically (60-fold) in individuals who had been exposed to AF and had chronic hepatitis infection compared to those with neither the chemical nor viral exposures. The underlying mechanism for this interaction remains poorly understood.

The relationship between AF exposure and human HCC is further highlighted by the recent molecular biological studies on the p53 tumor suppressor gene, the most common mutated gene detected in many human cancers. The initial results came from three independent studies of p53 mutations in HCCs occurring in populations exposed to high levels of dietary AF and found high frequencies of G→T transversions, with clustering at codon 249. A positive correlation has been observed between population estimates of AF exposure

and the proportion of HCC cases with a p53 249^{ser} mutation detected in plasma.

It is estimated that between 25 200 and 155 000 HCC cases worldwide per year, or 5–28% of all global HCC cases, could be attributable to AF. This was determined by a quantitative cancer risk assessment, and AF exposure data from around the world. Most of the AF-induced HCC cases were determined to be in sub-Saharan Africa, Southeast Asia, and China; where populations suffer from both high hepatitis B virus (HBV) prevalence and largely uncontrolled AF exposure in food.

AF exposure assessment has evolved significantly over the last two decades, largely due to the characterization of biomarkers for both AF exposure and effect. Before use of biomarkers, the primary way to estimate AF exposure was to estimate how much corn, nuts, and other contaminated foods people consumed on average; and to measure or assume AF levels in these foods. The difficulties associated with attempting to measure foodborne AF has been ameliorated with the use of biomarkers that resolve several problems related to obtaining accurate AF exposure data. Several serum- and urine-based biomarkers of AF exposure, internal dose, and biologically effective dose have been validated in experimental models and epidemiological studies. These include urinary AFM₁ as a biomarker of exposure, urinary AF-mercaptopuric acid and serum AF-albumin adduct as biomarkers of internal dose, and urinary aflatoxin-N7-guanine as a biomarker of biologically effective dose.

Use of these biomarkers has greatly assisted AF-related epidemiological and exposure assessment efforts. Moreover, quantitative relationships have been developed between certain biomarkers and actual AF exposure in the diet. However, obtaining and storing blood and urine samples to measure biomarker levels can be challenging for a variety of physical, technological, and cultural reasons. Nonetheless, these biomarkers have proven and will continue to be valuable tools in AF exposure assessment; and hence risk assessment and the estimation of global burden of disease.

Fumonisin

Occurrence

The fumonisins are a class of mycotoxins produced by *Fusarium verticillioides* and *Fusarium proliferatum*. Fumonisin are primarily contaminants in corn. Six fumonisins have been isolated and characterized from *F. verticillioides*. They are designated as fumonisin B₁ (FB₁), B₂, B₃, B₄, A₁, and A₂, respectively. Only FB₁ and FB₂ appear to be toxicologically significant and have been studied to any extent. FB₁ and FB₂ were first isolated in 1988 and invariably occur together, with FB₂ at levels of 15–35% of FB₁. Levels of FB₁ have annual variation, but are consistently in the 0.5–2 ppm range in US cornmeal, and have been reported as high as 150 ppm in corn destined for human consumption in South Africa. Regulatory limits for fumonisins in commodities are now being promulgated worldwide. In the US, FDA has set guidelines to industry for total fumonisins ranging from 2 to 4 ppm in human food products, and from 5 to 100 ppm in animal feeds depending on the sensitivity of the species (horses and rabbits are more sensitive to fumonisin toxicity; poultry less so).

Toxicity

Fumonisin-contaminated corn has been associated with several animal diseases, including leukoencephalomalacia (LEM) in horses, pulmonary edema in swine, neurotoxicity, and feed refusal in multiple species, and hepatotoxicity in horses, swine, and rats. Several studies have provided some possible insights into the mechanisms of toxicity. The fumonisins bear considerable structural similarity to the long-chain (sphingoid) base backbones of sphingolipids. It has been demonstrated that incubation of rat hepatocytes with fumonisins inhibited sphingolipid biosynthesis. FB₁ increased the amount of the biosynthetic intermediate sphinganine, which suggests that fumonisins inhibit the conversion of sphinganine to N-acyl-sphinganines. It was subsequently shown, using mouse cerebellar neurons in culture, that FB₁ inhibited ceramide synthase in mouse brain microsomes with a competitive-like kinetic behavior with respect to both sphinganine and stearyl-CoA. Thus, disruption of the *de novo* pathway of sphingolipid biosynthesis may be a critical event in the diseases that have been associated with consumption of fumonisins.

Carcinogenicity in Animals

Rats fed a diet supplemented with maize contaminated with the *F. verticillioides* that had caused an outbreak of LEM in horses with all developed hepatic nodules, cholangiofibrosis, or cholangiocarcinomas within 6 months. The carcinogenicity of FB₁ has also been directly assessed in a study where a semipurified diet containing 50 mg kg⁻¹ of pure (>90%) FB₁ was fed to rats. Ten out of 15 FB₁-treated rats (66%) developed primary HCC and they estimated the no-observable-effect-level for liver cancer induction by FB₁ in male rats at 0.8 mg kg⁻¹ bw per day. Although fumonisin does not directly damage DNA, it may stimulate oxidative damage and lipid peroxidation, or the disruption of sphingolipid biosynthesis could contribute to carcinogenesis through an altered balance of cell death and replication.

Human Health Effects

In an initial study conducted in high- and low-risk regions of Transkei (South Africa), esophageal cancer rates were correlated with the proportion of maize samples infected by *F. verticillioides*. In a follow-up study, the mean proportion of maize kernel infected with *F. verticillioides* in both healthy and moldy maize samples from households in the high-incidence esophageal cancer area were significantly higher than those in the low incidence area. FB₁ and FB₂ levels in healthy maize samples from the low-risk area were approximately 20 times lower than those in healthy samples from high-risk areas.

Chinese studies have likewise found geographical associations between fumonisin exposure and esophageal cancer incidence. The frequency of *F. verticillioides* contamination was significantly higher in food samples from five counties at high risk of esophageal cancer than in three counties at lower risk. Others found that averaged daily dietary FB₁ intake was significantly higher in a Chinese region with high esophageal cancer incidence (Huaian) compared with two regions with low esophageal cancer incidence (Huantai and Fusui).

Although these studies, as well as those conducted in South Africa, demonstrate correlations between high esophageal cancer rates and fumonisin exposure, the mechanism by which fumonisin induces this cancer has not yet been elucidated.

More recently, fumonisin exposure has been associated with neural tube defects (NTDs) in human infants. Fumonisin-induced depletion of glycosphingolipids impairs expression and function of particular folate receptors, which can contribute to adverse pregnancy outcomes. In a study of the sphinganine-to-sphingosine ratio, presumed to be correlated with fumonisin exposure, in the serum of postpartum women living along the Texas–Mexico border, it was found that increasing levels of fumonisin exposure were associated with increasing odds ratios for NTD occurrences. Moreover, maternal recall of corn tortilla intake revealed that higher tortilla consumption was associated with increased odds ratios for NTDs among babies. A recent study has linked fumonisin exposure with infant growth impairment in Tanzania, with infants consuming above the provisional maximum tolerable daily intake (PMTDI) of 2 µg kg⁻¹ bodyweight being on average 1.3 cm shorter and 328 g lighter than those consuming fumonisins below the PMTDI.

Recent work in Centane, South Africa, has established urinary FB₁ as a biomarker for future fumonisin exposure assessment; as this biomarker was correlated with FB₁ intake in home-grown corn porridge. Moreover, FB₁ could be reduced by a simple intervention of hand sorting and washing.

Ochratoxins

Occurrence

Ochratoxins are a group of structurally related metabolites produced by *A. ochraceus* and related species, as well as *P. verrucosum* and certain other *Penicillium* species. The major mycotoxin in this group, ochratoxin A (OA), appears the only one of major toxicological significance. Chemically, OA contains an isocoumarin moiety linked by a peptide bond to phenylalanine. OA have been detected in many food commodities worldwide, including cocoa, coffee, dried vine fruits, wine, and blood sausage; but is found primarily in grains grown in northern temperate areas resulting in contamination of breads and cereal products. In addition to cereals, animal products such as sausage can be significant human dietary sources of OA. High OA contamination in foods was measured in Croatia and surrounding nations where Balkan endemic nephropathy (BEN) is highly prevalent. BEN is now believed to be caused by aristolochic acid contamination through *Aristolochia* pollen cross-contaminating harvested grains; however, average concentrations of OA are higher in foods from nephropathic regions. Many countries have set regulatory limits for OA ranging from 1 to 50 ppb for food and from 100 to 1000 ppb for animal feeds.

Toxicity

The toxicity of OA varies considerably with dose and between species. Dogs and pigs are the most sensitive species. Synergistic effects of OA with other mycotoxins, such as citrinin

and penicillic acid, on the LD₅₀ were seen in mice following intraperitoneal injection. OA is nephrotoxic to a number of animal species; the presence of OA in feed is believed to be the most important cause of spontaneous mycotoxic porcine and poultry nephropathy. OA also produces hepatic toxicity at high doses. OA is teratogenic in mice, rats, and hamster, and the major target in the fetus is the developing central nervous system. OA is immunosuppressive at low doses, affecting immune function at both the level of antibody synthesis and natural killer cell activity. The toxic mechanism of OA has been shown to be inhibition of protein synthesis by competition with phenylalanine in the phenylalanyl-tRNA synthetase-catalyzed reaction. OA also inhibits other enzymes that use phenylalanine as a substrate such as phenylalanine hydroxylase. The effect of OA on protein synthesis is followed by an inhibition of RNA synthesis, which might affect proteins with a high turnover. OA was also found to enhance lipid peroxidation *in vivo*.

Carcinogenicity

The mechanism causing the carcinogenicity of OA is unclear. Various hypotheses were listed by the Joint Expert Committee on Food Additives (JECFA) of the Food and Agriculture Organization and World Health Organization at its most recent evaluation. Genotoxicity occurred in evaluated animal studies only under high OA exposure levels, which could be indicative of oxidative damage. A direct genotoxic mode of action remains unconfirmed, although a recent study provides information about covalent adducts between DNA and OA. The IARC has classified OA as a Group 2B possible human carcinogen.

Trichothecenes

The trichothecenes are a family of more than 150 structurally related compounds produced by several fungal genera (*Fusarium*, *Cephalosporium*, *Myrothecium*, *Stachybotrys*, and *Trichoderma*). Chemically, they are sesquiterpenes characterized by a double bond at position C-9, an epoxide ring at C-12, and various patterns of hydroxy and acetoxy substitutions at position C-3, C-4, C-15, C-7, and C-8. There are four naturally occurring trichothecene mycotoxins deoxynivalenol (DON), nivalenol, T-2 toxin, and diacetoxyscirpenol) produced in food and feed by *Fusarium* species.

DON

DON is probably the most widely distributed *Fusarium* mycotoxin, and is produced primarily by *Fusarium graminearum* and *Fusarium culmorum*. It occurs primarily in cooler temperate regions in wheat, corn, barley, and oats. Currently, several countries have set guidelines or official tolerance levels for DON in food and feed, ranging from 0.005 to 4 mg kg⁻¹ depending on the commodity, with lower tolerances for baby food. DON inhibits protein synthesis and causes immunomodulation in multiple species. Rarely, acute mycotoxicoses in human populations caused by ingestion of highly DON-

contaminated food have been reported in China, India, and some other countries. New technologies are now being applied to assess human exposure to DON and this has been used to measure routine exposures in the UK.

T-2 Toxin

T-2 toxin is produced primarily by *F. sporotrichioides* and has been reported in many parts of the world. It is formed in large quantities under the unusual circumstance of prolonged wet weather at harvest. Natural contamination of foods and feeds by T-2 toxin in the US has been reported in only one incident involving heavily molded corn. An official tolerance level of 0.1 mg kg⁻¹ was established for T-2 toxin in grains in Russia.

The toxic effects of T-2 toxin in various animal species include weight loss, decreased feed conversion, feed refusal, vomiting, bloody diarrhea, severe dermatitis, hemorrhage, decreased egg production, abortion, and death. Histologic lesions consist of necrosis and hemorrhage in proliferating tissues of the intestinal mucosa, bone marrow, spleen, testis, and ovary. T-2 toxin can affect cellular immune response in animals, and inhibit protein and DNA synthesis. It has been implicated in a variety of animal and human toxicities, such as alimentary toxic aleukia, Msleni joint disease, scabby grain toxicosis, and Kashin-Beck disease.

Other Mycotoxins

Zearalenone

Zearalenone (ZEN) is produced primarily by *F. graminearum* and is among the most widely distributed *Fusarium* mycotoxins. It is associated mainly with corn, wheat, barley, sorghum, and other grains. JECFA has set a PMTDI of 0.5 µg kg⁻¹ bw. ZEN has estrogenic effects in domestic pigs and experimental animals, is teratogenic to mice and rats, and induces chromosomal anomalies in cultured rodent cells. Its carcinogenicity was tested by administration in the diet in one experiment in mice and in two experiments in rats. An increased incidence of hepatocellular adenomas was observed in female mice and of pituitary adenomas in mice of each sex. No increase in the incidence of tumors was observed in rats.

Sterigmatocystin

Sterigmatocystin is produced by several species of *Aspergillus*, *Penicillium luteum*, and a *Bipolaris* species. Chemically, sterigmatocystin resembles the AFT and is a precursor in the biosynthesis of AFT. It has been detected at low concentrations in green coffee, moldy wheat, and in the rind of hard Dutch cheese. Sterigmatocystin is a hepatotoxin and is less potent than the AFT. It was mutagenic in the Ames test, the *Rec* assay, and the *Bacillus subtilis* assay. It can covalently bind to DNA and form DNA adducts. It has been proven that sterigmatocystin is carcinogenic to rats and mice, mainly inducing liver tumors.

Patulin

Patulin is produced primarily by *P. expansum*. Commodities found contaminated with patulin mainly are fruits and fruit

juices in Europe and North America. Patulin is appreciably stable in apple and grape juices, although at levels below those causing potential threat to humans. Multiple countries have set regulatory limits for patulin in fruit juice, ranging from 30 to 50 ppb. The toxicity of patulin has been studied in many experimental models including chicken, quail, cat, cattle, rabbit, mice, and rats. The toxic effects on these animals were found to be edema and hemorrhage in brain and lungs, capillary damage in the liver, spleen, and kidney, paralysis of motor nerves, and convulsions. Patulin is also an immunosuppressive agent which inhibits multiple aspects of macrophage function.

Summary

Collectively, the mycotoxins represent a diverse array of foodborne toxins that induce a spectrum of biological effects in human and animal populations. Many of these effects were initially determined because of acute toxicities that make the exposure–effect association easier to determine. With the advent of modern biomarker strategies, chronic effects – particularly cancers – in both humans and animals are now being elucidated. These biomarkers can also help in more accurately assessing human exposures to mycotoxins, to improve the quality of risk assessment in the future. As we continue to study this class of compounds and explore their toxicities, we will continue to discover the biological effects of these vast and structurally different chemicals.

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Other Contaminants

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Background

Food may be contaminated with many chemicals that pose the potential for toxicological consequences in humans consuming the contaminated food items. In addition to the presence of contaminants such as mycotoxins, pesticide residues, and heavy metals, food may contain numerous organic contaminants that enter the food supply from environmental sources or as a result of chemical reactions that occur during food processing. This review focuses on four types of food contaminants: (1) dioxins (including dibenzofurans and PCBs), (2) acrylamide, (3) perchlorate, and (4) bisphenol A. Each of these classes has been subject to considerable regulatory scrutiny, scientific study, and popular media coverage. It is likely that concerns regarding the presence of these contaminants in the food supply will continue throughout the next decade or longer and that significant efforts will be made to reduce human exposure to these substances from food. This review discusses how these types of food contaminants enter the food supply, the types of food items on which they are most likely to occur, and the potential toxicological consequences resulting from exposure to these contaminants.

Dioxins

Dioxins are organic chemicals that comprise a family of ubiquitous environmental contaminants. Technically speaking, the dioxins of potential toxicological concern are polychlorinated dibenzo-p-dioxins (PCDDs). They are related, both structurally and toxicologically, to polychlorinated dibenzofurans (PCDFs) and to polychlorinated biphenyls (PCBs). Structures of generic PCDDs, PCDFs, and PCBs are shown in **Figure 1**. Owing to their structural and toxicological similarity and to avoid confusion, all three related groups of chemicals are considered to represent “dioxins” for the purposes of this review. Specific chemicals belonging to this family will be referred to as congeners. Collectively, there are

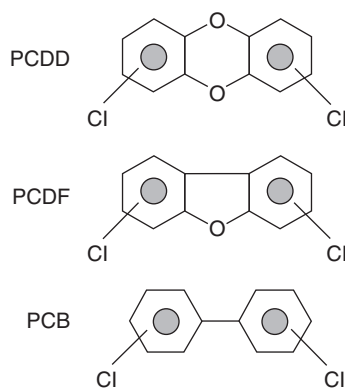


Figure 1 Structures of PCDDs, PCDFs, and PCBs.

more than 200 dioxin-related congeners and each possesses unique toxicological and chemical properties.

Occurrence in the Environment and in Food

PCDDs and PCDFs are primarily introduced into the environment as byproducts of combustion processes. These byproducts have been identified in the exhaust gases from sources such as cigarette smoke, industrial and municipal waste incinerators, power plants burning coal, oil, or wood, and automobiles. In addition to these human sources, PCDDs and PCDFs are also produced naturally by combustion in forest fires and from volcanic eruptions.

Historically, PCDDs and PCDFs have also been produced as impurities during organic chemical synthesis. The most notable and most toxic dioxin congener, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), has been shown to be produced in the synthesis of the herbicide 2,4,5-T, one of the herbicide components of Agent Orange notoriously used in the Vietnam War. Although 2,4,5-T is now banned for use in the U.S. because of TCDD and other dioxin impurities, health concerns over the exposure of military veterans to Agent Orange and to TCDD continue to be raised today. PCDDs and PCDFs can also be produced through the use of chlorine to bleach wood pulp, although most bleaching processes now use nonchlorine agents such as hydrogen peroxide.

PCBs have been produced synthetically since the 1930s and have been widely used for industrial applications such as dielectric fluids in transformers (due to their inflammability) and capacitors in electrical machinery. Like their PCDD and PCDF counterparts, PCBs are extremely persistent in the environment and are of toxicological concern. As a result, the synthesis and industrial use of PCBs were significantly curtailed in the 1970s although environmental residues of PCBs are still commonly detected today.

Although dioxin release into the environment has been known to occur for several decades, data are still limited with respect to the degree by which dioxins contaminate the food supply. Dioxin analysis in the laboratory is still extremely expensive as methods must identify hundreds of different congeners, detection limits are required in the sub-part per trillion range, and significant precautions must be taken to minimize exposure of laboratory personnel to the analytical standards used for dioxin congeners.

Dioxins are highly fat-soluble and have been shown to accumulate in the fat of birds, fish, and food animals. The U.S. Environmental Protection Agency (EPA) has estimated that over 95% of human exposure to dioxins results from dietary intake of animal fats. The major food sources for dioxin exposure include fish, poultry, meats, milk, and milk products. Dioxins are excreted in human breast milk and result in exposures to nursing infants.

Historically, it has been shown that human dioxin exposures, as determined by analyzing human tissues and

environmental samples, has decreased significantly since 1987 due to engineering controls to limit dioxin emissions during combustion processes and to increased regulatory control over other sources of dioxin exposure. Dietary dioxin exposures to UK consumers were reduced by nearly two-thirds from 1982 to 1992 whereas subsequent studies showed even lower exposures in 1997. Nevertheless, dioxins are still ubiquitous in the environment and human exposure still occurs.

Toxicological Considerations

Dioxin exposure at significant dose levels has been linked to a large number of adverse health effects. Large acute exposures, resulting from chemical accidents or occupational exposure to dioxins, have caused a severe skin condition known as chloracne. A variety of other skin effects, such as rashes and discoloration, have also been attributed to acute dioxin exposures, as has liver damage.

Concerns from chronic exposure to dioxins include cancer, reproductive effects, and developmental effects. The most toxic dioxin congener, TCDD, was classified by the International Agency for Research on Cancer as a human carcinogen.

From a biochemical standpoint, PCDDs, PCDFs, and PCBs appear to cause their toxic effects through chemical binding to a specific cellular receptor known as the Ah-receptor. Specific dioxin congeners vary dramatically with respect to their abilities to bind with the Ah-receptor; TCDD binds extremely effectively whereas other congeners are more limited in their binding capabilities. The degree to which various dioxin congeners bind with the Ah-receptor seems to be directly related to the number and location of chlorine atoms on the congeners.

Assessing the potential human health risks from exposure to dioxins presents significant challenges. Dioxin levels on specific food items can be quite variable, and, as discussed previously, data concerning dioxin levels on foods are frequently not available.

Another difficulty encountered in assessing dioxin risks is to appropriately account for exposures to the various congeners and to account for the toxicological differences among congeners. This is most appropriately achieved through a Toxic Equivalency Factor (TEF) approach that assigns a potency factor to each of the congeners relative to that of the most toxic dioxin TCDD. As an example, the TEF for TCDD is 1 and the TEF for 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin (with chlorines added to the 1 and 2 positions and otherwise similar to TCDD) is 0.1, based on findings that 1,2,3,4,7,8-hexachlorodibenzo-p-dioxin is ten times less capable of binding to the Ah-receptor than is TCDD. To calculate a total dioxin exposure, the dietary contributions of each of the dioxin congeners are multiplied by their corresponding TEFs and summed to determine a TCDD equivalent exposure.

According to the World Health Organization, a Tolerable Daily Intake (TDI) for TCDD was established at 10 pg TCDD per kg bodyweight per day in 1990 although revisions by the World Health Organization reduced the TDI range to 1–4 pg kg⁻¹ d⁻¹ in 1999. The EPA has recently proposed a reference dose for TCDD equivalents at 0.7 pg kg⁻¹ d⁻¹ in response to a 2006 report of the National Academy of Sciences

indicating a need to establish a reference dose. A 1997 UK survey of dioxin consumer exposure provided an upper bound of 1.8 pg TCDD equivalent per kg per day. Surveys from other countries, using slightly different TEF approaches, yielded exposures of 0.7 pg kg⁻¹ d⁻¹ for Italy, 1.4 pg kg⁻¹ d⁻¹ in Norway, 2.4–3.5 pg kg⁻¹ d⁻¹ in Spain, and 0.2 pg kg⁻¹ d⁻¹ in New Zealand.

The U.S. Food and Drug Administration has been monitoring finfish, shellfish, and dairy products for dioxins since 1995 and initiated dioxin analysis of foods analyzed in its Total Diet Study in 1999. Specific findings from FDA's annual Total Diet Study can be obtained by the FDA although human exposure estimates, in terms of the amount of TCDD equivalent exposure per kilogram of body weight per day, have not been published by the FDA.

The EPA recommends that consumers follow the existing Federal Dietary Guidelines to reduce fat consumption and, subsequently, dioxin exposure. Such guidelines suggest that consumers choose fish, lean meat, poultry, and low or fat free dairy products while increasing consumption of fruits, vegetables, and grains. Dioxin exposure can be further minimized by trimming visible fat from meats, removing the skin of fish and poultry, reducing the amount of butter or lard used in cooking, and replacing cooking methods such as frying with methods including boiling or oven broiling.

Acrylamide

Acrylamide is a widely used and versatile industrial chemical. Its most common use is as a coagulant in water treatment and purification. It is also used as a soil conditioner, in the sizing of paper and textiles, in ore processing, and as a construction aid for the building of tunnels and dam foundations.

Acrylamide is considered by the International Agency for Research on Cancer to be "probably carcinogenic to humans" based on the results of several animal carcinogenicity studies. As a result, there has been widespread concern about the potential risks from exposure to acrylamide among industrial, manufacturing, and laboratory workers. Consumer exposure to acrylamide in treated drinking water has posed a much lower concern because drinking water is subject to special treatment techniques that control the amount of acrylamide in drinking water.

Swedish researchers developed laboratory techniques that allowed for the detection of biological reaction products (hemoglobin adducts) of acrylamide in human blood samples; results from their studies allowed correlations to be made between occupational activities and acrylamide exposures. The findings that acrylamide occurred in tobacco smoke and that smokers had increased levels of hemoglobin adducts relative to nonsmokers provided a suggestion that acrylamide may be formed during incomplete combustion of organic matter or during heating. Interestingly, the researchers found significant levels of hemoglobin adducts in blood samples of nonsmoking humans not exposed occupationally to acrylamide. This led to speculation that the human diet could contain significant quantities of acrylamide. In April of 2002, Swedish researchers published results of research that demonstrated the presence of acrylamide in several common foodstuffs, with the

highest levels found in fried and baked foods. These findings stimulated worldwide interest in identifying the potential mechanisms for acrylamide formation in foods, in assaying a wide variety of foods for acrylamide levels, and in developing risk assessment and risk mitigation procedures.

Occurrence in Food

The findings from the initial Swedish study indicated that the highest levels ($150\text{--}4000\ \mu\text{g kg}^{-1}$) of acrylamide were detected in carbohydrate-rich foods such as potato and in heated commercial potato products (potato chips) and crispbread. Moderate levels ($5\text{--}50\ \mu\text{g kg}^{-1}$) were measured in protein-rich foods that were heated, whereas unheated or boiled foods showed no detectable acrylamide ($<5\ \mu\text{g kg}^{-1}$).

The governments of several countries throughout the world performed similar analyses of acrylamide in foods and findings were fairly consistent with those reported in the Swedish study. The FDA analyzed dozens of foods for acrylamide levels and concluded that the highest levels were observed in french fries (29 samples, range $117\text{--}1030\ \mu\text{g kg}^{-1}$) and in potato chips (40 samples, range $117\text{--}2762\ \mu\text{g kg}^{-1}$). Multiple samples from different lots of the same commercial food products showed significant variability with the highest levels often several times greater than the lowest levels. Commercial potato products that could be prepared by baking or by other methods showed much higher levels of acrylamide in the baked products. Acrylamide levels in baby food ranged from below the detection level ($<10\ \mu\text{g kg}^{-1}$) to $130\ \mu\text{g kg}^{-1}$. All infant formula samples had levels below $10\ \mu\text{g kg}^{-1}$, and acrylamide levels in dairy products were also low.

The widespread findings of acrylamide in foodstuffs throughout the world provided the basis for numerous studies designed to elucidate the mechanisms for acrylamide formation in foods. It has been demonstrated that acrylamide can be formed from classical Maillard reactions as well as from reaction of the fatty acid oxidation product acrolein with ammonia and subsequent oxidation steps. The most plausible explanation for the relatively high acrylamide levels in fried potato products comes from a mechanism involving the reaction of the amino group of the amino acid asparagine with the carbonyl group of a reducing sugar such as glucose during baking and frying. This mechanism is shown in **Figure 2**. Potatoes are high in asparagine and in reducing sugars, and are commonly prepared for consumption by frying or baking; all of these factors help explain the relatively high levels of acrylamide in heated potato products.

Although it is clear that humans have been consuming significant amounts of acrylamide in their diets for a long time, the relatively new discovery of acrylamide as a food contaminant has raised several questions. Significant efforts are currently being made to better understand the levels of acrylamide throughout the food chain and to estimate dietary exposure to acrylamide. In addition, there is much emphasis on developing food processing approaches that can reduce acrylamide formation.

Many methods have been used to estimate daily dietary exposure to acrylamide. Probabilistic and semiprobabilistic methods have estimated mean UK exposure to be 0.61 micrograms of

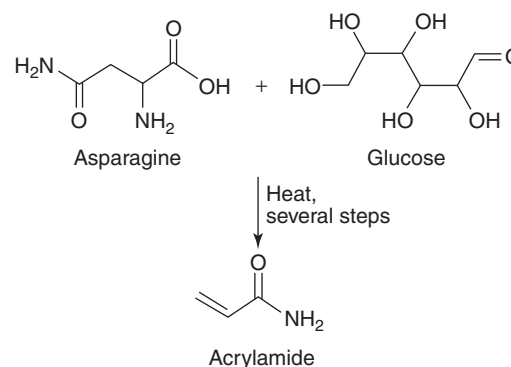


Figure 2 Proposed mechanism for acrylamide formation in heated foods.

acrylamide per kilogram of body weight per day ($\mu\text{g kg}^{-1}\text{ d}^{-1}$). Acrylamide exposure estimates for the US population have been estimated to be $0.44\ \mu\text{g kg}^{-1}\text{ d}^{-1}$ for the overall population and $0.86\ \mu\text{g kg}^{-1}\text{ d}^{-1}$ for children ages 3–12 years based on typical food consumption patterns and results from blood sampling of acrylamide-hemoglobin adducts. A different study in the US, using physiologically based pharmacokinetic modeling, provided an acrylamide exposure estimate of $0.4\ \mu\text{g kg}^{-1}\text{ d}^{-1}$. The U.S. Environmental Protection Agency has established a chronic oral reference dose for acrylamide at $2\ \mu\text{g kg}^{-1}\text{ d}^{-1}$ with respect to neurotoxicity; exposure of $0.4\ \mu\text{g kg}^{-1}\text{ d}^{-1}$ represents 20% of the chronic oral reference dose.

Toxicological Considerations

Laboratory toxicology studies have indicated that acrylamide is carcinogenic and also has been associated with the development of reproductive toxicity, genotoxicity and neurotoxicity. Epidemiological and analytical studies of people exposed to acrylamide in the workplace have indicated that acrylamide does indeed enter the bloodstreams of the workers and can be detected and quantified as hemoglobin adducts, thus indicating both exposure and absorption of acrylamide. Such studies have not, however, indicated increases in cancer rates among those exposed occupationally to acrylamide. To date, the only documented toxicological effect observed in epidemiological studies of workers exposed to acrylamide is neurotoxicity. This effect is primarily an acute effect caused by large exposures to acrylamide for relatively short periods of time, leading to nervous system damage, weakness, and incoordination of limbs.

From a biochemical standpoint, it is likely that the health effects caused by high levels of exposure in humans and in laboratory animals may result from a Michael-type nucleophilic addition reaction of amino acids (both amino and sulfhydryl groups), peptides, and proteins to acrylamide because of the presence of the α,β -unsaturated conjugated structure in acrylamide. This is a common toxicological pathway for many reactive compounds. It is likely that high doses of acrylamide may overwhelm the defensive mechanisms of the body such as glutathione conjugation and could cause reaction with biologically significant nucleophiles, leading to mutations and possible carcinogenicity.

Several recent epidemiology studies have been conducted to examine the relationship between dietary acrylamide exposure and cancer. A few have indicated the potential for dietary acrylamide to cause human cancers, particularly for postmenopausal endometrial and ovarian cancers. Most other epidemiological studies have been negative whereas inverse relationships between cancer and acrylamide exposure have been suggested for lung cancer and bladder cancer in women, and prostate and oro and hypopharyngeal cancer in men.

Regulatory limits for acrylamide in food have yet to be established because dietary acrylamide risk assessments are still being developed. In the meantime, the FDA recommends that consumers eat a balanced diet that includes a wide variety of foods low in trans fat and saturated fat while rich in high-fiber grains, fruits, and vegetables.

Perchlorate

Perchlorate exists as an anion (ClO_4^-) with a central chlorine atom surrounded by four oxygen atoms arranged in a tetrahedron. Perchlorate is manufactured in the US and is used as the primary ingredient of solid rocket propellant. Perchlorate wastes from the manufacture or improper disposal of perchlorate-containing chemicals are frequently detected in the soil and in the water. Levels of perchlorate have been detected in 58 California public water systems and in water samples from 18 states.

The widespread water contamination by perchlorate and its potential to cause health effects in those consuming contaminated drinking water led four U.S. agencies – the EPA, the Department of Defense, the Department of Energy, and the National Aeronautics and Space Administration – to ask the U.S. National Academy of Sciences to convene a study on “Toxicological Assessment of Perchlorate Ingestion.” This report was issued in January 2005 and provided a recommendation, since adopted by the EPA, that the chronic oral reference dose for perchlorate be set at $0.7 \mu\text{g kg}^{-1} \text{d}^{-1}$.

Occurrence in Food

Although the primary concerns from perchlorate contamination result from drinking water consumption, recent evidence has indicated that perchlorate may contaminate food items as well. A small survey of 22 lettuce samples purchased in Northern California showed perchlorate contamination in 4 samples. A subsequent study of California lettuce showed detectable perchlorate levels in all 18 samples tested. The toxicological significance of such findings has not been established but the studies clearly indicate that perchlorate can enter lettuce, presumably from growing conditions in which perchlorate has contaminated water or soil.

Milk also has been shown to be subject to perchlorate contamination. A small survey of seven milk samples purchased in Lubbock, Texas indicated that perchlorate was present in all of the samples at levels ranging from 1.12 to $6.30 \mu\text{g l}^{-1}$. To put such findings in perspective, the State of California has adopted an action level of $4 \mu\text{g l}^{-1}$ for perchlorate in drinking water whereas the EPA has yet to establish a specific drinking water limit.

The FDA's Total Diet Study was used to develop dietary estimates of perchlorate exposure based on samples collected between 2003 and 2006. Estimates of exposure for 14 age/sex subgroups using smallest lower bound to largest upper bound average perchlorate intakes ranged from 0.08 to $0.39 \mu\text{g kg}^{-1} \text{d}^{-1}$, all below the EPA's reference dose of $0.7 \mu\text{g kg}^{-1} \text{d}^{-1}$. Exposures were highest in infants and in children.

Toxicological Considerations

Perchlorate is thought to exert its toxic effects at high doses by interfering with iodide uptake into the thyroid gland. This inhibition of iodide uptake can lead to reductions in the secretion of thyroid hormones that are responsible for the control of growth, development, and metabolism. Disruption of the pituitary–hypothalamic–thyroid axis by perchlorate may lead to serious effects such as carcinogenicity, neurodevelopmental and developmental changes, reproductive toxicity, and immunotoxicity. Specific concerns relate to the exposures of infants, children, and pregnant women because the thyroid plays a major role in fetal and child development.

The ability of perchlorate to interfere with iodide uptake is due to its structural similarity with iodide. In recognition of this property, perchlorate has been used as a drug in the treatment of hyperthyroidism and for the diagnosis of thyroid or iodine metabolism disorders.

Ammonium perchlorate was found to be nongenotoxic in a number of tests, which is consistent with the fact that perchlorate is relatively inert under physiological conditions and is not metabolized to active metabolites in humans or in test animals.

Workers exposed to airborne levels of perchlorate absorbed between 0.004 and 167 mg perchlorate per day. These workers showed no evidence of thyroid abnormality and a No Observed Adverse Effect Level was established at 34 mg absorbed perchlorate per day. Perchlorate does not accumulate in the human body and 85–90 percent of perchlorate given to humans is excreted in the urine within 24 hours.

The National Academy of Sciences' perchlorate study identified inhibition of iodide uptake by the thyroid in humans as the most important toxicological endpoint, and cited a No Observed Adverse Effect Level (NOAEL) in humans at $7 \mu\text{g kg}^{-1} \text{d}^{-1}$. The reference dose for chronic oral perchlorate exposure of $0.7 \mu\text{g kg}^{-1} \text{d}^{-1}$ was determined by applying a 10-fold uncertainty factor to the NOAEL.

Bisphenol A

Bisphenol A (BPA) is an industrial chemical frequently used to produce food packaging materials such as plastic containers for food and drinks, baby bottles, and lining materials for food and beverage cans. The use of BPA has recently received considerable regulatory and public scrutiny resulting from potential concerns about BPA exposure to infants and children through their diets.

An Expert Panel Report of the National Toxicology Program estimated that infants and children receive greater exposures to BPA through their diets than do adults, with infant exposures ranging from $0.2 \mu\text{g kg}^{-1} \text{d}^{-1}$ to $24 \mu\text{g kg}^{-1} \text{d}^{-1}$.

These levels are below the established Tolerable Daily Intake level of $50 \mu\text{g kg}^{-1} \text{d}^{-1}$. The same report mentioned that there was some concern about BPA's potential effects on fetal and infant brain development.

Regulatory opinions concerning the safety of BPA vary dramatically. A World Health Organization panel did not advocate for additional regulations on BPA whereas the Australian and New Zealand Food Safety Authority does not consider health risks from BPA consumption by infants using baby bottles containing BPA. Health Canada did not anticipate adverse effects from BPA exposure but cautioned that the overall margin of safety was not large enough and developed a plan to ban the import and sale of baby bottles containing BPA.

The U.S. Food and Drug Administration (FDA) is carrying out in depth studies to further assess the potential risks of BPA in the diet. Although the studies are being conducted, the FDA supports industry activities to stop producing BPA-containing baby bottles, is looking into the development of alternatives to BPA for the linings of infant formula cans, and is seeking further public comment and external input on BPA scientific issues. At the present time, FDA does not recommend that families change the use of infant formula or foods as it considers the benefits of good nutrition as outweighing the potential risk from BPA exposure.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Food Safety: Heavy Metals; Mycotoxins – Occurrence and Toxic Effects; Pesticides. Phytochemicals: Health Effects

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Pesticides

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Glossary

Acceptable Daily Intake (ADI) The amount of a pesticide that can be taken in each day throughout a person's life based on all known facts that no harm will result. It includes a substantial safety factor.

Endocrine disruptors Chemicals that interfere with the endocrine (hormone) system of animals, including humans.

Maximum Residue Limits (MRLs) Statutory limits set on individual active ingredient and foodstuff combinations, based on residue levels that occur when the pesticide is used according to instructions on the label and following good agricultural practice (GAP).

Introduction

Pesticide is a generic term that covers a wide range of natural and synthetic chemicals (more than 700 in total) that are used to protect crops from attack from pests, both before and after harvest. There are many different sorts of pests. The term includes insects, slugs and snails, nematode worms, mites, rodents, weeds, molds, bacteria, and viruses. The chemicals can be applied before and during growth of the plant or on to the stored crop as, for example, fumigants, which are used to kill pests that have infested stored cocoa or grain. Chemicals used to treat pests on animals are not included; they are considered as veterinary medicines.

The pesticide formulation used by the farmer will include the pesticide chemical itself and a number of other chemicals that enable it to be applied and to work as effectively as possible. These will include solvents, adhesives, and surface-active agents such as emulsifiers. In some cases other chemicals, known as 'safeners', are applied to minimize the damage done to the crop while maintaining the effectiveness of the spray on the target. It is estimated that worldwide usage of pesticides is approximately 2.5 million tons with a cost of at least US\$25 billion.

Why Do We Need Pesticides?

Food crops are subject to attack by a multitude of pests and diseases and pesticides are applied to minimize the damage to the crop. It has been estimated that without protection world cereal crop yields would fall by between 46% and 83%. History is littered with records of crop failures and famine caused primarily by rodents, insects, or fungi. Some of these events have had a wide-ranging and long-lasting effect, like the 1845–46 Irish potato famine and the 1917–18 German 'turnip winter', the latter so called because the potatoes rotted and turnips were the only stored root crop that was available to feed the population through the winter. Both these events, in which 1.5 million and 700 000 people died, respectively, were caused by potato blight, infection by the fungus *Phytophthora*

infestans. Famine caused by massive swarms of locust is still all too common in Northern Africa and Arabia. Less spectacular but as disastrous is the loss of an estimated 30% of harvested crops in India to rodents.

In addition to the loss of the crop, pesticides are used to control agents which make the crop toxic rather than healthy. Two examples are the toxins caused by fungi. When an insect bores into a peanut it allows spores of the fungus *Aspergillus flavus* to enter and grow, producing aflatoxins, a series of carcinogens. When rye (*Secale cereale*) grows in damp conditions a fungus, *Claviceps purpurea*, can grow on the seed. If this seed is subsequently ground into flour and made into bread it can cause consumers to suffer hallucinations, gangrene, and death. Outbreaks amounting to epidemics were common in the Middle Ages in Europe and one occurred as recently as 1951 in France.

A second reason relates not so much to quantity as to quality. Supermarkets in the developed nations offer a wide range of fresh produce at competitive prices. Consumers do not like holes made by slugs and snails in their fresh lettuce. They do not expect scab marks on their apples, or holes made by small maggots in their carrots. Flour millers do not expect to have to clean the grain from weed seeds before milling. Even small defects can dramatically reduce the value of the crop, or indeed make it unsaleable, and the need for a competitive price requires minimal labor input so that application of pesticide is essential.

Types of Pesticides

There are currently approximately 600 pesticides, both natural and synthetic. Natural pesticides include both chemicals derived from plant sources and biological agents such as parasitic wasps, mites, bacteria, and chemicals contained within or exuded by plants or bacteria. Although there is no inherent reason why natural products should be any safer than synthetic ones (after all, insect venoms and toxins and poisonous plants are natural), it appears that the risks do lie in their potential impact on the environment rather than on their effect in food. There are also increasing numbers of cases where

plants have been given a gene which expresses a natural pesticide (see *Bacillus thuringiensis*, below).

Naturally derived pesticides make up only a few percent of the world pesticide market, but a great deal of work is being devoted to the screening of natural sources and this proportion will certainly increase. The most successful natural product development so far has been that of the pyrethrin insecticides, of which 33 are currently available.

The largest classes of pesticides are pyrethrins, organochlorines, organophosphates, and carbamates, although there are many smaller classes with only one or two members. The chemical structures of the key members of the major groups are given in Table 1.

Important Pesticide Groups

This list covers the important pesticide groups and some individual pesticides but does not attempt to be comprehensive.

Pyrethrins

Pyrethrins are chemically related to pyrethrin, which is a secondary metabolite found in the flowers of the pyrethrum plant (*Chrysanthemum cinerariaefolium*). Dried pyrethrum flowers were used as an insecticide in ancient China and in the middle ages in Persia. The dried flowers are still used. Current production is approximately 20 000 tons per annum centered in Kenya and Tanzania. The pyrethrins are effective insecticides, having very low dose rates and rapid knockdown of insects but being harmless to mammals under all normal conditions. Natural pyrethrins break down rapidly under the influence of oxygen and UV light. This limits their use in agriculture, but recently synthetic analogs have been developed to overcome these problems. Starting from the structure of the natural product a large number of synthetic compounds have been made. It is worth noting how they differ in effectiveness: deltamethrin is a broad-range insecticide; allethrin is particularly toxic to house flies (*Musca domestica*) but much less effective with other insects; flumethrin is active against cattle ticks; whereas others are acaricides or miticides with little or no insecticidal activity.

B. thuringiensis

B. thuringiensis is a widely distributed bacterium that during sporulation produces a crystal inclusion which is insecticidal when ingested by the larvae of a number of insect orders. Susceptible orders include Lepidoptera, Diptera, and Coleoptera. The action of *B. thuringiensis* was first observed in 1901 as the cause of a disease of silkworms. Several strains of the bacterium have been identified with activity against a range of insects including cabbage looper, tobacco budworm, mosquito, black fly, and more recently nematodes, ants and fruit flies. Although the bacterium appears an ideal insecticide (having a toxicity 300 times greater than synthetic pyrethroids), it requires careful use. It is most effective against neonates and early larval instars so that spraying must be timed for egg hatch. It also has no contact activity and must be

ingested so the plant must be well covered to ensure the insect receives a lethal dose. Furthermore it has a half-life in the field as short as 4 h, so careful timing is essential for it to be effective. Despite these limitations, it has been shown to be an important component of crop management programs.

One way of overcoming the problems of application of *B. thuringiensis* is to incorporate the gene responsible for expression of the protein into the crop plant. This has been achieved with maize (*Zea mays*) to protect against the European corn borer, with cotton (*Gossypium hirsutum*) to protect against a range of budworms and bollworms, and with potato (*Solanum tuberosum*) against Colorado beetle. (Cotton may seem irrelevant in a text on food but cottonseed oil is used extensively in cooking oils, margarines, and industrial fats.) This genetic modification has great benefits but care has to be taken that the food product has not changed in some unpredictable way. All genetically modified foods have to be extensively tested and cleared by regulatory agencies before release.

Neem Oil

This is an oil obtained from the neem tree, *Azadirachta indica* A. Juss. It has been used as an insecticide in India and Africa but is increasingly being developed as a significant commercial product. It contains a number of compounds, one of the most active being azadirachtin, which is an insect antifeedant but also shows growth inhibitory and endocrine disrupting effects. Commercial development of this product and its individual components is likely to result in a series of products as significant as those from pyrethrum.

Microbial Phytotoxins

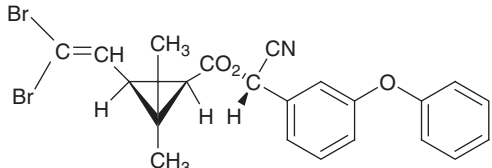
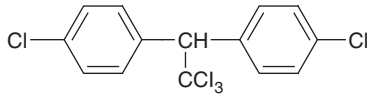
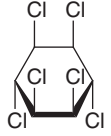
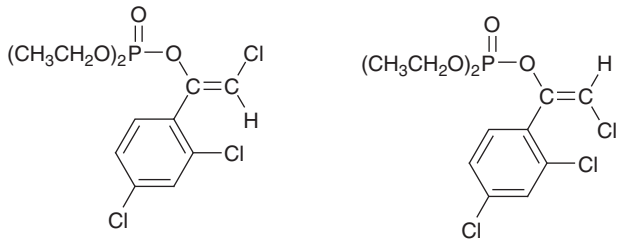
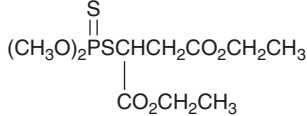
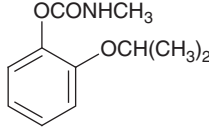
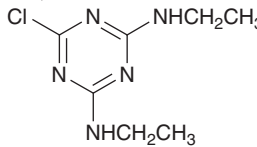
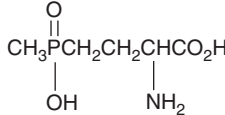
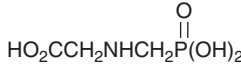
These are herbicides and include the highly commercially successful glufosinate, a synthetic form of phosphinothricin, first isolated from *Streptomyces hygroscopicus*, a soil-borne microbe. This compound is a potent, irreversible inhibitor of glutamine synthetase which is used in plants for photosynthesis. Many attempts have been made to make synthetic variants of phosphinothricin without success. Other members of this group include anisomycin and herbexidiene, derived from other *Streptomyces* strains. The veterinary insecticide, avermectin is derived from *Streptomyces avermitilis*.

Organochlorines

The organochlorines were the first group of synthetic insecticides and without them the dramatic decrease in malaria observed in the 1950s would have been impossible. The best known of this class is Dichlorodiphenyltrichloroethane (DDT) but others include 2,4 DD, hexachlorobenzene, and lindane. Of these only lindane (γ -hexachlorocyclohexane, see Table 1) is still in use in the developed world.

These compounds are very slow to break down in the environment and one result of this persistence was the decline in bird numbers graphically described by Rachel Carson in the book *Silent Spring*. The problem was that DDT was concentrated through the food chain and predator birds in particular were failing to raise chicks. Since the organochlorine pesticides

Table 1 Chemical structure and acceptable daily intake (ADI) of some pesticides

Compound	Class	Structure	ADI (mg per kg body weight)
Deltamethrin	Pyrethrin		0.01
DDT	Organochlorine		0.02
Lindane (HCH)	Organochlorine		0.008
Chlorlenvinphos	Organophosphate (mixture of two isomers)		0.002
Malathion	Organophosphate		0.02
Propoxur	Carbamate		0.02
Simazine	Tnazine		0.005
Glufosinate			0.02
Glyphosate			0.3

and other sources of organochlorines in the environment have been largely phased out, numbers of many species of birds have increased. It is recognized that pesticides are still having an adverse influence on numbers of some birds that inhabit farmland. However, this is not a straightforward effect. In the case of the gray partridge, for example, it is because herbicides have reduced the number of weeds, which in turn has reduced the number of insects that feed on the weeds, resulting in fewer insects for the chicks to eat.

The mechanism of action of the organochlorines is not known in detail although they appear to act on the central nervous system. In humans the organochlorine compounds tend to accumulate in the body fat and in mothers' milk. In 2009 the use of lindane in agriculture was banned under the Stockholm convention on persistent organic pollutants. It is classified by WHO and the Environmental Protection Agency (US) as moderately acutely toxic. Symptoms of headaches and dizziness – neurological effects – have been

reported in agricultural workers who had been chronically exposed to lindane. There is also concern about its potential carcinogenicity.

Organophosphorus Compounds

Organophosphorus compounds generally contain both sulfur and phosphorus linked to carbon atoms. Their discovery was a by-product of the development of nerve gases. The group includes parathion, malathion, dimethoate, diazinon, and chlorfenvinphos. They are used as herbicides, insecticides, and fungicides. They break down quickly in the environment and do not concentrate in body fats, although they may be stored for some time. However, their mode of action – inhibition of acetylcholine esterase – means that they affect both insects and mammals and their use depends on the effective dose in the target species being below the sensitivity of other species.

Acute effects of sublethal doses of organophosphates in man include sweating, salivation, abdominal cramps, vomiting, muscular weakness, and breathing difficulties. Concern has also been expressed about long-term effects following acute exposure. Research suggests that some victims may show reductions in some neurobehavioral tests when tested some months after exposure. There are also concerns that people who do not appear to have suffered acute poisoning have subsequently developed debilitating illnesses. Symptoms include extreme exhaustion, mood changes, memory loss, depression, and severe muscle weakness.

Carbamates

Carbamates are derived from carbamic acid and are used against both insects and weeds. They are also acetylcholine esterase inhibitors. They are very reactive and are used up rapidly after application.

Methyl Bromide

Methyl bromide was for many years the fumigant of choice for destroying insects in stored crops, but the internationally supported Montreal Protocol on Substances that Deplete the Ozone Layer agreed to phase out the production and use of this compound as part of the general restriction on volatile organohalogen compounds because of their damaging effect on the ozone layer. It is being replaced by a number of less environmentally damaging compounds, including phosphine, although none currently available is as effective or as cheap as methyl bromide.

Phosphine

Phosphine has been used as a fumigant for many years. It is highly reactive and leaves no residues but great care has to be taken in its application because it is very toxic to humans.

Control of Pesticides

Control over pesticides is exercised in two ways: stringent testing on new pesticides before they are permitted and measurement of the residue in the crop.

Testing Pesticides

There are a number of national and international bodies that approve new pesticides within their areas of responsibility. These include Codex Alimentarius, the European Union, and the US Food and Drug Administration (USFDA). Within the European Union, registration of pesticides has been harmonized under Directive 91/414 EEC. Annex 1 of this directive identifies all active ingredients permitted in pesticides. Within the UK, pesticide registration is carried out under the Control of Pesticide Regulations 1986 and is the responsibility of the Chemicals Regulation Directorate (CRD) of the Health and Safety Executive. In the USA the Food Quality Protection Act of 1996 replaced both the Food, Drug, and Cosmetic Act and the Insecticide, Fungicide, and Rodenticide Act to provide a comprehensive regulatory scheme for pesticides. Regulation is primarily the responsibility of the Environmental Protection Agency (EPA).

To gain approval for use, pesticides are subjected to an extensive testing program including toxicity tests on mammals, plants, insects, fungi, birds, bees, fish, earthworms, and other soil organisms. The toxicity studies include effects of pesticides on fetuses and infant animals. There are also environmental tests which include laboratory tests on the breakdown and movement of the chemical in plants, soil, water, air, mammals, birds, and fish. These latter tests determine the rate of decay in the various species. Laboratory tests are followed by prolonged field trials to determine the fate of the chemical and its breakdown products in the environment and to estimate how the pesticide is concentrated up the food chain. On an average it takes approximately 10 years to develop a new pesticide at a cost of approximately £50 million. The complete dossier of results has to be submitted to the approval body who determine whether the tests have been sufficiently rigorous to allow an acceptable daily intake (ADI) of the pesticide to be set. The ADI is defined as the amount of a pesticide that can be taken in each day throughout a person's life with the practical certainty, on the basis of all known facts, that no harm will result. This is determined on the basis of the highest level at which the pesticide has no observable effect in animal tests. This is then reduced by a factor of 10 in case humans are more sensitive than the animals used in the tests, and by a further factor of 10 to allow for cases where some humans may be more sensitive than others. In some cases, where the data show unusual effects, the safety factor can be increased from 100 to 500 or 1000. In practice the amount of pesticides to which the population is exposed is far below this level.

Table 1 includes the ADI for a number of the more common pesticides. There is no evidence that there are any cases where the combined effects of two pesticides are greater than the sum of their individual effects, in other words there is no evidence of synergy in toxicology between different pesticides.

Once maximum residue limits (MRL, see below) for foodstuffs have been set on the basis of GAP, a total dietary intake is determined by considering all commodities in which the pesticide is likely to be used, and assuming the upper range of consumption, all foodstuffs at the MRL and no losses during transport, storage or food preparation. This figure is then compared with the ADI. For all permitted pesticides in the UK the figure is below the ADI.

MRL

MRLs are statutory limits set on individual active ingredient and foodstuff combinations. They are based on residue levels which result when the pesticide is used according to the instructions on the label and in accordance with GAP. MRLs may be used to ensure that the pesticides are only being used in accordance with GAP. Many countries have codes of good operating practice with training for farmers and operators to ensure that pesticides are used at optimal levels. Some countries rely on the Codex Alimentarius Committee on Pesticide Residues to establish MRLs, whereas others set their own. (Codex Alimentarius is an international body which has more than 120 countries as members and their standards are increasingly being accepted as the basis of world trade in foodstuffs.)

In the USA the FDA used to set tolerances for pesticide/foodstuff combinations but under the 1996 Act it sets a level for each pesticide in all foods based on the principle of a reasonable certainty of no harm. This is defined as a lifetime cancer risk of less than one in a million. There is also a requirement that residue tolerances must be specifically determined as being safe for children.

Within the EU, individual member states have historically set their own MRLs which differ from state to state. Directive 76/895 established a common MRL setting regime and a series of subsequent directives has fixed the levels for a series of pesticides in fruit, vegetables, cereal products, and products of animal origin. There is an ongoing program to harmonize the levels throughout the Union.

Most industrialized countries have pesticide surveillance programs which cover both home-produced and imported commodities and these report annually. The EU has an annual specific coordinated program to check compliance in nominated combinations of pesticide and foodstuff. MRLs require sophisticated equipment for their determination because the levels are so low and the minimum detectable limit depends on the foodstuff. For example, the tolerance for aldrin and dieldrin (two organochlorines) in the USA is between 0.05 and 0.1 mg kg⁻¹ (parts per million), depending on the foodstuff. There are more than 600 different active ingredients available commercially. Because so many laboratories around the world have developed sophisticated rapid analytical techniques to allow them to screen pesticides by class so that retailers, food manufacturers, and governments can carry out analyses as a matter of routine.

The MAFF 7th Report of the UK Working Party on Pesticide Residues in 1996 showed 68% of samples had no detectable residue, 31% had residues below the MRL, and <1% were over the MRL. Similar results were obtained by the FDA who

report results with relation to the tolerance to the pesticide/commodity combination. In 1995, of more than 9000 samples analyzed, 64% had no detectable residues, 34% had residues below the tolerance, <1% had residues over the tolerance, and <1% had residues for which there is no tolerance in that particular pesticide/commodity combination.

In all cases where MRLs or tolerances are exceeded follow-up action is taken. For home-produced materials, this involves investigation of the grower and prosecution if necessary. For imports, exceeding the level causes the consignment to be refused entry.

Maximum levels of pesticides are also set for drinking water. Pesticides get into water from spraying, runoff, percolation, or from treatment of fish in aquaculture. Good practice is increasingly being developed to minimize the levels in raw water and treatment works are developing systems to reduce incoming levels to levels acceptable for drinking water.

Endocrine Disruption

The possibility that a number of chemicals discharged into the environment as a result of human activity may disrupt the endocrine system of a wide range of mammals has recently been given considerable prominence. Among the chemicals cited are the organochlorine pesticides, most of which have now been withdrawn for other reasons. Although there is no doubt that there are a significant number of cases of endocrine disruption, the evidence to point to any particular chemical as a cause is lacking. It is also worth noting that deliberate endocrine disruption is a mechanism of a number of natural insecticides which act so as to inhibit development of juvenile larvae to adults. Fortunately these pesticides are reactive and usually have a short life in the field.

It is also true that there are many naturally occurring endocrine disruptors, including the phytoestrogens present in vegetables, notably soya beans, peas, beans, cabbage, and hops. However, since this issue is very serious a considerable amount of work has now been initiated and its results will have implications for future testing of pesticides.

Future Prospects

In many parts of the world it is recognized that there has been too great a reliance on pesticide use and not enough on improving agricultural practices. There is increasing pressure to move toward minimizing pesticide usage in order to both improve the environment and to reduce cost. This is being done by using newer, more specific pesticides and by adopting improved agricultural practices and integrated pest management (a combination of biological and chemical control).

Biological control is not new. In the 1930s *Macrocentrus homonae* was introduced into Sri Lanka from Indonesia to control the tea small leaf roller (*Adoxophyes*) with such success that no chemical control measures are needed for this pest even today. More recently, there have been some impressive results from using predator insects, for example, in the control of cassava green mite (*Mononychellus tanajoa*) in West Africa and white fly in European greenhouses.

In terms of agricultural practice, improved crop hygiene, crop rotation, better understanding of optimal timing of application, and varying sowing dates, together with the development of more powerful and more discriminating pesticides has brought about a decrease in pesticide inputs. This is seen dramatically in the case of oil seed rape (canola). Less than 1% of the weight of herbicide applied to this crop in 1983 was applied in 1993.

Unfortunately pests develop resistance to individual pesticides over time and research is continually needed to develop both new pesticides and resistant varieties of crops to keep the pests in check. There has been some success with new pesticides having new modes of action such as the anti-feedants and antimolting agents, but this will be a continuing battle for the foreseeable future.

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FOOD SECURITY

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Abbreviations

DFID	United Kingdom Department for International Development
FANTA	Food and Nutrition Technical Assistance

HFIAS	Household Food Insecurity Access Scale
IPC	International food security Phase Classification
UNICEF	United Nations Children's Fund
WFP	United Nations World Food Programme

Glossary

Cost of the diet A linear programming tool that estimates the lowest cost diet that meets all nutrient requirements of individuals of a household, using data on which foods are locally available, their nutrient content and their price. This cost estimate can be compared to income or expenditure data to determine which proportion of the population would be able to afford an adequately nutritious diet.

Dietary diversity Extent to which the diet includes foods from different food groups. The greater the diversity, the more likely the diet meets nutrient requirements.

Food security When all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life.

Livelihood A livelihood comprises the capabilities, assets (stores, resources, claims and access), and activities required for a means of living.

Malnutrition Malnutrition is a broad term commonly used as an alternative to undernutrition but technically it also refers to 'overnutrition'. Please refer to definition of undernutrition below. People are also malnourished if they

are overweight or obese as a result of consuming too many calories, while intake of vitamins and minerals may still be inadequate. Overweight and obesity increase the risk of cardiovascular disease and diabetes.

Undernourishment The number of undernourished people is estimated at national level, and aggregated at global level, and represents the number of people that have to survive on less than 2100 kcal day⁻¹, which is estimated from food balance sheets (food production minus export plus import).

Undernutrition Undernutrition is defined as the outcome of insufficient food intake and repeated infectious diseases. It includes being underweight for one's age, too short for one's age (stunted), thin for one's height (wasted) and deficient in vitamins and minerals (micronutrient malnutrition).

Window of opportunity The first 1000 days of a child's life, from conception until 24 months of age, during which ensuring appropriate nutrition is essential to enable the child to have the best start in life and develop to its full potential.

Introduction

Food security is a very important determinant of whether people can lead an active and healthy life, because it determines their access to foods required to meet nutrient needs. This article reviews the definition of food security, the indicators used to measure food security depending on the level at which it is studied, how it links to nutrition and health as well as to livelihoods, what it is affected by, the consequences of food insecurity, and measures that are taken to mitigate these causes and consequences. Special attention will be paid to why it is important that food security assessments also include an estimate of the extent to which nutrient needs are being met, and the approaches and indicators, which can be used for that purpose.

Framework and Definitions

Food Security

Food security was defined, at the World Food Summit in 1996, as "when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life".

Food security has many dimensions and can be studied at different levels. The different dimensions include food availability, access to, and utilization of food. Recognizing the fact that food security is not constant the stability of the food supply is also an important dimension of food security. The utilization of food refers to what happens with food at the

household level, i.e., allocation among different members, preparation, and utilization by the body, i.e., digestion, bioavailability, etc., which is closely linked to health status as well as age and physiological status. The different levels at which food security can be studied include individual, household, community, district, province, country, and region. Depending on the level of focus, different dimensions, aspects, and indicators can be used.

Meeting Nutrient Needs

It is important to note that the definition makes explicit reference to safe and nutritious food to meet dietary needs, i.e., meeting every individual's nutrient requirements for leading an active and healthy life. Whether an individual's nutrient requirements are met depends on food consumption as well as on disease, as the latter increases nutrient needs and also affects the way nutrients are metabolized and used by the body.

The United Nations Children's Fund (UNICEF) framework for causes of malnutrition is focused on the individual level (see Figure 1 in which it has been integrated). It specifies that

direct causes of malnutrition are food consumption and health or disease; underlying causes are access to food, caring practices, hygiene conditions, and health-care services; basic causes are human, economic, and organizational resources and controls, including education, governance, etc. Whereas access to food determines food consumption and thus nutrient intake, and hygiene conditions and health-care services determine health and disease and thus nutrient needs and utilization, caring practices affect how accessible food is used, i.e., food distribution among household members, food preparation, feeding frequency, breastfeeding practices, and how health-care services are utilized. Simply put, caring practices reflect the choices made from among the options available to the individual or the household, and are also influenced by knowledge and empowerment.

Food Security as a Component of Livelihood Security

The United Nations World Food Programme (WFP) Food and Nutrition Security Conceptual Framework proposed in 2009 (see Figure 1) combines the UNICEF framework and the

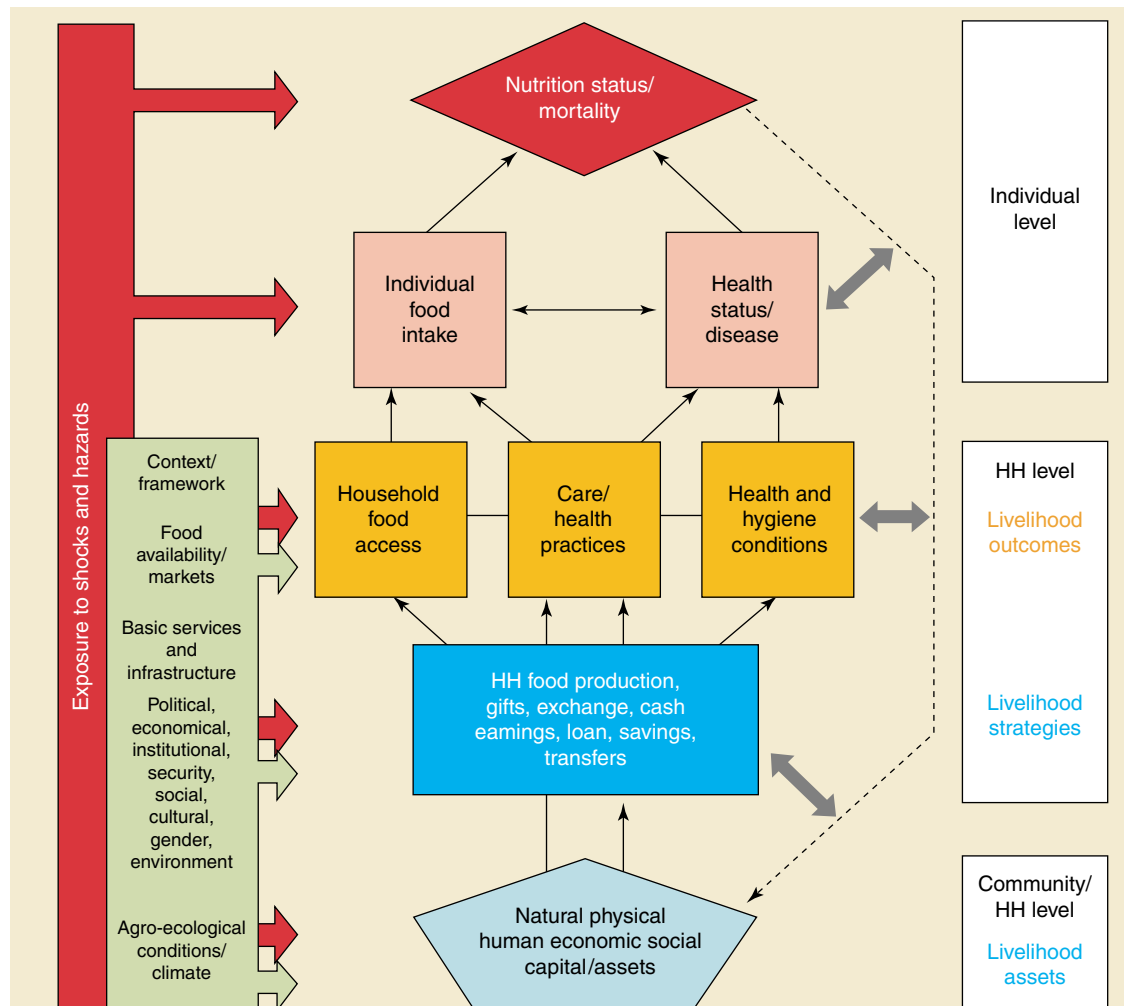


Figure 1 WFP Food and Nutrition Security Conceptual Framework (based on UNICEF conceptual framework for causes of malnutrition and DFID sustainable livelihoods framework). Reprinted with permission from WFP (2009) *Comprehensive Food Security and Vulnerability Analysis Guidelines*. Rome, Italy: WFP. Available from www.wfp.org

United Kingdom Department for International Development (DFID) Sustainable Livelihoods Framework, because food security is closely linked to household livelihood security, which can be described as the ability of a household to meet the basic needs of its members. Livelihood can be described as comprising the capabilities, assets (stores, resources, claims, and access), and activities required for a means of living. A livelihood is sustainable when it can cope with and recover from stress and shocks, maintain or enhance its capabilities and assets, and provide sustainable livelihood opportunities for the next generation; when it contributes net benefits to other livelihoods at the local and global levels in the long and short term. The livelihood unit is usually the household. Household livelihood security is defined as adequate and sustainable access to income and resources to meet basic needs. Basic needs include food, proper nutrition, clean water, health and health facilities, economic and educational opportunities, housing, physical safety, and time for community participation and social integration. Households use six main tangible and intangible forms of capital, i.e., human (skills, knowledge, health, and nutritional status), financial (income, credits, savings, and liquid assets), natural (crops grown), physical (assets and land), social (support networks), and political (participation in community decisions, power).

As shown in the framework, we distinguish livelihood assets, strategies and outcomes, and the outcomes that affect an individual's food consumption (i.e., nutrient intake), health or disease state (i.e., nutrient needs and nutrient utilization), and care and health practices (i.e., choices from among available options).

Policies, institutions, and organizations affect livelihood assets and strategies. Policies can be split into the following categories: macroeconomic policies, i.e., measures aimed at stabilizing an economy; social policies, which aim to protect and improve health, nutrition, education of the disadvantaged; sectoral policies that focus on specific areas within an economy, such as agriculture, health, water, sanitation, and environment. At the interface between these policies and households are the institutions and organizations that implement or affect the policies, i.e., the state, formal civil society, informal civil society, and the private sector.

Shocks and hazards, which can range from death of a family member to increased food prices, droughts, floods or armed conflict, can affect different and multiple dimensions of the framework, with different intensity and for a variable period of time.

Thus, the Food and Nutrition Security Conceptual Framework shows how many different factors affect food availability, food access, food utilization and stability of food supplies, which together determine food security, and how food security in turn is a component and function of livelihood security, which is a function of livelihood assets and strategies and is affected by both macro- and microlevel factors. It is also important to note the rich multidisciplinary nature of the topic of food security.

Indicators and Classification of Food Security Status

The level at which food security is of interest, i.e., household, community, district, or country, determines which factors should be assessed.

International Food Security Phase Classification (IPC) for Countries

For example, the IPC provides guidance to countries for classifying a country's regions and subregions. It emphasizes that as much as possible existing and relevant information should be used, and that there is no fixed set of indicators and methods to refer to.

Table 1 shows the five stages of food security that are distinguished in this IPC classification together with proposed indicators and their cut-offs for the different stages. Some indicators reflect context and livelihood assets, such as civil security, 'structural' (underlying hindrances to food security), livelihood assets/capital; some reflect vulnerability, such as hazards, destitution/displacement; others reflect underlying causes of malnutrition such as water access/availability, food access/availability, dietary diversity; the top ones reflect outcome, i.e., crude mortality rate ($\times/10\,000\text{ day}^{-1}$), disease, acute malnutrition (wasting), and stunting. Some of these apply to the national or subnational situation (civil security, hazards, and structural hindrances), whereas others have to be collected from individuals (nutritional status) or households (food and water access). This means that information from different sources needs to be gathered, reviewed, and classified in order to arrive at a food security classification of a country's regions or subregions.

The IPC partners have substantial experience with the collection and interpretation of these indicators and their classification. Here, we will focus on the aspects of food security that are most directly linked to food intake and nutrient adequacy, because these are most closely linked to the outcomes, nutritional status, and health.

Indicators of Nutrient Intake at the National, Household, and Individual Level

Nutritional status and health are the ultimate outcomes of food security that are very much of interest because they determine human capital at present and in the future. It is very important to recognize the fact that foods are a source of nutrients and that the human body requires approximately 40 different nutrients for growth, development, and health. Reaching an adequate intake for each of these nutrients requires consumption of a diverse diet, including plant and animal source foods as well as fortified foods. Also different groups in the population require these nutrients in different amounts, depending on their age (growth spurts), physiological status (pregnancy and menstruation), health, and physical activity.

The indicators of food intake in the IPC classification are food access/availability, expressed as $\text{kcal capita}^{-1}\text{ day}^{-1}$, and dietary diversity, expressed as deficient or sufficient. These two specific indicators are usually collected either at the household level or, in the case of $\text{kcal capita}^{-1}\text{ day}^{-1}$, estimated at the national level. The latter indicator is also used for Millennium Development Goal no. 1, i.e., the proportion of the population that is undernourished, which presents the proportion of the population that has to survive on less than $2100\text{ kcal capita}^{-1}\text{ day}^{-1}$ and is estimated from food balance sheet data (i.e., total kcal available from food production and net import or export) compared to total population size. (The other

Table 1 International food security phase classification reference table

Phase classification		Key reference outcomes <i>Current or imminent outcomes on lives and livelihoods. Based on convergence of direct and indirect evidence rather than absolute thresholds. Not all indicators must be present for classification.</i>	
1	Generally food secure	Crude mortality rate	<0.5/10 000/day
		Acute malnutrition	<3% (w/h <-2 z-scores)
		Stunting	<20% (h/age <-2 z-scores)
		Food access/availability	Usually adequate (> 2100 kcal ppp day), stable
		Dietary diversity	Consistent quality and quantity of diversity
		Water access/avail.	Usually adequate (> 15 litres ppp day), stable
		Hazards	Moderate to low probability and vulnerability
		Civil security	Prevailing and structural peace
		Livelihood assets	Generally sustainable utilization (of 6 capitals)
2	Moderately/borderline food insecure	Crude mortality rate	<0.5/10 000/day; U5MR <1/10 000/day
		Acute malnutrition	>3% but <10% (w/h <-2 z-score), usual range, stable
		Stunting	>20% (h/age <-2 z-scores)
		Food access/availability	Borderline adequate (2100 kcal ppp day); unstable
		Dietary diversity	Chronic dietary diversity deficit
		Water access/avail.	Borderline adequate (15 litres ppp day); unstable
		Hazards	Recurrent, with high livelihood vulnerability
		Civil security	Unstable; disruptive tension
		Coping	'Insurance strategies'
		Livelihood assets	Stressed and unsustainable utilization (of 6 capitals)
		Structural	Pronounced underlying hindrances to food security
3	Acute food and livelihood crisis	Crude mortality rate	0.5–1/10 000/day, U5MR1–2/10 000/day
		Acute malnutrition	10–15% (w/h <-2 z-score), > than usual, increasing
		Disease	Epidemic; increasing
		Food access/availability	Lack of entitlement; 2100 kcal ppp day via asset stripping
		Dietary diversity	Acute dietary diversity deficit
		Water access/avail.	7.5–15 litres ppp day, accessed via asset stripping
		Destitution/displacement	Emerging; diffuse
		Civil security	Limited spread, low intensity conflict
		Coping	'Crisis strategies'; CSI > than reference; increasing
		Livelihood assets	Accelerated and critical depletion or loss of access
4	Humanitarian emergency	Crude mortality rate	1–2/10 000/day, >2x reference rate, increasing; U5MR > 2/10 000/day
		Acute malnutrition	>15% (w/h <-2 z-score), > than usual, increasing
		Disease	Pandemic
		Food access/availability	Severe entitlement gap; unable to meet 2100 kcal ppp day
		Dietary diversity	Regularly 3 or fewer main food groups consumed
		Water access/avail.	<7.5 litres ppp day (human usage only)
		Destitution/displacement	Concentrated; increasing
		Civil security	Widespread, high intensity conflict
		Coping	'Distress strategies'; CSI significantly > than reference
		Livelihood assets	Near complete and irreversible depletion or loss of access
5	Famine/humanitarian catastrophe	Crude mortality rate	> 2/10 000/day (example: 6000/1 000 000/30 days)
		Acute malnutrition	> 30% (w/h <-2 z-score)
		Disease	Pandemic
		Food access/availability	Extreme entitlement gap; much below 2100 kcal ppp day
		Water access/avail.	< 4 litres ppp day (human usage only)
		Destitution/displacement	Large scale, concentrated
		Civil security	Widespread, high intensity conflict
		Livelihood assets	Effectively complete loss; collapse

Source: Reprinted with permission from Integrated Food Security Phase Classification (2008) *IPC User Guide*, Version 1.0. The IPC in the Central and Eastern Africa Region project. Nairobi: FAO. Available from www.ipcinfo.org (accessed on 17 January 2011).

indicator for MDG1 is underweight, i.e., the proportion of children aged 0–59 months that have a weight that is too low for their age (< -2 SD of the median of the reference population)).

However, nutrient needs are different for different individuals. For that reason, adequacy of food intake, in terms of energy (kcal), dietary diversity, and if possible also specific nutrients (such as micronutrients, protein, and fat), are best collected at the individual level and expressed per population group, in order to identify which groups are most at-risk from a nutritional point of view. However, collecting these data at the individual level is very labor intensive and requires detailed food composition data. Furthermore, there are several ways of collecting and interpreting these data. Therefore, very often outcome indicators are collected at the individual level, i.e., nutritional status and health, together with one or more household level indicators of access to food and possibly dietary diversity.

Household Food Insecurity Access Scale

Food and Nutrition Technical Assistance (FANTA) has developed a Household Food Insecurity Access Scale (HFIAS) that builds on the experience with the US Household Food Security Module and the Radimer/Cornell scale. It has been well validated and is increasingly being used. **Table 2** shows the nine questions asked of households, which range from having experienced a small degree of food insecurity, i.e., worrying about not having enough food, to not having had any food for a consecutive 24 h period. The reference period is the past 4 weeks and each question is followed by a question about how frequently this specific experience occurred in the previous 4 weeks. This scale thus assesses access to food, mainly in terms of meeting self-perceived adequacy of quantity and whether there was deterioration from what the household is used to. It has recently evolved to a smaller subset of questions that are used to define the severity of household hunger (Household Hunger Scale). It is important to note that neither of the scales enquire about dietary diversity or individual foods consumed, nor do they estimate individual nutrient intake.

Dietary Diversity

Dietary diversity provides an indication of the potential adequacy of nutrient intake, because a diet that is sourced from a

greater variety of food groups provides a wider range of nutrients (e.g., carbohydrates, fat, protein, fat soluble vitamins, water soluble vitamins, minerals, essential fatty acids, sulfur containing amino acids, etc.).

Methods for assessing dietary diversity ask how frequently, usually during the previous 7 day or 24 h, foods from different food groups were consumed. The number of food groups can range from 7 to 15, for example staples, lentils and legumes, oils and fats, leafy vegetables, colored vegetables, fruits, dairy products, eggs, meat and poultry, fish, and sugary foods and drinks.

These answers can be processed and used in different ways. For example, when the questions refer to a 7 day period, the total number of days that different food groups are consumed can be added together, thus if there are nine food groups, the total score would be $9 \times 7 = 63$, and households that consumed three foods every day would have a score of 21. One can also decide to count the food groups that provide more nutrients than just energy (i.e., in the above example leave out staples, oils and fats, and sugary foods and drinks), or calculate a score that reflects just the frequency of consumption of particular food groups, such as animal source foods. For categorizing the obtained scores into different levels of dietary diversity, knowledge of the local situation is required and preferably a relationship should have previously been demonstrated between differently derived scores and nutritional status, for example of children under 5 years, so that the best cut-offs can be chosen.

Whereas good correlations have been shown between household dietary diversity and household expenditure on different food categories as well as between household dietary diversity and children's nutritional status (stunting and micronutrient deficiencies), this relationship has not been established for every population from which dietary diversity data are being collected. This means that part of the use and interpretation of dietary diversity data has to refer to findings in other settings and be based on knowledge and judgment of the local situation.

Common Pitfalls of Analyzing Causes of Malnutrition

As shown above, food security is generally assessed at the household level or higher, i.e., at the community, subregion or country level, whereas its main outcome, nutritional status, is

Table 2 Questions of the FANTA household food insecurity access scale

No.	Question
1	In the past 4 weeks, did you worry that your household would not have enough food?
2	In the past 4 weeks, were you or any household member not able to eat the kinds of foods you preferred because of a lack of resources?
3	In the past 4 weeks, did you or any household member have to eat a limited variety of foods due to a lack of resources?
4	In the past 4 weeks, did you or any household member have to eat some foods that you really did not want to eat because of a lack of resources to obtain other types of food?
5	In the past 4 weeks, did you or any household member have to eat a smaller meal than you felt you needed because there was not enough food?
6	In the past 4 weeks, did you or any household member have to eat fewer meals in a day because there was not enough food?
7	In the past 4 weeks, was there ever no food to eat of any kind in your household because of a lack of resources to get food?
8	In the past 4 weeks, did you or any household member go to sleep at night hungry because there was not enough food?
9	In the past 4 weeks, did you or any household member go a whole day and night without eating anything because there was not enough food?

Source: Reprinted with permission from Coates J, Swindale A, and Bilinsky P (2007) *Household Food Insecurity Access Scale (HFIAS) for Measurement of Household Food Access: Indicator Guide*, vol 3. Washington, DC: Food and Nutrition Technical Assistance (FANTA) Project, Academy for Educational Development. Available from www.fanta.org

assessed at the individual level. Depending on which indicators are used to assess food security, the conclusion that food security is not a problem whereas stunting, for example, affects 30% of the children under five, is very common. In these cases, the cause of malnutrition is then often sought in the nonfood-related factors of the framework for causes of malnutrition, i.e., hygiene, health and caring practices, rather than in a deficient intake of specific nutrients. This reasoning fails to recognize that as long as the food security indicator does not assess adequacy of nutrient intake, but just focuses on access to food, which can be any food, 'food' may still be the main problem causing malnutrition.

For this reason it is very important to include dietary diversity as an indicator of food security, and preferably not only at household level, but also for specific groups such as children under five, and to analyze the data in such a way that it is a good proxy for nutrient adequacy of the population group of interest. For example, in the case of under fives, they require animal source foods, including dairy foods, as well as fortified foods in their diet in order to meet their nutrient requirements, especially of bioavailable minerals and type II nutrients that are important for growth. The intake of foods from these groups should thus be specifically assessed and reported.

Another way of assessing whether food consumption could possibly meet nutrient requirements is through linear programming. Based on availability of foods, their nutrient composition and their price, the lowest cost diet that would meet all nutrient requirements of a family composed of different members, e.g., a child younger than 24 months, a school-age child, a lactating mother, a father and a grandparent, can be calculated. This price can then be compared to income or food expenditure data in order to determine what proportion of the population could in theory afford a diet that meets all nutrient requirements. It is important to note that in practice, it is likely that a larger proportion does not meet all the nutrient requirements, because food choices are also affected by cultural practices and taste preferences that may not necessarily concur with best choice from a nutrient content point of view. This method, which is also known as the Cost of the Diet tool, has been developed by Save the Children UK and is known as the Cost of the Diet tool.

Knowing the causes of malnutrition is a must for being able to address it. Thus, when the prevalence of malnutrition is high, it is important to assess the level of food insecurity, including dietary diversity, and possibly also whether households could in theory afford a diet that could meet the nutrient requirements of all its members at the lowest cost. In addition to these factors that largely determine food consumption and hence nutrient intake, factors that affect nutrient needs, i.e., health or disease, as well as caring practices, which affect the choices made from among the possibilities available, are also important.

Factors Affecting Food Security

The conceptual framework shows that many different factors can affect livelihoods and food security, as well as the degree of vulnerability to changes thereof. Factors that affect food availability generally have to do with climate change,

seasonality affecting crop production, or large-scale political changes such as wars. Food access is to a large extent determined by purchasing power, which is a function of income and food prices as well as of prices of other commodities such as fuel (for cooking and transport). The stability of food supplies is usually the first aspect of food security that changes and can be regarded as an early warning sign that livelihoods and food security status are likely to deteriorate. Last but not least, food utilization is affected by household dynamics (intra-household distribution), behavioral factors, and health or disease (utilization by the body), both of which are less likely to change unless household structure and livelihood change substantially.

Relatively new and emerging challenges for food security include climate change and increasing natural disasters (affecting food availability) as well as high and fluctuating food prices and the global financial crisis (affecting access to food) and rapid urbanization (reliance on cash economy, smaller social networks to assist in coping with shocks, etc.).

With the food price increase in 2008, the global economic crisis in 2009, and the rising food prices in 2011, it is important to determine in which countries the population is at greatest risk of suffering from food insecurity, and to identify measures for mitigating the causes and consequences in the best possible way. It has been found that countries that were more linked to the global economy, more dependent on import and where a large proportion of the population were net-buyers of food, were more at-risk.

Consequences of Food Insecurity

The consequences of food insecurity are several. Very importantly, due to decreased dietary diversity and also quantity, meeting nutrient needs becomes more and more difficult and therefore nutritional status and health deteriorate. This has short-term consequences, such as increased morbidity and mortality, as well as long-term consequences because an entire generation of young children can be affected for the rest of their lives.

The latter is due to the fact that the window of opportunity for development of a young child is concentrated within its first 1000 days of life, starting from conception until 24 months of age. When the child is undernourished in this period of his or her life, he or she is at increased risk of morbidity and mortality, and later in life of delayed school enrolment, poorer school performance including earlier drop-out, lower income earning potential, and higher risk of chronic disease (diabetes, cardiovascular disease, and obesity).

Food insecurity also has consequences for other livelihood priorities, such as education of children, health-care seeking behavior, asset ownership, etc., which in-turn also relates to vulnerability to shocks. **Figure 2** shows consequences for livelihood and food consumption when households descend into stress and distress, and the concurrent health consequences.

Health-care seeking and treatment adherence behaviors that are particularly affected by food insecurity are those of HIV/AIDS and tuberculosis, as populations affected by both overlap geographically. Firstly, these diseases are likely to increase food insecurity as household members fall ill and are

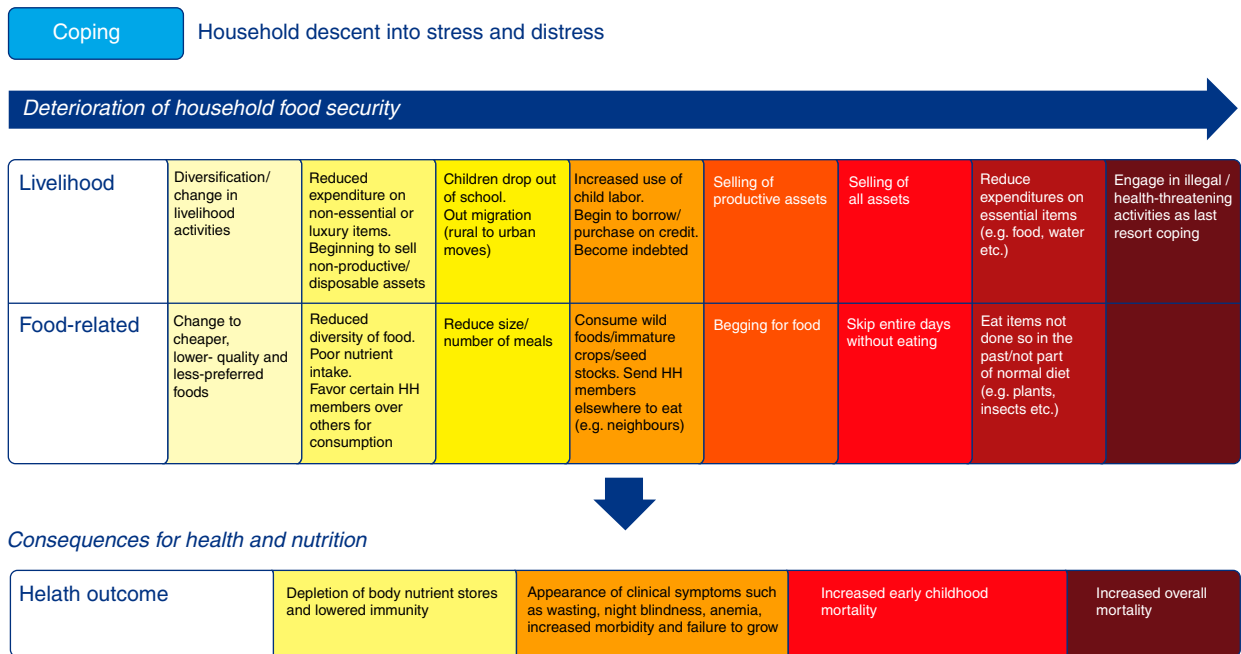


Figure 2 Changes in livelihood, food selection and consumption, and nutritional status and health outcome when households descend into stress and distress. Reprinted with permission from Klotz C, de Pee S, Thorne-Lyman A, Kraemer K, and Bloem MW (2008) Nutrition in a perfect storm: Why micronutrient malnutrition will be a widespread health consequence of high food prices. *Sight and Life Magazine* 2: 6–13.

hence not able to contribute to food production or earn an income. Secondly, their food insecurity affects diagnosis, treatment uptake and treatment adherence because that incurs costs, for example for transport, which hence competes with other priorities such as buying food for the family. Thirdly, some side effects of treatment such as nausea can be overcome by consumption of more palatable foods, which requires access to a variety of foods. Fourthly, recovery from malnutrition, which is what many people present with as they seek a diagnosis and enroll for treatment, requires treatment of the HIV and opportunistic infections as well as consumption of nutritious foods that need to be acquired.

Mitigating the Consequences of Food Insecurity

Measures to mitigate food insecurity can be several and depend on the causes, on who is affected and how, and on what changes can be realized in the particular context. Depending on whether the deterioration of food security is related to food availability, access, utilization or stability of supplies, different measures can be taken.

If the problem is related to food production, i.e., food availability, large-scale multiyear agricultural measures may be required, for example to mitigate impact of climate change, increase yields, improve irrigation, develop storage, transport and markets for produce, etc. Although this is often focused on staple crops, there can also be a component focused on dietary diversity and household level food production including vegetables, fruits, small livestock, community fish ponds, etc. These types of interventions are, in addition to being income-generating, also more nutrition-oriented,

especially when combined with an education component to emphasize the importance of consuming a diverse diet.

Food access problems are typically experienced by households that largely depend on cash purchases, and are thus vulnerable to changes in income and changes in food prices. Urban households as well as rural households that are net-buyers because they do not produce enough food throughout the year were hit hardest by the recent high food prices and the global financial crisis. They faced lower or loss of income, increased food prices, lower subsidies or higher taxes on food and fuel, and also reduced public spending on health, water and sanitation.

Mitigating these kinds of consequences can either be at the 'blanket' level, i.e., by reducing prices, or removing tax, for specific staple foods (bread and maize), which apply to everyone, or they can target the most vulnerable, for example through safety-net programs that provide free or subsidized foods to households that are identified to be most in need. Such identification can for example be done through self-selection, i.e., participation in public work programs in return for which a basic food supply is provided, or through applying specific selection criteria such as female- or child-headed households, income below a percentage of the minimum wage, etc. The assistance provided can be in the form of food, vouchers for food, fuel or transport, or cash. Depending on the context, one or the other may be more suitable.

However, because of the far-reaching consequences of food insecurity and poor dietary diversity on nutrition and health, food assistance for such households should not only focus on meeting caloric needs and having enough meals per day, but also on meeting nutrient needs, especially of the most vulnerable, i.e., young children and pregnant and lactating

women. This may require making specific products available that can increase their diet's content of specific nutrients. Such products can be in paste or powder form and if their aim is to improve intake of specific nutrients in addition to the prevailing, and affordable, home diet, an amount of 1–20 g day⁻¹ (i.e., up to 125 kcal day⁻¹) should generally be sufficient.

Conclusions

In summary, food security is achieved when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life. Food security has many different dimensions, i.e., food availability, food access, food utilization and stability of food supply, and is closely linked to livelihood security, i.e., the ability to provide for the basic needs of the family such as food, shelter, clean water, education, etc. The fact that food security can be studied at many different levels, from the national to household or individual level, means that the outcome of food security, i.e., nutritional status and health, and food availability and access to food are often the areas of focus. However, when a population is affected by malnutrition, and access to food seems to be in order, it is very important to determine whether nutrient needs are actually being met. This can be done by including measures of dietary diversity, nutrient intake, food expenditure and ability to afford a lowest cost diet that could meet all nutrient requirements. It would be most appropriate to refer to 'Food and Nutrient Security'.

See also: Biochemical Indices. Dietary Intake Measurement: Methodology. Dietary Surveys: Surveys of Food Intake in Groups

and Individuals. **Hunger. Nutritional Assessment:** Anthropometry; Clinical Examination. **Nutritional Surveillance:** Developing Countries

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FRUCTOSE

Absorption and Metabolism

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Abbreviations

ATP	adenosine triphosphate
CO ₂	carbon dioxide
DHAP	dihydroxyacetone phosphate

GLUT-5	glucose transporter-5
HFCS	high-fructose corn syrup
PFK	phosphofructokinase
VLDL	very low density lipoprotein

Introduction

Fructose, a monosaccharide, is naturally present in fruits and is used in many food products as a sweetener. This article reviews the properties and sources of fructose in the food supply, the estimated intake of fructose in Western diets, the intestinal absorption of fructose, and the metabolism of fructose and its effect on lipid and glucose metabolism. The health implications of increased consumption of fructose are discussed, and inborn errors of fructose metabolism are described.

Properties and Sources of Fructose

Fructose has a fruity taste that is rated sweeter than sucrose. Sweetness ratings of fructose are between 130% and 180% (in part dependent on the serving temperature), compared to the standard, sucrose, rated at 100%. Both sucrose and fructose are used extensively in foods to provide sweetness, texture, and palatability. These sugars also contribute to the appearance, preservation, and energy content of the food product.

Natural sources of dietary fructose are fruits, fruit juices, and some vegetables. In these foods, fructose is found as the monosaccharide and also as a component of the disaccharide sucrose (Table 1). However, the primary source of fructose in Western diets is sugars added to baked goods, candies, soft drinks, and other beverages sweetened with sucrose and high-fructose corn syrup (HFCS). HFCS is produced by hydrolyzing the starch in corn to glucose, using α -amylase and glucoamylase. This is followed by treatment with glucose isomerase to yield a mixture of glucose and fructose. The process typically produces an HFCS composed of 42% fructose, 50% glucose, and 8% other sugars (HFCS-42). By fractionation, a concentrated fructose syrup containing 90% fructose can be isolated (HFCS-90). HFCS-42 and HFCS-90 are blended to produce HFCS-55, which is 55% fructose, 41% glucose, and 4% other sugars. HFCS-55 is the preferred sweetener used by the soft

drink industry, although HFCS-42 is also commonly used as a sweetener in many processed food products. Concentrated fruit juices, such as apple juice and white grape juice, are also used to sweeten beverages. The amount of fructose in fruit juices varies, as does its proportion with glucose, but clearly the use of concentrated apple juice provides more fructose relative to glucose (Table 1), as compared to either sucrose or HFCS-55. Nevertheless, considering the variety of sweeteners commonly available, it is likely that fructose constitutes close to 50% of energy from added sweeteners.

As a result of the addition of sweeteners and sugars to so many food products, the consumption of fructose has increased from the mid-1970s to the mid-2000s. Sugars added to the diet are difficult to quantify accurately, but based on food intake survey data, total fructose consumption provides approximately 8–12% of adult energy intake, or 40–60 g day⁻¹, based on a 2 000 kcal diet. Individuals who are avid consumers of soft drinks, such as adolescent males, typically consume more than two times the average intake, or more than 100 g day⁻¹, of fructose from added sweeteners. Considering the US population as whole and using both food disappearance data and food survey data, total fructose consumption has increased by approximately 25% over the course of three decades.

Absorption of Fructose

Dietary fructose is ingested as the simple monosaccharide and also as part of the disaccharide sucrose. Sucrose is hydrolyzed by sucrase at the intestinal brush border to yield one molecule of glucose and one of fructose. Glucose is rapidly absorbed via a sodium-coupled cotransporter and arrives at the liver via the portal circulation. Fructose absorption occurs by facilitated diffusion, enabled by a fructose-specific hexose transporter, GLUT-5. This transporter is found in the jejunum on the brush border membranes. Fructose enters the portal circulation from the enterocytes using the basolateral transporter, GLUT-2,

Table 1 Sucrose, glucose, and fructose contents of fruits, vegetables, and sweeteners

<i>Food item</i>	<i>Serving size</i>	<i>Sucrose (g)</i>	<i>Glucose (g)</i>	<i>Fructose (g)</i>
Apple	1 medium	2.86	3.35	8.14
Apple juice	1 cup	4.22	6.20	13.89
Banana	1 medium	2.82	5.88	5.72
Blueberries	1 cup	0.16	7.08	7.21
Cantaloupe	1/8 melon	3.00	1.06	1.29
Cherries	1 cup	0.18	7.71	6.28
Grapes	1 cup	0.24	11.52	13.01
Oranges	1 medium	5.99	2.76	3.15
Peaches	1 medium	4.66	1.91	1.50
Pears	1 medium	1.29	4.58	10.34
Plums	1 medium	1.04	3.35	2.03
Pineapple	1 cup diced	8.48	2.70	3.18
Raspberries	1 cup	0.25	2.29	2.89
Strawberries	1 cup	0.20	3.39	4.15
Watermelon	1/16 melon	3.46	4.52	9.61
Avocado	1 fruit	0.10	0.14	0.14
Broccoli	1 cup	0.09	0.43	0.60
Carrots, baby	10 small	2.70	1.00	1.00
Corn, sweet	1 ear	1.85	0.45	0.43
Cucumber	1 cup	0.00	0.75	0.89
Onions	1 slice	0.44	0.74	0.44
Peas, green	1 cup	7.24	0.17	0.57
Potatoes	1 medium	0.36	0.70	0.58
Spinach	1 cup	0.02	0.03	0.04
Sweet potato	1 medium	3.28	1.25	0.91
Tomatoes	1 medium	0.00	1.54	1.69
Honey	1 tbsp	0.19	7.51	8.60
Maple syrup	1 tbsp	11.26	0.47	0.18
Molasses	1 tbsp	5.88	2.38	2.56

Source: <http://www.nal.usda.gov/fnic/foodcomp>.

which also transports glucose and galactose. Expression of GLUT-5 increases within hours of exposure to a fructose-enriched diet, indicating that the transporter is regulated by luminal signals. However, consumption of a large amount of pure fructose can exceed the capacity of intestinal fructose absorption, resulting in diarrhea. Several studies have shown that when a single dose of 50 g of fructose is consumed by healthy adults, more than half experience malabsorption, and in some studies malabsorption is also observed with a 25-g dose. Fructose malabsorption results in abdominal bloating, flatulence, and diarrhea. However, the intestinal absorptive capacity for fructose increases when glucose is consumed along with fructose. Thus, coingesting glucose to roughly balance fructose, as occurs when most fruits or sucrose are consumed, largely alleviates problems of fructose malabsorption. In addition, fructose absorption increases during sustained fructose consumption, suggesting adaptation to increased fructose intake.

Fructose Metabolism

The predominant site of fructose metabolism is the liver, where fructose enters the intermediary pathways of carbohydrate metabolism. Fructose is readily extracted by the liver because of the presence of an active hepatic enzyme system for metabolizing fructose, and the majority of ingested fructose is

cleared in a single pass through the liver. Thus, the concentration of fructose circulating in the blood is low after consumption of moderate amounts of fructose. Other tissues that take up small quantities of fructose include the kidney, skeletal muscle, and adipose tissue. The GLUT-5 transporter is expressed in these tissues but at relatively low levels.

In the liver, fructose is phosphorylated and forms fructose-1-phosphate. This reaction requires ATP and is catalyzed by fructokinase (EC 2.7.1.4), an enzyme with high affinity and specificity for fructose. Fructose-1-phosphate is then cleaved by hepatic aldolase (aldolase B) (EC 4.1.2.13) to form dihydroxyacetone phosphate (DHAP) and glyceraldehyde. DHAP is an intermediate metabolite in both the gluconeogenic and glycolytic pathways. Thus, a portion of the original fructose carbon structure forms glucose, and, in fact, a small increase in circulating glucose occurs after ingestion of fructose. The glyceraldehyde intermediate is phosphorylated by triokinase (EC 2.7.1.28) to form glyceraldehyde-3-phosphate, another intermediate in the glycolytic pathway. The triose phosphate compounds provide substrate for glycolysis and oxidative metabolism, formation of glycogen, and synthesis of glucose and fatty acids. With the formation of the triose phosphates, the metabolism of fructose and glucose converges. However, before this step, there are important differences in fructose and glucose metabolism that impact both carbohydrate and lipid metabolism. The initial reaction that primes fructose for entry to the glycolytic pathway allows it to bypass the critical

rate-limiting step of glycolysis. This critical step precedes the formation of triose phosphates; glucose carbons pass through an intermediate step where fructose-6-phosphate is converted to fructose-1,6-bisphosphate. This reaction is catalyzed by the allosterically regulated enzyme phosphofructokinase (PFK) (EC 2.7.1.11) and is the most important control point in the glycolytic sequence. Among the multiple effectors of PFK are ATP and citrate; these products of glucose oxidation exert an inhibitory effect on the enzyme (**Figure 1**). The allosteric inhibition of PFK effectively reduces the rate of glycolysis and decreases hepatic glucose uptake overall. In contrast, the entry of fructose carbons through the pathway proceeds without this limitation.

Fructose and Lipid Metabolism

When large amounts of fructose are ingested, the glycolytic pathway becomes saturated with intermediates. Under these circumstances, the intermediates become substrates for triacylglycerol synthesis: DHAP can be converted to glycerol, and acetyl CoA can enter the lipogenic pathway to form fatty acids that are then esterified to the glycerol molecule to form triacylglycerols. During the initial step of lipogenesis, malonyl-CoA is formed. This intermediate serves to inhibit the transport of fatty acids into the mitochondria where they are oxidized. By this regulatory mechanism, esterification of the newly synthesized fatty acids is reinforced. Studies have shown that the ingestion of fructose results in increased synthesis of fatty acids, compared to ingestion of a comparable

amount of glucose. The increased availability of fatty acids and subsequent triacylglycerol synthesis results in production and secretion of triacylglycerols from the liver, in the form of very low density lipoproteins (VLDLs). Studies in animals have demonstrated that when large quantities of fructose or sucrose are consumed, an increase in blood triacylglycerol concentration occurs. Similar findings have been observed in humans, although some humans appear to be more susceptible to fructose consumption than others. For example, the lipogenic sequence may be accentuated in humans with pre-existing hypertriacylglycerolemia or in those who are insulin resistant. Because high circulating triacylglycerol levels have been identified as a risk factor for coronary heart disease, long-term exposure to high levels of dietary fructose may contribute to a chronic, unfavorable lipid profile and increase the risk of coronary heart disease.

Fructose and Glucose Metabolism

With fructose ingestion, there is an increased flux through the glycolytic pathway, with formation of pyruvate and lactate. As fructose-1-phosphate is formed, at the initial priming stage of glycolysis, it feeds forward and enhances the activation of pyruvate kinase (EC 2.7.1.40), thereby facilitating the passage of fructose carbon to pyruvate and lactate. With fructose ingestion, it is common to observe increases in blood lactate concentrations.

In the postprandial state, fructose serves to promote the formation of glycogen, but only when it is consumed along

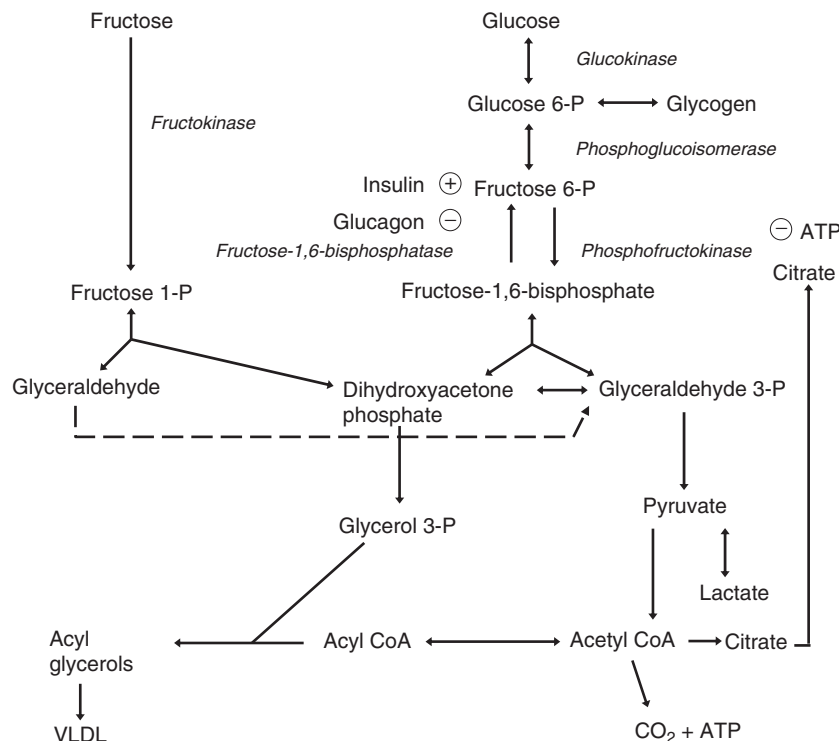


Figure 1 The intermediary pathways and fructose metabolism. Reproduced from Bray GA, Nielsen SJ, and Popkin BM (2004) Consumption of high-fructose corn syrup in beverages may play a role in the epidemic of obesity. *American Journal of Clinical Nutrition* 79: 537–743, with permission from American Society for Nutrition.

with glucose. This occurs through the activation of glycogen synthase (EC 2.4.1.11) and the inhibition of glycogen phosphorylase (EC 2.4.1.1).

In the starved state, fructose actively serves as a substrate for gluconeogenesis and glucose production. The gluconeogenic pathway and the glycolytic pathway share many common intermediates and enzymes, but the direction of the carbon flux through these pathways is controlled by several allosteric enzymes unique to each pathway. Because fructose enters the glycolytic pathway beyond the major gluconeogenic–glycolytic pivotal point (the interconversion between fructose-6-phosphate and fructose-1,6-bisphosphate), it does not exert an inhibitory effect on the gluconeogenic rate-limiting enzyme, fructose-1,6-bisphosphatase (EC 3.1.3.11). Consequently, there is no inhibition of gluconeogenesis by fructose as fructose carbons proceed through the glycolytic pathway. When a large quantity of fructose is infused intravenously, hepatic glucose production and output increases.

Consumption of large amounts of fructose is also associated with an impairment of glucose disposal. Prolonged feeding of fructose or sucrose to animals impairs insulin signaling and induces insulin resistance. Less is known about the effect of fructose ingestion on glucose tolerance and insulin resistance in humans, as the scientific literature contains conflicting results. However, the lipogenic effects of fructose may contribute to insulin resistance indirectly because increased blood levels of triacylglycerols and fatty acids and deposition of lipid in the liver and skeletal muscle have been implicated in the etiology of insulin resistance.

In a recently published study, the effects of fructose and glucose consumption on lipid and carbohydrate metabolism were compared in a group of older overweight/obese men and women. With consumption of large quantities of fructose-sweetened beverages over a period of 10 weeks, there was an increase in *de novo* lipogenesis, 24 h postprandial circulating triglycerides, LDL cholesterol, apolipoprotein-B, small, dense LDL cholesterol, oxidized LDL, and remnant lipoproteins, and a 20% decrease in insulin sensitivity that did not occur with consumption of glucose-sweetened beverages. Important metabolic differences between men and women in response to fructose ingestion have been reported, with women being less responsive to the metabolic dysfunction associated with large doses of fructose. The metabolic effects of dietary fructose and mechanisms by which fructose consumption increases visceral adiposity and causes adverse alterations in lipid profile and insulin sensitivity have been discussed in several recent reviews.

Fructose and Diabetes

Historically, in the nutritional management of diabetes mellitus, the ingestion of fructose was recommended as a sweetener for diabetics because it causes smaller increases in blood glucose following ingestion compared to similar amounts of glucose, sucrose, or starches. In fact, fructose, in small quantities, increases the hepatic uptake of glucose and promotes glycogen storage, probably by stimulating the activity of hepatic glucokinase (EC 2.7.1.2). Also, in individuals with type 2 diabetes mellitus, the addition of a small amount of fructose

to an oral glucose tolerance test improves the glycemic response, indicating improved glycemic control. It must be emphasized, however, that the consumption of large quantities of fructose is not recommended, particularly for diabetic patients who, as a group, are at increased risk for cardiovascular disease, because of potentially adverse effects of fructose on lipid metabolism, body weight regulation, and oxidative stress that may contribute to diabetic complications.

Fructose Consumption, Body Weight, and Obesity

With the increase in fructose intake, primarily as sugar-sweetened beverages, occurring coincidentally with the increase in prevalence of overweight and obesity over the past two decades, it is important to examine the evidence that links fructose consumption and body weight gain. In epidemiological studies, consumption of larger amounts of soft drinks and sweetened beverages is associated with greater weight gain in women and increased energy intake and higher body mass index in children. In experimental studies when fructose- or sucrose-sweetened beverages are added to the diet, subjects do not compensate for the additional energy provided by these beverages by reducing energy intake from other sources, and total energy intake increases. Possibly, this lack of compensation may be explained by the lack of a significant effect of fructose ingestion on the secretion of hormones involved in the long-term regulation of food intake.

In a controlled clinical trial, the consumption of large quantities of glucose- or fructose-sweetened beverages along with the usual diets *ad libitum* led to a weight gain of approximately 1.5 kg after 8 weeks. However, intra-abdominal (visceral) fat increased significantly in the subjects consuming fructose-sweetened beverages, whereas the increase in body fat was primarily due to an increase in subcutaneous fat in the glucose-sweetened beverage-consuming group.

Comparison of the effects of ingesting fructose- and glucose-sweetened beverages with meals indicates that fructose ingestion results in smaller increases in blood glucose and insulin concentrations following the meals. In addition, circulating leptin concentrations are lower, and the normal suppressive effect of meal consumption on ghrelin concentrations is attenuated with fructose beverages. Glucose, insulin, leptin, and ghrelin are all involved in the long-term control of food intake and body weight regulation through the central nervous system. Because these key signals are absent or weakened with fructose consumption, chronic consumption of a diet high in fructose could contribute, along with dietary fat and inactivity, to increased energy intake, weight gain, and obesity.

Inborn Errors of Fructose Metabolism

Several genetically based abnormalities in fructose metabolism have been described in humans. Fructokinase deficiency leads to high levels of fructose in the blood and urine. In the absence of fructokinase, fructose can be metabolized to fructose-6-phosphate by hexokinase (EC 2.7.1.1), although at a

low rate. Consequently, no serious health problems are associated with this abnormality.

The aldolase A, B, and C enzymes catalyze the reversible conversion of fructose-1-diphosphate into glyceraldehyde-3-phosphate and dihydroxyacetone phosphate, and deficiencies in the A and B enzymes have been identified. Aldolase A is expressed in embryonic tissues and adult muscle. Possibly owing to the importance of this enzyme in fetal glycolysis, its deficiency results in mental retardation, short stature, hemolytic anemia, and abnormal facial appearance. There is no known treatment for aldolase A deficiency. Aldolase B is expressed in liver, kidney, and intestine, and a deficiency of this enzyme is more common and can be exhibited at first exposure to fructose during infancy or can have its onset in adulthood. Upon ingestion of fructose-containing foods, vomiting and failure to thrive are apparent. Hypoglycemia (in some cases severe), increased blood uric acid, and liver dysfunction also occur. This disorder can be treated effectively by eliminating sources of fructose, sucrose, and sorbitol from the diet. However, left untreated, severe organ damage can occur, presumably caused by the accumulation of fructose-1-phosphate, which is possibly toxic to liver and kidney cells, but also results in depletion of the cellular phosphate pool.

Deficiency of fructose-1,6-bisphosphatase is also considered a genetic disorder of fructose metabolism. This enzyme has a critical role in the enzyme complex regulating glycolysis and gluconeogenesis. Deficient individuals exhibit hypoglycemia, acidosis, ketonuria, hyperventilation, and often hypotonia and hepatomegaly. The urinary excretion of many organic acids is altered, notably urinary glycerol is elevated and is useful in the diagnosis of this disease. The treatment includes avoidance of dietary fructose, sorbitol, and prolonged fasting.

D-Glyceric aciduria is caused by D-glycerate kinase (EC 2.7.1.31) deficiency. Only 10 cases have been documented, with symptoms ranging from none to metabolic acidosis, failure to thrive, psychomotor retardation, spastic tetraparesis, and seizures. The absence of significant symptoms in some suggests that this enzyme deficiency is essentially benign, and other associated enzyme deficiencies may underlie the more severe symptoms.

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FUNCTIONAL FOODS

Health Effects and Clinical Applications

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Glossary

FOSHU Foods for Special Health Uses, The name applied to government-approved functional foods in Japan.

Functional foods Foods with health benefits that exceed the nutritional value of the food.

Phytochemicals Plant-derived chemicals with physiological or pharmacological effects when ingested as food.

Polyphenols Phytochemicals with a chemical structure that includes multiple ring structures and double bonds.

Prebiotics Indigestible dietary carbohydrates that support the growth of beneficial intestinal bacteria.

Probiotics Ingested live bacterial cultures with health-promoting benefits.

Introduction

Functional foods are foods with health benefits that are in addition to those attributable to the nutritional value of the food. The term is usually applied to foods that have been modified or combined in order to enhance the health benefits but may include any food that naturally possesses components with demonstrable pharmacological activity. Functional foods are most often selected because they contain ingredients with hypolipidemic, immune-modulating, antioxidant, anti-inflammatory, antitoxic, or ergogenic effects. The most widely studied functional ingredients are plant-derived phenolic chemicals, probiotic bacteria, and fiber or other poorly digested carbohydrates, but peptides, colostrum, egg yolk, and other nonplant foods may also serve as functional food sources. Although the pharmacological activity of most of these substances is well established *in vitro* or in small mammals, establishing clinical effects in humans poses a challenge for functional food research.

Concept and Definition

The concept of functional foods derives from the observation that certain foods and beverages exert beneficial effects on human health that are not explained by their nutritional content (i.e., macronutrients, vitamins, and minerals). The definition of functional foods varies among countries for reasons that are historical, cultural, and regulatory. In its broadest use, functional foods are food-derived products that, in addition to their nutritional value, enhance normal physiological or cognitive functions or prevent the abnormal function that underlies disease. In most countries, a functional food must take the form of a food or beverage, not a

medication, and should be consumed the way a conventional food or beverage is consumed. If the ingredients are incorporated into pills, sachets, or other dosage forms, they are considered dietary supplements or nutraceuticals, not functional foods. In Japan and Australia, the functional food appellation has been applied only to food that is modified for the purpose of enhancing its health benefits; in China, Europe, and North America, any natural or preserved food that enhances physiological function or prevents disease might be considered a functional food. If food is modified, there is lack of international consensus as to whether a vitamin or mineral-enriched food (e.g., folate-fortified flour or calcium-fortified orange juice) should be considered a functional food, or whether functional foods are described by the presence of their nonnutritive components (e.g., fiber or polyphenols).

History

If the broadest definition is employed, the use of functional foods for promoting health and relieving symptoms is as old as the practice of medicine. Specific dietary recommendations for treating or preventing various types of illness have been documented in Hippocratic and Vedic texts and the canons of traditional Chinese medicine. Traditional Chinese remedies frequently contain recipes for combining specific foods with culinary and nonculinary herbs to produce healing mixtures. Folk medicine, East and West, has always depended on functional foods. Peppermint (*Mentha piperita*) tea has a long history of use for digestive complaints. Peppermint oil contains spasmolytic components that block calcium channels in smooth muscle. Cranberry (*Vaccinium macrocarpon*) juice contains proanthocyanidins that may inhibit the attachment of *Escherichia coli* to the epithelium of the urinary bladder,

explaining its efficacy in prevention of bacterial cystitis and its traditional use for treatment of urinary infection.

Herbs and spices are added to food to enhance flavor and initially were used to inhibit spoilage. Many of these have documented medicinal uses that render them functional foods, broadly defined. Thyme (*Lamiaceae* spp.) was used to treat worms in ancient Egypt. Thyme oils possess potent antimicrobial properties. Ginger (*Zingiber officinale* root), cinnamon (*Cinnamomum* spp. bark), and licorice (*Glycyrrhiza glabra* root) are common ingredients in Chinese herbal tonics and have been widely used in Western folk medicine for treating digestive disorders. Ginger contains more than 400 biologically active constituents. Some have antimicrobial, anti-inflammatory, or antiplatelet effects; others enhance intestinal motility, protect the intestinal mucosa against ulceration, and dilate or constrict blood vessels. Cinnamon oil contains cinnamaldehyde and various phenols and terpenes with antifungal, antidiarrheal, vasoactive, and analgesic effects. Recent research has identified phenolic polymers in cinnamon with actions that increase the sensitivity of cells to insulin, leading to the recognition that regular consumption of cinnamon may help to prevent type 2 diabetes. The most studied component of licorice, glycyrrhizin, inhibits the enzyme 11β -hydroxysteroid dehydrogenase type 2, potentiating the biological activity of endogenous cortisol. Glycyrrhizin also inhibits the growth of *Helicobacter pylori*. Glycyrrhizin and its derivatives may account for the anti-inflammatory and antitumor effects of licorice.

Fermentation is a form of food modification initially developed for preservation. The health-enhancing effects of fermented foods have a place in folk medicine. Several fermented foods have health benefits that exceed those of their parent foods and can be considered functional foods, broadly defined. These include red wine, yogurt, and tempeh. Red wine is a whole fruit alcohol extract that concentrates polyphenols found primarily in the seed and skin of the grape. Its consumption is associated with possible protection against heart disease, perhaps because red wine polyphenols inhibit the production of free radicals and lipid peroxides that result from the simultaneous ingestion of cooked meat. Fresh yogurt contains live cultures of lactic acid-producing bacteria that can prevent the development of traveler's diarrhea, antibiotic-induced diarrhea, rotavirus infection, and vaginal yeast infection, decrease the incidence of postoperative wound infection following abdominal surgery and restore the integrity of the intestinal mucosa of patients who have received radiation therapy. Tempeh is made from dehulled, cooked soy beans fermented by the fungus *Rhizopus oligosporus*. Not only is its protein content higher than the parent soy bean but it also has antibiotic activity *in vitro* and the ability to shorten childhood diarrhea *in vivo*.

Modification of a food to make it less harmful by removing potential toxins or allergens may create a functional food. Using this criterion, infant formula, protein hydrolysates, low-sodium salt substitutes, low-fat dairy products, and low-erucic-acid rapeseed oil (canola oil) might be considered functional foods.

If the most restrictive definition of functional foods is employed, the functional food movement began in Japan during the 1980s, when the Japanese government launched

three major research initiatives designed to identify health-enhancing foods to control the rising cost of medical care. In 1991, a regulatory framework, Foods for Special Health Uses (FOSHU), was implemented, identifying those ingredients expected to have specific health benefits when added to common foods, or identifying foods from which allergens had been removed. FOSHU products were to be in the form of ordinary food (not pills or sachets) and consumed regularly as part of the diet. Initially, 11 categories of ingredients were identified for which sufficient scientific evidence indicated beneficial health effects. The Japanese Ministry of Health recognized foods containing these ingredients as functional foods. They were intended to improve the intestinal function, reduce blood lipids and blood pressure, enhance calcium or iron absorption, or serve as noncariogenic sweeteners (see Table 1). In addition, low-phosphorus milk was approved for people with renal insufficiency and protein-modified rice for people with rice allergy. Ingredients that have subsequently received FOSHU approval include chitosan for reducing cholesterol, peptides derived from milk or fish that are alleged to reduce blood pressure, and soy isoflavones for prevention of osteoporosis.

Interest in the development of functional foods quickly spread to North America and Europe, where the concept was expanded to include any food or food component providing health benefits in addition to its nutritive value. In Europe, functional food proponents distinguished functional foods from dietetic foods, which are defined by law. European dietetic foods are intended to satisfy special nutritional requirements of specific groups rather than to enhance physiological function or prevent disease through nonnutritive influences. They include infant formula, processed baby foods (weanling foods), low-calorie foods for weight reduction, high-calorie foods for weight gain, ergogenic foods for athletes, and foods for special medical purposes such as the treatment of diabetes or hypertension. In the United States, functional food proponents have distinguished functional foods from medical foods, defined by law as special foods designed to be used under medical supervision to meet nutritional requirements in specific medical conditions. In both domains, functional foods have been viewed as whole foods or food components with the potential for preventing cancer, osteoporosis, or cardiovascular disease; improving immunity, detoxification, physical performance, weight loss, cognitive function, and the ability to cope with stress; inhibiting inflammation, free-radical pathology and the ravages of aging; and modulating the effects of hormones. Researchers have sought to validate biomarkers that demonstrate functional improvement in response to dietary intervention, identify the chemical components of functional foods responsible for those effects, and elucidate the mechanism of action of those components. The scientific substantiation of claims is a major objective.

In China, functional foods (referred to as health foods) have been viewed as part of an unbroken medical tradition that does not separate medicinal herbs from foods. More than 3000 varieties of health foods are available to Chinese consumers, most derived from compound herbal formulas for which the active ingredients and their mechanism of action are unknown, all claiming multiple effects on various body

Table 1 Some ingredients conferring FOSHU status on Japanese functional foods

<i>Ingredient</i>	<i>Physiological function</i>
Dietary fiber	Improve gastrointestinal function
Psyllium seed husk	
Wheat bran	
Hydrolyzed guar gum	
Oligosaccharides	Improve gastrointestinal function and mineral absorption
Xylo-, fructo-, isomalto-	
Soy-derived	
Polydextrose	
Bacterial cultures	Improve gastrointestinal function
Lactobacilli	
Bifidobacteria	
Soy protein isolates	Reduce cholesterol levels
Diacylglycerols	Reduce triglyceride levels
Sugar alcohols	Prevent dental caries
Maltitol	
Palatinose	
Erythritol	
Green tea polyphenols	Prevent dental caries
Absorbable calcium	Improve bone health
Calcium citrate malate	
Casein phosphopeptide	
Heme iron	Correct iron deficiency
<i>Eucommiaceae</i> (tochu) leaf glycosides	Reduce blood pressure
Lactosucrose, lactulose, indigestible dextrin	Improve gastrointestinal function

systems, with little experimental evidence for safety and efficacy but widespread acceptance due to their history of use.

Edible Plants and Phytochemicals

Because their consumption is known to enhance health, vegetables, fruits, cereal grains, nuts and seeds are the most widely researched functional foods. The health benefits of a plant-based diet are usually attributed to the content of fiber, vitamins, magnesium and of a variety of plant-derived substances called phytonutrients or phytochemicals. Among phytochemicals, plant sterols have enjoyed the widest commercial use. Plant sterols compete with cholesterol for absorption and transport. When added to margarine or yogurt, plant sterols at a dose of two grams per day significantly decrease cholesterol levels in blood.

Most other phytochemicals have been studied for their effects as antioxidants or inducers or inhibitors of metabolic enzymes. The effects of phytochemicals on gene expression (nutrigenomics) is a growing area for research. The results of *in vitro* studies have led to early clinical trials. Virgin olive oil, for example, contains phenolic phytochemicals that are removed by processing. Olive oil phenolics are believed to contribute to some of the health benefits of the Mediterranean diet. Human studies have shown that consumption of virgin olive oil, but not processed olive oil, reduces blood levels of inflammatory prostaglandins and decreases gene expression of several proinflammatory proteins in blood cells. Heating processed olive oil produces oxidized fatty acids that are mutagenic (capable of damaging cellular DNA). Heating of virgin olive oil does not create mutagenic fatty acids,

presumably because of the antioxidant or antimutagenic effect of phenolic compounds. Extracting oil from olives in a way that maximizes the concentration of phenolics enhances the role of olives as a functional food.

Phytochemicals associated with health promotion and disease prevention are described in [Table 2](#). The most studied food sources of these phytonutrients are soy beans (*Glycine max*) and tea (*Camellia sinensis* leaves), but olives (*Olea europaea*), tomatoes (*Lycopersicon esculentum*), broccoli (*Brassica oleracea*), garlic (*Allium sativum*), turmeric (*Curcuma longa*), tart cherries (*Prunus cerasus*), and various types of berries are also receiving considerable attention as functional food candidates. An overview research on soy, tea, and turmeric illustrates some of the clinical issues encountered in the development of functional foods from edible plants.

Soy protein extracts have been found to lower cholesterol in humans, an effect that appears to be related to amino acid composition. Soy protein extracts frequently contain non-protein isoflavones, which have received considerable attention because of their structural similarity to estrogen. Soy isoflavones are weak estrogen agonists and partial estrogen antagonists. Epidemiological and experimental data indicate that isoflavone exposure during adolescence and adulthood may diminish the incidence of premenopausal breast cancer. A study from China found that soy consumption among women with breast cancer was associated with decreased cancer recurrence. *In vitro* studies show conflicting effects. On the one hand, soy isoflavones induce apoptosis of many types of cancer cells; on the other hand, estrogen receptor-bearing human breast cancer cells proliferate in tissue culture when exposed to isoflavones. Although the widespread use of soy in Asia is cited in support of the safety of soy foods, the intake of

Table 2 Phytochemicals associated with health promotion and disease prevention

Group	Typical components	Biological activities	Food sources
Carotenoids	α - and β -carotene, cryptoxanthin, lutein, lycopene, zeaxanthin, astaxanthin	Quench singlet and triplet oxygen, increase cell-cell communication	Red, orange, and yellow fruits and vegetables, egg yolk, butter fat, margarine, wild salmon
Glucosinolates, isothiocyanates	Indole-3-carbinol, sulforaphane	Increase xenobiotic metabolism, alter estrogen metabolism	Cruciferous vegetables, horseradish
Inositol phosphates	Inositol hexaphosphate (phytate)	Stimulate natural killer cell function, chelate divalent cations	Bran, soy foods
Isoflavones	Genistein, daidzein	Estrogen agonist and antagonist, induce apoptosis	Soy foods, kudzu
Lignans	Enterolactone, enterolactone	Estrogen agonists and antagonists, inhibit tyrosine kinase	Flax seed, rye
Phenolic acids	Gallic, ellagic, ferulic, chlorogenic, coumaric	Antioxidant, enhance xenobiotic metabolism	Diverse fruits, vegetables
Phytoalexins	Resveratrol	Antioxidant, platelet inhibition, induce apoptosis	Red wine, grape seed
Polyphenols	Flavonoids, chalcones, catechins, anthocyanins, proanthocyanidins	Antioxidant, enhance xenobiotic metabolism, inhibit numerous enzymes	Diverse fruits, vegetables, red wine, tea
Saponins	Glycyrrhizin, ginsenosides, diosgenin	Antimicrobial, immune boosting, cytotoxic to cancer cells	Legumes, yams, nuts, herbs
Sterols	β -sitosterol, campesterol	Bind cholesterol, decrease colonic cell proliferation, stimulate T-helper-1 cells	Nuts, seeds, legumes, cereal grains
Sulfides	Diallyl sulfides	Antimicrobial, antioxidant	Garlic, onions

isoflavones among Asian women consuming soy regularly is in the range of 15–40 mg day⁻¹, significantly less than the isoflavone content of a serving of soymilk as consumed in the United States. In clinical trials, soy isoflavones have not been effective in relieving hot flashes of menopausal women but do diminish the increased bone resorption that causes postmenopausal bone loss. In premenopausal women, soy isoflavones may cause menstrual irregularities. The successful development of soy derivatives as functional foods will require that these complex and diverse effects of different soy components in different clinical settings be better understood.

Regular consumption of tea, green, or black, is associated with a decreased risk of heart disease and several kinds of cancer. These benefits are attributed to tea's high content of catechin polymers, especially epigallocatechin gallate (EGCG), which has potent antioxidant and anti-inflammatory effects, that may lower cholesterol in hyperlipidemic individuals and alter the activity of several enzymes involved in carcinogenesis. Catechin content is highest in young leaves. Aging and the fermentation used to produce black tea oxidize tea catechins, which polymerize further to form the tannins, theaflavin, and thearubigin. Although EGCG is a more potent antioxidant than theaflavin, clinical trials have shown that consumption of black tea has more pronounced anti-inflammatory effects than consumption of green tea. Oolong tea, made from leaves that are partly fermented, may have its own unique anti-inflammatory effects that have shown benefit in the treatment of eczema. Black and oolong tea, derived from tea leaves by controlled fermentation, may emerge as functional foods, whereas EGCG, which is not well absorbed from the intestinal tract, becomes the basis for a nutraceutical or pharmaceutical preparation.

Turmeric extracts are the subject of intensive research, because flavonoids derived from turmeric (curcuminoids) have potent pharmacologic effects *in vitro* that may convey anti-inflammatory and antineoplastic benefits. However, when taken orally curcuminoids are rapidly conjugated with organic acids in the small intestine, severely limiting absorption. Coadministration of piperine, an alkaloid found in black pepper and a potent inhibitor of conjugase enzymes, dramatically increases absorption of curcumin, but does not prevent further conjugation by enzymes in the liver. The difficulty in achieving pharmacologically active levels of curcumin after ingestion has directed research on curcuminoids away from their incorporation into functional foods and toward the use of specialized delivery systems that bypass intestinal and hepatic conjugation.

Probiotics and Prebiotics

Probiotics are live microbes that exert health benefits when ingested in sufficient quantities. Species of lactobacilli and bifidobacteria, sometimes combined with *Streptococcus thermophilus*, are the main bacteria used as probiotics in fermented dairy products. Most probiotic research has been done with nutraceutical preparations, but a number of clinical trials employing yogurt have demonstrated alleviation of lactose intolerance, prevention of vaginal candidosis in women with recurrent vaginitis and of respiratory infections in vulnerable groups like young children and the elderly, and reduction in the incidence or severity of gastrointestinal infections.

Prebiotics are nondigestible food ingredients that stimulate the growth or modify the metabolic activity of intestinal bacterial species that have the potential to improve the health

of their human host. Criteria associated with the notion that a food ingredient should be classified as a prebiotic are that it remains undigested and unabsorbed as it passes through the upper part of the gastrointestinal tract and is a selective substrate for the growth of specific strains of beneficial bacteria (usually lactobacilli or bifidobacteria), rather than for all colonic bacteria, inducing intestinal or systemic effects through bacterial fermentation products that are beneficial to host health. Prebiotic food ingredients include bran, psyllium husk, resistant (high amylose) starch, inulin (a polymer of fructofuranose), lactulose, and various natural or synthetic oligosaccharides, which consist of short-chain complexes of sucrose, fructose, galactose, glucose, maltose, or xylose. The best-known effect of prebiotics is to increase fecal water content, relieving constipation. Bacterial fermentation of prebiotics yields short-chain fatty acids (SCFAs) that nourish and encourage differentiation of colonic epithelial cells. Absorbed SCFAs decrease hepatic cholesterol synthesis. Fructooligosaccharides (FOSs) have been shown to alter fecal biomarkers (pH and the concentration of bacterial enzymes like nitroreductase and β -glucuronidase) in a direction that may convey protection against the development of colon cancer.

Several prebiotics have documented effects that are probably independent of their effects on gastrointestinal flora. Whereas the high phytic acid content of bran inhibits the absorption of minerals, FOSs have been shown to increase absorption of calcium and magnesium. Short-chain FOSs are sweet enough to be used as sugar substitutes. Because they are not hydrolyzed in the mouth or upper gastrointestinal tract, they are noncariogenic and noninsulogenic. Bran contains immunostimulating polysaccharides, especially β -glucans and inositol phosphates, which have been shown to stimulate the macrophage and natural killer cell activity *in vitro* and in rodent experiments. The poor solubility and absorption of β -glucans and inositol phosphates are significant barriers to clinical effects in humans.

Immune Modulators

Several substances produced by animals and fungi have been investigated for immune-modulating effects. Fish oils are the most studied. As a source of *n*-3 fatty acids, fish oil consumption by humans has been shown to influence the synthesis of inflammatory signaling molecules such as prostaglandins, leukotrienes, and cytokines. In addition to direct effects on prostanoid synthesis, *n*-3 fats have also been shown to directly alter the intracellular availability of free calcium ions, the function of ion channels, and the activity of protein kinases. Generally administered as nutraceuticals rather than as functional foods, fish oil supplements have demonstrated anti-inflammatory and immune suppressive effects in human adults. A high intake of the *n*-3 fatty acids eicosapentaenoic (20: 5*n*-3) and docosahexaenoic (22: 6*n*-3) acid (DHA) from seafood or fish oil supplements has also been associated with prevention of several types of cancer, myocardial infarction, ventricular arrhythmias, migraine headaches, and premature births, and with improved control of type 2 diabetes mellitus, inflammatory bowel disease, rheumatoid arthritis, cystic fibrosis, multiple sclerosis, bipolar disorder, and

schizophrenia. 20: 5*n*-3 but not 22: 6*n*-3 is effective for schizophrenia and depression; 22: 6*n*-3 but not 20: 5*n*-3 improves control of blood sugar in diabetics. The benefits of fish oil supplements have prompted efforts at increasing the *n*-3 fatty acid content of common foods by adding fish oil or flax oil extracts. Consumption of these has been associated with decreased levels of some inflammatory biomarkers, including thromboxane B₂, prostaglandin E₂, and interleukin 1-beta.

Feeding flax seed meal or fish meal to hens enriches the *n*-3 fatty acid content of the yolks of the eggs they lay. Consumption of these eggs increases the *n*-3 fatty acid content of plasma and cellular phospholipids and produces an improved blood lipid profile when compared with consumption of standard eggs. Egg yolk is not only a source of fatty acids, but also of carotenoids and immunoglobulins. The xanthophyll carotenoids zeaxanthin and its stereoisomer lutein are readily absorbed from egg yolk. Their consumption is associated with a decreased incidence of macular degeneration and cataract. Immunizing hens to specific pathogens and extracting the antibodies present in their egg yolks yields a functional food that has been shown to prevent enteric bacterial or viral infection in experimental animals.

Bovine colostrum, the milk produced by cows during the first few days postpartum, has a long history of use as a functional food. Compared to mature milk, colostrum contains higher amounts of immunoglobulins, growth factors, cytokines, and various antimicrobial and immune-regulating factors. Some studies suggest that consumption of bovine colostrums may reduce the incidence of diarrheal disease in infants and the symptoms of respiratory infection in adults. Specific hyperimmune bovine colostrums, produced by immunizing cows to pathogenic organisms like *Cryptosporidium parvum*, *H. pylori*, rotavirus, and *Shigella* spp., may prevent or treat infection by these organisms.

Human studies have also shown that consumption of bovine colostrum can improve anaerobic athletic performance and prevent the enteropathy induced by use of nonsteroidal anti-inflammatory drugs.

Mushrooms play a major role in traditional Chinese medicine and as components of contemporary Chinese health foods. Many *Basidiomycetes* mushrooms contain biologically active polysaccharides in fruiting bodies, cultured mycelium, or culture broth. Most belong to the group of β -glucans that have both beta-(1→3) and beta-(1→6) linkages. Although they stimulate macrophages and natural killer cells, the anticancer effect of mushroom polysaccharide extracts appears to be mediated by thymus-derived lymphocytes. In experimental animals, mushroom polysaccharides prevent oncogenesis, show direct antitumor activity against various cancers, and prevent tumor metastasis. Clinical trials in humans have been limited to the use of purified polysaccharides like lentinan (from *Lentinus edodes* or shiitake), krestin (from *Coriolus versicolor*), or schizophyllan (from *Schizophyllum commune*), often administered by injection. Mushroom extracts may fulfill their potential more as medicines than as functional foods.

Designer Foods

An important direction in the development of functional foods is the combination of numerous ingredients to achieve a

specific set of goals, rather than efforts to uncover the potential benefits of a single food source. Optimal cardiovascular health involves control of blood pressure, reduction of oxidant stress, cholesterol, triglycerides and fibrinogen, and protection of the vascular endothelium. Designer foods alleged to enhance cardiovascular health include combinations of ingredients that have been shown to have these effects when given individually in laboratory experiments. Soy protein powder, oat β -glucan, and plant sterols and stanols, for example, may have an additive hypolipidemic effect. Chlorogenic acid (CGA), a phenolic compound found in coffee beans, apples, pears, parsley, tomatoes, blueberries, eggplant, and numerous other fruits and vegetables, has well-documented hypotensive effects in humans. It is thought to control blood pressure by protecting the enzyme that synthesizes nitric oxide, which relaxes the walls of arteries. Fish protein hydrolysates contain peptides that reduce blood pressure by inhibiting angiotensin converting enzyme (ACE), a target for many anti-hypertensive drugs. Combining lipid lowering components with fish oils, CGA and bioactive peptides may produce designer food for reducing cardiovascular risk factors. Despite excellent commercial prospects for functional foods targeted at cardiovascular health, there is a lack of clinical trial data to support short or long-term benefits with regard to health outcomes.

Sports nutrition is an established arena for designer foods, with numerous products available that lay claim to improving performance. Oral rehydration products for athletes were one of the first categories of functional foods for which scientific evidence of benefit was obtained. Oral rehydration solutions must permit rapid gastric emptying and enteral absorption, improved fluid retention, and thermal regulation, to enhance physical performance and delay fatigue. Carbohydrates with relatively high glycemic index combined with whey protein concentrates or other sources of branched chain amino acids have been shown to enhance recovery of athletes. Caffeine, creatine, ribose, citrulline, L-carnitine, and branched chain amino acids have each been shown to improve exercise performance or diminish post-exercise fatigue. Whether combinations of these ingredients, blended into foods or beverages, will perform better than the individual ingredients will help to determine the design of future sports foods.

Although development of functional food combinations to achieve specific health-related goals has attracted a great deal of commercial attention, scientific support for effects of designer foods lags far behind commercial development.

See also: Alcohol: Absorption, Metabolism, and Physiological Effects. Microbiota of the Intestine: Probiotics. Phytochemicals: Classification and Occurrence. Protein: Quality and Sources. Tea

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Glossary

Beta-glucan Glucose polymer and a type of viscous fiber.

Brush-border The brush border of the small intestine is the epithelium covered by microvilli and it is the site of terminal carbohydrate absorption.

Cellulose Glucose polymer and a type of insoluble dietary fiber, soluble only in acids or alkali.

Daily Recommended Intakes (DRI) Values that are quantitative estimates of nutrient intakes to be used for planning and assessing diets for healthy people.

Dietary fiber Glucose polymer or carbohydrate not digested by upper gastrointestinal enzymes.

Disaccharide Two basic carbohydrate units.

Glycemic Index (GI) The physiologic classification of carbohydrate-containing foods based on postprandial blood glucose responses.

Hemicellulose Glucose polymer and a type of insoluble dietary fiber, soluble only in alkali.

High-performance liquid chromatography (HPLC) A laboratory technique that separates a mixture into its individual components.

Insoluble fiber Dietary fiber resistant to digestion and insoluble in cold and hot water. It is partially degraded by colonic bacteria.

Kilo Dalton (kDa) A unit used for indicating atomic or molecular mass. It has a value of $1.660538921(73) \times 10^{-27}$ kg.

Monosaccharides Basic carbohydrate unit (e.g., glucose, galactose, fructose).

Oligosaccharides More than two basic carbohydrate units.

Resistant starch Starch resistant to digestion in the upper gastrointestinal tract, classified into three kinds: RS1, RS2, and RS3.

Short-chain fatty acids SCFA with less than six carbon chains, byproducts of bacterial fermentation of food components in the colon (e.g., acetic, propionic, and butyric acids).

Soluble fiber Glucose polymer or dietary fiber resistant to digestion but soluble in cold and hot water.

Starch Glucose polymers or carbohydrates formed by at least 1000 glucose units joined by glycosidic bonds, present in grains, legumes, and tubers.

Viscous fiber A type of soluble fiber which becomes viscous on contact with water.

Glucose and its polymers are important energy sources for living organisms and structural components of plants. Because of the diversity of compounds in which glucose occurs, it may be helpful to first discuss nomenclature.

Nomenclature and Chemical Structure

Glucose

The compound D-glucose (Greek *glukus*, sweet) or dextrose is 2,3,4,5,6-pentahydroxyhexaldehyde, more conventionally expressed as $C_6H_{12}O_6$, with a molecular weight of 180.16 kDa. Glucose is readily soluble in water in powder form. Below 50 °C, α -D-glucose hydrate is the stable form; at 50 °C the anhydrous form is obtained; and at higher temperatures, α -D-glucose is obtained. Glucose is also present in the diet as part of the disaccharides sucrose (glucose and fructose), lactose (glucose and galactose), and maltose (glucose).

Glucose Oligosaccharides

Oligosaccharides (Greek *oligo*, few) are sugar polymers; the term usually refers to compounds containing 2–9 units but may include polymers containing up to 19 units. The dimer, trimer, and tetramer forms in which glucose molecules are joined by $\alpha(1-4)$ glucosidic linkages are referred to as maltose, maltotriose, and maltotetraose, respectively, because these substances are the products of starch digestion in the malting process. Sucrose $\alpha(1-2)$, maltose, and lactose $\beta(1-4)$ are common dietary disaccharides.

Starches

Starches are large-molecular-weight, α -linked polymers of glucose ($C_6H_{10}O_5$) $_n$. Most starches show a mixture of $\alpha(1-4)$ and $\alpha(1-6)$ linkages. The $\alpha(1-4)$ -linked polymer forms a linear structure that allows for hydrogen bonding between polymer chains and a more compact starch structure. Introduction of $\alpha(1-6)$ linkages results in branch points and a more open structure that allows the $\alpha(1-4)$ -linked backbone with the hemiacetal bond in the alpha configuration to coil like a spring into a helical form. Branched starches with the $\alpha(1-6)$ linkage are more readily hydrated and digested compared to the $\alpha(1-4)$ -linked linear starch. The $\alpha(1-4)$ -linked starches are referred to as amylose starch, and $\alpha(1-6)$ -linked starches are amylopectin starches.

Resistant Starch

Resistant starches are defined by their resistance to digestion in the human upper gastrointestinal tract. As with the term dietary fiber, the definition is largely physiological. One proposed classification divides resistant starches into three classes: RS₁, RS₂, and RS₃. The first class, RS₁, is starch that escapes small intestinal digestion owing to the food form and incomplete enzymatic attack (e.g., large particle size or compact nature of food, or starch entrapment by dietary fiber). The second, RS₂, includes the more crystalline starches that resist

digestion (e.g., high-amylose starches that resist gelatinization). The RS₃ starches are retrograded starches (e.g., high-amylose starches that upon cooling after cooking form a compact, hydrogen-bonded crystalline structure that excludes water).

Cellulose

Like starch, cellulose is a (1–4)-linked glucose polymer ($C_6H_{10}O_5$) $_n$, but in this instance the glucose molecules are β -linked, allowing the development of a linear polymer with strong intra-chain hydrogen bonding. Cellulose polymers may consist of as many as 10 000 glucose monomer units. Cellulose is both resistant to small intestinal digestion and insoluble in cold or hot water and mostly digested in strong acids and alkali. Cellulose is partially degraded by colonic bacteria. The proportion degraded is dependent on the source, with cellulose from vegetables generally broken down to a greater extent than cellulose from cereals such as wheat.

β -Glucans

In many ways, these predominantly $\beta(1-4)$ -linked glucose polymers are the cellulose equivalent of the starch amylopectin. Here, it is the $\beta(1-3)$ linkages interspersed throughout the polymer that prevent the formation of a compact structure otherwise achieved with the cellulose polymer where only the $\beta(1-4)$ linkages exist. As a result of the more open molecular structure of the β -glucan, unlike cellulose, it is readily hydrated and soluble in water, forming a solution of high viscosity. The viscosity, in turn, is dependent on the molecular weight and the presence of the $\beta(1-3)$ linkages. The greater the molecular weight is, the greater the viscosity. Thus, reduction of molecular weight by acid or enzymatic hydrolysis, which may also occur during food processing, may greatly reduce viscosity. The common feature shared by cellulose and the β -glucans is that both are resistant to digestion by small intestinal enzymes. However, whereas cellulose is only partially fermented by the colonic bacteria, β -glucans are completely fermented.

Hemicellulose

The term hemicellulose should not be taken to imply a class of $\beta(1-4)$ -linked glucose polymers. The similarity with cellulose lies not in the chemical structure but in the fact that hemicellulose is also insoluble in hot or cold water or hot dilute acid. It is, however, soluble in dilute alkali. The polymeric structure is heterosaccharitic with two or more sugars (e.g., arabinoxylans found in cereals), with a relatively small molecular size (50–200 saccharide units).

Occurrence

Glucose is the primary carbohydrate energy source of vertebrates. In healthy humans, fasting blood glucose levels are approximately 3.5–5.5 mmol l⁻¹ and increase in the postprandial state to values considerably less than 10 mmol l⁻¹ (the renal threshold for complete reabsorption, above which

glucose spills over into the urine). Blood levels higher than 7.8 mmol l^{-1} 2 h after a 75-g glucose load is one of the diagnostic criteria for diabetes. Glucose is stored as glycogen, an α -linked polymer, predominantly in the liver and muscles (animal starch). On average, a 70-kg man may store 500 g of glycogen. Glucose can also be synthesized *de novo* by gluconeogenesis from the gluconeogenic amino acids, odd-numbered fatty acids, lactate, glycerol, and pyruvate.

Erythrocytes, renal tissue, and nervous tissue require glucose as an energy source. In erythrocytes and renal tissue, the glucose is not oxidized but is returned to the liver as part of the Cori cycle for glucose synthesis. The brain oxidizes glucose and requires 140 g per day. From this figure the carbohydrate requirement was derived in the recent Dietary Reference Intake (DRI) assessment (Table 1). Despite this modest requirement, carbohydrate is still recommended to comprise between 45% and 65% of dietary calories.

Glucose is present in fruits and vegetables and, although less sweet on a per gram basis than fructose or sucrose, it is responsible together with fructose and sucrose for the sweet taste of vegetables and fruit. With the exception of fruit such as green banana, seeds (grain and dried legumes), and tubers, in which starch is the major carbohydrate form, foods containing glucose, fructose, and sucrose in various ratios comprise the major available (i.e., absorbable in the small intestine)

Table 1 Estimation of brain glucose requirements of adult humans

Glucose consumption ($\mu\text{mol per } 100 \text{ g brain}$ per min)	Estimated brain weight (g)	Brain glucose consumption	
		mg min ⁻¹	g d ⁻¹
31–38	1450	81–99	117–142

Source: Reproduced from Sokoloff L (1977) Relation between physiological function and energy metabolism in the central nervous system. *Journal of Neurochemistry* 29: 13–26; Scheinberg P and Stead EA (1949) The cerebral blood flow in male subjects as measured by the nitrous oxide technique. Normal values for blood flow, oxygen utilization, glucose utilization, and peripheral resistance, with observations on the effect of tilting and anxiety. *Journal of Clinical Investigation* 28: 1163–71, and Hatazawa J, Brooks RA, Di Chiro G, and Bacharach SL (1987) Glucose utilization rate versus brain size in humans. *Neurology* 37: 583–588. "DRI for carbohydrates": http://www.nal.usda.gov/fnic/DRI/DRI_Energy/265-338.pdf.

carbohydrate sources. The relative proportions of the sugars have not been generally determined, and data are not available for many foods.

The main sources of dietary starch are cereal grains, dried legumes, and tubers. The major portion of the available carbohydrate in these foods is starch. Starches contain both $\alpha(1-4)$ and $\alpha(1-6)$ linkages (i.e., amylose and amylopectin) (Figure 1). In most studies amylose predominates, with a ratio of amylose to amylopectin of 2–3:1. In general, legumes contain higher amylose levels than do cereals. Cultivars of corn have been bred with high amylose levels.

Resistant starches comprise a small proportion of most industrialized Western diets. Increased starch malabsorption may result from coarse milling or increasing particle size of cereal grains (e.g., whole-grain pumpernickel or bulgur wheat). Such foods may be said to contain resistant starch (RS₁). Resistant starches that are crystalline in nature and resist hydration (RS₂) are found in green banana, high-amylose corn, and relatively high-amylose legumes (peas, beans, and lentils). Starches, especially high-amylose starches that are cooked and then allowed to cool, undergo retrogradation with more crystalline realignment. These starches (RS₃) are produced in common foods such as potato, rice, and bread. Resistant starches in this category are produced commercially from high-amylose cornstarch by enzymatically debranching the remaining $\alpha(1-6)$ linkages and allowing the resulting $\alpha(1-4)$ -linked starch to retrograde into a highly crystalline, digestion-resistant starch.

Cellulose is an important structural component of plant cell walls. In human nutrition, it forms an important part of the insoluble dietary fiber component reported in food composition tables. However, values for the actual proportion of the total dietary fiber that is composed of cellulose are only available in special food composition tables for a relatively small number of foods.

From the standpoint of human nutrition, β -glucans are found predominantly in cereals, notably oats and barley, with trace amounts in wheat. In oats, the β -glucan is concentrated in the outer bran layer and may comprise 50% of the dietary fiber value and possibly 8% or 9% of the so-called oat bran derived from standard milling practices. In barley, the β -glucan is more dispersed through the endosperm, and thus a bran concentrate is less easy to achieve. In both cases, high β -glucan

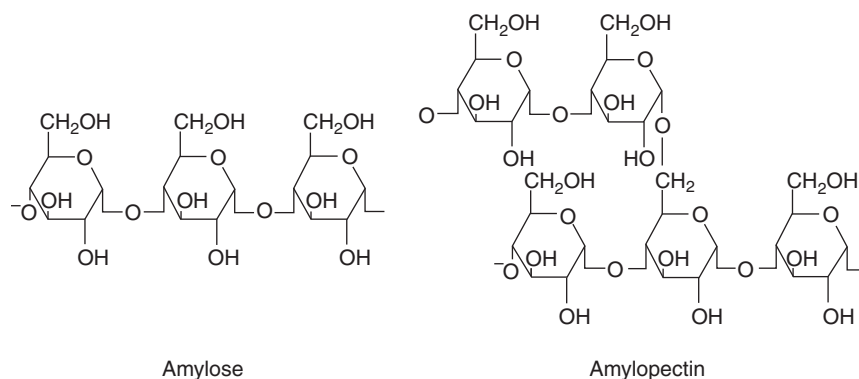


Figure 1 Partial structures of amylose (linear) and amylopectin (branched) starches.

cultivars may greatly increase the yield of β -glucan. In addition, wet processing techniques may yield a higher concentration of β -glucan bran and purified β -glucan oat gum.

Analysis

Analysis of glucose may involve chemical, enzymatic, electrochemical, and high-performance liquid chromatography (HPLC) systems. Before the introduction of enzyme-based analyses, chemical techniques were based on the reducing ability of glucose, and techniques employing copper sulfate were popular. Such techniques were influenced by other reducing sugars and reducing substances, including uric acid and vitamin C. With the introduction of the more specific glucose oxidase-based tests, the chemical tests were abandoned, although there was debate over the potential carcinogenicity of the early chromogens, *O*-dianizadine and *O*-toluidine. Later, more specific, hexokinase-based enzyme assays were introduced. Current methods for rapid determination of blood glucose, which no longer require prior precipitation of plasma proteins, involve electrochemical detection. These methods rely on silver electrodes to detect electrons generated by the oxidation of glucose by glucose oxidase contained in membranes on the surface of the electrodes. For determination of glucose and α -limit dextrins resulting from starch digestion, HPLC techniques have proved useful.

Much attention has been given to the analysis of the glucose polymers – starches, resistant starches, cellulose, and β -glucans – in the context of dietary fiber. The ultimate assessment depends on the use of specific enzymes or enzyme systems to break the macromolecules down to their

component glucose and other sugars when mixtures containing other polymers (dietary fibers) are being analyzed. These are then assessed by gas chromatography or HPLC and the ratios of the sugars determined. More routine assessment may involve a variety of chemical techniques combined with enzymatic digestion and, in the case of a popular Association of Official Analytical Chemists (AOAC)-approved technique for dietary fiber analysis, with a gravimetric determination. However, there is debate as to whether the resistant starch, which in the gravimetric AOAC technique is analyzed as dietary fiber, should be included as fiber or whether it is physiologically distinct. It is also debated whether a determination of β -glucan is sufficient without knowledge of its viscosity and molecular weight – factors that determine its physiological effect.

Physiology

The physiology of the gastrointestinal absorption of (and the energy retrieved from) the glucose molecule along the length of the gastrointestinal tract in its various forms is discussed in the following sections, together with the influence of other dietary factors (Figure 2).

Absorption

In its simplest form, glucose ingested by mouth is rendered isotonic in the stomach by the gastric juices and expelled through the pylorus into the duodenum, where active transport takes place at the brush border by way of a sodium-linked glucose transporter. The absorbed glucose that is taken up by way of the portal vein suppresses hepatic glucose output but

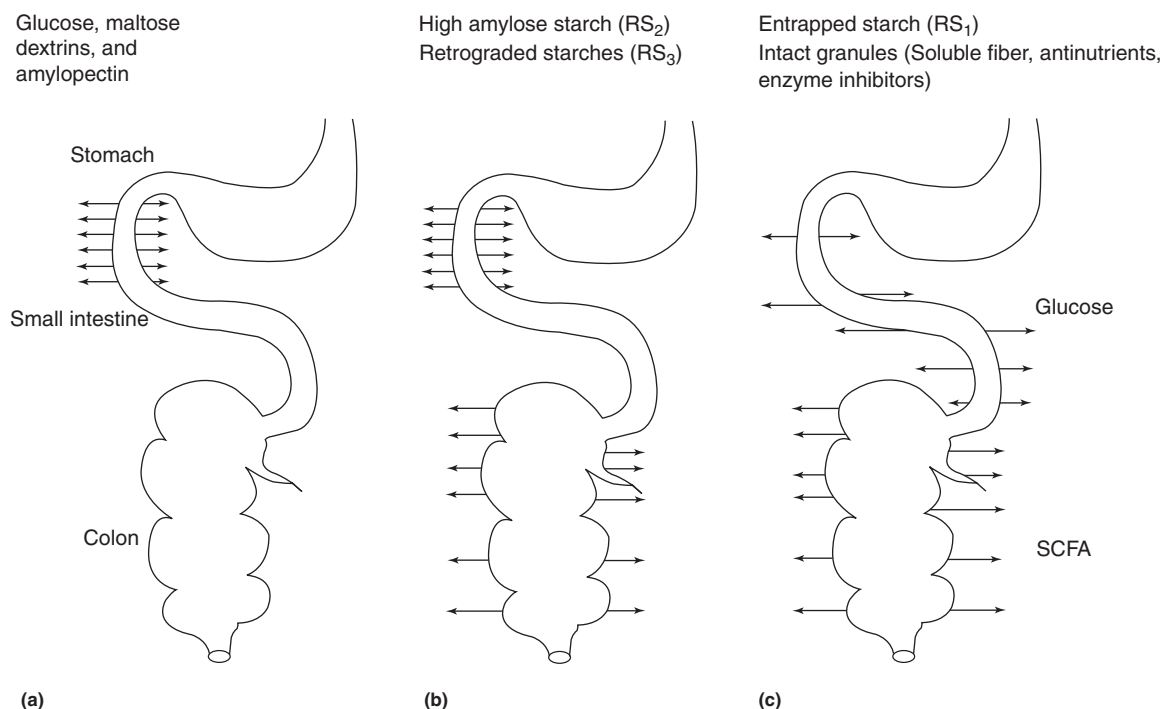


Figure 2 Effect of different forms of glucose on glucose absorption and short-chain fatty acid (SCFA) production and uptake from the gut.

does not markedly alter the glucose balance across the liver. The major part of the absorbed glucose is taken up by muscle and also adipose tissue under the action of insulin. Similarly, sucrose, maltose, and lactose are both split and absorbed at the brush border by the brush border enzymes sucrose – isomaltase, maltase, and lactase. Although sucrose deficiency is exceedingly rare, hypolactasia is common in adult life in most of the world's populations, with the exception of those of north European origin. Thus, unlike sucrose malabsorption, small-intestinal lactose malabsorption is common, with significant amounts of lactose entering the colon, resulting in gas production, short-chain fatty acid (SCFA) synthesis, and, in some instances, osmotic diarrhea.

On the other hand, purified, fully hydrated, cooked amylopectin starch commences digestion in the mouth under the action of salivary amylase. Enzyme activity ceases under the acidic conditions of the stomach and resumes in the duodenum under the action of pancreatic amylase. Amylolitic

digestion in both mouth and stomach results predominantly in the production of free glucose, maltose, maltotriose, and the α -limit dextrans of greater polymeric length. Free glucose is taken up by the brush border glucose transporter, and the uptake of maltose and maltotriose is effected by brush border enzymes, notably the sucrase–isomaltase complex. In both situations, absorption in the small intestine is considered to be complete.

However, foods as eaten do not usually comprise pure glucose and pure amylopectin starch as their carbohydrate components. Many factors influence small-intestinal absorption in terms of both rate and amount (Table 2). Some of these factors were previously discussed in connection with amylose and resistant starch.

Food Components

Insoluble fiber may form a coat around starchy foods, limiting the penetration of enzymes and thus reducing the rate and

Table 2 Factors influencing glycemia and gastrointestinal events

Factors influencing the availability of carbohydrate	Physiological effect					
	Glycemia	Stomach, gastric emptying	Small intestine, absorption rate	Motility	Colon Bacterial fermentation	Fecal bulk
Food components						
Fiber						
Soluble (viscous)	—	— — —	— —	— ?	++ +	+
Insoluble	0 ?	+	+	+	+	+++
Macronutrients						
Protein–starch interaction	—	— ?	— —	?	+	?
Fat–starch interaction	—	—	— —	?	+	?
Starches						
Amylopectin	+	?	++	?	0	0
Amylose	—	—	— — —	?	++	+
Sugars and glucose polymers						
Glucose	++	— ?	+	0	0	0
Maltose	++	— ?	+	0	0	0
Maltodextrins	++	0	+	0	0	0
Antinutrients						
Phytates	—	?	—	?	+	?
Tanins	—	?	—	?	+	?
Saponins	?	?	—	?	+	?
Lectins	—	—	—	?	+	?
Amylase inhibitors	—	0	—	?	+	?
α -glucosidase inhibitors	—	0	—	?	+	?
Food processing						
Cooking						
Starch gelatinisation	+	0	++	?	0	0
Starch retrogradation	—	?	— —	?	+	+
Parboiling (e.g., rice)	—	?	—	?	?	0
Particle size						
Milling	+	+	+	?	0	0
Crushing	+	?	+	?	0	0
Flaking	+	?	+	?	0	0
Extruding	—	?	—	?	0	0

+, increase, promote; —, inhibit, reduce; 0, no effect; ?, uncertain.

amount digested. Viscous soluble fibers may also reduce the rate of absorption through prolonging gastric emptying and by acting as a barrier to diffusion in the small intestine. Starch–protein interactions (as seen with gluten in wheat products) and starch–fat interactions have been shown to reduce the rate of digestion, and fat is known to slow gastric emptying. A number of the so-called antinutrients⁷ present in foods, notably lectins, phytates, and tannins, have been shown to reduce the digestibility of foods. For example, it is considered that phytate, by binding calcium ions that catalyze starch digestion by amylase, reduces the rate of small-intestinal starch digestion.

Food processing may influence the rate of digestion by removing or reducing the level and activity of inhibitory food components. It may also modify the structure of the food or its components to make the food more available to digestive enzyme attack. Examples are cooking, resulting in starch gelatinization, and reducing the particle size (and hence increasing the surface area available to digestive enzymes) by milling, crushing, or flaking. On the other hand, processing may also reduce digestibility by parboiling, cooking with retrogradation of the starch, and extrusion, as in the production of pasta, producing a more compact physical structure.

Increasing the frequency of meals and reducing their size spreads the nutrient load over time and hence prolongs the time spent in the absorptive state. It is perhaps the clearest model of slowing the rate of absorption and is referred to again to explain the metabolic consequences of reducing the absorption rate.

Finally, enzyme inhibitors of carbohydrate absorption have been developed for pharmacological use in the treatment of diabetes, and these work by reducing the rate of carbohydrate uptake from the small intestine. One example of this class of substances is acarbose, an α -glycoside hydrolase inhibitor that has antiamylase and antisucrease–isomaltase activity and thus inhibits both intraluminal and brush border carbohydrate digestion and absorption of starch, sucrose, and maltose.

Possible Effects of Prolonging Absorption Time of Carbohydrate

The question remains as to what physiological effects are produced when carbohydrate is absorbed more slowly (Table 3). Studies have demonstrated the effectiveness of carbohydrate-absorption enzyme inhibitors (acarbose) in treating diabetes and in decreasing diabetes-related vascular complications. Additionally, acarbose treatment has been shown to prevent the development of diabetes in high-risk subjects when treated over a 3-year period and through the reversal of impaired glucose tolerance. A further way to reduce the rate of absorption of carbohydrate without altering its composition is to change the rate of ingestion of carbohydrate substrates.

A number of metabolic benefits are observed when glucose is sipped slowly rather than drunk as a bolus or when starchy meals are eaten more frequently but in smaller amounts. Studies by Ellis in the 1930s first demonstrated a reduction in insulin requirements in patients with diabetes when glucose and insulin were administered in small, frequent doses.

Table 3 Possible effects of prolonging absorption time of carbohydrates

Flatter postprandial glucose profile
Lower mean insulin levels post-prandially and throughout the day
Reduced gastric inhibitory polypeptide (glucose-dependent insulinotropic peptide) response
Reduced 24-h urinary C peptide output
Prolonged suppression of plasma free fatty acids
Reduced urinary catecholamine output
Lower fasting and postprandial serum total and LDL cholesterol levels
Reduced hepatic cholesterol synthesis
Lower serum apolipoprotein B levels
Lower serum uric acid levels
Increased urinary uric acid excretion

Because then, a range of metabolic benefits has been ascribed to increased meal frequency (the nibbling versus gorging phenomenon). Early studies reported lower total cholesterol levels with increased meal frequency. Subsequent studies showed low-density lipoprotein (LDL) cholesterol reduction in subjects eating from six to as many as 17 meals daily, compared to three meals a day, for periods of 2–8 weeks. An extreme model of slowing absorption, in which 17 meals daily were fed, demonstrated lower levels of apolipoprotein B in addition to total and LDL cholesterol. Population-based studies also indicated that total cholesterol levels were lower in those who ate more meals daily. Studies using stable isotopes showed that cholesterol synthesis was reduced at greater meal frequencies. Furthermore, mevalonic acid excretion (a water-soluble marker of cholesterol synthesis) suggested that the change in cholesterol levels was also related to the change in urinary mevalonic acid output. Because insulin is known to stimulate HMG-CoA reductase activity, a rate-limiting enzyme in cholesterol synthesis, the depressed cholesterol synthesis was attributed to the lower insulin levels observed. In addition, the reduction in serum cholesterol levels on nibbling may have resulted from increased bile acid losses owing to more frequent bile acid cycling through the gut following increased meal frequency.

Studies of noninsulin-dependent diabetes have shown blunting of glucose and insulin spikes postprandially with increased meal frequency. In nondiabetic subjects, the major effect of reducing the absorption rate (by sipping glucose over 3 h instead of taking the same amount of glucose as a bolus within 5 min) was to reduce insulin secretion. In addition, insulin suppression of free fatty acids and branched-chain amino acid levels was prolonged, and no counter-regulatory response was observed following the glucose challenge.

Finally, serum uric acid, an independent risk factor for coronary heart disease, was reduced and increased urinary uric acid excretion was seen with increased food frequency. As with the reduction in serum cholesterol levels, the effects of lower insulin levels were used to explain these differences. It was suggested that insulin promoted renal reabsorption of uric acid, as demonstrated in the context of sodium reabsorption and hypertension in hyperinsulinemic states.

Further effects of food frequency on diabetes have been assessed. It has been suggested that increased food frequency may limit obesity by reducing adipose tissue enzyme levels. Acute studies in humans failed to show an increased

thermogenic response with increased meal frequency. Nevertheless, when satiety was assessed in acute studies, fluctuations in satiety were less over the whole day; long-term studies have yet to be undertaken. Concern still remains that snacking may increase body weight in susceptible individuals. Despite these concerns, the demonstration that increased meal frequency can improve certain aspects of lipid and carbohydrate metabolism makes it a valuable model for other methods of spreading the nutrient load (e.g., reducing the rate of glucose absorption).

Colonic Function

A portion of starch, together with dietary fiber including cellulose and β -glucan, enters the colon and is fermented by the colonic microflora, which promote growth of the fecal biomass and the production of SCFA, hydrogen, and methane. The extent to which this occurs varies from individual to individual and is based on the nature of the resistant starch and the source of the cellulose (e.g., vegetable cellulose is more readily fermented than cereal cellulose). Although some individuals may have starch in their feces, the majority of subjects show little or no fecal starch. Furthermore, all the β -glucan is broken down by bacterial action in the colon. A large proportion of the cellulose escapes colonic bacterial fermentation and contributes directly to fecal bulk. Thus, a significant proportion of glucose molecules are not absorbed in the small intestine but enter the colon and are salvaged after conversion to SCFAs. The SCFAs are rapidly absorbed and contribute to the host's energy metabolism. They are usually produced in the ratio of 60% acetate, 20% propionate, and 20% butyrate, but the relative ratios of these three fatty acids vary depending on the substrate and the rate of fermentation. Of the three SCFAs, only acetate appears in the peripheral circulation to any significant extent. Propionate is of interest since it is gluconeogenic and has been suggested to inhibit hepatic cholesterol synthesis. Propionate is largely extracted by the liver at first pass. Butyrate, on the other hand, is taken up and used by colonocytes. The slower the fermentation, the higher the butyrate levels. Starches have been claimed to increase colonic butyrate and in some instances propionate production, and butyrate may have antineoplastic properties.

The Glycemic Index

The widely differing effects of different carbohydrate foods in raising the blood glucose concentration postprandially have

long been recognized. The glycemic index (GI) classification was proposed to indicate the rates at which different starchy foods were digested, the hypothesis being that selection of foods with lower glycemic indices would contribute to prolonging the absorption of nutrients and thus improve the glycemic profile and reduce levels of fasting blood lipids. The GI is a physiological classification which ranks carbohydrate containing foods based on their ability to raise postprandial glycemia, hence it is a measure of carbohydrate quality (i.e., slow vs. fast carbohydrate absorption). Evidence from large epidemiological studies indicates that diets containing low as compared to high GI carbohydrate foods may reduce the risk of chronic diseases including diabetes, cardiovascular disease, and cancer. Although inconsistencies in the current findings still need to be resolved, a recent meta-analysis of observational studies resulted in a 14% risk reduction of chronic diseases and 40% risk reduction of type 2 diabetes with low GI diets. Randomized controlled trials have found that low compared to higher GI diets were effective in reducing body fat in overweight and obese individuals.

A number of clinical trials have documented improved glycemic control in both type 1 and 2 diabetics on low as compared to high GI index diets as judged by serum fructosamine and glycosylated hemoglobin levels in studies lasting from 2 weeks to 6 months. Furthermore, some studies also noted reductions in serum lipids with low GI diets. Many high-fiber foods that lower LDL cholesterol levels also have low glycemic indices (barley, beans, lentils, etc.). Extensive glycemic index tables have been published that will help in food selection for therapeutic and study purposes.

Many of the traditional starchy foods from different cultures have a low glycemic index (Table 4). Finally, results of cohort studies suggest that consumption of foods with a low glycemic index, especially in the context of a high-fiber diet, protects from the development of type 2 diabetes. Therefore, the question is whether the rapid increase in diabetes in cultures in transition from traditional to Western lifestyle patterns is in part owing to the high glycemic index of the diets eaten, in addition to the excess consumption of energy and reduced physical activity.

Calculation of the Glycemic Index

The GI has been defined as the area under the blood glucose response curve for 50 g available carbohydrate from the test food divided by the area under the blood glucose response

Table 4 Glycemic foods of staples from different cultures

Food	Average GI ^a	Culture
White bread rolls	100	North American, European
Pumpernickel	70–90	North European
Pasta	50–70	Mediterranean
Cracked wheat (tabouli)	60–70	Mediterranean, Middle Eastern
Beans, lentils, dried peas	40–70	Southern United States, Latin American, Middle Eastern, Indian, Oriental
Parboiled long-grain rice	70	Asian, North African

^aGlycemic index (GI) is rounded to the nearest 10%.

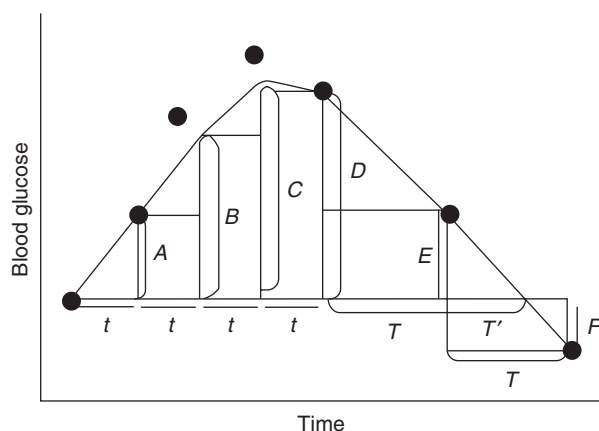


Figure 3 Schematic representation of postprandial blood glucose response. (Reproduced with permission from Wolever TMS and Jenkins DJA (1986) The use of the glycemic index in predicting the blood glucose response to mixed meals. *American Journal of Clinical Nutrition* 43: 167–172.)

curve for 50 g carbohydrate from the standard source, multiplied by 100. The standard carbohydrate source is typically white bread or a glucose drink. On the bread scale the glucose GI is approximately 130. Other food GI values can be adjusted accordingly to allow direct comparison of the two scales.

The area under the blood glucose curve includes the area above the fasting level only. Any area beneath the fasting level is ignored. The incremental area under the blood glucose response curve is the sum of the areas of the triangles and rectangles. In **Figure 3**, A–F represent the blood glucose increments above the baseline value (fasting level) at sequential time points, where t and T represent different time intervals between blood samples.

When the blood glucose concentration at F falls below the fasting concentration (**Figure 3**), only the area above the fasting level is included in the total area represented by the triangle ET , where T' represents the portion of the time interval T when the blood glucose level between E and F is above the fasting level.

The overall equation simplifies to

$$\text{Area} = \left(A + B + C + \frac{D}{2} \right) t + \frac{(D + E)T}{2} + \frac{E^2 T}{2(E + F)}$$

If the last blood glucose concentration F is above the fasting level, then the term $(E + F)T/2$ is substituted for the last term in the equation, namely $E^2 T/2(E + F)$. An example of the incremental area calculation is shown in **Table 5**.

Calculation of Mixed Meal or Total Day's Glycemic Index

Each carbohydrate component is expressed as a percentage of the total carbohydrate in the meal or day and multiplied by the relevant GI. The sum of these values represents the meal's or the day's GI.

Table 5 Example of calculation of incremental area under the blood glucose response curve for glycemic response when the last glucose value falls below baseline

Time (min)	Corresponding letter on Figure 3	Blood glucose (mg dl ⁻¹)	Blood glucose increment (mg dl ⁻¹)
0	—	100	—
15	A	120	20
30	B	140	40
45	C	160	60
60	D	150	50
90	E	120	20
120	F	90	–10

Calculation: $\text{Area} = (20 + 40 + 60 + 25) \times 15 + (25 + 10) \times 30 + (20^2 \times 30 / 2 \times (20 + 10)) = 3425 \text{ mg min dl}$.

Source: Reproduced with permission from Wolever TMS and Jenkins DJA (1986) The use of the glycemic index in predicting the blood glucose response to mixed meals. *American Journal of Clinical Nutrition* 43: 167–172.

Calculation of Glycemic Load

Glycemic load is the diet GI multiplied by daily dietary carbohydrate intake in grams per day.

See also: Diabetes Mellitus: Classification and Chemical Pathology. Fiber: Resistant Starch and Oligosaccharides. Glucose: Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level

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Glucose Tolerance

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Glossary

Hepatic glucose output A sum of glucose leaving the liver and the net effect of glucose uptake and glucose production.

IFG (impaired fasting glucose) A condition where fasting glucose is elevated above the normal limit but not reaching the threshold for type 2 diabetes.

IGT (impaired glucose tolerance) A condition when 2-h glucose after OGTT is elevated above the normal

limit but does not reaching the threshold for type 2 diabetes.

IVGTT (intravenous glucose tolerance test) Standardized test where glucose is given intravenously and blood samples are taken regularly during the following hour.

OGTT (oral glucose tolerance test) Standardized test where glucose is given orally and blood samples taken at 2 h.

Definition and Impact of Glucose Tolerance

The prevalence of type 2 diabetes is steadily increasing and it has been estimated that the prevalence will further increase during the coming years. It has been assumed that within 25 years, if the trend is not altered, more than 25% of the global adult population older than 65 years of age will be affected by diabetes. The prevalence also shows ethnic differences, with prevalence ranging by a factor of 10 between different populations. The high prevalence of diabetes also implies increasing prevalence of diabetic complications, resulting in high and significant morbidity. Diabetes is also a major risk factor for cardiovascular diseases, which are responsible for the majority of deaths in diabetes. Altogether, this makes diabetes a major burden for global health and health economy.

A most important factor underlying the morbidity in diabetes, its complications and concurrent cardiovascular diseases, is hyperglycemia. Importantly, even at such a low degree as not to reach the limit criteria for diabetes, hyperglycemia is related to morbidity. Lifestyle changes and pharmacological interventions to reduce or even normalize the hyperglycemia exist, and consistent adherence to such regimen will reduce the morbidity. However, hyperglycemia is initially without symptoms and therefore usually remains undetected for a long period of time. Therefore, it is important to have reliable methods for the detection of hyperglycemia in its initial stages for proper actions to be taken. Such detection relies on analysis of the circulating glucose in the fasting state or after a challenge. These detections imply that hyperglycemia is subdivided into two different entities. The first entity is fasting hyperglycemia. This is mainly due to inappropriate release of glucose from the liver, which is caused by excessive glucagon levels in combination with low insulin levels or deficient action of insulin to restrain glucose release from the liver. The second entity of hyperglycemia is postchallenge hyperglycemia, which occurs after meal or glucose ingestion. This is called 'glucose intolerance' and is equivalent to an impairment to dispose glucose after a challenge. Several modes to diagnose glucose intolerance exist but the gold standard for its diagnosis is the oral glucose tolerance test (OGTT). This article describes this test, its advantages and limitations, and its potential role

for early detection of patients with increased risk for developing type 2 diabetes and cardiovascular diseases. The article also summarizes the basic mechanisms determining glucose tolerance as well as epidemiological and clinical aspects of glucose intolerance, including the potential of treating the condition for prevention of diabetes and cardiovascular diseases.

Glucose Tolerance Tests

History and Definition of Oral Glucose Tolerance Tests

A major breakthrough in the understanding of glucose intolerance as a risk factor for development of type 2 diabetes and cardiovascular diseases was the introduction of a worldwide standardization of the OGTT in the 1970s. By this introduction, glucose tolerance became a standardized entity, which enabled studies in metabolism, physiology, and clinical medicine with detection of risk factors as well as progressive follow-up studies using a standard recognized worldwide. At the same time, and also of significant importance for the generation of present-day knowledge within the field, was the introduction of the clinical entity impaired glucose tolerance (IGT), which replaced the then commonly used term 'borderline' diabetes. IGT as an entity was introduced simultaneously with the suggestion that glucose tolerance in a clinical test should be determined following ingestion of 75 g glucose, with a blood sample for the measure of glucose to be taken after 2 h.

The evaluation of the standardized OGTT in the clinical setting thus relies on a single 2-h glucose value. This value during the 75-g OGTT usually displays a normal distribution slightly skewed to the right. **Figure 1** shows the distribution pattern of 2-h glucose levels obtained from 802 Caucasian subjects in Malmö, Sweden. From this distribution, normal values may be defined statistically from mean and variance values for statistical definition of the distribution. The mean value is $\approx 7 \text{ mmol l}^{-1}$ and standard deviation is $\approx 1 \text{ mmol l}^{-1}$. By defining reference values as 95% confidence intervals, the cutoff value for normality would be approximately

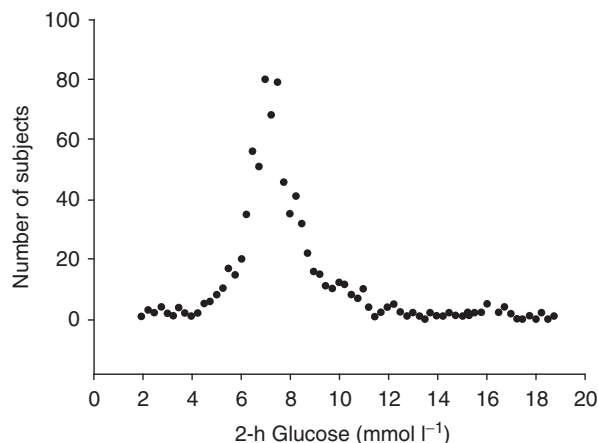


Figure 1 Distribution of the 2-h glucose value in OGTT performed in 802 Caucasian women, all aged 58 years, in Malmö, Sweden.

9 mmol l⁻¹ and, hence, values higher than 9 mmol l⁻¹ would indicate diabetes. By using such a definition of diabetes, a large number of subjects would have the disease, the clinical relevance of which is doubtful. Therefore, the definition of diabetes has instead been based on prospective studies evaluating the risk for microvascular disease and the cutoff-levels have been defined as levels substantially increasing this risk. Therefore, a cutoff value of 11.1 mmol l⁻¹ glucose has been used for the definition of type 2 diabetes.

OGTT was frequently used during the 1980s and 1990s for the clinical diagnosis of type 2 diabetes and in epidemiological studies, which markedly increased our knowledge of these conditions. By the end of the 1990s, however, definitions of IGT and clinical tests to be performed were again discussed. This resulted in revised cutoff levels and the introduction of a new entity called impaired fasting glycemia (IFG), which is defined as high fasting glucose. Current cutoff values for the 2 h glucose after 75 g glucose is 7.8–11.0 mmol l⁻¹ for IGT and ≥ 11.1 mmol l⁻¹ for type 2 diabetes. It was also suggested that fasting glucose was sufficient for the diagnosis of glucose intolerance and type 2 diabetes. The suggestion that a fasting sample is sufficient for the diagnosis of abnormal glycemia has been questioned, however, mainly because studies have shown that such a strategy will reduce the numbers at risk who are diagnosed and detected. This is because a large proportion of subjects with IGT have a normal fasting glucose but an elevated 2-h glucose value. In fact, there are populations with IFG alone, IGT alone, and IFG and IGT together, and these populations may represent risks for diabetes and cardiovascular diseases. Consequently, those having a high 2-h glucose value but a normal fasting glucose, who also have increased risk for cardiovascular diseases, will be missed by the suggested strategy. A study from Sweden identified this dilemma because it was demonstrated that out of 414 subjects with abnormal fasting or 2-h glucose values during an OGTT, only 140 (34%) had elevation of both values. The largest group comprised subjects with high 2-h glucose values but normal fasting glucose values (i.e., IGT but not IFG), which were seen in 235 subjects (57%), whereas only 39 subjects (9%) had high fasting but normal 2-h glucose values (i.e., true IFG). The individual subgroups were shown to have similar

risk factor patterns in terms of degree of obesity, blood pressure, and lipid levels. Therefore, a proper strategy to detect early cases at risk for diabetes and cardiovascular diseases, involves an OGTT.

Procedures and Evaluation of the Oral Glucose Tolerance Test

Glucose tolerance is defined as the ability to dispose of a glucose load, and therefore glucose intolerance is defined as an impaired ability for glucose disposal. The gold standard technique is to challenge with an oral glucose load, with measurement of circulating glucose before and after the challenge – the OGTT. As routinely performed, this test determines the ability to dispose of glucose after oral administration of 75 g glucose. The test is standardized such that it is performed in the morning after a 12-h overnight fast and blood samples are taken before the glucose load and after 2 h. Furthermore, the diet during the 3 days preceding the test should contain at least 250 g carbohydrates per day and the subjects should rest during the test in a semirecumbant position without smoking. The glucose given should be dissolved in 250–300 ml fluid, and sometimes fruit-flavored water is used to improve the taste. There has been much debate about how to take the blood sample. The original diagnostic criteria used values obtained from plasma derived from blood taken venously in tubes containing additives for prevention of coagulation. However, valid results are also obtained when glucose is measured in whole blood and when capillary samples are taken, although cutoff levels need to be adjusted for the different glucose concentrations in these samples. Arterial samples are also possible but rarely, if ever, used. Sometimes, mainly for research purposes, more frequent samples are taken and the test may last 3 h; however, for clinical purposes, the routine OGTT lasts 2 h, with a sample taken at that time point.

As shown in Figure 2, in a normal person, circulating levels of glucose increase within the first 15 min after the oral ingestion of glucose to reach a peak after 30 min. Thereafter, a progressive decline occurs, with the 2-h value usually approximately 25% higher than the fasting value. Usually, it takes 3 h for a return to baseline glucose levels. In subjects with IGT, there is usually also a peak at 30 min, albeit at a higher level than in normal subjects, but the main difference versus normal subjects is that the glucose disposal is impaired, which results in a higher 2-h glucose value. In diabetics, there is usually not a peak at 30 min but a continuous rise throughout the 2-h study period.

Limitations of the Oral Glucose Tolerance Test

An important limitation of the OGTT is the variability in results when the test is repeated. The coefficient of variance (CV) is $\approx 15\%$ which is higher than that for most other clinical tests. This high variance is not dependent on CV in the measurement of glucose, which is a procedure with very small error and CVs ($< 3\%$). Instead, biological variation explains the high CV in OGTT. Such factors, which are poorly understood, may be preceding diet, exercise, emotions, stress, drugs

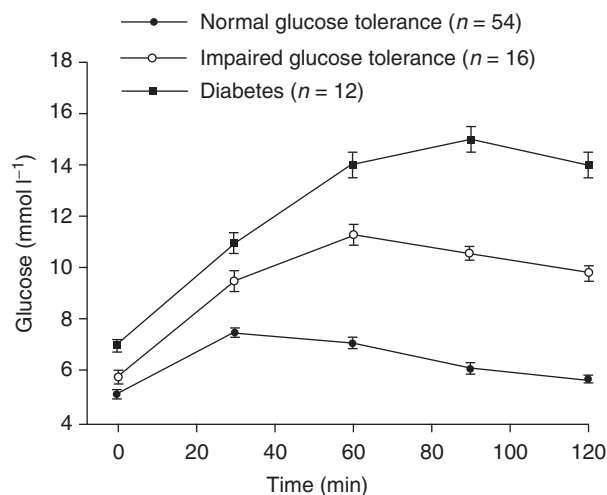


Figure 2 Venous plasma glucose levels during OGTT in subjects with normal or impaired glucose tolerance or type 2 diabetes who are treated with lifestyle modifications. Means \pm SEM are shown.

taken for various diseases, and gender, which are all factors influencing gastric emptying, carbohydrate absorption, islet hormone secretion, hepatic glucose production, and peripheral glucose uptake. Because of the high variability in the 2-h glucose value, a diagnosis of IGT or diabetes, particularly if intervention is planned, should not be based on a single OGTT. Instead, a clinical recommendation is to perform two OGTTs and use the mean of the two 2-h glucose values as the diagnostic value. The time interval between the two OGTTs should not exceed 3 months.

Differential Tests for Glucose Intolerance

As an alternative to OGTT, glucose tolerance may also be determined by administering glucose intravenously. In the intravenous glucose tolerance test (IVGTT), glucose is injected intravenously, usually at a dose of 0.3, 0.5, or 1 g kg⁻¹, and circulating glucose is determined before and 8, 10, 15, 20, 30, 40, 50, 60, and 80 min after injection. Glucose tolerance is estimated from the elimination rate, where a glucose elimination constant (kg) is calculated. The theory behind this is that the glucose elimination after intravenous glucose displays an exponential function (i.e., after logarithmic transformation of the data, the elimination is linear). kg is thus calculated as the slope for the glucose curve following logarithmic transformation of the individual glucose values and is calculated from the formula $kg = (0.693 \times 100)/t_{1/2}$ where $t_{1/2}$ is the half-time of glucose elimination (in minutes). The unit for kg is percentage of glucose decay per minute. Figure 3 shows this variable. Before OGTT was routinely used, IVGTT was undertaken more frequently. Unless very specific questions are asked, it is currently not used in clinical practice because it is more cumbersome to perform and it identifies only some of the metabolic processes underlying glucose tolerance, mainly insulin secretion, insulin sensitivity, and glucose uptake. Thus, other important aspects, such as glucagon secretion, release of incretin hormones, and hepatic glucose output, which are involved in the overall glucose tolerance and included in the

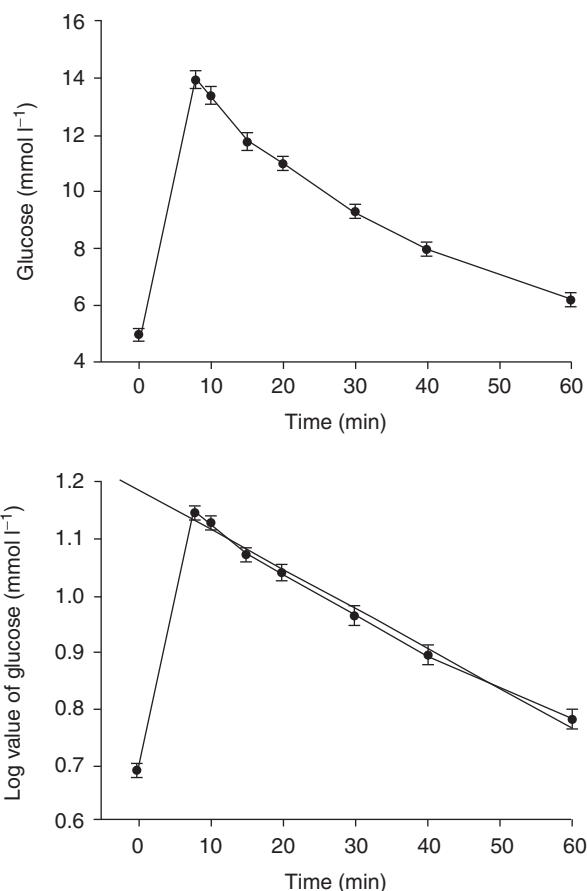


Figure 3 Glucose levels during IVGTT in 41 healthy subjects with normal glucose tolerance. Glucose (0.3 g kg⁻¹) was injected intravenously at time 0. Linear regression curve for the logarithmic values from minutes 8 to 60 is shown; the kg value in these subjects is $1.61 \pm 0.08\% \text{ min}^{-1}$. Means \pm SEM are shown.

2-h glucose value after OGTT, contribute only marginally to the kg after IVGTT.

It has also been suggested that measurement of HbA1c (i.e., the fraction of glycosylated hemoglobin) may be used for the diagnosis of type 2 diabetes. The rationale is that hemoglobin is irreversibly glycosylated in proportion to the glucose level, and therefore the level of HbA1c should reflect the mean of the glucose concentrations during the preceding 2 or 3 months. However, although this theoretical assumption is true, replacing diagnostic tools based on glucose with those based on HbA1c needs further analysis.

Metabolic Basis for Oral Glucose Tolerance

Oral ingestion of glucose initiates a series of metabolic perturbations, which comprise the 2-h glucose value. These metabolic perturbations are complex and involve glucose entering the bloodstream, changes in neural activity and incretin and islet hormone secretion, suppression of hepatic glucose production, and stimulation of peripheral glucose uptake. From a quantitative standpoint, of most importance with regard to the 2-h value are the changes in islet hormone secretion, which

include stimulation of insulin secretion and inhibition of glucagon secretion, and the suppression of hepatic glucose production. In fact, there is an inverse linear relation between the inhibition of hepatic glucose production and the 2-h glucose value and, similarly, a linear inverse relation between stimulation of the early (first 30 min) insulin secretion and 2-h glucose. A first series of events in the OGTT is initiated during the anticipation of the oral glucose ingestion, through olfactory stimuli and through receptors located in the oral cavity. This response is called the cephalic phase and activates sensory nerves. These give input to the central nervous system. This information is integrated in the hypothalamus for initiation and adjustment of a vagal nerve response to release insulin from the pancreatic islets. Therefore, when analyzed in detail, there is an increase in circulating insulin after glucose or meal ingestion already before glucose levels become elevated.

After passage of glucose through the oral cavity, glucose passes to the stomach and through a regulated mechanism is delivered into the gut. Because glucose is a monosaccharide, it is readily absorbed in the small intestine and reaches the splanchnic venous drainage. Glucose then passes to the portal vein and the liver. In the portal vein, glucose activates glucose-sensitive receptors, which through afferent sensory nerves send signals centrally to the brain for further integration with the previous signals in the hypothalamus for adjustment of efferent nerve activity. Furthermore, glucose in the liver inhibits hepatic glucose production, which is high after the overnight fast. Then, glucose passes to the general circulation to reach the pancreatic islets and the peripheral cells.

The glucose load to the gut also stimulates the release of the intestinal incretin hormones, such as glucose-dependent insulinotropic polypeptide (GIP) and glucagonlike peptide-1 (GLP-1). These hormones both activate local sensory nerves and pass through the circulation to reach the pancreas, where they stimulate insulin secretion and, in the case of GLP-1, inhibit glucagon secretion. In the pancreatic islets, vagal activation, intestinal hormones, and glucose stimulate insulin secretion, and glucose, GLP-1, and insulin inhibit glucagon secretion. These islet responses are of major importance for a normal glucose tolerance, and defects in these islet responses are major determinants of IGT and type 2 diabetes. Following passage of insulin into the venous drainage of the pancreas, the islet hormones reach the portal vein and the liver, and a main function of insulin is to potently suppress hepatic glucose production.

The suppression of hepatic glucose production by insulin is a major process with regard to the degree of hyperglycemia during the test; in subjects with inappropriately high hepatic glucose production, the glucose level after oral glucose is high. This explained why subjects with inappropriate insulin secretion have prandial hyperglycemia. This suppression of hepatic glucose production is augmented by the reduction in circulating levels of glucagon, which is initiated by direct and indirect actions of glucose and GLP-1 on the glucagon-producing cells and also by the action of insulin to inhibit glucagon secretion. After the liver, glucose and insulin reach the peripheral circulation and peripheral cells, where glucose is transported across the cell membranes and therefore leaves the circulation. In most cells, the insulin sensitivity of the cell is of major importance for the delivery rate of glucose. However, insulin-independent mechanisms also exist, even in tissues, which are also insulin

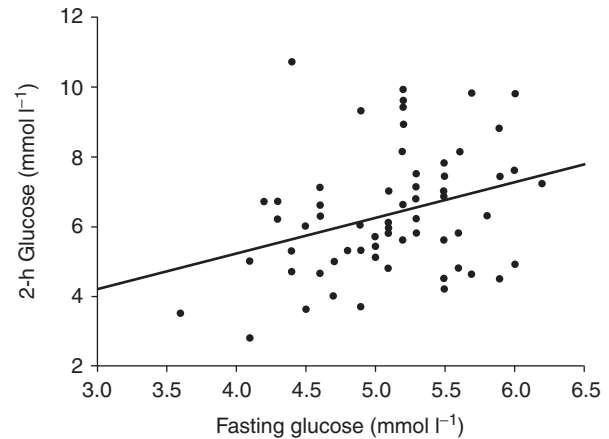


Figure 4 Correlation between fasting glucose and 2-h glucose during an OGTT in nondiabetic subjects ($r=0.32$; $P=0.008$).

sensitive, and glucose uptake is thus also dependent on glucose. Of most importance for glucose disposal after oral glucose is the muscle cells, which have a high capacity for glucose uptake. From all these processes, the glucose level at 2 h can be determined.

The metabolic processes underlying glucose tolerance are different from those underlying the fasting glucose value. Fasting glucose is mainly determined by hepatic glucose delivery, which in turn is governed by the ability to maintain normal basal insulin and glucagon levels. Therefore, mechanisms underlying IFG include defective insulin secretion, defective suppression of glucagon secretion, defective sensitivity in the liver for the action of insulin, and defective peripheral glucose disposal at low glucose levels, which is a sign of insulin resistance. Although mechanisms underlying fasting and 2-h glucose values differ, there is a high correlation between fasting and 2-h glucose values in normal subjects, as shown in **Figure 4**. Nevertheless, there is a limited overlap between IGT and IFG in a population; in fact, most subjects with IGT have normal fasting glucose, and most subjects with IFG have a normal 2-h glucose value. This suggests that different pathophysiological processes underlie IGT and IFG, which in turn suggests that OGTT should be undertaken more frequently than performed today.

Clinical Aspects of IGT

Epidemiology of IGT

After the introduction of the OGTT procedure in clinical practice and as a research tool in the 1980s, several studies on the prevalence of IGT and type 2 diabetes were performed in different populations. It became apparent that the prevalence of these conditions varied markedly among different populations. Studies during the past 15 years have further increased our knowledge because they have included additional populations and demonstrated that the prevalence of IGT and type 2 diabetes is steadily increasing over time. Also, an ethnic difference seems to exist, with extremely high rates in some Pacific island and North American Indian populations and

a low prevalence in South American Indian and Bantu populations.

Clinical Consequences of IGT

IGT is an important risk factor for development of type 2 diabetes. In general, the risk of transition of IGT into type 2 diabetes ranges from 1–2% to 5% and as high as 15–20% per year. The risk is higher for those older than 50 years of age. There is also evidence that hyperglycemia, even at levels not reaching the threshold for type 2 diabetes, is associated with a substantial risk for the development of cardiovascular diseases. One explanation for this is that glucose initiates metabolic perturbations of importance for developing angiopathy, such as tissue peroxidation, production of plasminogen activation inhibition-1, and impairment of endothelial function, such as nitric oxide production. Another explanation is that hyperglycemia is associated with a number of risk factors for cardiovascular diseases, such as high blood pressure, hyperinsulinemia, dyslipidemia, and microalbuminuria. In fact, if hyperglycemia is present, the risk for developing cardiovascular diseases for each of the other risk factors is augmented. Attempts to define cutoff values of glucose for cardiovascular risks have been problematic, however, probably due to the fact that the risk is continuously increased across the glucose ranges. Hence, the use of defined cutoff values is more a convenient practical issue, which is important in a clinical setting, but offers limitations from a theoretical standpoint. Furthermore, prospective studies have shown that an individual with IGT has an increased risk not only for type 2 diabetes but also for cardiovascular diseases and hence mortality. This indicates that attempts should be made to prevent IGT from progressing to cardiovascular diseases and type 2 diabetes.

Treatment of IGT

During recent years, the issue of whether IGT may be treated to prevent progression of type 2 diabetes or cardiovascular diseases has gained considerable interest. On the one hand, it has been argued that it is important to prevent progression of IGT. On the other hand, it has been argued that treating such a large population group as those with IGT would be risky. More fundamentally, the optimal preventive intervention for IGT is not known. The intervention may include lifestyle changes, notably increased physical activity and dietary regulations. Such interventions have been shown to be efficient in highly motivated populations and study centers. However, whether generalization of these results to the general population is possible is not known. Another mode of intervention is pharmacological treatment using compounds to stimulate insulin secretion and inhibit glucagon secretion, suppress hepatic glucose production, or enhance insulin sensitivity. These two strategies are not mutually exclusive, however, and introducing pharmacological intervention without giving lifestyle advice is not appropriate in a clinical setting.

Data from large population studies on the prevention of progression of IGT have been emerged. Two studies, the Finnish diabetes prevention study and the Diabetes Prevention Program, have shown that lifestyle changes (i.e., individualized diet and exercise counseling) in subjects with IGT reduced the incidence of diabetes by more than 50%. In

addition, in the Diabetes Prevention Program, it was shown that metformin (which reduces glucose output from the liver) reduces the risk by approximately 30%. This suggests that pharmacological treatment of IGT prevents development of type 2 diabetes. Several large studies are ongoing and results are expected within a few years.

Whether interventional programs on IGT are valid also for the prevention of cardiovascular diseases is not clearly established, mainly because long-term studies have not been performed. The Study to Prevent Non-insulin-dependent Diabetes Mellitus (STOP-NIDDM), however, showed that acarbose, which reduces glucose absorption from the gut, reduced cardiovascular events by more than 30% during a 3-year study period. This suggests that cardiovascular diseases may be prevented by treating IGT. It should be noted, however, that for prevention of cardiovascular diseases and mortality, more studies and longer follow-up periods are required.

Conclusion

Glucose intolerance increases the risk of developing type 2 diabetes and cardiovascular diseases, and it could be diagnosed with an OGTT. Lifestyle changes reduce the risk for progression of the condition.

See also: Diabetes Mellitus: Classification and Chemical Pathology; Etiology and Epidemiology. Glucose: Metabolism and Maintenance of Blood Glucose Level

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Metabolism and Maintenance of Blood Glucose Level

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Glossary

Glucagon A polypeptide hormone secreted by the A cells of the pancreatic islets of Langerhans that raises the concentration of glucose in the blood by stimulating glycogenolysis in the liver. It also stimulates insulin secretion.

Glucose A ubiquitous monosaccharide that provides 60–80% of dietary energy as the simple sugar, combined with fructose or galactose in the form of sucrose and lactose, respectively, or predominantly as its polymer, starch.

Gluconeogenesis The process by which simple precursors such as lactic acid and glycerol can be built up to produce new glucose molecules in the liver.

Glucose pool An expression of the amount of glucose in the body to which glucose molecules can be added from the food after eating and from the liver during fasting and removed into the tissues under the influence of insulin.

Glycogenesis The enzymatic process by which glycogen is formed from glucose.

Glycogenolysis The breakdown of glycogen, the polymeric form of glucose in which it is stored in the liver, to produce glucose that is secreted into the blood.

Homeostasis The concept introduced by Claud Bernard, to explain how the body maintains a number of vital functions such as temperature, blood sodium, and glucose concentration within narrow limits despite large changes in the environment.

Incretin One of several hormones released by glandular cells in the intestinal mucosa in response to the ingestion of food and which stimulate insulin secretion only in the presence of a higher than fasting blood glucose concentration.

Insulin A polypeptide hormone secreted by the B cells of the pancreatic islets of Langerhans that enables glucose to enter insulin-sensitive cells and from which it is otherwise excluded. It also suppresses glucagon secretion.

Glucose

Glucose is the only simple sugar found in most body fluids in anything more than trace amounts and, for all practical purposes, is confined to extracellular water. Lactose and fructose are the major sugars in milk and semen, respectively. This article reviews the major factors determining the concentrations of glucose in blood under everyday physiological and pathological conditions.

Body Glucose Pool

The body of an adult subject seldom contains less than 8 g, or more than 28 g, of glucose at any one time (corresponding to blood glucose concentrations of 3.5–10 mmol l⁻¹) despite enormous fluctuations in demand and supply. This quantity of glucose can be looked upon as constituting a hypothetical body pool (**Figure 1**) confined within a glucose space equal in volume to the combined water in blood and interstitial fluid, i.e., some 35% of total body water.

Glucose enters the cells by facilitated transport utilizing one or more genetically determined glucose transporter proteins depending on the tissue. The brain, for example, uses mainly GLUT1, whereas muscle and adipose tissue use insulin-sensitive GLUT4. Upon entering a cell, glucose is immediately phosphorylated and consequently removed from the pool.

Although its subsequent conversion into carbon dioxide and water or other metabolites (most notably glycerol, fatty acids, and the glycomoieties of mucopolysaccharides and

glycoproteins) is the only way that glucose ordinarily leaves the glucose pool, its loss in the urine may become a major factor in diabetes mellitus and is responsible for polyuria, one of its major symptoms.

Glucose enters the glucose pool from food via the hepatic vein or, in the postabsorptive subject, by release of glucose from preformed glycogen or molecules newly synthesized by liver and, to a lesser extent, kidney cells.

Glucose Space

The glucose space is constant in any individual, and, consequently, the amount of glucose in the pool is directly proportional to its concentration in the blood (see *Blood Glucose* below). It is this concentration that is homeostatically controlled through a series of rather complicated control mechanisms, the most important of which involve individual pancreatic islets of Langerhans that function semi-autonomously.

When pool size increases above a threshold, corresponding to a concentration in blood of approximately 10 mmol l⁻¹, glucose filtered at the glomeruli exceeds the tubular capacity to reabsorb it, and, consequently, glucose spills over into the urine. Although temporary increases in glucose pool size (hyperglycemia) are not immediately harmful, decreases in glucose pool size (hypoglycemia) are. Indeed, they are potentially so dangerous that many defense mechanisms have evolved to prevent or overcome it (see *Counterregulatory Hormones* below).

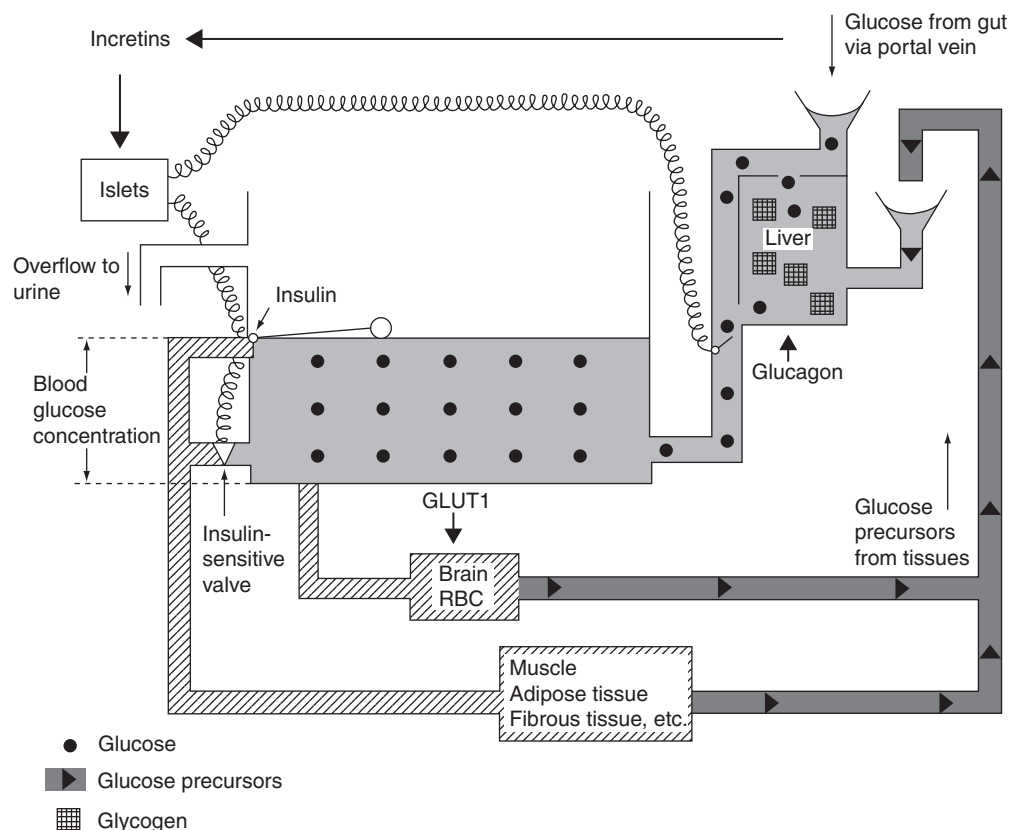


Figure 1 Schematic representation of blood glucose concentration and its relationship to the body glucose pool. The central system represents the hypothetical glucose pool, the actual size of which is represented by the horizontal axis (i.e., volume of distribution multiplied by blood (and extracellular fluid) glucose concentration). The postulated homeostatic switch is the cells of the endocrine pancreas, which respond to blood glucose concentration modulated by intestinal hormonal (incretin) and neural factors, which are themselves controlled by messages received from the gut (enteroinsular axis) and the autonomic nervous system. RBC: red blood cells.

Blood Glucose

The brain is the only important drain on the glucose pool in the fasting subject when plasma insulin levels are minimal. It consumes glucose at the rate of approximately 78 mg per gram of tissue per day. This amounts, in an adult man, to approximately 110 g day⁻¹ or 75 g day⁻¹ in a 1-year-old child due to its relatively large brain. A much smaller loss is into the erythron, which consists mainly of the bone marrow and red blood cells (RBCs). Estimates of glucose turnover suggest that approximately 9 g of glucose enters and leaves the glucose pool every hour in the average overnight-fasting healthy subject.

The concentrations of glucose in venous and arterial blood are similar in the fasting subject because peripheral tissues such as muscle, skin, and connective tissue do not extract significant amounts of glucose from the blood under these circumstances. In the recently fed subject, however, glucose uptake by peripheral tissues increases markedly under the influence of insulin released in response to the ingestion of a meal. This can produce a difference of 2 mmol l⁻¹ or more in arterial and venous blood glucose concentrations.

Experimentalists and clinicians alike still often ignore this fact, even though it was described more than 90 years ago. It not only has implications as regards our understanding of the physiology of glucose homeostasis, but sometimes has

unfortunate consequences for patients who may, if only venous blood is sampled, be misdiagnosed as suffering from hypoglycemia (i.e., blood glucose < 3.0 mmol l⁻¹) when it is not really present. It is, after all, arterial, and not venous, blood glucose that is homeostatically controlled and relevant to brain physiology.

Venous blood is much easier to obtain than arterial blood and explains why, despite its theoretical disadvantages, it is so often used in studies of glucose homeostasis and clinical practice. Fresh flowing finger-prick or earlobe-capillary blood more accurately reflects arterial blood glucose levels but is difficult to obtain in more than small amounts. In studies of glucose homeostasis, when collection of arterial blood would not be ethical, so-called arterialized venous blood collected from heat-distended veins on the back of the hands is often used as a surrogate.

Blood glucose concentrations generally lie within the range 3.5–6.0 mmol l⁻¹ in healthy fasting adult subjects and seldom rise above 11 mmol l⁻¹ in arterial blood, or 10 mmol l⁻¹ in venous blood, even after a large carbohydrate-rich meal. Glucose and other simple sugars given in solution produce greater rises in blood glucose than equal or larger amounts of glucose-yielding carbohydrate taken as part of a solid mixed meal. Conversely, prolonged starvation for as long as several weeks rarely causes the blood glucose concentration to fall below

3 mmol l⁻¹ except in children and adults with impaired gluconeogenesis.

The remarkable ability of the body to regulate the size of the glucose pool under such widely diverse conditions depends mainly on two organs – the liver and the pancreas – although during prolonged starvation, the kidneys become important generators of new glucose molecules.

Effects of Feeding on Blood Glucose

Glucose

Glucose and the two lesser dietary monosaccharides – fructose and galactose – enter the circulation through the intestinal mucosa. The speed with which they can be absorbed is limited by the rate of transfer from the intestine but rarely exceeds 50 g (0.28 mol) of carbohydrate, as glucose, per hour. This comparatively massive influx of glucose into a pool of 20 g ordinarily produces a remarkably small perturbation in blood glucose, as the rate of removal from the glucose pool rises to match glucose input.

In healthy people, arterial blood glucose concentrations generally return to fasting levels within 2 h of eating a carbohydrate-rich meal. This remarkable feat of homeostasis is achieved through the prompt, but appropriate, release of insulin into the circulation. This is a consequence of stimulation of pancreatic B cells (the source of insulin) and suppression of glucagon secretion by a rising arterial blood glucose concentration augmented by nervous impulses originating in the brain (cephalic phase), mouth, gut wall, and portal vein, as well as the insulinotropic hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), released by endocrine cells in the intestinal mucosa and whose importance in glucose homeostasis has only recently been fully appreciated.

Under the influence of food-induced insulinemia and suppressed glucagon secretion, the liver reduces its rate of glucose input into the pool and increases its rate of extraction. Peripheral insulin-sensitive tissues, such as fat, muscle, and skin, start removing glucose at an accelerated rate. As a result, the arterial blood glucose concentration falls and the stimulus to insulin secretion declines.

Ordinarily, the rates of change of glucose inflow from the gut into the glucose pool and the outflow of glucose into the tissues are so well aligned that arterial blood glucose levels rarely fall below fasting levels after a meal, and then only temporarily. Under the somewhat unnatural conditions resulting from ingestion of large amounts of glucose in solution on an empty stomach, a 'reactive hypoglycemia' may result from persistence of insulin action on peripheral tissues after plasma insulin has fallen to basal levels and all the glucose has been absorbed from the gut. Very occasionally, but usually not, the fall in arterial blood glucose concentration may be sufficiently severe to produce symptoms of glucose deprivation to the brain (neuroglycopenia), even in a perfectly healthy individual given a simple sugar solution on an empty stomach.

Disposal of an Oral Glucose Load

The exact disposition of glucose absorbed from the gut after a carbohydrate-rich meal by healthy subjects varies widely from

individual to individual, and depends on the size, composition, and physical nature of the meal.

All in all, some 70% of a 70-g oral glucose load is taken up by the peripheral tissues, where most of it is used, within 4 h of ingestion, to generate energy by oxidation to carbon dioxide and water. The remaining 30% is removed by the liver during its passage from the gut to the periphery and converted into glycogen, triglycerides, or other metabolites.

When volunteers were given a meal consisting of glucose (1 g kg⁻¹ of bodyweight) as a 45% solution on an empty stomach, their normal basal release of glucose from preformed glycogen in the liver was reduced from 9 g h⁻¹ to approximately 2.2 g h⁻¹, i.e., by approximately 75%. This persisted for a period (~3 h) during which glucose was being absorbed from the gut. In other words, although there is a small net uptake of glucose by the liver following a carbohydrate-rich meal, the liberation of glucose from preformed glycogen does not cease completely. Put differently, glycogenolysis and gluconeogenesis take place simultaneously, though at different rates, depending on whether glucose is or is not being absorbed, as well as on the amount and nature of the hormones released by the pancreas and the intestine in response to the presence of food.

Fructose and Galactose

Galactose and fructose are absorbed from the gut by mechanisms different from one another: galactose shares a transporter mechanism with glucose, whereas fructose has a less efficient one of its own. Once in the portal circulation, both sugars are rapidly taken up by the liver and largely converted into glycogen. This, together with glycogen formed from glucose, provides a small store of carbohydrate that is released as glucose into the body pool during the period when absorption from the gut is no longer occurring and gluconeogenesis has not yet become fully reestablished.

Starches and their hydrolytic products are converted into glucose in the gut lumen and brush border of the mucosa at a rate dependent on their exact composition. Some starches are absorbed as rapidly as performed glucose, whereas others are absorbed much more slowly. Sucrose is cleaved into glucose and fructose, and lactose into glucose and galactose, before absorption into the body. Intraluminal hydrolysis is rarely a factor in limiting the rate of absorption of simple sugars. Each moiety is dealt with separately.

Postabsorptive Stage

The exact duration of the absorptive phase that follows ingestion of a meal depends on many factors. These include the rate of gastric emptying as well as the size, composition, and physical nature of the food. It is unlikely, however, that an average adult eating three meals a day is truly post-absorptive, i.e., absorbing no glucose at all through the intestinal mucosa, for more than a few hours in any 24-h period, and this is mainly between 02.00 and 08.00. During this brief fasting period, glucose lost from the glucose pool by a constant drain into the brain and erythron is replaced by glucose

from the liver. This is derived either from reserves of glycogen built up during the absorptive phase of a meal or from new glucose molecules formed from glucose precursors such as lactate, pyruvate, glycerol, and alanine, brought to it in the blood from peripheral tissues. Gluconeogenesis increases under the influence of rising levels of glucagon and fatty acids, both of which are a consequence of falling plasma insulin levels.

The amount of glycogen in the liver varies with the nature of the diet, the size and composition of the last meal, and its timing. The average amount of glycogen in the liver after an overnight fast is approximately 44 g (range 15–80 g) and does not increase much after a meal; in other words, gluconeogenesis is already well under way within 12 h of eating the last meal. Nevertheless, after 36 h without food, liver glycogen stores may fall to as low as 4–8 g. Paradoxically, more prolonged fasting has little additional effect: indeed, hepatic glycogen stores may actually be replenished as the brain shifts from using glucose to β -hydroxybutyrate as its main source of energy.

Glycogen probably never disappears from the liver completely except *in extremis*, and there is evidence that it may be an intermediary in the production of glucose by the gluconeogenic pathway. Striatal muscles lack glucose-6-phosphatase, and, consequently, although they contain substantial amounts of glycogen, they cannot convert and release it into the blood as glucose. They can, however, release its main breakdown product, lactate, into the blood for reconversion into glucose in the liver.

Gluconeogenesis

The mechanism whereby the liver and, to a smaller but significant extent, the kidneys make new glucose molecules from chemically simpler compounds is referred to as 'gluconeogenesis.' Much attention has been paid to the role of specific hormones, such as glucagon and cortisol, and the enzymes they affect, in determining the rate of gluconeogenesis. The supply of glucose precursors is also important. In humans, lactate is probably the most important precursor, especially during exercise. Others, in descending order of importance, are alanine, pyruvate, glycerol, and, finally, some glucogenic amino acids, including glutamate. The last named is especially important in gluconeogenesis in the kidney. Fatty acids, apart from propionate, do not serve as glucose precursors to any significant degree.

The contribution made by alanine to gluconeogenesis may have been exaggerated in the past, though it does have a role in transporting three-carbon skeletons derived from muscle glycogen to the liver during fasting for conversion into glucose. In effect, it behaves as a shuttle to the liver for amino acids released by proteolysis of nonstructural muscle proteins during periods of prolonged fasting and starvation.

Gluconeogenesis is inhibited by eating, mainly through an increase in insulin and decrease in glucagon action. It is enhanced by fasting, probably by the reverse procedure. Alcohol specifically inhibits gluconeogenesis, from lactate but not alanine, by adversely changing the redox potential within hepatocytes and consequently reducing the availability of

nicotinamide adenine dinucleotide (NAD), which is an essential component in the formation of glucose from lactate. The inhibition of gluconeogenesis by alcohol can be so profound that severe hypoglycemia may develop in fasting subjects, especially children, who are totally dependent on gluconeogenesis for glucose needed by their brain and erythron.

Hormones and Glucose Homeostasis

Insulin is ordinarily the only major hormone capable of lowering blood glucose levels (Table 1). It does so by inhibiting glycogen breakdown in the liver and inhibiting gluconeogenesis and by encouraging glucose to leave the glucose pool by entering peripheral tissues. It achieves this mainly by activating the glucose transporter protein GLUT4: an action that is enhanced by exercise and hyperglycemia. Consequently, insulin lowers blood glucose by two independent mechanisms. Which of the two actions predominates depends on the circumstances: one of the most important is the concentration of insulin in the blood; another is whether it is of exogenous or endogenous (pancreatic) origin. Exogenous insulin reaches peripheral tissues at a higher concentration than blood perfusing the liver and is unaccompanied by C-peptide. Endogenous insulin, however, reaches the liver at a higher concentration than peripheral tissues and is accompanied by C-peptide, for which there is increasing evidence of synergism with insulin action and its absence from the circulation is probably in part responsible for some of the vascular complication of diabetes.

Insulin released into the portal circulation is partially or completely removed by the liver. Insulin injected into skin, muscle, or a vein reaches insulin-sensitive tissues in the periphery at a concentration equal to, or greater than, the liver. Not all tissues on which insulin acts are equally sensitive to its actions: fat cells, for example, are more sensitive to its antilipolytic actions than muscle cells are to its glucose uptake-stimulating properties.

At the concentration at which insulin normally circulates in the peripheral blood of fasting subjects ($\sim 30 \text{ pmol l}^{-1}$), it depresses, but does not completely suppress, the unbridled release of fatty acids from lipocytes. At this concentration, insulin does not encourage glucose uptake by striatal muscle – which falls almost to zero. At insulin concentrations seen in peripheral blood in the absorptive phase of a meal ($\sim 150\text{--}600 \text{ pmol l}^{-1}$), its effect on peripheral glucose uptake is pronounced and responsible for the marked arteriovenous glucose difference observed at this time. Similar plasma insulin levels are achieved during insulin therapy for diabetes.

The release of insulin from the B cells of the pancreatic islets is highly dependent on the concentration of glucose in the blood perfusing them. At blood glucose levels below approximately $3.5\text{--}4.0 \text{ mmol l}^{-1}$, insulin secretion is minimal but nonetheless essential. This means that as the arterial blood glucose falls toward its basal level in the postabsorptive state, plasma insulin levels also fall. They never fall low enough, however, in the nondiabetic subject, to permit uncontrolled liberation of glucose by the liver or fatty acids by adipocytes. This does, of course, happen when the B cells are destroyed

Table 1 Hormones that affect blood glucose concentrations

<i>Hormones concerned with glucose homeostasis</i>				
<i>Hormone</i>	<i>Source</i>	<i>Stimuli</i>	<i>Inhibitors</i>	<i>Main effect on glucose homeostasis</i>
Insulin	B cells of islets	Hyperglycemia, incretins (i.e., GIP and GLP-1), glucagon, some amino acids, e.g., arginine, leucine: parasympathetic nervous system, i.e., vagus	Hypoglycemia, sympathetic nervous system and adrenaline, somatostatin	Reduces blood glucose concentration by inhibition of hepatic glycogenolysis and gluconeogenesis, permitting peripheral glucose uptake
GIP	K cells of duodenum, jejunum, and ileum	Actively absorbed sugars, e.g., glucose and galactose, actively absorbed fats, especially polyunsaturated	Glucagon, insulin	Incretin: stimulates insulin secretion only in presence of hyperglycemia
GLP-1	L cells of the ileum and colon	Ingested food, whether absorbed or not	Glucagon, insulin	Incretin: stimulates insulin secretion only in presence of hyperglycemia: inhibits glucagon secretion. Inhibits gastric emptying: reduces appetite
Glucagon	A cells of islets	Hypoglycemia, adrenaline, some amino acids, e.g., arginine	Insulin, hyperglycemia	Increases glycogenolysis in liver (not peripheral tissues), enhances gluconeogenesis
Adrenaline	Adrenal medulla	Hypoglycemia, through sympathetic nervous stimulation, physical and mental stress		Increases glycogenolysis in liver and peripheral tissues, inhibits insulin secretion, stimulates glucagon secretion, impairs peripheral glucose utilization, and increases lipolysis (i.e., raises plasma NEFA levels)
Cortisol	Adrenal cortex	Hypoglycemia through hypothalamic release of ACTH		Decreases peripheral glucose uptake, induces insulin resistance, and permits hepatic glycogenesis
Growth hormone	Anterior pituitary	Hypoglycemia, through hypothalamus, ghrelin released from stomach following ingestion of food	Hyperglycemia, somatostatin, alcohol	Decreases peripheral glucose uptake, increases adipocytes lipolysis
Vasopressin	Hypothalamus and posterior pituitary	Hypoglycemic stress, dehydration	Hypo-osmolality: alcohol	Stimulates hepatic glycogenolysis

and insulin secretion ceases completely; consequently, both gross hyperglycemia and ketosis, which are the hallmark of insulin-dependent diabetes, develop.

During prolonged starvation (20 days or more without food), small amounts of insulin reach the liver. The amounts reaching the adipocytes are, however, insufficient to prevent seemingly uncontrolled lipolysis leading to hyperketonemia comparable with that seen in diabetic ketoacidosis ($\sim 10\text{--}20\text{ mmol l}^{-1}$). The situation differs, however, from that obtained in diabetes, in that the restraining effect of insulin on hepatic gluconeogenesis and glycogenolysis remains. Consequently, blood glucose levels are normal during prolonged fasting rather than grossly elevated as in insulin deficiency resulting from B-cell malfunction.

A consequence of the reduction of insulin release as blood glucose levels fall after absorption of a meal is liberation of the A cells, which are 'downstream' of B cells in the islet, from the suppressive effect of (endogenous) insulin on their own release of glucagon. On reaching its target organ, glucagon promotes glycogenolysis and gluconeogenesis in the liver, in

effect reversing the effect of insulin during the absorptive phase. In other words, each islet functions as a miniature homeostat.

Counterregulatory Hormones

Although it is possible to explain the control of blood glucose largely by means of the servoregulatory control of insulin and glucagon secretion described earlier, the body also has many neural and hormonal mechanisms at its disposal to correct or overcome any fall in blood glucose to below the critical level necessary for maintenance of normal brain function. The sensors for this regulatory function are located in at least two anatomically distinct sites within the brain and in the portal vein itself. The most important mechanisms involved are the following:

- Stimulation of the sympathetic and parasympathetic nervous systems, which in turn lead, respectively, to release of adrenaline from the adrenal medulla and noradrenaline

from nerve terminals in the liver, and glucagon from the pancreas.

- Secretion of vasopressin by the posterior pituitary gland; adrenocorticotrophic hormone (ACTH), growth hormone, and prolactin by the anterior pituitary gland; and cortisol by the adrenal cortex.

They produce their hyperglycemic effects in a variety of ways that can be summarized as follows:

1. Increasing the liberation of glucose by the breakdown of preformed glycogen in the liver, e.g., glucagon, adrenaline, noradrenaline, and vasopressin.
2. Increasing gluconeogenesis in the liver, e.g., glucagon, cortisol.
3. Decreasing peripheral glucose utilization by peripheral tissues, e.g., growth hormone, adrenaline, cortisol, and prolactin.

Glycosuria

Upwards of 100 g of glucose are normally filtered from the blood at the glomeruli of the kidneys each day, more than 99% of which is reabsorbed by the kidney tubules. As a result, healthy people lose less than 150 mg of glucose in their urine each day, an amount too small to be detected by most simple screening procedures for glycosuria. When, for any reason, the amount of glucose filtered at a glomerulus is more than that which can be reabsorbed by the tubules, glucose appears in the urine at a concentration many times greater than that in the blood.

The commonest cause of significant glucosuria, i.e., the presence of glucose in the urine at a concentration greater than 0.15 g l^{-1} (0.8 mmol l^{-1}), is a blood glucose concentration of 10 mmol l^{-1} or more. At or above this concentration, the amount of glucose filtered at the glomerulus is more than that which its associated kidney tubule can transport back into the circulation. Once this reabsorptive threshold has been exceeded, any increase in filtered glucose load is reflected by a large increase in the amount of glucose eliminated in the urine. The osmotic diuresis so produced is associated with an increased excretion of water, sodium, chloride, and potassium and is often the first clue to the existence of hyperglycemia, the characteristic hallmark of diabetes.

Glycosuria can occur at normal blood glucose levels when, for example, blood flow through the glomerulus is increased, such as during pregnancy, or when, owing to either an inherited or acquired defect of renal tubular glucose transport, even the normal amount of glucose filtered at the glomerulus cannot be reabsorbed. Conversely, in people in whom blood flow through the glomeruli is reduced or in whom glomerular filtration is impaired, but in whom normal tubular glucose reabsorption is retained, even gross hyperglycemia may not produce glycosuria.

Fructose and galactose do not normally appear in urine because their concentration in blood is never sufficiently high – except in disease – for them to do so.

See also: Diabetes Mellitus: Classification and Chemical Pathology; Dietary Management. Etiology and Epidemiology. Fiber: Resistant Starch and Oligosaccharides. Fructose: Absorption and Metabolism. Glucose: Glucose Tolerance. Lactose Intolerance. Sucrose: Dietary Sucrose and Disease

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GLYCEMIC INDEX

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In the past 10 years, a number of important epidemiological and experimental studies have linked glycemic index to postprandial glucose metabolism, insulin resistance, and cardiovascular risk factors. The World Health Organization and the Food and Agriculture Organization recommended that the physiological effects of dietary carbohydrates be classified according to their glycemic index. This review examines the historical and scientific background of the glycemic index.

Background and Definition

In 1939, Conn and Newburgh noted how different carbohydrate-containing foods could have the same macronutrient

composition but different glycemic responses. Insulin responses elicited by different carbohydrates also vary. These observations led to the first classification of carbohydrate foods according to their glycemic response, which then allowed different dietary carbohydrates to be exchanged within a meal without altering postprandial glucose levels. The 'glycemic index' was introduced as a means of quantifying the glycemic response of different dietary carbohydrates.

Glycemic indexes of several foods are published in international nutritional tables, the most recent of which was published in 2002. Methodology on their derivation is available from previous reviews. Glycemic index of a food is a measure of postprandial glucose response after a 50-g load of available carbohydrate from the food (**Figure 1**) and provides a standardized comparison of a carbohydrate's 2-h postprandial glucose response with that of glucose (**Table 1**). Low glycemic index carbohydrates have lower 2-h incremental areas under the glucose curve than glucose, whereas high glycemic index foods have higher areas. Although the insulin response is not used to define glycemic index, the lower the glycemic index of a food, the more attenuated is the insulin response to a standard test meal. It has been argued that it is the insulin response to foods and not the glycemic response that is important in the pathogenesis of insulin resistance and related metabolic disturbances and disease risk. Although still an area of debate in general, glycemic index is a surrogate marker of the insulin response to different carbohydrates, with the possible exception of dairy products. Indeed, the insulin response in nondiabetic subjects to a wide range of foods (glycemic indexes between 32 and 100) is highly correlated. The exception to this is possibly dairy products which have an insulin response, high than predicted but the glycemic index. This remains unexplained at present. Dietary carbohydrates stimulate insulin secretion both directly by stimulating the

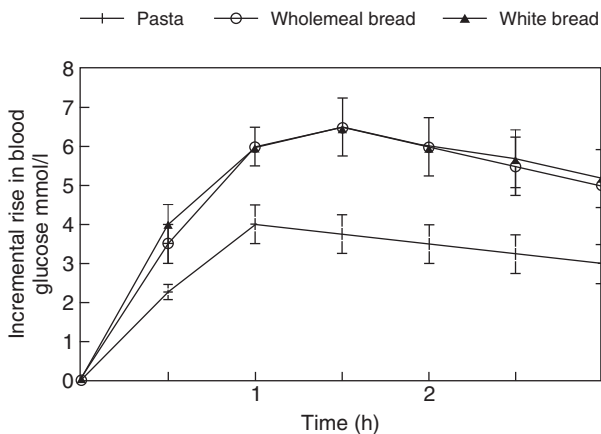


Figure 1 Mean blood glucose increment after equiavailable carbohydrate meals. Data with permission from Jenkins DJ, Wolever TM, and Jenkins AL (1988) Starchy foods and glycemic index. *Diabetes Care* 11: 149–159.

Table 1 The glycemic index model

Incremental area under blood glucose response curve for the test food containing 50 g available carbohydrate
Corresponding area after equicarbohydrate portion of glucose
Calculation of the glycemic index of a mixed meal containing three separate carbohydrate-containing foods
Glycemic index/mixed meal $(GI_1)(PCF_1) + (GI_2)(PCF_2) + (GI_3)(PCF_3)$
Where
The three carbohydrate-containing foods are 1, 2, and 3
The glycemic index for each carbohydrate-containing food is GI_1 , GI_2 , and GI_3
The carbohydrate content is C_1 , C_2 , and C_3 (g)
The total meal carbohydrate (TMC) is $[C_1 + C_2 + C_3]$ g
The proportion of carbohydrate from each food (PCF) is $PCF_1 = C_1/TMCg$, $PCF_2 = C_2/TMCg$, and $PCF_3 = C_3/TMCg$

pancreatic β cell and indirectly through their secretion effect. The pattern of insulin secretion caused by different carbohydrates reflects their different intestinal transit times.

Type of Dietary Carbohydrate and the Glycemic Index

The glycemic index of a carbohydrate is influenced by its rate of intestinal absorption, which in turn is influenced by its composition, tertiary structure, type of starch, and susceptibility to enzymatic digestion.

Chain Length and Composition

Complex carbohydrates are polymeric chains of repeating monosaccharide units. Starches comprise repeating glucose units. The glycemic indexes of different starches are determined by their susceptibility to enzymatic digestion, and not chain length. White bread and pasta have similar chain lengths, but bread has a higher glycemic index due to its tertiary structure and solubility that ensures greater exposure to salivary and pancreatic amylases.

Short-chain carbohydrates are rapidly absorbed; however, when they contain nonglucose sugars, the glycemic index is lowered proportionally. The disaccharides sucrose and lactose consist of 50% glucose and 50% fructose or galactose, respectively, and both have a lower glycemic index than maltose, the disaccharide formed from two molecules of glucose.

Amylose and Amylopectin

The starches in cereal grains, rice, potatoes, and all green plants are composed of repeating glucose units arranged in straight (amylose) and branched-chained (amylopectin) polysaccharides. The absorption rate, and hence the glycemic index, of these starches is influenced by the ratio of amylose to amylopectin. The more compact structure of amylose than amylopectin results in a smaller surface area being available for amylase digestion. Amylose-enriched starches therefore have lower glycemic indexes than those enriched in amylopectin.

Relationship of Insoluble and Soluble Nonstarch Polysaccharides (NSPs) (Fiber) to Glycemic Index

Nondigestible complex carbohydrates are commonly known as dietary fibers; the more correct terminology is NSPs. NSPs are either soluble or insoluble. Clinical studies have shown that diets rich in soluble fiber/NSPs, such as guar gum, pectin, and sugar beet fibers; and lower postprandial blood glucose and insulin levels. Guar gum, a β -galactomannan from the Indian locust bean, also reduces postprandial lipemia. Non-soluble NSPs have no effect on dietary glycemic index.

Soluble NSPs, such as pulse vegetables, whole fruits, oats, and barley, form gelatinous gels within the stomach that delay gastric emptying and enzymatic digestion, the latter by forming a physical barrier around the carbohydrate. Insoluble NSPs have little effect on gastric emptying and no effect on glucose absorption. High fiber/NSP diets are therefore not necessarily synonymous with low glycemic foods. Cellulose is the most widely used NSP in household cereals, whole meal bread, and

brown rice; and because it is insoluble, these foods have the same glycemic index whether replete or deplete of their dietary fiber/NSPs. For unknown reasons, All-bran is an exception, and despite its high insoluble fiber content, it has a low glycemic index.

The solubility of dietary fiber/NSPs have benefits on postprandial glycemia and hyperinsulinemia. The reason for this is multifactorial including slowing of gastric emptying, a physical barrier to amylase, possible thickening of the unstirred layer and positive effects on gut incretin hormones such as glucagon-like peptide-1 and gastric inhibitory polypeptide. The lack of effect on increasing nonsoluble fiber NSPs on glucose and insulin should not detract from important effects on bowel function and bowel pathology.

Cell Structure, Food Preparation, and Processing

Cooking and food preparation can modify the glycemic index. Highly processed convenience foods tend to have high glycemic indexes. When cooking and processing disrupt the cell wall, the starch granules are broken open, optimizing amylase digestion and increasing the glycemic index. Cooked pulse vegetables have low glycemic indexes because their cell walls are resistant to cooking. The intact cereal grains of pumpernickel rye bread, granary bread, and bulgur wheat all have low glycemic indexes. However, when granary bread is processed to wholemeal bread, these grains are disrupted and the glycemic index rises. Cooling can paradoxically lower the glycemic index of certain starches, such as potatoes, due to the formation of retrograde starches that are resistant to amylase digestion.

Effects of the Upper Gastrointestinal Tract

For many foods, their glycemic index is determined by the process of chewing and swallowing. Chewing can reduce food particle size, which increases absorption rates. This explains why boiled and mashed potatoes have different glycemic indexes. Chewing can also change the constituency of the food such that with bread the particle size is reduced to such an extent that it behaves more as a fluid on swallowing and is therefore very rapidly absorbed. In contrast, pastas retain their structure on swallowing and are more slowly absorbed. The rate of gastric emptying also influences the glycemic index, with lower glycemic index foods being retained in the stomach for longer periods than high glycemic index foods.

Concerns Related to the Glycemic Index

Whereas the 1998 World Health Organization (WHO)/Food and Agriculture Organization dietary carbohydrate guidelines and the 1998 European dietetic guidelines advocate greater use of the glycemic index, the American Diabetes Association's evidence-based guidelines are more cautious, suggesting a 'B'-level evidence grade. This is basically due to the lack of long-term studies that there is only one randomized control trial which had a study period of longer than 6 months. Also there remains concerns regarding the effects of mixed meals are difficult to predict. Against this is the observation in well-

conducted randomized control trials, blood glucose during the low glycemic index diet is lower than the high glycemic index diet. Studies of the long-term efficacy of low glycemic index diets in diabetes, obesity, and coronary risk groups using randomized control methodology are under way and will report during the next 5 years. The issue regarding the predictability of the glycemic index of mixed meals remains a matter of debate, but evidence suggests that the glycemic index of a mixed meal is reasonable when the fat content is low and deteriorates as the fat content of the meal rises. However, this academic debate should not detract from the fact that the evidence from randomized control trial suggests positive benefits on glucose, insulin, and lipids from low glycemic index diets.

Reproducibility

Within-Subject Variation

The variability of the glycemic response for a given food for any individual is similar to that seen for the oral glucose tolerance test. In one study, a 25% coefficient of variation (CV) within individuals was seen when 11 healthy subjects had their glycemic response to different carbohydrates tested on eight separate occasions. In another study, the CV of the glycemic response in 22 healthy subjects given 50 g of white bread was 22%. This variability is reduced when the glycemic response is expressed in terms of the 'glycemic index.'

Between-Individual Variation

The variability of the glycemic responses between individuals is larger than that within individual subjects. In a study that included 11 nondiabetic individuals; 10 noninsulin-treated type 2 diabetic subjects, 12 insulin-treated type 2 diabetic subjects, and 14 type 1 diabetic subjects; the CV between individuals within each group was 26%, 34%, 23%, and 34%, respectively. From this it can be seen that comparing the absolute glycemic responses both within and between subjects is unreliable. However, this problem is considerably lessened when the glycemic response to any given food is expressed as a percentage of that individual's glycemic response to a standardized food substance, which in the case of the glycemic index is usually 50 g of glucose. By expressing the glycemic response of a test food against an equal amount of a standard carbohydrate in an individual, variations that occur with age, sex, body mass index, and ethnicity, as well as medical conditions such as diabetes are minimized. By using the glycemic index, the between-individual CV of the glycemic response is reduced approximately from 40% to 10%.

Reproducibility of the Glycemic Index

The glycemic index measurement of certain foods can vary between individuals. For example, one study reported that the glycemic index of lentils ranged between 23 and 70 for different subjects. However, this large variability can be minimized to approximately 10% when both the food to be tested and the standard, usually white bread, are each measured in triplicate.

Problems Arising From Different Methodologies Used to Calculate the Glycemic Index

Before 1998, the WHO nutritional report that standardized the methodology of assessing the glycemic index, different groups used different techniques to calculate the area under the glucose curve and to assess the postprandial glycemic response. The biggest change has been the standardization of the standard used from white bread to glucose. To allow comparison to historic data, published glycemic index tables provide conversion factors or present tables using different methods.

Mixed Meals and Other Nutrients

Carbohydrate foods are frequently taken as part of a mixed meal, and the addition of fat and protein to a carbohydrate-containing meal tends to lower the glycemic response. Although the addition of protein or fat to carbohydrate foods reduces the glycemic response, the relative response of one carbohydrate to another remains, such that lentils will always have a lower response than white bread when part of a mixed meal.

The glycemic index of a mixed meal can be calculated from the different proportions of each of the carbohydrate-containing foods and their individual glycemic index values. For example, when bread and beans are mixed in equal portions, the resulting glycemic response is midway between that of bread alone and that of beans alone. A formula for calculating the glycemic index of mixed meals has been derived by Wolever and Jenkins ([Table 1](#)). For accuracy, this method requires all individual carbohydrate components of a mixed meal to be pretested. Other methods of calculating the glycemic responses of mixed meals relying on a single measurement of the area under the glycemic curve for the mixed meal or an estimation that does not account for all the carbohydrate-containing foods will be less accurate. To be fair, this remains an area of debate; a recent study suggested that the ability to predict the glycemic index of a mixed meal is poor, particularly those with a high fat content.

The Second Meal Effect

Dietary carbohydrates can influence the glycemic response of a second meal consumed during the postprandial period. The blood glucose response to a lunchtime meal is lower when taken after a low glycemic index breakfast than after a high glycemic index breakfast. Similarly, the glycemic response of a second meal taken during the postprandial period after lunch or dinner is influenced by the glycemic index of the preceding meal.

Wolever attributed the differences in the glycemic response to a second meal during the postprandial period to differences in intermediary metabolism and insulin action associated with rapidly and slowly absorbed carbohydrates. Rapidly absorbed carbohydrates produce large increases in blood insulin levels that result in blood glucose levels decreasing sufficiently quickly to stimulate several counterregulatory hormones that inhibit insulin action and glucose disposal. Both carbohydrate drinks and meals consumed rapidly rather than sipped or eaten slowly are associated with significantly higher serum

concentrations of glucagon, catecholamines, growth hormone, and nonesterified fatty acid (NEFA) levels postprandially. The addition of guar to a meal, which slows glucose absorption and lowers the glycemic response, reduces postprandial NEFA and β -hydroxybutyrate levels and improves insulin action. In contrast, nibbling high glycemic index foods between meals increases the glycemic response of a subsequent meal.

Clinical Significance of Postprandial and Fasting Hyperglycemia in Diabetic and Nondiabetic Populations

As with fasting blood glucose levels, postprandial hyperglycemia in nondiabetic populations is a predictor of insulin resistance and cardiovascular disease (CVD). The combined 20-year mortality data on men from the Whitehall, Paris prospective, and Helsinki policemen studies showed that the highest quintile compared with the lowest for the 2-h postplasma glucose load was associated with a 2.7 increased risk of CVD mortality. The fasting glucose values were less predictive for CVD, with only the top 2.5% conferring a 1.8-fold increased mortality risk. During a 7-year period, elderly women with isolated postprandial hyperglycemia and a 2-h value more than 11.1 mmol l^{-1} and fasting value less than 7.0 mmol l^{-1} on a 75-g oral glucose tolerance test had an approximately threefold increased risk of heart disease compared with women whose 2-h values were less than 11.1 mmol l^{-1} .

In established diabetes, postprandial glycemia appears to have a stronger relationship with microvascular and macrovascular disease than fasting blood glucose. Similarly, in gestational diabetes adverse pregnancy outcome is more closely related to postprandial glycemia than fasting and premeal glycemic values.

Benefits of Low Glycemic Index Carbohydrates on Diabetic Control

This is the area in which there is most evidence of clinical efficacy. Two independent systematic reviews of the world evidence demonstrated the efficacy of low glycemic index diets on glycemic control in both type 1 and type 2 diabetes. Clinical studies have shown that after 3 months of a diet containing low glycemic index carbohydrates, glycemic control is improved in both type 1 and type 2 diabetes. With low glycemic diets, postprandial glucose and insulin concentrations decrease in type 2 diabetic subjects, whereas both postprandial glucose values and insulin requirements decrease in type 1 diabetic subjects. Good glycemic control and favorable lipid and fibrinolytic profiles have also been reported in individuals with either type 1 or 2 diabetes who habitually consume low glycemic index dietary carbohydrates. It remains to be shown whether these diets bestow long-term benefits on micro- or macrovascular complications.

Benefits of Low Glycemic Index Carbohydrates on CVD Risk Factors

High glycemic index foods induce postprandial hyperinsulinemia, which is a powerful predictor for metabolic risk

factors and CVD in epidemiological studies. Both cross-sectional and prospective population studies have shown favorable lipid profiles in association with high carbohydrate diets. Initially, these benefits were attributed to a high-fiber content. However, when the glycemic index is controlled for, it is the low glycemic index diets rather than high-fiber content that have the greatest influence on high-density lipoprotein (HDL) cholesterol, insulin sensitivity, and fibrinolytic parameters. In a cross-sectional study on more than 2000 middle-aged subjects, the glycemic index was a stronger determinant of HDL cholesterol than any other dietary factor, be it carbohydrate or fat. In this study, the HDL cholesterol of the women whose habitual diet was within the lowest quintile for glycemic index was 0.25 mmol l^{-1} higher than that for women whose dietary carbohydrate was within the highest quintile. Extrapolating from the Framingham data that showed a 3% decrease in female and a 2% decrease in male cardiovascular morbidity to be associated with a $0.026 \text{ mmol l}^{-1}$ increase in HDL cholesterol, one would predict a 29% difference in coronary heart disease (CHD) morbidity between women in the lowest and highest quintile for dietary glycemic index. A similar calculation for men with dietary carbohydrates in the lowest and highest quintile for glycemic index found a 7% decrease in CHD morbidity associated with the 0.09 mmol l^{-1} difference in HDL cholesterol concentrations. Low glycemic index diets have also been shown to lower serum cholesterol and triglyceride levels in hyperlipidemic subjects.

Glycemic Index and the Prevention of Type 2 Diabetes

Changes in diet and physical activity levels, both alone and in combination, reduce the progression of impaired glucose tolerance to diabetes. Two large US prospective population studies have demonstrated a doubling of the relative risk of developing type 2 diabetes for both men and women when the habitual diet is characterized by a high glycemic index and high fat content. A similar protective effect against diabetes has been reported in populations consuming high-fiber foods and high quantities of fruit, and one would predict that these diets would also have a low glycemic index.

Obesity and Glycemic Index

Obesity contributes to the pathogenesis and morbidity of type 2 diabetes. Obesity is associated with changes in carbohydrate and fat metabolism that are central to the development of insulin resistance. Although low glycemic index diets enhance insulin sensitivity and improve metabolic cardiovascular risk factors, they will not reduce weight unless part of an energy-deficient diet. However, in obese subjects, when low glycemic carbohydrates are incorporated into a hypocaloric diet, there is a greater decrease in insulin resistance than can be accounted for by weight loss alone. Evidence from both animal and human studies demonstrates a change in body composition (decrease in fat but no change on overall weight) when exposed to a low glycemic index diet.

Pregnancy and Glycemic Index

Throughout pregnancy in well-nourished urbanized women consuming typical Western diets, glucose tolerance deteriorates. During pregnancy, African women living in traditional rural populations and consuming high-carbohydrate/low glycemic index diets do not invariably experience deterioration in their glucose tolerance. Clinical studies in the West show that women consuming similar high-carbohydrate/low glycemic index diets throughout pregnancy also have no deterioration of glucose tolerance despite the physiological increase in insulin resistance that occurs secondary to maternal and placental hormones. When the proportion of dietary carbohydrate increases above 50% in women with gestational diabetes, if no emphasis on low glycemic index carbohydrates is given, glucose tolerance will deteriorate.

Proposed Mechanism by which Dietary Carbohydrates/Glycemic Index Influence Insulin Resistance

Adipocyte metabolism is central to the pathogenesis of insulin resistance and dietary carbohydrates influence adipocyte function. The previous simplistic view that insulin resistance resulted from the downregulation of the insulin receptors in response to hyperinsulinemia is being replaced by the hypothesis that high circulating NEFA levels both impair insulin action and reduce pancreatic β cell secretion. It is plausible that low glycemic index carbohydrates reduce insulin resistance by their ability to reduce adipocyte NEFA release. There is evidence of a loss of suppression of hormone-sensitive lipase (HSL), an enzyme that breaks down triglyceride to free fatty acids and glycerol, to small physiological amounts of insulin and, to a lesser extent, insulin insensitivity of lipoprotein lipase. HSL is normally very sensitive to small increases in insulin levels and is totally suppressed at much lower concentrations than those required for glucose uptake. In insulin-resistant subjects, HSL is less sensitive to small changes in insulin levels and adipocyte NEFA release is increased. A relationship between increased adipocyte NEFA release and insulin resistance has been shown in subjects with CHD. The metabolic consequences of increased circulating NEFA are multiple and are beyond the scope of this review, but they include adverse lipoprotein and coagulation changes and have been reported to affect insulin secretion and have a lipotoxic effect on the β cell. Accumulation of triglyceride within the β cell also impairs insulin secretion.

Many of the metabolic benefits associated with low glycemic index carbohydrates can be attributed to their ability to reduce adipocyte NEFA release. Low glycemic index foods have been consistently shown to reduce insulin resistance, and animal studies have shown that improvements in fat and muscle insulin sensitivity are accompanied by decreases in fatty acid synthetase activity, adipocyte size, and lipid storage. Although human studies have shown that low glycemic index diets consumed for 3 weeks increase adipocyte insulin sensitivity, no direct effect on adipocyte metabolism has been identified.

Low glycemic index diets attenuate the insulin response for approximately 4 h postprandially. This slightly high

postprandial insulin is insufficient to affect glucose transport but does suppress the insulin-sensitive enzyme, HSL, and thus ensures prolonged suppression of postprandial NEFA output. The ability of low glycemic carbohydrates to do this is in stark contrast with high glycemic diets that can cause an elevation of NEFA release postprandially by stimulating the counter-regulatory hormones, as discussed previously. Low glycemic meals taken in the evening can effectively suppress circulating NEFA concentrations and hepatic glucose output throughout the night. These metabolic effects are predicted to promote insulin sensitivity.

Our own work has shown that insulin-resistant adults with a history or who are at risk of CHD improve their adipocyte insulin sensitivity after consuming a low glycemic index diet for 3 weeks and their circulating NEFA levels decline. These human studies complement animal work showing that low glycemic index diets improve insulin sensitivity by modulating adipocyte metabolism.

Conclusion

The glycemic index of a diet is an indicator of postprandial metabolism, which is important in contributing to cardiovascular risk. Dietary carbohydrates are absorbed and metabolized differently and therefore influence postprandial glucose, insulin, and NEFA concentrations differently. In Western society, the proportion of the day that we spend in the postprandial state is increasing as the tendency to snack throughout the day replaces sit-down meals. The known detrimental consequences of high glycemic foods and snacks on postprandial metabolism should encourage us to advocate low glycemic diets to counter the current epidemic of insulin resistance-related diseases, notably CVD and diabetes. The relevance of the glycemic index to these two major preventable diseases of the Western world argues strongly for its greater acceptance in current nutritional guidelines.

See also: Carbohydrates: Chemistry and Classification; Regulation of Metabolism; Requirements and Dietary Importance. **Fiber:** Physiological and Functional Effects; Resistant Starch and Oligosaccharides. **Fructose:** Absorption and Metabolism. **Glucose:** Chemistry and Dietary Sources. **Obesity:** Complications. **Pregnancy:** Nutrient Requirements; Safe Diets

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GROWTH AND DEVELOPMENT

Physiological Aspects

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Glossary

AGA Appropriate for gestational age.

IGF Insulin-like growth factor.

IUGR Intrauterine growth restriction.

LGA Large for gestational age.

Macrosomia Larger than normal body size due to excess fat.

Monotocous Singleton fetus within the same uterus (e.g., in most human pregnancies).

Polytocous Multiple fetuses within the same uterus (e.g., in rats, with 8–10 pups/litter).

Programming An insult, when applied at a critical or sensitive stage in development, produces lasting, even lifelong, effects on the structure or function of the organism.

SGA Small for gestational age.

Introduction

Growth and development refers to the growth of the individual in size as assessed by anthropometric measurements of body weight, length, circumference (head and body), and body weight/length ratio (or Ponderal Index), as well as changes in body composition, primarily the amount of fat and muscle. This review focuses on growth and development of the fetus. Most of the relevant concepts about growth and development apply to the fetal period of development and this period encompasses the greatest changes in body proportion and composition during the life of the individual. Fetal growth occurs by increase in cell number and size. In the first third of gestation, during the embryonic period, growth occurs primarily by increased cell number (hyperplasia); in the middle third of gestation, cell size also increases (hypertrophy) while the rate of cell division becomes stable. In the last third of gestation, the rate of cell division declines while cell size continues to increase.

Many terms are used to describe variations in growth. For example, human newborns are classified as having normal birth weight (greater than 2500 g), low birth weight (less than 2500 g), very low birth weight (less than 1500 g), or extremely low birth weight (less than 1000 g). Obviously, classification by weight alone says little about growth rate, as most infants with less than normal birth weights are the result of a shorter than normal gestation, i.e., they are born preterm (<37 weeks of gestational age). Furthermore, it is inappropriate to label newborns as abnormally grown when their birth weight is less than some arbitrarily determined 'normal' birth weight, but their mother was quite small to begin with; such newborns are considered constitutionally small but not abnormal. Classifying newborns according to duration of gestation (e.g., preterm, term, or post term)

on the basis of birth weight also is erroneous, because infants with intrauterine growth restriction (IUGR) are smaller and macrosomic infants of diabetic mothers are larger than normal at any gestational age. Interestingly, though, both IUGR and macrosomia predispose the fetus to the same later-life disorders of obesity, insulin resistance, and type 2 diabetes, despite their very different patterns of intrauterine growth.

Growth of Fetal Size

Under usual conditions, the fetus grows at its genetic potential. Small fetuses of small parents or large fetuses of large parents do not reflect fetal growth restriction or fetal overgrowth, respectively; in fact, their rates of growth are normal for their genome. If the mother is unusually small, however, she might limit fetal growth by 'maternal constraint,' which represents a limited uterine size (primarily endometrial surface area) and thus the capacity to support placental growth and nutrient supply to the fetus. A clear example of maternal constraint is shown in **Figure 1**, depicting the reduced rate of fetal growth of multiple fetuses in a species – human – that optimally supports only one fetus.

Fetal weight tends to increase exponentially in the middle part of gestation but then slows during the latter third of gestation, producing the typical S-shaped curve of fetal weight versus gestational age that is derived from cross-sectional measurements of newborn weights at different known gestational ages (**Figure 2**). The length of gestation is more strongly related to the growth of neural tissue (range $0.015\text{--}0.033\text{ g}^{1/3}\text{ day}^{-1}$ a 2.2-fold range) than to the growth of the fetal body (range $0.033\text{ to }0.25\text{ g}^{1/3}\text{ day}^{-1}$ a 7.6-fold range). The physiological significance of this relationship is not known, but intrauterine development

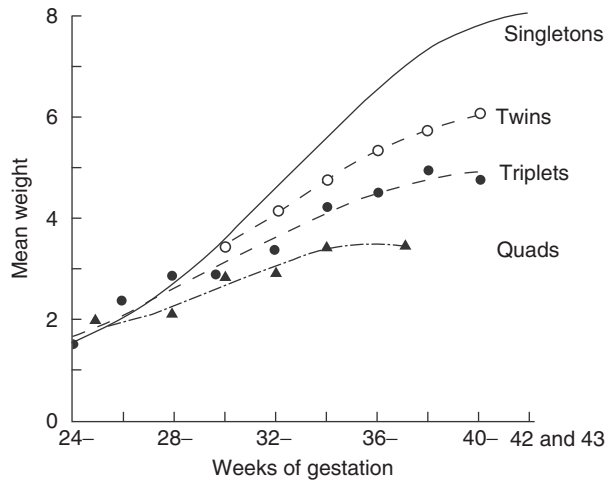


Figure 1 Mean birth weight of single and multiple fetuses related to duration of gestation. Adapted from McKeown and Record (1952) *Journal of Endocrinology* 8: 386 and Ounsted M and Ounsted C (1973) On fetal growth rate. Clinics in developmental medicine: No. 46. Spastics International Medical Publications, p. 17. London: William Heinemann Medical Books Ltd, with permission from Society for Endocrinology.

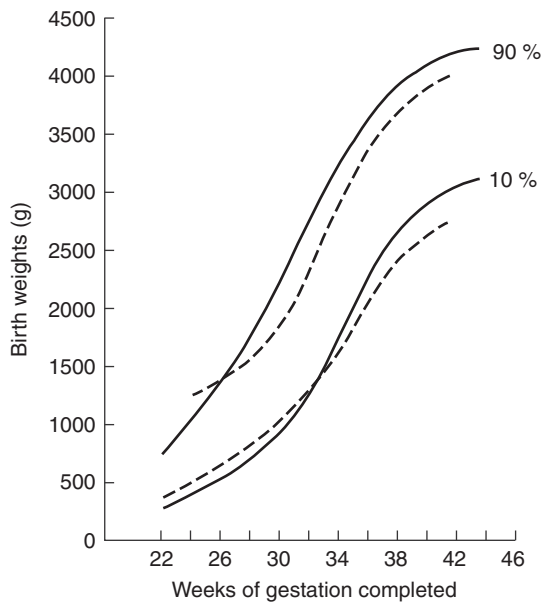


Figure 2 Birth-weight percentiles for gestational age. Solid lines represent California total singleton live births, 1970-76; dotted lines represent Colorado General Hospital (Denver, Colorado) live births, 1948-60. Reproduced from Creasy RK and Resnik R (1999) Intrauterine growth restriction. In: Creasy R and Resnik R (eds.) *Maternal-Fetal Medicine*, 4th edn., pp. 569-584. Philadelphia: W. B. Saunders Co.

of a large brain in humans is made possible by a slow rate of somatic growth.

Developmental Change of Fetal Body Composition

Fetal growth during the last third of gestation requires large increases in nutrient supplies and appropriate utilization of

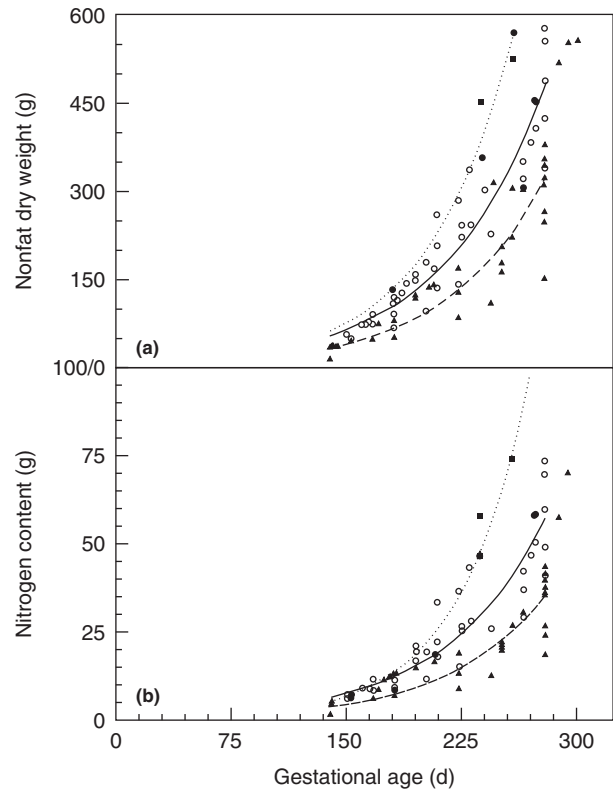


Figure 3 Nonfat dry weight (a) and nitrogen content (b) are plotted against gestational age for LGA (■,), AGA (○, —), and SGA (▲, ----) infants. Reproduced from Sparks JW (1984) Intrauterine growth and nutrition. *Seminars in Perinatology* 8(2): 74-93.

these nutrients. Nutrient substrate supply is coupled with increased development of anabolic hormones and growth factors in fetal tissues and fetal plasma to produce increased nitrogen and carbon deposition in protein, carbohydrate deposition in glycogen, and fatty acid, glycerol, and triglyceride deposition in adipose tissue. Growth of these tissues gradually replaces water in the fetal extracellular space.

Chemical composition studies of normal human infants are limited. Based on data from 15 studies that included 207 infants, nonfat dry weight and nitrogen content (predictors of protein content) show a linear relationship with fetal weight and an exponential relationship with gestational age (Figure 3). As gestation proceeds, larger fetuses grow faster than smaller fetuses, and protein accretion follows accordingly.

Water

Fetal water content increases directly with body weight, but not proportionally to body weight, as fetal body water, expressed as a fraction of body weight, decreases with advancing gestation. The relatively larger growth of adipose tissue in the human fetus compared with all other species further dilutes the body concentration of water. Extracellular water, as a fraction of fetal body weight, also decreases more than intracellular water as gestation advances; this is

mainly due to increasing cell number and increasing cell size rather than the intracellular concentrations of osmotic substances.

Nonfat Dry Weight

Comparative aspects of chemical and physical growth in fetuses of six different species are summarized in **Table 1**. Despite growth rate variations of up to 20-fold and weight-specific fat content variances at term of up to 16-fold among these species, nonfat dry weight and protein weight-specific contents (as percentages of total weight at term) are constant. Protein concentration is approximately 12% in all species at term and fetal protein content is linearly related to fetal weight; thus, protein accretion in the fetal rat occurs approximately 23 times as fast as it does in the human. These species-related differences in growth rate are remarkable and require marked differences in the capacity of the placenta to transport nutrients to the fetus.

Nitrogen Balance, Protein Turnover, and Protein Synthesis

According to animal data, only approximately 80% of the nitrogen content of the fetus is found in protein; the remainder is found in urea, ammonia, and free amino acids. Additional nitrogen requirements for urea excretion and for other possible nitrogen excretion products are not known for human fetuses.

Isotopic tracers of selected amino acids, especially essential amino acids such as leucine and lysine, have been used to measure fetal protein synthesis, breakdown, and accretion. Limited human data is consistent with data in the fetal sheep, the only species studied in significant detail. **Figure 4** shows results of experiments in fetal sheep over the second half of gestation, comparing fractional protein synthesis rates derived from tracer data and fractional body growth rates derived from body composition data. Whole body weight-specific protein turnover rate is higher in the early-gestation fetus, primarily from increased rates of amino acid uptake from the placenta (exogenous entry of amino acids into the fetal circulation) and protein synthesis. These processes produce a 50% higher rate of net protein accretion in the mid-gestation fetus.

Mechanisms underlying the decrease in protein synthesis rate over gestation are not well understood, but they appear to be intrinsic to the fetus and not to a limitation of nutrient

supply by the placenta. At least a partial explanation can be offered according to the changing proportion of body mass contributed by the major organs (**Table 2**). Based on the increased mass of skeletal muscle with advancing gestation, fetal whole body fractional protein synthesis rate should be lower, as skeletal muscle has a relatively lower fractional protein synthetic rate in late gestation than in earlier gestation. A direct relationship between anabolic growth promoting substances acting as principal regulators of fetal protein synthesis rate and thus fetal growth rate cannot be made, however, as plasma concentrations or secretion rates of these substances increase in the fetus as gestation proceeds, while protein synthetic rates decline.

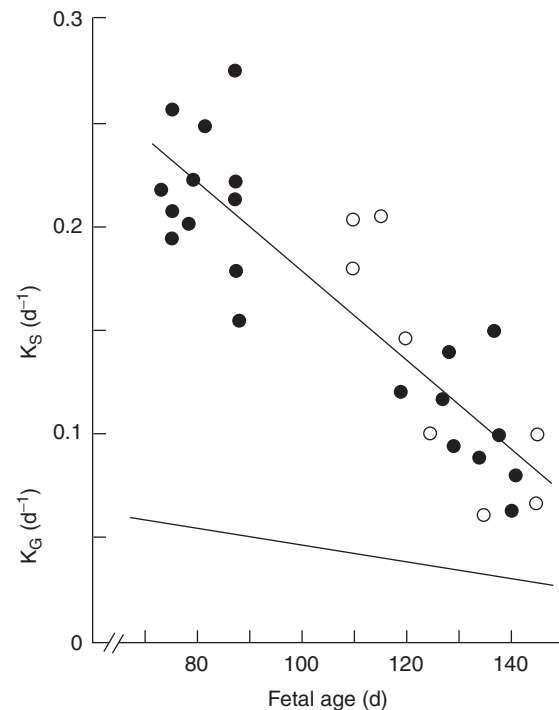


Figure 4 Fractional rate of protein synthesis (K_s) over gestation in fetal sheep studied with leucine (●) and lysine (○) radioactive tracers compared with the fractional rate of growth (K_g) in the lower portion of the figure (—). Reproduced from Hay Jr WW (2004) Fetal requirements and placental transfer of nitrogenous compounds. In: Polin RA, Fox WW, and Abman SH (eds.) *Fetal and Neonatal Physiology*, 3rd edn., p. 509. Philadelphia: W. B. Saunders Co.

Table 1 Growth characteristics and chemical composition at term of selected mammals and a representative human fetus

	Human	Monkey	Sheep	Pig	Rabbit	Rat
Gestation (days)	280	163	47	67	30	21.5
Number of fetuses	1	1	1	3–5	4–6	10–12
Growth rate ($\text{g day}^{-1} \text{kg}^{-1}$)	15	44	60	70	300	350
Fetal weight (g)	3500	500	4000	100	60	5
Dry weight (g per % body wt)	1050/30	125/25	760/19	25/25	9/15	.2/4
Nonfat dry weight (g per % body wt)	490/14	-	640/16	14/14	-	-
Protein (g per % body wt)	420/12	-	480/12	12/12	7.2/12	.6/12

Source: Reproduced from McCance and Widdowson (1985) In: Falkner F and Tanner JM (eds.) *Human Growth*, 2nd edn., vol. 1, p. 139. New York: Plenum Press.

Glycogen

Many tissues in the fetus, including brain, liver, lung, heart, and skeletal muscle, produce glycogen over the second half of gestation. Liver glycogen content, which increases with gestation, is the most important store of carbohydrate for systemic glucose needs immediately after birth, because only the liver contains sufficient glucose-6-phosphatase for release of glucose into the circulation. Skeletal muscle glycogen content increases during late gestation and forms a ready source of glucose for glycolysis within the myocytes. Lung glycogen content decreases in late gestation with loss of glycogen-containing alveolar epithelial cells, development of type II pneumocytes, and onset of surfactant production. Cardiac glycogen concentration decreases with gestation as cellular hypertrophy develops, but cardiac glycogen appears essential for postnatal cardiac energy metabolism and function. At term, fetal liver glycogen concentration in most species is approximately $80\text{--}120\text{ mg g}^{-1}$, at least twice the adult concentration. In the relatively slowly growing human fetus, glycogen synthesis rates are low (approximately $2\text{ mg day}^{-1}\text{ g}^{-1}$), representing less than 2% of estimated whole body glucose utilization rates.

Macrosomic fetuses of diabetic mothers have very high body and organ contents of glycogen. In IUGR fetuses,

placental insufficiency and decreased placental glucose supply to the fetus tend to decrease fetal organ glycogen content. This could be augmented by acute hypoxic stress, especially right before birth, that would increase catecholamine and glucagon secretion, both of which would increase glycogen breakdown. More recent studies have shown, however, that the tendency to reduced glycogen content in IUGR fetuses is balanced or even exceeded by increased glycogen formation produced by increased insulin sensitivity and cellular glucose uptake capacity. These increases represent compensatory adaptations in IUGR fetuses to the chronically low glucose concentrations.

Fat

Fetal fat content as a fraction of fetal weight varies several fold among species (Figure 5). The fat content of newborns at term of almost all land mammals is 1–3% and is considerably less than that of the human, 15–20%. Differences in body fat content among species are due primarily to the capacity of the placenta to transfer fat to the fetus and to the capacity of the fetus to synthesize triglycerides, produce fat, and store fat in adipose tissue cells that human fetuses have in relatively large abundance. Even in those species that take up fat from the placenta and deposit fat in fetal tissues, such as in human fetuses, the rate of fetal fatty acid oxidation is presumed to be low, because plasma concentrations of fatty acids are low and the carnitine palmitoyl transferase enzyme system is not sufficiently developed to deliver long chain fatty acids to the respiration pathway inside the mitochondria.

In the human fetus, calories produced by the complete oxidation of glucose, lactate, and amino acids can fully meet energy required for maintenance metabolism. In the human fetus between 26 and 30 weeks gestation, nonfat and fat components contribute equally to the carbon content of the fetal body. After that period, fat accumulation considerably

Table 2 Fetal organ weight as percent of body weight

	50%Gestation	67%Gestation	90%Gestation
Liver	6.5	5.1	3.1
Kidneys	1.6	1.2	0.7
Heart	0.9	0.8	0.8
Brain	3.4	2.9	1.7
Hindquarters	14.5	15.1	22.0

Source: Reproduced from Bell AW, et al. (1987) *The Journal of Nutrition* 117: 1181–1186.

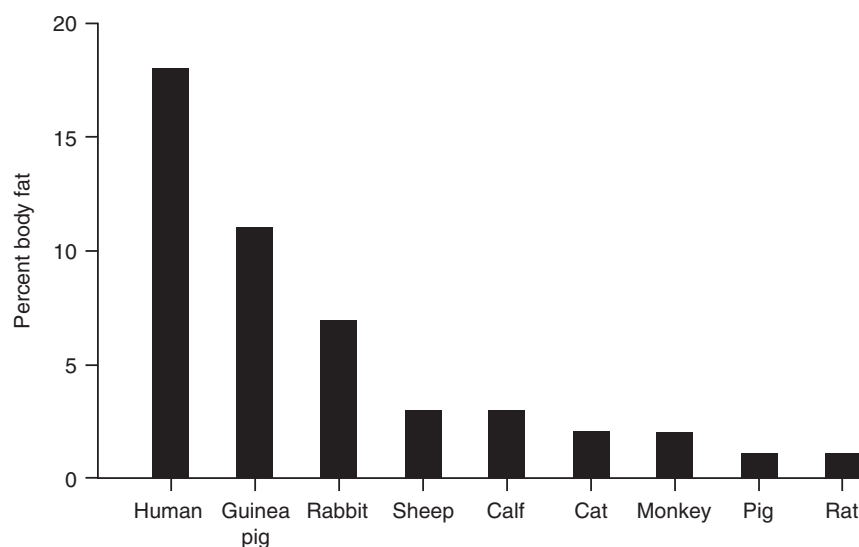


Figure 5 Fetal fat content at term as a percent of fetal body weight among species. Reproduced with permission from Hay Jr WW (2003) *Nutrition and development of the fetus: Carbohydrate and lipid metabolism*. In: Walker WA, Watkins JB, and Duggan C (eds.) *Nutrition in Pediatrics*, 3rd edn., pp. 449–470. Hamilton: B. C. Decker, Inc.

exceeds that of the nonfat components. At 36 weeks gestation, 1.9 g of fat accumulates for each gram of nonfat daily weight gain, and by term, the deposition of fat accounts for over 90% of the carbon accumulated by the fetus. The rate of fat accretion is approximately linear between 36 and 40 weeks gestation, and by the end of gestation, fat accretion ranges from 1.6 to 3.4 g day⁻¹ kg⁻¹. By term, the fat content of the human fetus is 15–20% of body weight, ranging from less than 10% in IUGR fetuses to 25% or more in macrosomic infants of diabetic mothers.

Caloric Accretion in the Fetus

Fat has a high energy content, 9.0 kcal g⁻¹ (values vary according to fatty acid content, hydration state, and methods used to determine caloric values), and a very high carbon content, approximately 78%. Thus, differences in fetal fat concentration among species lead to large differences in calculated caloric accretion rates and carbon requirements of the fetal tissues for growth. The caloric concentration of nonfat dry weight is fairly consistent across species and also within species at different developmental stages, indicating that the ratio of protein to nonprotein substrates in the tissues is relatively constant. Thus, caloric accretion rate of any fetus can be estimated from the growth curve of the fetus in question and the changing fat and water concentrations.

Data for caloric accretion and caloric distribution in the human fetus are shown in Table 3. Because growth of fat and nonfat (protein plus other) tissues are metabolically linked through energy supply that is used for protein synthesis and the production of anabolic hormones that promote positive protein, fat, and carbohydrate growth, restriction of nutrient supply is likely to produce growth deficits of all tissues, not just fat (i.e., growth restriction involves limitation of muscle growth as well as fat and glycogen). Indeed, chronic experimental selective caloric (glucose) restriction in the fetal sheep leads to increased protein breakdown as well as to lower rates of fetal growth and lipid content. In contrast, as shown by the growth curves in Figure 6 from human infants born preterm at different times over the last third of gestation, there is a bias toward thinner, small for gestational age (SGA) infants with less fat content, indicating that in a species that does lay down considerable fetal fat during late gestation, differences in intrauterine growth rate reflect fat deposition more than the growth of nonfat tissues. IUGR fetuses of all species, however, also show reduced growth of skeletal muscle. An important long term adverse consequence of reduced muscle growth might involve diminished whole body insulin action and a tendency to hyperglycemia and type 2 diabetes, as skeletal muscle is the principal insulin-sensitive tissue in the body.

Mineral Accretion in the Fetus

Fetal calcium content is best correlated with fetal body length; this is true for both AGA and SGA infants. Using this index, fetal calcium content increases exponentially with a linear increase in length. Using this estimate, the human fetal rate of calcium accretion is approximately 85 mg day⁻¹ kg⁻¹. Accretion of other minerals varies more directly with body weight, and according to the distribution of the minerals into extracellular (e.g., sodium) or intracellular (e.g., potassium) spaces.

Regulation of Fetal Growth

Regulation of fetal growth represents a mix of genetic mechanisms and environmental influences through which the genetic factors are expressed and modulated. The single most

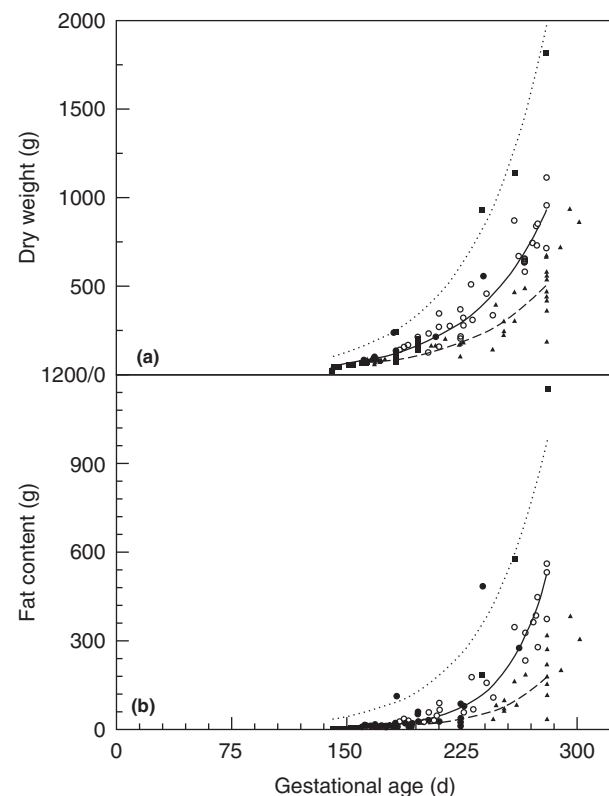


Figure 6 Dry weight (a) and fat content (b) plotted against gestation age in the same newborn human infants shown in Figure 3 for LGA (■,), AGA (○, —), and SGA (▲, ----) infants. Reproduced from Sparks JW (1984) Intrauterine growth and nutrition. *Seminars in Perinatology* 8(2): 74–93.

Table 3 Calculation of the caloric distribution in the term human infant

Wet weight	Wet weight	Fat	Nonfat wet weight	Nonfat dry weight
Weight (g)	3450	386	3064	511
Total calories (kcal)	5950	3650	2300	2300
Caloric concentration (kcal g ⁻¹)	1.73	9.45	.75	4.5

Source: Reproduced from Ziegler EE, et al. (1979) *Growth* 40: 329–341.

important environmental influence that affects fetal growth is the nutrition of the fetus. Nutrient supply to the fetus and the resulting increases in fetal tissue and plasma concentrations of anabolic hormones and growth factors are regulated by maternal health, maternal nutrition, uterine growth (including uterine blood flow and endometrial surface area), and placental growth and function.

Genetic Factors

Maternal genotype is more important than fetal genotype in the overall genetic regulation of fetal growth. Table 4 presents estimates of the quantitative contribution of fetal and parental factors to fetal growth and birth weight at term. The more modest regulation by the paternal genotype acts through its contribution to trophoblast development and thus placental-to-fetal nutrient transport capacity. More specific gene targeting studies have shown the importance of genomic imprinting on fetal growth. For example, in mice normal fetal and placental growth require that the IGF-II gene be paternal and the IGF-II receptor gene be maternal, and paternal disomy producing IGF-II gene over-expression results in fetal overgrowth whereas maternal disomy producing IGF-II under-expression results in fetal dwarfism. In humans, isopaternal inheritance of IGF-II alleles is associated with the Beckwith-Wiedemann syndrome that includes hyperinsulinism and fetal macrosomia.

Nongenetic Maternal Factors

There is a high correlation between birth weights of siblings that extends to cousins. The nongenetic, maternal nature of this effect is demonstrated by embryo transfer and cross-breeding experiments. For example, a small-breed embryo transplanted into a large-breed uterus will grow larger than a small-breed embryo remaining in a small-breed uterus. Furthermore, partial reduction in fetal number in a polytocus species such as the rat produces greater than normal birth weights in the remaining offspring. Conversely, embryo transfer of a large-breed into a small-breed uterus will result in

a newborn that is smaller than in its natural large-breed environment. Such evidence demonstrates that fetal growth is normally constrained, and that this constraint comes from the maternal environment. This is a physiological process and includes the maternal-specific capacity of uterine size, placental implantation surface area of the uterus, and uterine circulation, which together support the growth of the placenta and its capacity to transport nutrients to the fetus to support fetal growth.

Maternal Nutrition

Normal variations in maternal nutrition have relatively little effect on fetal growth, because they do not markedly alter maternal plasma concentrations of nutrient substrates or the rate of uterine blood flow, the principal determinants of nutrient substrate delivery to and transport by the placenta. Human epidemiological data from conditions of prolonged starvation, as well as nutritional deprivation in experimental animals, indicate that even severe limitations in maternal nutrition only limit fetal growth by 10–20%. Restriction of caloric and protein intakes to less than 50% of normal for a considerable portion of gestation are needed before marked reductions in fetal growth are observed; such severe conditions often result in fetal loss before the impact on late gestation fetal growth rate and fetal size at birth are manifested. Similarly, fetal macrosomia is common in pregnancies complicated by gestational diabetes mellitus in which maternal plasma hyperglycemia and hypertriglyceridemia plus fetal hyperinsulinemia combine to produce excessive fetal adiposity. More recent observations of increasing fetal birth weight and macrosomia in relation to increased maternal obesity, a potentially serious adverse impact of the world wide obesity epidemic, remain mechanistically unexplained, although there is some evidence that obese pregnant women tend to be hypertriglyceridemic as well as slightly hyperglycemic, both of which would contribute to increased lipid and glucose supply to the fetus and fetal fat production.

The Placenta

The placenta exerts strong control over fetal growth by providing nutrients directly or in metabolically altered form and amount. Naturally and experimentally, placental growth precedes fetal growth, and failure of placental growth is directly associated with decreased fetal growth. There is considerable variation in this control, however. For example, experiments in sheep that limited placental growth did not result in proportionately reduced fetal weight, indicating that either the capacity of the smaller placenta to transport nutrients to the fetus increased adaptively or that the fetus developed increased capacity to extract nutrients from the placenta and direct those nutrients to growth. More characteristically, though, limitation in placental function to transfer nutrients to the fetus directly limits fetal growth. In fact, fetal growth restriction is seen as a natural and reproductively successful (though not perfect) compensatory adaptation to nutrient limitation. There is a direct relationship between fetal weight and placental weight

Table 4 Factors determining variance in birth weight

	Percent of Total Variance
<i>Fetal</i>	
Genotype	16
Sex	2
	18
<i>Maternal</i>	
Genotype	20
Maternal environment	24
Maternal age	1
Parity	7
	52
<i>Unknown</i>	30

Source: Derived from Penrose LS (1954) Proceedings of the 9th International Congress of Genetics Part 1, p. 520, and Milner RDG and Gluckman PD (1996) Regulation of intrauterine growth. In: Gluckman PD and Heymann MA (eds.) *Pediatrics and Perinatology*, 2nd edn., p. 285. London: Arnold.

in humans, although functional interactions between placenta and fetus also are important to fetal growth and development.

Growth of the Placenta and its Transport Capacity

Placental nutrient transfer capacity increases over gestation by increased placental growth, primarily of membrane surface area, allowing for the increase in nutrient supply required for the growing fetus. Placental size, morphology, and membrane transporter abundance are regulated by imprinted paternally-derived genes, such as the placental-specific *Igf2-H19* gene complex. A larger paternal versus maternal *Igf2* gene allele supply leads to a larger placenta and the potential for a larger fetus. Activity of the imprinted genes also can be affected by epigenetic modification, which allows for considerable environmental influence over gene expression. Thus, DNA methylation can limit placental-specific *Igf-2* gene activity, leading to IUGR of the placenta and, in turn, the fetus.

Maternal Endocrine Influences on Fetal Growth

Changes in maternal circulating growth hormone and growth hormone-like peptides such as placental lactogen, which increase during pregnancy, have combined effects that induce maternal insulin resistance and lead to higher circulating concentrations of glucose and lipids. These in turn are transported in increased amounts to the fetus where, combined with their stimulatory effects on fetal insulin and IGF-I and II, promote fetal adiposity (or macrosomia, as in the infant of the diabetic mother) and limit fetal protein breakdown, both of which promote fetal growth.

Fetal Endocrine and Autocrine/Paracrine-Acting Growth Factors Influences on Fetal Growth

Growth hormone, which classically acts as the major regulator of postnatal growth, has no demonstrable influence on fetal growth. Fetal insulin does regulate fetal growth, although the complete absence of insulin does not abolish fetal growth. In sheep, for example, fetal pancreatectomy in late gestation limits fetal growth rate only by 20–30%, and pancreatic agenesis in humans produces IUGR fetuses that are 30–50% less than normal weight near term. Insulin infusions into the fetus and excessive fetal insulin secretion enhance fetal glucose utilization and produce increased adiposity. Such hyperinsulinemic conditions also limit protein breakdown, which supports increased protein accretion, but overall there is little impact of excess insulin to promote excess growth of nonfat lean body mass in the fetus. The primary action of fetal insulin may be to promote glucose utilization and, in turn, enhance protein accretion by providing more energy substrate to fuel protein synthesis and to substitute glucose carbon for amino acids to fuel oxidative metabolism. For example, reducing fetal glucose supply lowers fetal weight and oxygen consumption to the same extent, indicating that oxidative metabolism of glucose was responsible for the growth. Similarly, removal of insulin from the fetus increases fetal glucose concentration and the transfer of glucose from mother to fetus *via* the placenta, which reduces fetal glucose oxidative metabolism and

results in reduced net fetal carbon accretion and reduced fetal growth.

Insulin also directly activates its signal transduction pathway *via* mTOR and the activity of the MAP-kinase pathway, which, when augmented by increased amino acid supply, promote the incorporation of amino acids into protein synthesis. Recent studies in the fetal sheep have shown that amino acid infusion, independent of insulin, increases skeletal muscle concentrations of mTOR and eIF4E, the key regulatory proteins for ribosomal synthesis of amino acids into protein. In contrast, increases in phosphorylated mTOR and 4EBP1 were only demonstrated when insulin concentrations also were increased. These observations indicate that amino acids can independently up-regulate particular signal transduction proteins during late gestation fetal growth and emphasize, as does the data showing insulin activation of the MAP-kinase pathway, that nutrient–hormonal interaction is central for regulation of growth.

Both IGF-I and -II regulate fetal growth. Mice lacking the IGF-I gene have markedly reduced rates of fetal growth in late gestation. IGF-II knockouts also have delayed fetal growth that is more pronounced in early to mid-gestation. IGF-I receptor knockout mice are more growth restricted than either IGF-I or IGF-II knockouts alone. These IGF-I receptor knockouts are growth restricted to the same extent as mice in which both IGF-I and -II genes are deleted, confirming that receptor activation is the principal growth-regulating step in IGF-I and -II action. Infusions of IGF-I into fetal sheep demonstrate limited insulin-like effects on fetal glucose metabolism, but they do limit fetal protein breakdown, particularly when sustained hypoglycemia occurs. IGFs also regulate fetal growth by regulating placental growth. IGF-II gene knockout mice have smaller placentas and, in turn, lower IGF-I and -II binding proteins. IGF binding proteins modulate the effects of IGF-I and -II on fetal growth. Circulating IGF-II receptor limits IGF-II effects by binding most of it in the circulation. IGFBP-1 and -2 levels are relatively high in fetal plasma, perhaps limiting the effectiveness of IGF-I, whereas IGFBP-3 is low in the plasma of fetuses with IUGR, perhaps due to simultaneous insulin deficiency.

Interpretation of Growth Curves

Cross-sectional growth curves have been developed from anthropometric measurements in populations of infants born at different gestational ages. Such curves have been used to estimate whether growth of an individual fetus or preterm newborn is within or outside of the normal range of fetal growth, which is defined as between the 10th and 90th percentile. Fetuses and newborns infants who are within the 10th and 90th percentiles for weight versus gestational age are considered appropriate for gestational age (AGA), those who are less than the 10th percentile are considered SGA, and those who are greater than the 90th percentile are considered large for gestational age (LGA). In general, SGA infants come from small parents (particularly the mother) and LGA infants come from large parents (again, particularly when the mother is big as well as the father) or from mothers who have gestational diabetes.

Standard fetal and preterm neonatal growth curves represent the third trimester in humans. Each curve is based on local populations with variable composition of maternal age, parity, socio-economic status, race, ethnic background, body size, degree of obesity or thinness, health, pregnancy-related problems, and nutrition, as well as the number of fetuses per mother, the number of infants included in the study, and how well measurements of body size and gestational age were made. Estimates of gestational age of newborn infants often are imprecise because of variable maternal postimplantation bleeding and irregular menses, onset and appearance of physical features of maturation in the infant, and inter-observer assessments of an infant's developmental stage.

Mathematical analyses of various growth curves have been used to determine growth rates over relatively short gestational periods or at discrete gestational ages. The data used in the Lubchenco growth curves (Figure 2), for example, reflect a simple exponential function showing fetal weight increasing at approximately $15 \text{ g day}^{-1} \text{ kg}^{-1}$ for average sized infants; this rate will be slower for smaller infants and faster for larger infants.

More recent growth curves have been developed from serial ultrasound measurements of fetal growth in normal pregnancies, providing continuous rather than cross-sectional growth patterns. The growth of a preterm infant is better correlated with serially-determined fetal growth rates than with cross-sectional neonatal growth curves. Serial ultrasound measurements of fetal growth also more accurately determine how environmental factors can inhibit (e.g., maternal under nutrition globally, or hypoglycemia specifically) or enhance (e.g., maternal over nutrition globally or hyperglycemia and hypertriglyceridemia specifically) the rates of fetal growth.

Extremes of Growth and Development: Intrauterine Growth Restriction and Macrosomia

Human birth weights at term have been steadily increasing since the 1970s throughout much of the developed world, although in developed countries, this increase has been tempered by the increased number of preterm infants born as multiple births following *in vitro* fertilization procedures. The relative proportion of infants with IUGR has not changed much whereas the proportion of those who are excessively large with macrosomia has increased.

Intrauterine Growth Restriction

In developed countries, 3–7% of newborns are classified as IUGR. This is an underestimate, however, as it is based on IUGR infants who are SGA, whereas fetal growth restriction can occur within the 10th and 90th percentiles of weight and length. Most IUGR infants experienced suboptimal nutrient supply and consequently a restriction of fetal growth as a result of some form of placental insufficiency. Generally, such infants have less subcutaneous fat and skeletal muscle, with low weight/length ratios or low Ponderal Index values ($\text{Ponderal Index} = \text{weight (grams)} / [\text{length (cm)}]^3$).

Table 5 Risks of specific types of fetal and neonatal morbidity and mortality in IUGR infants

Problem	Pathogenesis/pathophysiology
Intrauterine death	Chronic hypoxia Placental insufficiency Growth failure Malformation Infection Infarction/abruption Preeclampsia
Asphyxia	Acute hypoxia/abruption Chronic hypoxia Placental insufficiency/preeclampsia Acidosis Glycogen depletion
Meconium aspiration	Hypoxia
Hypothermia	Cold stress Hypoxia Hypoglycemia Decreased fat stores Decreased subcutaneous insulation Increased surface area Catecholamine depletion Chronic hypoxia
Persistent pulmonary hypertension	
Hypoglycemia	Decreased hepatic/muscle glycogen Decreased alternative energy sources Heat loss Hypoxia Decreased gluconeogenesis Decreased counterregulatory hormones Increased insulin sensitivity
Hyperglycemia	Low insulin secretion rate Excessive glucose delivery Increased catecholamine and glucagon effects
Polycythemia/hyperviscosity	Chronic hypoxia Maternal–fetal transfusion Increased erythropoiesis
Gastrointestinal perforation	Focal ischemia Hypoperistalsis
Acute renal failure	Hypoxia/ischemia
Immunodeficiency	Malnutrition Congenital infection

Source: Adapted from growth and development 5th edition.

IUGR imposes increased risks of specific types of fetal and neonatal morbidity and mortality (Table 5).

Possible Adult Disorders Resulting from Intrauterine Growth Restriction

Interest in IUGR has been enhanced recently by retrospective epidemiological, clinical follow-up, and animal studies that indicate long-term consequences in adult life of IUGR offspring, including higher incidences of obesity, insulin resistance, impaired glucose tolerance, enhanced hepatic glucose production, pancreatic insulin secretion deficiency, type 2 diabetes mellitus, hypertriglyceridemia, and cardiovascular disease, particularly hypertension. These conditions, often

called the Metabolic Syndrome, may represent an example of 'programming' in which an insult, when applied at a critical or sensitive stage in development, produces lasting, even lifelong, effects on the structure or function of the organism. Mechanisms responsible for these later-life morbidities are not fully established. There is relatively consistent evidence in human IUGR infants and those produced experimentally in animal models of diminished pancreatic growth and development, which might present later in life as pancreatic insufficiency when the adult starts and then continues eating a diet rich in simple carbohydrates and lipids. IUGR fetuses tend to have increased peripheral insulin and glucose sensitivity, which would augment fat production and obesity with such a diet. Over time, however, peripheral insulin resistance develops, assumed to be the result of obesity with reduction in the insulin signal transduction pathways in both adipocytes and myocytes that result from specific products of intracellular fat metabolism, leading to glucose intolerance. When coupled with pancreatic insufficiency, this would lead to type 2 diabetes. A common theme among these observations is that excessive weight gain of adipose tissue starting at any weight percentile and continuing or even starting after birth is the strongest predictor of the Metabolic Syndrome disorders. Hypertension in adulthood in individuals who experienced IUGR, also part of the Metabolic Syndrome, may be the result of restricted renal and adrenal development.

Macrosomia

At the other end of the birth weight spectrum are macrosomic, LGA infants. These infants were exposed to excess nutrient supply in utero, principally of carbohydrates and lipids. Macrosomic newborns have increased specific morbidities primarily associated with metabolic complications of maternal diabetes mellitus during pregnancy and associated birth complications and birth injuries as a result of excessive fetal size.

Macrosomia is defined in a newborn as a birth weight more than two standard deviations above the mean percentile for gestational age or a birth weight greater than 4000 g at term. Neonatal macrosomia has a strong ethnic predisposition affecting up to 50% of Latino and Native American pregnant women versus 19% of African-American pregnant women. Macrosomia is characteristic of infants of diabetic mothers (IDMs) who were hyperglycemic during pregnancy. The diabetes can be long standing, but the most common group producing macrosomic infants are women with gestational diabetes mellitus (GDM). The percentage of pregnant women who have some form of GDM has been increasing worldwide and now is well about the historical range of 3 to 5% of all pregnancies. The risk of macrosomia is not consistent across all classes of diabetes; it primarily reflects the degree and duration of maternal hyperglycemia and hypertriglyceridemia and particularly high spikes of these conditions following meals that are more common in gestational diabetes. Maternal hyperglycemia results in fetal hyperglycemia and hyperinsulinemia; maternal and fetal hypertriglyceridemia contribute to the effect of the excess glucose and insulin to produce excess fat deposition in the fetus.

Development of Type II Diabetes in Later Life in Macrosomic Offspring

IDMs, particularly those with macrosomia, have increased risk of developing type II diabetes earlier in life. Mechanisms responsible for this sequence of events include insulin resistance and insufficient insulin secretion (β -cell dysfunction) in response to hyperglycemia. Typically glucose intolerance from obesity and increased insulin resistance progress to fasting hyperglycemia and the inability of β -cells to compensate by increasing their rate of insulin secretion. This form of β -cell failure appears to be reversible over short periods by improved glycemic control, but long-term exposure to hyperglycemia can lead to β -cell exhaustion and specific inhibition of insulin secretion. The insulin resistance also extends to the liver where glucose production increases. This triad of insulin resistance, reduced β -cell insulin secretion, and increased hepatic glucose production produces type II diabetes.

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GROWTH MONITORING

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Glossary

Growth monitoring The serial weighing and measuring of the length/height (and head circumference if ≤ 2 years old) of a child and graphing both measurements on a growth chart.

Growth reference Simply describes the growth pattern of a defined population, without making any claims about the health status. In simple terms, a reference describes 'what is'.

Growth standard Defines a recommended pattern of growth that has been associated empirically with specified health outcomes and minimization of long-term risks of disease. It represents 'healthy' growth of a population and suggests a model or target pattern of growth for all children to achieve. In simple terms, a standard describes 'what should be'.

Growth surveillance Monitoring the growth status of a population. Usually measurements of height and weight are taken periodically on a representative sample of children to monitor trends in their growth status over time.

Growth velocity The average change in a specific anthropometric measure over a specific time period that varies depending on the child's age. Growth velocity is more sensitive indicator of small changes in growth status than attained growth, and more helpful when assessing changes in growth rates that are important in selected growth disorders and therapies.

Malnutrition Deficiencies, excesses or imbalances in intake of energy, protein and/or other nutrients. Contrary to

common usage, the term *malnutrition* correctly includes both undernutrition and overnutrition.

Promotion of optimal growth The process of weighing and measuring the length/height (and head circumference if ≤ 2 years old), assessing growth, and providing counselling and motivation for actions to improve abnormal patterns of growth.

Z-scores Also known as standard deviation (SD) scores, z-scores are a statistical unit used to describe how far a measurement is from the mean (average) or median. Percentiles are commonly used in the clinical setting because they indicate simply and clearly a child's position within the context of the standard or reference population. Use of z-scores is almost universal for population-based applications and research reporting. For comparison purposes, the 50th percentile is equal to a z-score of 0, the 15th and 85th percentiles approximate z-scores of -1 and $+1$ respectively, the 3rd and 97th percentiles approximate z-scores of -2 and $+2$ respectively, and the 1st and 99th percentiles approximate z-scores of -3 and $+3$, respectively.

Z-Score	Exact Percentile	Rounded Percentile	Z-Score	Exact Percentile	Rounded Percentile
0	50th	50th	+1	84.1	85th
-1	15.9	15th	+2	97.7	97th
-2	2.3	3rd	+3	99.9	99th
-3	0.1	1st			

Introduction

Growth monitoring and promotion of optimal growth are essential components of primary health care for infants and children. Serial measurements of weight, length/height for all children, and head circumference for infants and toddlers, compared with the growth of a large sample population of children depicted on an appropriate growth chart help confirm a child's healthy growth and development. It also allows early identification of potential nutritional or health problems and enables prompt action before a child's health is seriously compromised.

The consequences of poor nutrition during the early years include compromised immunity, cognitive problems, and stunted growth. Subsequent overnutrition may predispose to conditions such as obesity, diabetes, and metabolic syndrome

later in life. When potential problems are identified early, health professionals and parents can work together to initiate action before the child's nutritional status or health are significantly affected.

Objectives and Activities

The main objectives of growth monitoring are to do the following:

- Provide a tool for nutrition and health evaluation of individual children.
- Initiate effective action in response to abnormal patterns of growth.
- Teach parents how nutrition, physical activity, genetics, and illness can affect growth and, in doing so, motivate and

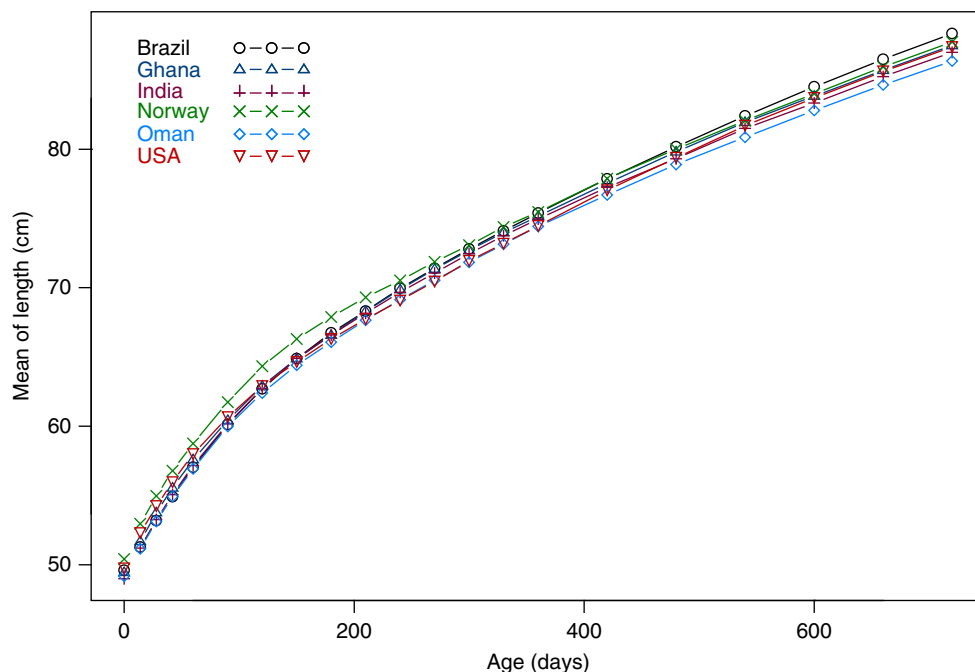
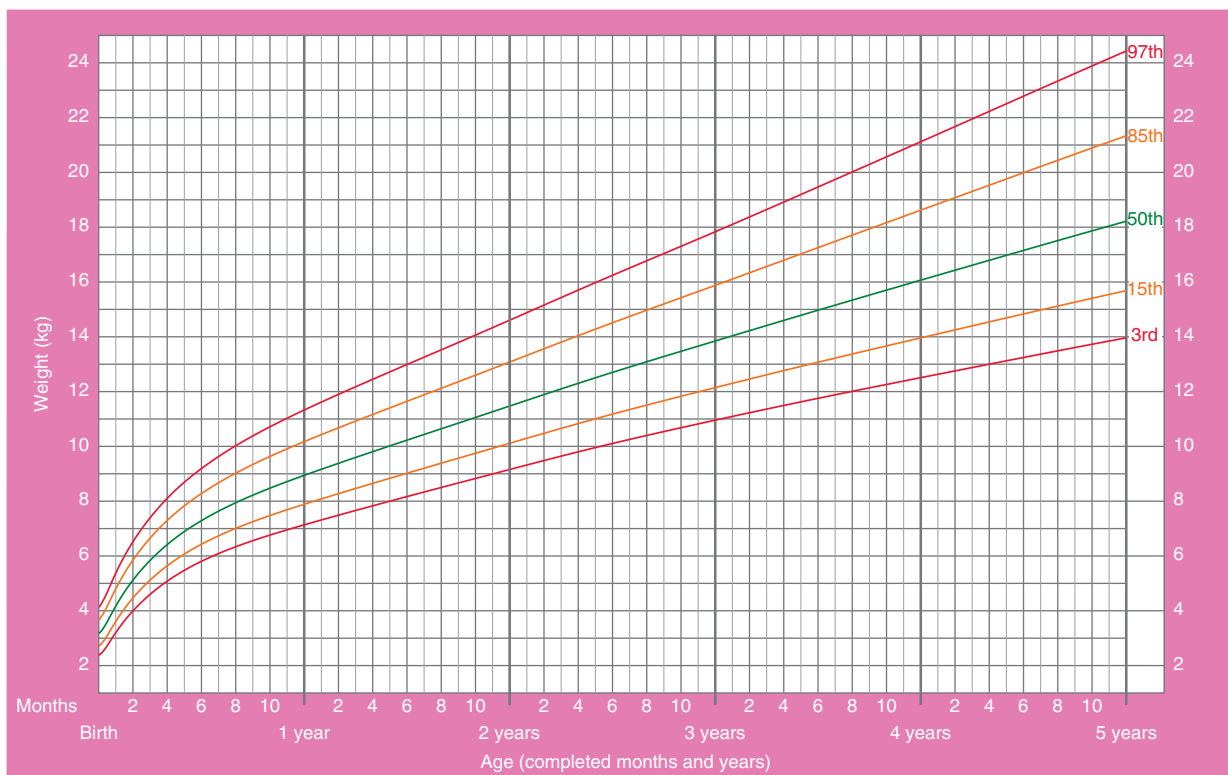


Figure 1 Mean length (cm) from birth through 2 years for each of the six sites in the WHO Multicentre Growth Reference Study. Reproduced from WHO.

Weight-for-age girls

Birth to 5 years (percentiles)



WHO Child Growth Standards

Figure 2 Weight-for-age for girls from birth to 5 years (percentiles). Reproduced from WHO Child Growth Standards for Preschool Age Children at <http://www.who.int/childgrowth>

facilitate individual initiative and improved child-care practices.

- Provide regular contact with primary health care services and facilitate their utilization.

Activities linked to growth monitoring at the individual level include the following:

- Accurately measuring weight, length or height, and head circumference.
- Precisely plotting measurements on the appropriate growth chart.
- Correctly interpreting the child's pattern of growth.
- Discussing the child's growth pattern with the parent(s)/caregiver and agreeing on subsequent action when required.

It is important to adapt the objectives, activities, and interventions in relation to the environment in which they are used. In practice, this makes growth monitoring a very different proposition in the developing and industrialized worlds due to differences in the burden of disease, resources, and training. The disease burden is much greater in the developing world, with diarrhea, malaria, respiratory infection, tuberculosis, and HIV all common. Here, effective growth monitoring can save lives. In contrast, in the industrialized world such conditions are less common and milder, and the focus is more

on growth disorders such as growth hormone deficiency or Turner's syndrome. This different focus also affects the target age range when children are monitored. Whereas in the developing world growth monitoring targets the preschool years, in the industrialized world it covers all childhood up to and including puberty.

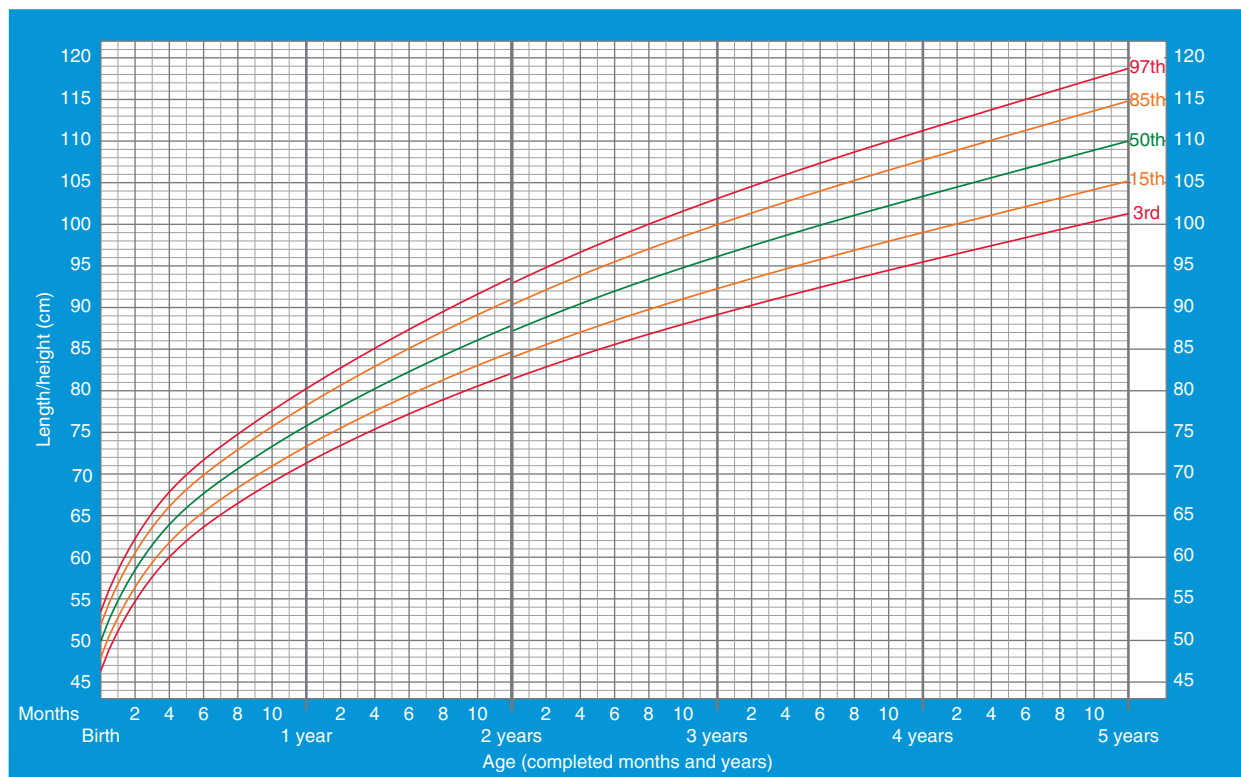
In addition to detecting disease and raising parental awareness at the individual level, growth monitoring in the sense of information gathering has potential benefits at the population level. It provides information about average child growth that is useful for comparison, policy, and planning. For example, monitoring the growth status of populations from different regions is useful for identifying areas where the prevalence of malnutrition is highest, which in turn allows resources and supports staff to be effectively targeted.

Monitoring Growth Successfully

The successful assessment of growth is founded on the selection of (1) an appropriate anthropometric indicator, (2) an appropriate reference population with which to compare the index child or community, and (3) appropriate cutoff points to interpret anthropometric measurements and classify children according to various degrees of malnutrition.

Length/height-for-age boys

Birth to 5 years (percentiles)



WHO Child Growth Standards

Figure 3 Length/height-for-age for boys from birth to 5 years (percentiles). Reproduced from WHO.

Anthropometric Indicators

In children the three most commonly used indicators to assess growth status are weight-for-age, length/height-for-age, and weight-for-length/height. Weight-for-age is the most commonly used and, in many developing countries, the sole anthropometric indicator used. During infancy, the measurement of weight is simple to do, the required equipment is reasonably cheap, and it provides a convenient global summary of the infant's size. Birth weight in particular is a useful proxy for fetal growth. An advantage of weight is that it relates closely to the mother's own perception of her child's size. However, although it is the easiest indicator to use where children's ages are known, weight-for-age lacks the biological specificity necessary to separate weight from length/height-related deficits or excesses in growth. Conversely, length/height-for-age and weight-for-length/height permit the distinction of stunted, wasted, and overweight children and allow the appropriate targeting of interventions.

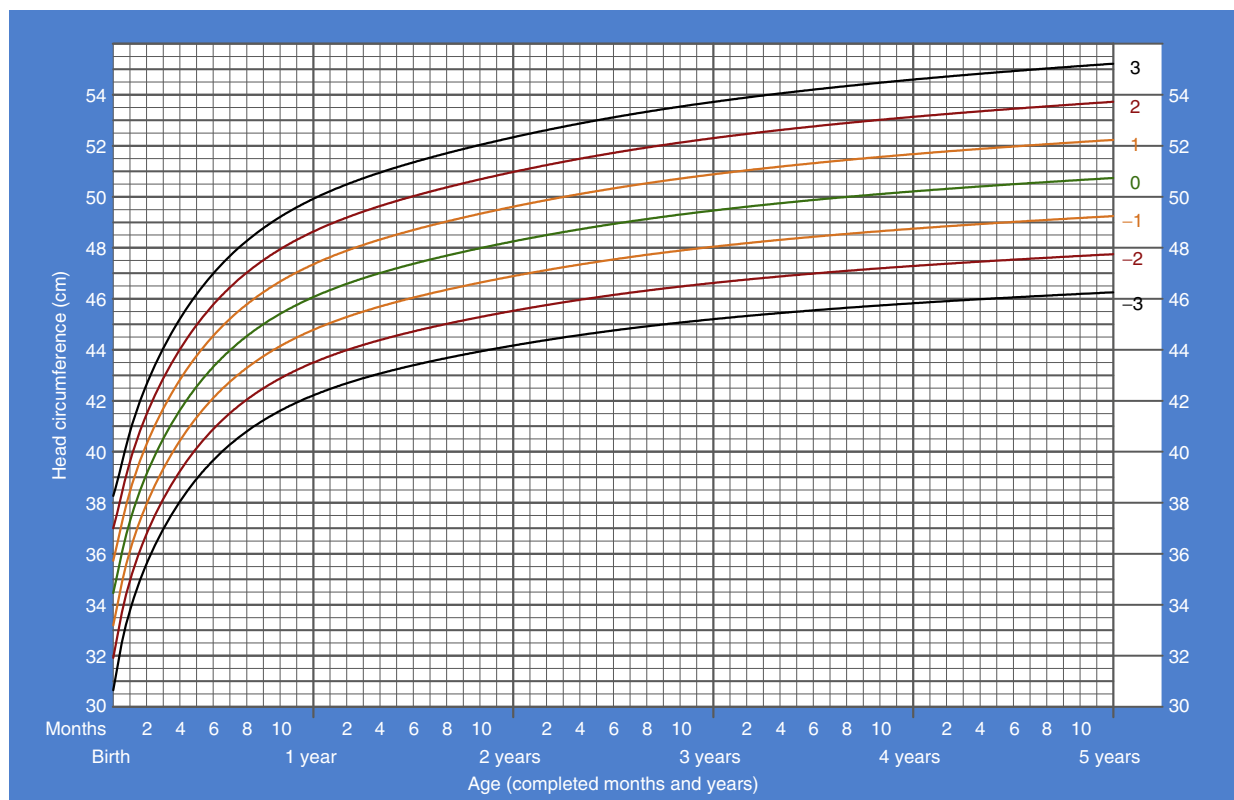
The routine collection of length/height measurements (recumbent length up to 2 years of age and standing height for older children) is important because this enables not only the assessment of weight-for-height, but also body mass index (BMI) (i.e., ratio of weight in kilograms to the square of height

in meters), a valuable indicator proposed for monitoring the increasing public health problem of overweight and obesity in childhood. During infancy length is more difficult to measure for several reasons. The optimal equipment is a length board with a sliding footboard, which is expensive and needs regular calibration. Simpler equipment such as a tape measure increases the measurement error dramatically. Most important, proper length measurement requires two trained observers – one to hold the infant's head against the headboard and the other to position the footboard and take the measurement. For these reasons, infant length is often measured either poorly or not at all. After infancy, monitoring length/height becomes much more important, particularly in the industrialized world, where the emphasis is on detecting primary growth disorders such as growth hormone deficiency or Turner's syndrome.

Head circumference is another important indicator in neonates and toddlers to detect abnormal patterns of growth due to conditions such as hydrocephalus. Other available anthropometric indicators that are used to describe growth status during childhood include mid-upper arm circumference, most commonly used in resource poor settings but less so in the industrialized countries. Other measurements such as triceps and subscapular skinfolds are useful proximate

Head circumference-for-age boys

Birth to 5 years (z-scores)



WHO Child Growth Standards

Figure 4 Head circumference-for-age for boys from birth to 5 years (z-scores). Reproduced from WHO.

measures of body fat but are not widely used due, in part, to technical difficulties and require skilled individuals to perform the measurements accurately and precisely.

Growth References and Standards

A clear understanding of the terms 'reference' and 'standard' and of the appropriate uses of those tools is important. The common use of reference and standard as synonyms is not surprising. The 'International Dictionary for Medicine and Biology' defines reference as "a standard against which techniques, measurements or other observations can be compared, or on which inferences or calculations can be based." Standard is given two definitions of interest, "a unit, level, or specification established as a reference for purposes of comparison or control, or for securing uniformity" and "serving as a model or magnitude against which similar entities, performances, or quantities may be compared." Thus reference appears to be defined as a standard, and standard as a reference.

In the context of growth assessments, however, the distinction between a reference and a standard is important from theoretical and practical perspectives. It is much more useful to conceptualize references as 'devices for grouping and analyzing data' and standards as devices that 'embody the concept

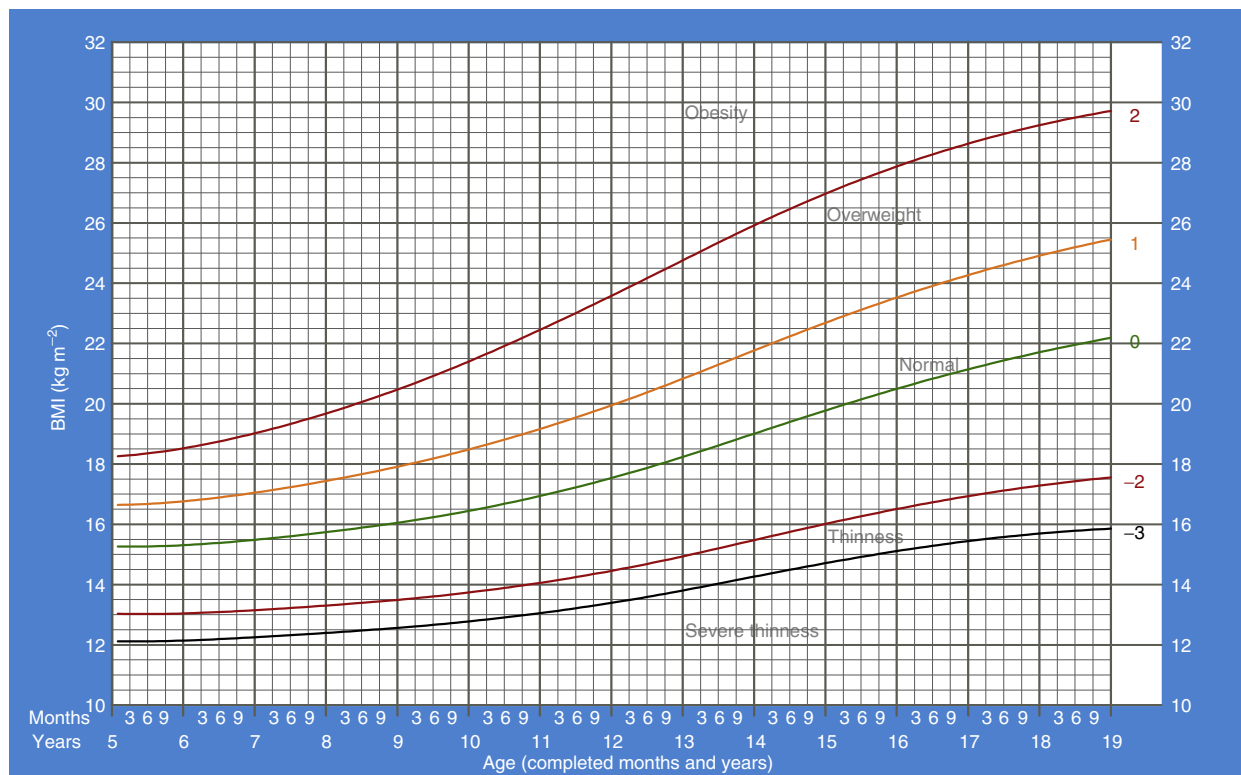
of a norm or target – that is a value judgment.' Thus standards should aid health professionals to judge current status and predict future outcomes. References should provide useful tools for the more limited purpose of making comparisons.

There is now broad international consensus about the utility of the World Health Organization (WHO) Child Growth Standards (www.who.int/childgrowth/en) for assessing the growth of preschool children. Based on an international sample of breastfed infants and young children, the WHO standards depict physiological human growth under optimal environmental conditions and can be used to assess children everywhere, regardless of ethnicity, socioeconomic status, and type of feeding. A salient outcome of the WHO growth standards' project is the striking similarity in linear growth of the child populations from the six countries (Brazil, Ghana, India, Norway, Oman, and the USA) that participated in the study (Figure 1). The WHO standards have been adopted by more than 120 countries and many researchers worldwide. Figures 2–4 present examples of generic WHO growth charts for selected commonly used indicators in the age group 0–60 months. Useful software to monitor the growth of individual children and populations can be found at www.who.int/childgrowth/software.

After 5 years of age, the use of the WHO reference for school-age children and adolescents provides a suitable reference for the 5–19 years age group for use in conjunction

BMI-for-age boys

5–19 years (z-scores)

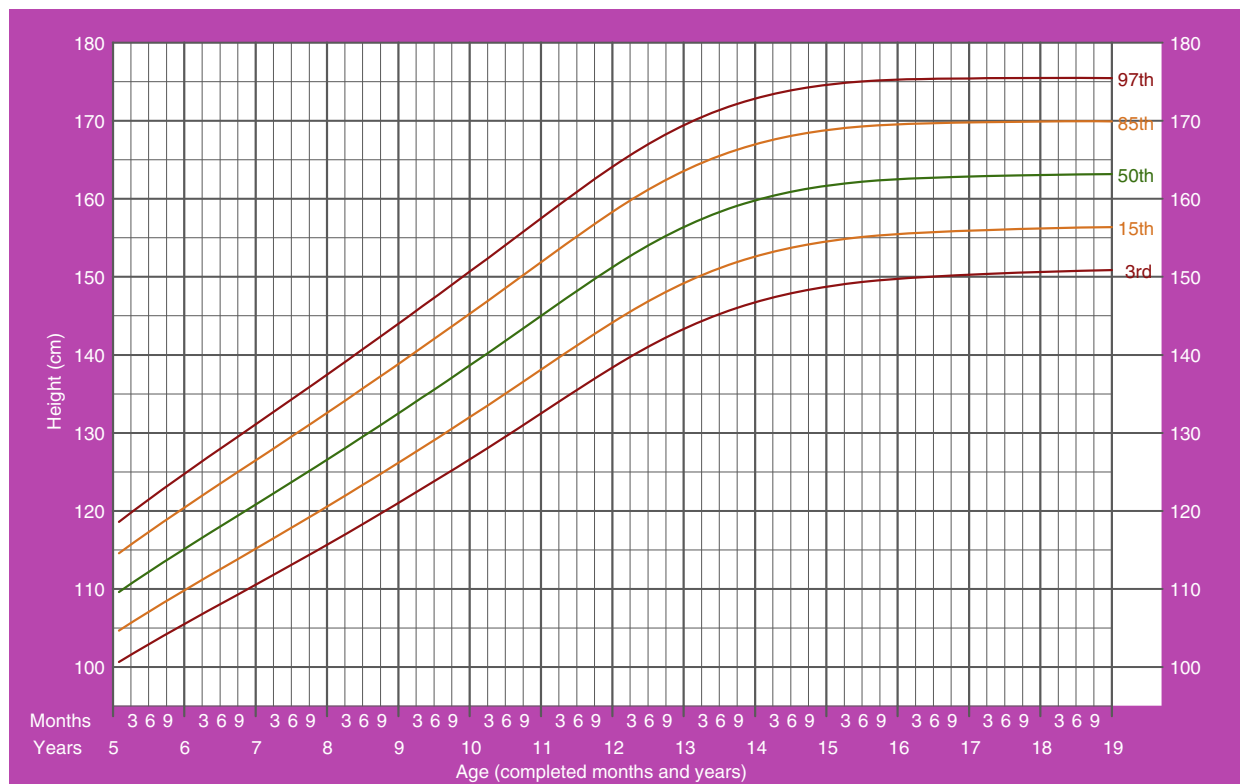


2007 WHO reference

Figure 5 BMI-for-age for boys from 5 to 19 years (z-scores). Reproduced from WHO (2007).

Height-for-age girls

5–19 years (percentiles)



2007 WHO reference

Figure 6 Height-for-age for girls from 5 to 19 years (percentiles). Reproduced from WHO (2007).

with the WHO Child Growth Standards from 0 to 5 years, and is recommended by WHO for both clinical and epidemiological use. The full set of tables and charts is available at www.who.int/growthref/en, including application tools such as software for clinicians and public health specialists. Figures 5–7 present examples of generic WHO growth charts for selected commonly used indicators in the age group 5–19 years.

Cutoff Points and Interpretation of Anthropometric Measurements

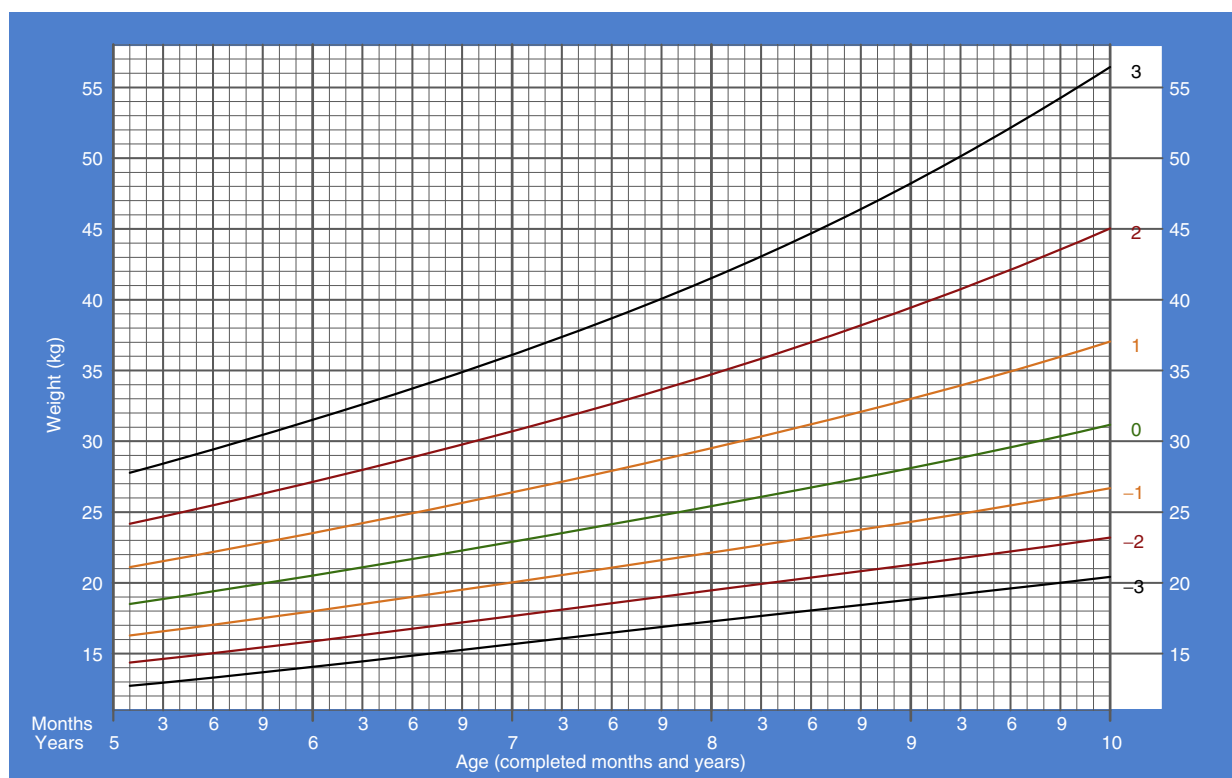
Cutoffs and the bases for their designation are among the principal factors that influence the interpretation of anthropometric measurements. They are intended to provide guidance for further assessment, referral, or intervention. Two basic approaches are used to designate cutoffs that are intended to reflect levels of adverse risks. A so-called statistical approach that is based on the mathematical estimation of presumed risk depends strictly on the cutoff's distance from a given measurement's central tendency, i.e., its mean or median. The other is a risk-based approach that relies on a demonstrable link between the selected cutoff and a designated health outcome (e.g., illness or death).

The statistical approach is the one most commonly used and is usually expressed in terms of percentiles or as multiples

of a standard deviation (i.e., Z-score) of the targeted measure's distribution. In the latter, a Z-score of zero corresponds to the measurement's mean. Z-scores of +1 and –1 correspond to values one standard deviation above or below the mean, respectively. Z-scores of +2 and –2 correspond to analogous values. The Z-score system has advantages that relate to the comparability of similar Z-scores across ages, sexes, and other traits that may be accounted for by this system. Briefly, a Z-score of +2 always designates the same relative position within a normal distribution. This has enormous advantages whenever judgments are based on comparisons of any sort whether applied to individuals or populations. The recommended cutoffs (percentiles and Z-scores) by the WHO for screening for undernutrition and overnutrition are presented in **Table 1**.

An important concept in the interpretation of anthropometric measurements is the need for serial measurements. One-time measurements, taken and plotted accurately on a growth chart, reflect a child's size and may be used to screen children for nutritional risk using the cutoffs indicated in **Table 1**. However, they do not provide adequate information to assess a child's growth. A series of weight and length/height measurements over time are more informative and reflect a child's growth pattern. In most children, serial length/height, and weight measurements follow consistently along a percentile or Z-score curve. This is referred to as 'tracking' on the

Weight-for-age boys
5–10 years (z-scores)



2007 WHO reference

Figure 7 Weight-for-age for boys from 5 to 10 years (z-scores). Reproduced from WHO (2007).

Table 1 Recommended cutoffs by the WHO for screening for undernutrition and overweight/obesity

Parameters	WHO child growth standards	WHO reference 2007
Age	Birth to 5 years	5–19 years
Underweight weight-for-age	<3rd centile < – 2 Z-scores	<3rd centile < – 2 Z-scores
Stunted length-for-age/height-for-age	<3rd centile < – 2 Z-scores	<3rd centile < – 2 Z-scores
Wasted weight-for-length/height or BMI-for-age	<3rd centile < – 2 Z-scores	<3rd centile < – 2 Z-scores
Overweight weight-for-length/height or BMI-for-age	>97th centile > + 2 Z-scores	>85th centile > + 1 Z-scores
Obese weight-for-length/height or BMI-for-age	>99.9th centile > + 3 Z-scores	>97th centile > + 2 Z-scores

Source: Reproduced from WHO.

growth chart. With the exception of the first two to three years of life when crossing percentile curves may be normal, and again in puberty, when the age of onset is variable, a sharp incline or decline in growth, or a growth line that remains flat, is potentially a sign of growth disturbance. A shift toward the 50th centile is likely a healthy change, whereas a shift away from the 50th percentile may signal a problem.

The interpretation of the commonly used anthropometric indicators is as follows:

Low weight-for-age: Weight-for-age reflects body mass relative to chronological age. It is influenced by both the child's height (height-for-age) and his or her weight (weight-for-height). Its composite nature makes interpretation complex. For example, weight-for-age fails to distinguish

between short children of adequate body weight and tall, thin children. However, in the absence of significant wasting in a community, similar information is provided by weight-for-age and height-for-age, in that both reflect an individual's or population's long-term health and nutritional experiences. Short-term changes, especially reductions in weight-for-age, reveal changes in weight-for-height. In general terms, the worldwide variation of low weight-for-age and its age distribution are similar to those of low height-for-age.

Low height-for-age: Stunted growth reflects a process of failure to reach linear growth potential as a result of sub-optimal health or nutritional conditions. On a population basis, high levels of stunting are associated with poor socioeconomic conditions and increased risk of frequent and early exposure to adverse conditions such as illness or inappropriate feeding practices. Similarly, a decrease in the national stunting rate is usually indicative of improvements in overall socioeconomic conditions of a country. The worldwide variation of the prevalence of low height-for-age is considerable, ranging from 5% to 65% among the less developed countries. In many such settings, growth faltering starts at birth and mainly happens during the first two years of life, after which the process of stunting slows down. Therefore, the age modifies the interpretation of findings: for children in the age group below 2 years, low height-for-age probably reflects a continuing process of 'failing to grow' or 'stunting'; for older children, it reflects a state of 'having failed to grow' or 'being stunted.' From the point of view of interventions, it is important to differentiate between these two groups.

In populations without significant stunting or wasting, low height-for-age in individual children is more likely to signal deprivation due to conditions not necessarily related to poverty. Thus, for example in industrialized countries, it is necessary to consider possibilities that include psychosocial deprivation, illness, metabolic conditions constraints, and other mechanisms that may result in growth failure.

Low weight-for-height: Wasting or thinness indicates in most cases a recent and severe process of weight loss, which is often associated with acute starvation or severe disease. However, wasting also may be the result of chronic unfavorable conditions. Provided there is no severe food shortage, the prevalence of wasting is usually below 5%, even in poor countries. The Indian subcontinent, where a higher prevalence of wasting is found, is an important exception. Prevalence between 10% and 14% is regarded as serious, and above or equal to 15% as critical. Typically, the prevalence of low weight-for-height reaches a peak in the second year of life. Lack of evidence of wasting in a population does not imply the absence of current nutritional problems: stunting and other deficits such as micronutrient deficiencies may be present. Given these characteristics wasting or thinness demands a careful assessment in all cases in which it is encountered.

High weight-for-height: Overweight is the preferred term for describing high weight-for-height. Even though there is a strong correlation between high weight-for-height and obesity as measured by adiposity, greater lean body mass can also contribute to high weight-for-height. On an individual basis, therefore, 'fatness' or 'obesity' should not be used to describe high weight-for-height. However, on a population-wide basis,

high weight-for-height can be considered as an adequate indicator of obesity, because the majority of individuals with high weight-for-height are obese. Strictly speaking, the term obesity should be used only in the context of adiposity measurements, for example skinfold thickness.

High BMI-for-age: Another measure of body mass relative to height, is emerging as a widely used indicator for classifying overweight and obesity. Although there is some reluctance to describe children as obese on the basis of BMI alone, i.e., without taking into account some more direct measure of body fat, recognition of the difficulties inherent in obtaining more proximate measures of body fat and lack of references to interpret them has resulted in BMI-for-age alone being used to define overweight and obesity. In its favor, increased BMI-for-age in childhood and adolescence is associated with higher percentages of body fat and known risk factors for cardiovascular disease.

Accuracy of Anthropometric Measures

The process of anthropometry requires attention to detail: suitable equipment that is regularly maintained and calibrated; observers who are trained in correct measurement technique; regular quality control sessions in which observers are checked, against both themselves and each other, for measurement precision and accuracy. Only in this way can accuracy and precision be maintained.

Variability in infant and child measurements can result from a number of influences: the setting in which the measurements are taken (e.g., home or clinic), the degree of filling of the stomach or bladder (in the case of weight), the behavior and cooperation of the child being measured, accuracy and precision of instruments, the anthropometrist's technical capacity (i.e., training, experience, and reliability), and the methods for recording data. Appropriate training and continual standardization, adherence to specified methods and procedures, and monitoring of data quality are essential for reducing measurement error and minimizing bias. Similarly, the use of appropriate measurement techniques is essential. A comprehensive description of the techniques used in the development of the WHO Child Growth Standards can be found under the Further Reading section.

Interventions

The action arising from growth monitoring may be quite specific (e.g., the identification and treatment of a particular growth disorder) or it may be more general (e.g., referral to a growth clinic, a dietician, or a therapeutic feeding center). If the mother is involved, it may alter her view of her child's health and so modify her child care. At the population level, it may affect the allocation of resources (e.g., between regions for malnutrition relief).

A Cochrane Review on growth monitoring attempted to answer the question 'Does it work?' In doing so, defined growth monitoring as "the regular recording of a child's weight, coupled with some specified remedial actions if the weight is abnormal in some way." The Review found only two

randomized clinical trials measuring the impact of growth monitoring, and they differed in their conclusions. One found that infants whose growth was monitored for 30 months were no healthier than age-matched controls, whereas the other showed that mothers trained to use a growth chart were more knowledgeable after 4 months. The review showed that there is little research on the subject, and even less evidence to justify its use, which is surprising given the enormous resources devoted each year to growth monitoring throughout the industrialized and developing worlds.

A possible reason for this lack of evidence is that growth monitoring is seen as intrinsically 'a good thing.' Parents are always interested to know how their children are growing, and the benefit to them of measuring the child regularly, although difficult to quantify, is assumed. Despite the Cochrane Review suggesting that growth monitoring in the developing world is ineffective, several potential outcomes are too diffuse to quantify (e.g., increased parental interest and education), and this needs to be recognized. The absence of an evidence base in favor of growth monitoring should not necessarily be interpreted as evidence that it lacks benefit. The benefits may simply be too subtle to detect using conventional trials.

See also: Growth and Development: Physiological Aspects. Low Birth Weight and Preterm Infants: Causes, Prevalence, and Prevention. Malnutrition: Secondary, Diagnosis and Management. Nutritional Assessment: Anthropometry. Nutritional Surveillance: Developed Countries; Developing Countries. Obesity: Childhood Obesity

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HEALTH DISPARITIES

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Glossary

Conditional cash transfer programs Government programs that provide a small monthly stipend to low-income families as long as they meet certain conditions.

Food security Access by all people at all times to enough food for an active, healthy life.

Health disparities Differences in health outcomes and risk factors among population subgroups.

Health inequities Differences in health which are not only unnecessary and avoidable but, in addition, are considered unfair and unjust.

Social determinants of health The conditions in which people are born, grow, live, work, and age, including the health system.

Health disparities refer to differences in health outcomes and risk factors among population subgroups. When deemed avoidable and unjust these differences represent health inequities. The root cause of health inequities is social injustice. Health inequities are more prevalent among vulnerable subgroups at a social disadvantage. Health inequities follow a social gradient and can be a function of ethnicity/race, immigration status, gender, sexual preferences, religion, and disability status.

The social determinants of health (SDH) are key for understanding the distribution of health inequities within and across countries. These refer to the physical and social conditions of the environments where individuals are born, grow, go to school, work, socialize, and age. Thus, efforts toward addressing health inequities need to go above and beyond disease control and antipoverty measures, and must improve the psycho-social environments where socially disadvantaged groups live and work. Fully addressing health inequities requires making the basic resources needed to improve the quality of life accessible to the most vulnerable. For example, improving the availability of nutritious foods, clean water, and better health care in a community does not guarantee that the most vulnerable within that community will have access to them because the control of access to these resources has been shown to be socially determined.

Nutrition and Health Inequities Epidemiology

Health and nutrition inequities have been well documented not only in developing but also in developed countries. Even in the USA, which is considered to be the wealthiest nation on earth, low-income groups (which are overrepresented by ethnic/racial minorities) are substantially more likely to be food insecure, overweight or obese, to have type 2 diabetes, and to lack health insurance (**Figure 1**).

Malnutrition Inequities

Decades of research have conclusively shown that undernutrition, even in its relatively mild forms, has negative consequences for human physical and intellectual development. Indeed, malnutrition explains over half of deaths among children under 5 years of age living in developing countries. Because human development is the basis for social capital, which in turn is the engine that drives national development, the problem of undernutrition is now recognized by key decision-making bodies and other stakeholders as a top priority that needs to be addressed. Food and Agricultural Organization (FAO) estimates that approximately 1 billion individuals worldwide experience caloric undernutrition. The problem is more severe in the poorest regions of the world including sub-

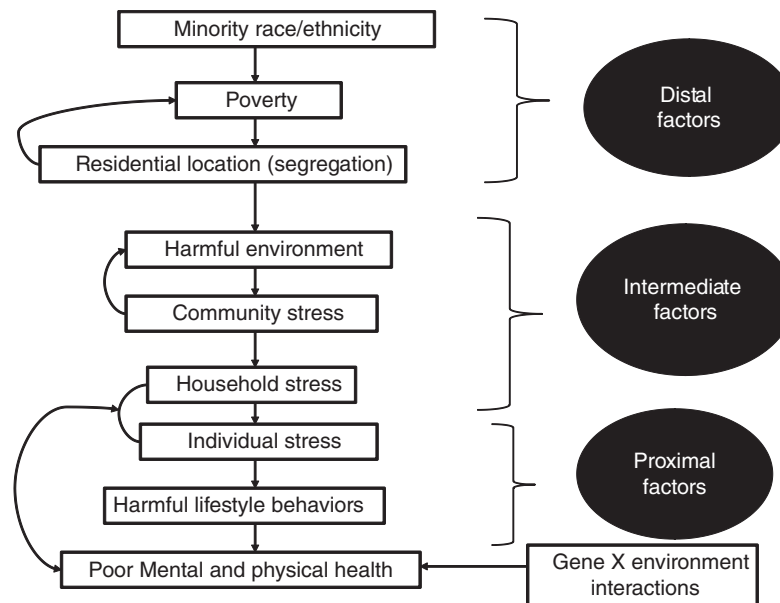


Figure 1 Socio-ecological model of health and disease.

Table 1 Key concepts and definitions

Health inequities	Differences in health which are not only unnecessary and avoidable but, in addition, are considered unfair and unjust.
Social gradient in health	Health outcomes, including life expectancy, improve as a function of the position of individuals along the socio-economic continuum.
Social determinants of health	The conditions in which people are born, grow, live, work, and age, including the health system. These circumstances are shaped by the distribution of money, power, and resources at global, national, and local levels, which are themselves influenced by policy choices. The social determinants of health are mostly responsible for health inequities.
Socio-ecological model of health	Individual's health and nutrition outcomes are the result of multilevel distal, meso, and proximal factors and the interactions among them. This model recognizes that individual lifestyle choices are strongly shaped by the environments where people grow and live (i.e., the social determinants of health).
Social capital	Quality of social relationships, sense of trust, belonging, and reciprocity in wider society.
Developmental Origins of Health and Disease hypothesis	Early life conditions, including nutritional and hormonal milieu to which individuals are exposed <i>in utero</i> and early childhood, impact longer-term health outcomes including obesity and chronic disease risk.
Food security	Access by all people at all times to enough food for an active, healthy life, and includes, at a minimum: (1) the ready availability of nutritionally adequate and safe foods and (2) an assured availability to acquire acceptable foods in socially acceptable ways (e.g., without resorting to emergency food supplies, scavenging, stealing, or other coping strategies).
Conditional Cash Transfer Programs	Government programs that provide a small monthly stipend to low-income families as long as they meet certain conditions. These have included keeping children in school, bringing them to get immunized and receive other primary health care services, and in some instances participation of their caregivers.

Saharan Africa, South and Southeast Asia. Epidemiological data also clearly show that within countries, socio-economic position is a powerful determinant of the risk of under-nutrition, including the risk of stunting in children. In addition, there are hundreds of millions of individuals that may have access to sufficient or even excessive amounts of calories but that do not have access to diets of adequate nutritional quality. As a result, low socio-economic groups are now facing a 'double burden' of malnutrition (i.e., excessive rates of both

under- and overnutrition) oftentimes coexisting within the same household. The problem of malnutrition has its roots on the SDH and is also largely explained by major inequities in the distribution of wealth, power, and resources. In addition, two of the key proximal determinants of malnutrition are lack of access to nutritious diets and inadequate health care. Thus, nutrition inequities go hand in hand with inequities related to access to food, health care, and other basic human needs (Tables 1–3).

Household Food Insecurity Inequities

Household food security is defined as 'access by all people at all times to enough food for an active, healthy life, and includes, at a minimum: (1) the ready availability of nutritionally adequate and safe foods and (2) an assured availability to acquire acceptable foods in socially acceptable ways (e.g., without resorting to emergency food supplies, scavenging, stealing, or other coping strategies).' Thus, food insecurity (FI) exists when there is 'limited or uncertain availability of nutritionally adequate and safe foods or

limited or uncertain ability to acquire acceptable foods in socially acceptable ways.' The US Census Bureau reports annual household FI rates since 1995 estimated from the 18-item Household Food Security Supplement Module (HFSSM) applied through the December Current Population Survey Food Security Supplement. The reference time period of the HFSSM is the 12 months preceding the survey and households are classified as having either food security, low food security, or very low food security based on the number of affirmative answers to the HFSSM items. In 2009, 14.7% of US households were food insecure (i.e., with either low or very low food security). This rate, which translates into 17.4 million households, is the highest ever recorded since 1995. US households are more likely to be food insecure if they are poor, are headed by a single female, and the head of household is Hispanic or African-American. Thus, the distribution of household FI is also a reflection of the strong socioeconomic inequities in the USA. Similar conclusions have been reached in countries as diverse as Colombia, Mexico, and Brazil, where the severity of FI and consumption of nutritious foods also follows a socioeconomic gradient.

Table 2 United Nations millennium development goals

1. Reduce extreme poverty and hunger
2. Universal primary education
3. Gender equality and women's empowerment
4. Reduce child mortality
5. Improve maternal health
6. Combat HIV/AIDS, malaria, and other diseases
7. Environmental sustainability
8. Global partnerships for development

Table 3 Addressing key distal, meso, and proximal determinants of health: Policy goals

<i>Issues</i>	<i>Policy goals</i>
The social gradient	More equity in income and wealth distribution through better access to adequate education, employment opportunities, housing, health care, and food security.
Stress	Improve neighborhood safety, built and social environment. Universal access to good-quality schools and health care (physical, mental, and dental).
Early life	Universal access to prenatal care, breastfeeding promotion and support, high-quality daycare and preschool programs, and adequate nutrition. Home visiting programs to support development of better infant feeding, parenting skills, and psycho-emotional health of the caregiver.
Social exclusion	Strong and enforceable civil rights laws that prevent discrimination based on individual's ethnicity/race, religion, gender, sexual orientation, age, disability status, and immigration status, among others.
Work	Develop good skills for job market through good-quality vocational, college, and postgraduate educational opportunities. Establish minimum wages based on reasonable estimates of what families need to have a quality of life compatible with the principles of human rights and dignity. Provide reasonable paid parental leave policies that protect the job security of parents. Improve safety of work environments. Global trade policies should be restructured to allow the less powerful to have a chance to fairly compete in local and global markets. Increase credit access to small producers and businesses.
Unemployment	Safety net with reasonable unemployment benefits and retraining opportunities.
Social support	Increase social capital in deprived neighborhoods/communities.
Addiction	Increase social capital in deprived neighborhoods/communities. Provide access to effective primary prevention and substance-abuse treatment programs. Limit advertisement of alcohol and tobacco products in low-income neighborhoods. Use effective taxation (pricing) policies to limit abuse of substances.
Food	Restructure food price policies so that nutrient-dense foods become more economically accessible to low-income groups. Restructure food systems in low-income areas to improve community access to healthy and nutritious foods, including fresh fruits and vegetables, legumes/pulses, and minimally processed grains. Limit advertisement of foods high in saturated/transfats, sodium, and refined sugars in low-income neighborhoods and through mass media marketing targeting children. Ensure that food assistance programs are consistent with evidence-based dietary guidelines across the life cycle. Provide effective instrumental nutrition and food safety education, including healthy cooking, in schools and diverse community settings.
Physical activity and transport	Increase community access to safe green areas. Improve use of public open spaces for families to walk, exercise, and interact socially with other community members. Redesign urban areas traffic systems to facilitate the routine use of bicycles when going to school or work. Provide access to safe, clean, and efficient public transportation for individuals to be able to transport to work, school, and public open spaces.
Health care	Health care access for all. Must emphasize primary prevention, screenings, and referrals and cover physical, mental, and dental health care needs.

Obesity Inequities

Obesity is a major public health problem in both developed and developing countries. Indeed, maternal–child obesity rates in Mexico are now as high as in the USA. Consistent evidence indicates that, within developed countries and increasingly among developing countries as well, poverty is associated with a higher obesity risk. This is not surprising as lack of access to healthy, nutrient-dense foods and opportunities for physical activity are important obesity risk factors. In the USA, 76.9% of Hispanic and 73.7% of non-Hispanic Black women, versus 67.5% of Non-Hispanic White adult women, are overweight or obese (i.e., body mass index (BMI) ≥ 25). The corresponding rates for morbid obesity (i.e., BMI ≥ 35) are 18.9%, 27.9%, and 16.6%, respectively. The prevalence of type 2 diabetes in the USA is about twice as high among Hispanic and Black than among White individuals, and hypertension is substantially more prevalent among Blacks. Because obesity is a major risk factor for chronic diseases, such as cardiovascular disease and type 2 diabetes, it is not surprising that the distribution of these chronic conditions is inversely associated with socio-economic status.

Ethnic/racial differences in obesity rates in the USA are evident since very early childhood. The obesity prevalence among 2- to 5-year-old White children is 10.7% compared with 14.9% among non-Hispanic Black and 16.7% among Mexican-American preschoolers. As expected these differences are reflected in a significantly greater prevalence of early life obesity risk factors including maternal depression, rapid weight gain during infancy, lower rates of exclusive breastfeeding, introduction of complementary feeding before recommended age, more restrictive maternal feeding style, higher intakes of sugar-sweetened beverages and fast food, and insufficient sleep during infancy. These findings are of concern as there is increasing evidence that the intrauterine and early childhood environments are powerful determinants of health outcomes later in life, including the development of obesity and chronic diseases. This is known as the ‘developmental origin of health and disease’ hypothesis.

Health Inequities

Health Care Access Inequities

Close to 50 million individuals living in the USA do not have health insurance and thus lack access to basic health care and oftentimes end up receiving needed medical attention in emergency room services. Millions more of low-income individuals who have limited insurance coverage also do not have access to adequate physical, mental, and dental health care. In the USA the risk of not having access to health care access is much higher among low-income families, even though the majority of these households have employed members. This risk increases considerably among non-citizens, many of whom are migrant farm workers, who are not fluent in English and live in isolated communities. Thus, social exclusion and lack of adequate health care policies are largely responsible for the highly visible health care access inequities in the USA. This is emphasized by the improved health care access and health outcomes that populations living in other developed countries have. These countries’ systems are characterized by a ‘not-

for-profit’ health insurance structure, coupled with a single payer health care system focused on primary and secondary prevention.

Life Expectancy Inequities

According to the World Health Organization (WHO) the probability of a man dying between the ages of 15 and 60 years ranges from 8.3% in Sweden to 90.2% in Lesotho. Life expectancy is almost 50 years longer in Japan than in Sierra Leone. These remarkable differences in life expectancy have also been documented within countries. In Australia, aboriginal people have a life expectancy that is 20 years less than the national average. In the USA, the difference between the counties with the highest and lowest life expectancy is 18.4 years for men and 14.3 years for women. In the USA, Black men live 6.3 years less and Black women live 4.5 years less than their White counterparts. In England, people living in the poorest neighborhoods die on average 7 years earlier, and their disability-free life expectancy is 13 years lower, compared with their counterparts living in the wealthiest neighborhoods. Furthermore, a clear social gradient has been documented within both developed and developing countries showing that social and economic position is a powerful determinant of health outcomes. Although genetic susceptibilities play a role in the development of disease within populations and population subgroups, the large between-group differences in health outcomes as a function of social and economic position are largely explained by the SDH and thus need to be addressed accordingly. It is clear that when more equitable development approaches are used, key maternal, child, and adult health indicators rapidly improve as a result, thus ruling out a ‘genetic’ explanation. For example, Brazil recently demonstrated how large-scale investments in social programs capable of reducing income and wealth disparities across socio-economic groups have led to improvements in public health indicators, especially among the most vulnerable.

Understanding the Determinants of Health and Nutrition Inequities

Socio-ecological Model of Health

Health inequities can best be explained by the socio-ecological model of health. This model posits that multilevel factors that range from the individual to the macrosocial level interact with each other in determining health outcomes. This model is consistent with the SDH construct. It recognizes how the social distribution of wealth, resources, and power strongly determine access to healthy lifestyle components, including health screenings and timely treatment. Communities with low access to quality education and employment opportunities are characterized by unhealthy environments. These environments have low social capital and poor infrastructure including limited availability of healthy foods (e.g., fresh fruits and vegetables), limited opportunities for leisure-time physical activities (e.g., safe green areas and sports facilities), and very limited access to quality primary, secondary, and tertiary health care. They are also characterized by high levels of crime, violence, and other psychosocial stressors. This stressful environment, coupled with an inadequate coping response

among low-income individuals, represents another major barrier to a healthy lifestyle.

Research conducted in the USA has shown that self-perceived discrimination related to skin color has a negative impact on health, which is independent of the socio-economic status. Self-perceived discrimination has been associated with high blood pressure, arterial plaques, and higher levels of inflammation markers and visceral fat. These are important risk factors in the development of cardiovascular disease. Because cardiovascular disease explains a substantial amount of health inequities between Blacks and Whites in the USA, racial discrimination needs to be understood and addressed also from the health inequities perspective.

Health inequities are likely to be substantially explained by the direct biological impact (e.g., resulting from poor nutrition, low levels of physical activity, substance abuse) as well as the higher levels of chronic stress experienced by socially disadvantaged communities. In other words, individual level health outcomes are the result of lifestyle choices (diet, physical activity, substance abuse, sex practices) that are strongly shaped by the social policies and physical and social environments where people grow, live, and work (i.e., the SDH). Thus, correcting health inequities requires addressing the macro (e.g., safety net policies, minimum wage, social exclusion), meso (e.g., neighborhood safety, access to healthy foods, good quality schools, and adequate health care), and proximal lifestyle determinants (e.g., dietary intake, physical activity, substance abuse, sex behaviors) of health, and the interaction among them.

Solutions

Major international organizations and governments worldwide now fully acknowledge the existence and pressing need for addressing the major health and nutrition inequities worldwide. The United Nations Millennium Development Goals (MDGs) call for significant reductions in extreme poverty and hunger as well as in major communicable diseases. The MDGs place strong emphasis on addressing gender inequities and other SDH including universal access to education.

The MDGs do not include specific goals related to inequities in chronic disease outcomes. However, there is now global consensus that the SDH also strongly determine the risk factors and health outcomes for chronic diseases. This is due to the evidence indicating that lower socio-economic position is linked with obesity, less access to healthy foods, and fewer opportunities for leisure-time physical activity, proper screenings, medical referrals, and good-quality health care.

Although the existence of health and nutrition inequities is increasingly being acknowledged, there is still no consensus on the best ways to approach them. The MDGs specifically call for emphasizing sustainable development approaches based on strong multisectorial partnerships. Thus far, most efforts have focused on communicable disease prevention, food assistance programs, conditional cash transfers provision of microcredits to groups of women, and universal health-insurance strategies.

Few developed countries have based their national development approaches on the SDH including access to

education, adequate work environments, social and economic security, and health care access. However, Scandinavian countries have taken this approach and as a result have some of the best health indicators in the world. The UK recently released a major report on health inequalities in England and the national and local governments addressing the social determinants findings. Perhaps, the most dramatic demonstration of the power of the social determinants approach is illustrated by developing countries such as Costa Rica where the average life expectancy is a year longer than in the USA, even though its GNP is one-third of that in the USA.

Conditional Cash Transfer Programs

Conditional cash transfer programs (CCTPs) have gained popularity as a pragmatic way of addressing the major food, education, and health care needs of the most poor. CCTPs provide a 'small' cash allowance to low-income households with the condition that they keep the children in school and bring them to primary health care centers. In some programs, caregivers are required to attend health and nutrition workshops. CCTPs originated in Latin America and have been shown to have positive effects on maternal-child health behaviors and outcomes. In countries as diverse as Mexico, Brazil, Colombia, Honduras, and Nicaragua, CCTPs have been shown to lift families out of extreme poverty into poverty. However, from the social determinants perspective, they represent only a partial solution as they do not address the underlying social gradient. In other words, CCTPs do not benefit poor and lower middle-class families that are above the program financial inclusion criteria and are not able to lift families from extreme poverty into the middle class. As other regions of the world, including Sub-Saharan Africa, consider incorporating CCTPs as a strategy for reducing extreme poverty and improving education, health, and nutrition outcomes, it is essential that health and education infrastructures are in place before the program is implemented so that participants can truly meet the program's 'conditions.'

Access to Nutritious Foods

A key indicator of dietary quality is the consumption of fresh fruits and vegetables. For this reason, food assistance programs heavily emphasize the need for improving access to these healthy and nutritious foods in socio-economically disadvantaged communities. The USA Supplementary Nutrition Assistance Program (SNAP), formerly known as the food stamp program, provides a cash transfer to low-income families earmarked for purchasing food but does not place any conditions as to the nutritional value of the foods purchased. One approach that SNAP has used for improving participants' consumption of fruits and vegetables is to provide nutrition education through the SNAP-Ed program. Because education by itself is not enough to meet this goal, SNAP is piloting fiscal incentives to foster the availability, access, and purchase of fruits and vegetables by program participants. An important incentive program being piloted is providing recipients with a 'bonus' or 'discount' incentive when using their SNAP benefits to purchase fruits and vegetables. At the same time, SNAP is supportive of the development of more points of access to fruits and vegetables in low-income areas, including corner

stores and farmers markets, and by facilitating the use of the program electronic benefit cards at these points of purchase. This example illustrates how much food systems as a whole need to be revamped for vulnerable communities to have more access to healthy and nutritious foods and for their residents to be able to purchase them. Culturally appropriate health and nutrition education then becomes crucial for ensuring that the products are actually consumed in recommended amounts. Otherwise, the impact of these major investments will not be able to impact on the obesity and health inequities in the USA.

In developing countries micronutrient fortification has become a major strategy for improving the nutritional status of their populations. These strategies include the provision to households with micronutrient powders (i.e., 'sprinkles') or of energy- and micronutrient-dense spreads to improve the nutritional value of foods given to young children. These approaches have been shown to be effective but to a very limited extent relative to the major health and nutrition deficits experienced by low-income individuals as a result of pervasive social inequities. Although some of these short- and medium-term biomedical-type solutions are needed to address under-nutrition in developing countries, the problem will not be solved unless policies based on the SDH and nutrition well-being inequities are implemented. These policies are expected to reduce economic and social inequities and, as a result, to improve access to better education, employment, and housing opportunities. This in turn would facilitate the adoption and dissemination of lifestyles compatible with good health and quality of life.

Physical Activity

Regarding opportunities for leisure-time physical activity, urban areas in Latin America have launched innovative 'public space use' and 'public transportation' initiatives. These include the development and maintenance of safe green areas for people engaged in individual and group leisure-time activities, the provision of low bicycle rental fees to move around downtown areas, and the closing of major city streets to vehicular traffic during weekends. Although few, if any, of these structural changes in the physical environments have been formally evaluated, given their emphasis on facilitating 'access for all' it is likely that these efforts represent a step in the right direction with regards to bringing more equity to opportunities for leisure-time physical activity. These opportunities for physical activity in public open spaces improve levels of social capital and air quality, and directly benefit the health of individuals through increased levels of physical activity.

Health Care Reform

Many nations have reformed their health care systems to provide 'universal' access to timely health care. This is the case of the 'seguro popular' effort in Mexico, the 'single health system' in Brazil, and more recently the effort by the USA government to have all individuals covered by health insurance based on a sliding scale system with subsidies for those who cannot afford it. In the USA, ongoing health care reform efforts also include forbidding health insurance companies from denying coverage to individuals

with preexisting medical conditions. This reform however excludes from its coverage millions of individuals living in the country who are non-citizens, highlighting the need to simultaneously address immigration reform. This illustrates how ignoring relevant SDH, in this instance the exclusion of individuals based on their immigration status, precludes a country from truly correcting major health care access inequities. In fact, it may make this inequity even worse for the most vulnerable.

Reduce Discrimination

Because of the impact that stress associated with self-perceived racial discrimination has on the risk of poor mental health and chronic diseases, it is important that individuals who are vulnerable to this type of discrimination are protected through civil rights laws and awareness of the general public. Racial discrimination is not always intentional and when this is the case it can be addressed through improvements in cultural sensitivity education and trainings.

Conclusion

Breaking the cycle between poverty, disease, and malnutrition requires a new way of thinking to move forward within a health and nutrition equity framework. This approach will indeed require substantially changing the social and political structures responsible for the inequitable distribution of power, wealth, and resources within and across countries. This is unlikely to happen without including the active participation of the most vulnerable communities in the shaping of this 'new' development paradigm. The WHO commission on SDH concluded that social inequities are of such a magnitude that they are responsible for excessive morbidity and the premature deaths of people on a massive scale. Correcting these inequities will take time. The commission however concluded that health outcomes can continue to improve and persistent health inequity gaps can be substantially narrowed within a generation, as long as social injustice is addressed through policies and programs based on the well-documented SDH. These efforts should consider establishing livable minimum wages, and should heavily invest in opening access to quality education, employment opportunities, and improved built environments. At a macro level it is essential to overhaul and restructure inequitable global trade policies. To be successful at reducing health and nutrition disparities, these combined efforts must result in a more equitable distribution of wealth, power, and resources between and within nations.

See also: Dietary Guidelines, International Perspectives. Early Origins of Disease: Fetal; Non-Fetal. Food Choice: Behavioral Aspects. Food Security. Hunger. Low Birth Weight and Preterm Infants: Causes, Prevalence, and Prevention. Nutrition Transition, Diet Change, and its Implications. Obesity: Prevention. Refugees: Nutritional Implications

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HOMOCYSTEINE

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Introduction

Homocysteine is a sulfur amino acid and an intermediate in the biochemical conversion of methionine to cysteine, a process called transsulfuration. The biochemistry of homocysteine was elucidated by Vincent Du Vigneaud and colleagues from the 1930s to the 1950s. In the early 1960s, the description and characterization of the inborn error of metabolism, homocystinuria, initiated a 50-year (and continuing) period of investigation that has revealed homocysteine as an independent risk factor for vascular disease. The association between elevated blood levels of homocysteine (hyperhomocysteinemia) and vascular disease may be similar in magnitude to the association between cholesterol and vascular disease, thereby implicating hyperhomocysteinemia as a significant public health concern. However, randomized control trials of B vitamins (folic acid with or without vitamin B₁₂ and vitamin B₆), which effectively lower blood homocysteine concentrations, have not demonstrated significant effects on cardiovascular disease incidence or related mortality. Hyperhomocysteinemia may therefore be a risk marker but not a causative factor in the pathogenesis of cardiovascular disease.

Structure and Forms

The structure of homocysteine is represented in [Table 1](#) along with the related structures of cysteine and methionine. The most prominent features of homocysteine and cysteine are the free sulfhydryl groups located at the end of the side chains of both amino acids. These sulfhydryl groups are highly susceptible to oxidation and formation of disulfide linkages with

other sulfhydryl compounds. The primary forms of homocysteine found in the blood ([Table 1](#)) consist of homocysteine in disulfide linkage with (1) cysteine residues within the primary sequences of albumin and other plasma proteins (protein-bound form), (2) free cysteines or cysteine-containing peptides (mixed disulfides), and (3) other homocysteine molecules (homocystine). Only approximately 1% of homocysteine in the blood is in the free-reduced form. Methionine, in contrast, does not have a free sulfhydryl group and, therefore, does not form disulfide compounds.

Biosynthesis and Metabolism

The biosynthesis and metabolism of homocysteine is presented in [Figure 1](#). The ultimate source of homocysteine is dietary methionine. Methionine is first activated by the addition of an adenosyl group (from adenosine triphosphate) to form S-adenosylmethionine (SAM). SAM is an important intermediate known as the universal methyl donor for its role as the methylating agent in a variety of essential reactions, including those involving DNA, RNA, proteins, membrane phospholipids, neurotransmitters, and the synthesis of creatine. A product of all SAM-dependent methylation reactions is S-adenosylhomocysteine (SAH), which in turn is metabolized to form adenosine and homocysteine. Homocysteine is then at a metabolic crossroad: It can be remethylated to form methionine or catabolized through cystathionine synthesis.

In remethylation, homocysteine reacquires a methyl group in a reaction catalyzed by the zinc-dependent enzyme, methionine synthase (5-methyltetrahydrofolate–homocysteine methyltransferase, EC 2.1.1.13), with methyltetrahydrofolate

Table 1 Structures and forms of homocysteine and related amino acids

Structures of homocysteine and related amino acids		Forms of homocysteine in blood	
$\begin{array}{c} \text{NH}_3^+ \\ \\ \text{H}-\text{C}-\text{CH}_2-\text{CH}_2-\text{SH} \\ \\ \text{COOH} \end{array}$	Homocysteine	Hcy-S-S-Cys-Albumin	Protein-bound form
$\begin{array}{c} \text{NH}_3^+ \\ \\ \text{H}-\text{C}-\text{CH}_2-\text{SH} \\ \\ \text{COOH} \end{array}$	Cysteine	Hcy-S-S-Cys Hcy-S-S-Hcy	Mixed disulfide Homocystine
$\begin{array}{c} \text{NH}_3^+ \\ \\ \text{H}-\text{C}-\text{CH}_2-\text{CH}_2-\text{S}-\text{CH}_3 \\ \\ \text{COOH} \end{array}$	Methionine	Hcy-SH	Free-reduced form

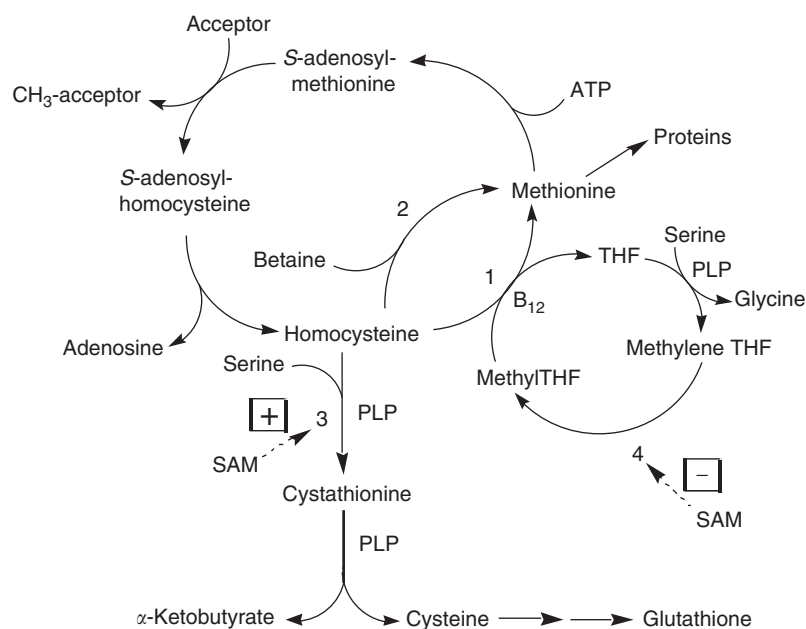


Figure 1 The biosynthesis and metabolism of homocysteine. Reactions that are regulated by SAM are indicated by positive and negative signs. Key enzymes: (1) methyltetrahydrofolate-homocysteine methyltransferase or methionine synthase, (2) betaine-homocysteine methyltransferase, (3) cystathionine β -synthase, and (4) MTHFR.

serving as the methyl donor and vitamin B₁₂ serving as a cofactor. This reaction occurs in all mammalian cells. Alternatively, homocysteine can be remethylated in a folate- and vitamin B₁₂-independent reaction using betaine as the methyl donor and catalyzed by betaine-homocysteine methyltransferase (EC 2.1.1.5). This reaction occurs primarily in the liver and to a lesser extent in the kidney and possibly in the brain.

Homocysteine catabolism occurs through cystathionine synthesis in a condensation reaction with serine. This reaction is catalyzed by cystathionine β -synthase (EC 4.2.1.22), which requires vitamin B₆ in the form of pyridoxal-5'-phosphate (PLP) as a cofactor. Cystathionine is then cleaved to form α -ketobutyrate and cysteine in a second PLP-dependent reaction catalyzed by cystathionase. Further metabolism of cysteine leads to the formation of glutathione or inorganic sulfate.

Regulation of Metabolism

An important aspect of homocysteine metabolism is that it is subject to allosteric control. In addition to serving as the universal methyl donor, SAM is also an activator of cystathionine β -synthase and an inhibitor of methylenetetrahydrofolate reductase (MTHFR, EC 1.7.99.5), the enzyme responsible for the synthesis of methyltetrahydrofolate (Figure 1). These allosteric functions serve to control whether homocysteine is recycled to form methionine or catabolized to form cystathionine. When dietary supply of methionine is high, that is, after a protein meal, intracellular SAM levels increase. The high concentration of SAM activates cystathionine β -synthase and inhibits MTHFR, thereby promoting homocysteine catabolism and limiting homocysteine remethylation. This serves to reduce the recycling of homocysteine when there is an adequate

dietary supply of methionine. Conversely, under fasting conditions when there is no dietary influx of methionine, intracellular SAM levels go down. Cystathionine β -synthase is then not activated and the inhibition of MTHFR is relieved, thus promoting homocysteine remethylation over catabolism. Consequently, this maintains intracellular methionine levels during times of limited dietary supply.

An additional level of control on homocysteine metabolism is exerted by oxidative stress, which reduces methionine synthase activity. This may occur by oxidative inactivation of the vitamin B₁₂ cofactor or by the oxidation of cysteine residues that are important for zinc binding. By inhibiting methionine synthase, oxidative stress tends to divert homocysteine toward cystathionine synthesis away from methionine synthesis. This serves to increase the synthesis of glutathione, a product of homocysteine metabolism through the transsulfuration pathway and an important intracellular antioxidant.

As discussed in the following Section (Hyperhomocysteinemia – Other Causes of Hyperhomocysteinemia), alterations in homocysteine metabolism also occur after menopause, in diabetes, and in hypothyroidism. These observations suggest that hormones, including estrogen, insulin, thyroxine, and thyroid-stimulating hormone, may directly or indirectly affect homocysteine metabolism. The mechanisms by which these hormones affect homocysteine metabolism are poorly understood.

Hyperhomocysteinemia

Under conditions of maximal metabolic efficiency, plasma levels of homocysteine range from 4 to 10 $\mu\text{mol l}^{-1}$. Metabolic blocks in homocysteine metabolism lead to accumulation of intracellular homocysteine with subsequent export into the blood. Depending on the magnitude of the metabolic

Table 2 Degrees of hyperhomocysteinemia

Total plasma homocysteine ($\mu\text{mol l}^{-1}$)	Designation
4–10	Normal
11–25	Mild to moderate
26–50	Intermediate
> 50	Severe

impairment, plasma homocysteine levels can rise to varying degrees, as defined in **Table 2**.

Genetic Defects

Severe elevations in plasma homocysteine (concentrations as high as several hundred micromole per liter) are observed in individuals with homozygous genetic defects affecting cystathionine β -synthase, MTHFR, or any of several enzymes responsible for the conversion of vitamin B₁₂ to its methionine synthase-associated cofactor form. These autosomal recessive genetic disorders, collectively termed homocystinuria because homocysteine accumulates in the urine as well as the blood, are associated with severe premature vascular disease, including thrombosis and atherosclerosis, mental retardation, dislocation of the eye lens (*ectopia lentis*), and skeletal malformations. Premature death (often in childhood) usually results from a major thrombotic or embolic event. Notably, one of the genetic defects that afflicts a significant proportion of homocystinuria patients reduces the affinity of cystathionine β -synthase for its vitamin B₆ cofactor, PLP. For these patients, the metabolic defect can be overcome to some extent with high-dose vitamin B₆ supplements, which significantly lower plasma homocysteine levels, reduce morbidity, and increase life expectancy. Interestingly, for other genetic defects involving cystathionine β -synthase that cause homocystinuria independent of the affinity of the enzyme for PLP, high-dose vitamin B₆ supplements nonetheless have a therapeutic effect despite having little or no influence on plasma homocysteine levels.

B-Vitamin Deficiencies

Hyperhomocysteinemia is also caused by B-vitamin deficiencies. Deficiencies of folate and vitamin B₁₂ lead to impaired remethylation of homocysteine causing mild, moderate, or severe elevations in plasma homocysteine, depending on the severity of the deficiency, as well as coexistence of genetic or other factors that interfere with homocysteine metabolism (see below, in this Section). As riboflavin is required for the synthesis of flavin adenine dinucleotide (FAD), and because FAD serves as a cofactor for MTHFR, riboflavin deficiency can also affect homocysteine remethylation, and, therefore, contribute to elevations in plasma homocysteine. Vitamin B₆ deficiency leads to impairment of homocysteine catabolism and thus also causes hyperhomocysteinemia. However, the nature of hyperhomocysteinemia caused by vitamin B₆ deficiency differs from that caused by folate and vitamin B₁₂ deficiencies. In vitamin B₆ deficiency, fasting blood levels of homocysteine are usually not elevated or only slightly elevated. Only after a protein meal or after consumption of an oral methionine load (see the Section on Measurement of Blood Levels) does plasma homocysteine

become abnormally elevated in vitamin B₆-deficient patients. In contrast, plasma homocysteine levels tend to be elevated regardless of prandial state in patients with folate or vitamin B₁₂ deficiency. The basis for these different manifestations is likely due to differential effects of the vitamin deficiencies on intracellular SAM levels and consequent disruption of the allosteric control of homocysteine metabolism.

Recently, there has been a growing interest in the concept of nutritional genomics. This refers to genetic variability among individuals and its effect on nutritional requirements. A prime example of this concept is a common polymorphism in MTHFR (677C→T), in which an alanine is replaced by valine at codon 222 in the primary sequence of the enzyme. Individuals with the homozygous variant (677 TT) of this gene (10–15% of the general population, lower in blacks, higher in Latinos and in some parts of Europe, e.g., southern Italy) have an enzyme that is thermolabile, with reduced affinity for its substrate (methylenetetrahydrofolate) and its cofactor (FAD). Consequently, 677 TT individuals require a higher intake of folate and riboflavin to maintain optimal enzyme activity than those with the wild-type isoform, of the enzyme (677 CC). This is reflected by the fact that blood homocysteine levels are higher in people with the 677 TT isoform than in those with the 677 CC isoform, but only when overall folate and riboflavin status is low. When overall folate and riboflavin status is high, no difference in homocysteine levels is observed between the isoforms.

The clinical and public health importance of the MTHFR polymorphism is that women with the 677 TT isoform are at increased risk of having a child with a neural tube defect (e.g., *spina bifida* and anencephaly). This risk can be reduced by folic acid supplements, an observation that underlies the decision by the US and Canadian governments to mandate folic acid fortification of grain products in 1998. This program has been highly successful, having reduced the prevalence of folate deficiency from more than 20% to approximately 1%, the prevalence of hyperhomocysteinemia by approximately 50%, and the incidence of neural tube defects by at least 20%. The success of the folic acid fortification program in USA and Canada spawned similar programs in more than 50 countries and territories throughout the world. Notable exceptions are all the countries of the European Union, which have been slow to adopt this intervention strategy. This is due to concerns about the feasibility of fortification, a hesitancy to impose mandatory fortification on the population, and lingering concerns over masking vitamin B₁₂ deficiency and the possibility of other unrecognized health consequences associated with excess folic acid intake.

Other polymorphisms in MTHFR and other enzymes involved in homocysteine metabolism (e.g., methionine synthase, methionine synthase reductase (EC 1.16.1.8), cystathionine β -synthase) have been identified and their overall influence on homocysteine metabolism, B-vitamin requirements, and disease risk have been and continue to be evaluated.

Other Causes of Hyperhomocysteinemia

Other pathophysiological causes of hyperhomocysteinemia include renal dysfunction and hypothyroidism. The kidney is

a major site of homocysteine metabolism and renal disease leads to a significant reduction in the body's overall capacity to metabolize this amino acid. The resulting moderate to severe hyperhomocysteinemia can be attenuated, in part, by high-dose B-vitamin supplements, which putatively maximize the residual renal metabolism as well as the metabolic capacities of the extrarenal organs. Mild elevations in homocysteine occur in patients with hypothyroidism, which resolve to normal with thyroid replacement therapy. This observation implies that thyroxine and thyroid-stimulating hormone influence homocysteine metabolism directly, perhaps through up- or downregulation of key homocysteine-metabolizing enzymes. Alternatively, homocysteine may become elevated in hypothyroid patients secondary to mild impairment of renal function that may accompany the disorder.

Patients with diabetes (both insulin-dependent and insulin-independent) tend to have mild hyperhomocysteinemia. However, this seems to be confined to those patients whose diabetic condition has progressed to involve renal insufficiency. Interestingly, in the absence of renal involvement, homocysteine levels in diabetic patients tend to be lower than normal. Insulin has been shown to inhibit homocysteine catabolism through cystathionine synthesis. Therefore, reduced insulin levels in diabetic patients may actually promote homocysteine catabolism, thus leading to lower plasma levels.

Premenopausal women tend to have lower plasma homocysteine than men of similar age, and homocysteine levels tend to rise in women after menopause. Hormone replacement therapy reduces homocysteine back to premenopausal levels. Moreover, homocysteine decreases in male-to-female transsexuals, and increases in female-to-male transsexuals, effects that are primarily related to the estrogen and androgen regimens that such individuals respectively follow. Taken together, these observations strongly suggest an influence of sex hormones on homocysteine metabolism, although the mechanisms are not well understood.

Drugs can also affect homocysteine metabolism and lead to elevations of homocysteine in the blood. Certain anticancer drugs, such as methotrexate, and antiepilepsy medications, such as valproate and carbamazepine, are inhibitors of folate metabolism. The resulting functional folate deficiency leads to hyperhomocysteinemia. The anti-Parkinsonian drug, levodopa or L-dopa, causes elevations in blood homocysteine levels by a different mechanism: A significant proportion of an oral dose of L-dopa is methylated by SAM, leading to increased intracellular synthesis of SAH and homocysteine. The excess synthesis of homocysteine can overwhelm the capacities of the homocysteine metabolic pathways, particularly when B-vitamin status is suboptimal, leading to hyperhomocysteinemia.

Homocysteine and Vascular Disease

The continuing interest in homocysteine is primarily related to its recognized status as an independent risk factor for cardiovascular, cerebrovascular, and peripheral vascular disease. This homocysteine theory of vascular disease is directly descendent from a seminal observation made by Kilmer McCully. In the early to mid-1960s, it was recognized that a prominent characteristic of patients with homocystinuria caused by defects in

cystathionine β -synthase were very high elevations of both homocysteine and methionine in the blood. Therefore, it was not clear whether the vascular complications of this disorder were the consequence of hyperhomocysteinemia or hypermethioninemia. McCully observed that a patient with homocystinuria caused by a defect in a vitamin B₁₂-metabolizing enzyme had hyperhomocysteinemia, but not hypermethioninemia. Nonetheless, this patient had similar (although not identical) vascular pathology to that observed in patients with homocystinuria caused by cystathionine β -synthase deficiency. From this, McCully concluded that the vascular culprit was homocysteine, and not methionine.

McCully's hypothesis was not immediately embraced. The prevailing theory of atherosclerosis at that time centered on cholesterol, and it proved difficult for McCully to convince his peers and national funding agencies of the potential importance of this new and competing hypothesis. Contributing to this was the lack of a reproducible animal model of homocysteine-induced vascular disease and a sensitive method to measure homocysteine in the blood. Consequently, McCully's hypothesis went into temporary obscurity.

In the mid-1970s, David and Bridget Wilcken reinvigorated McCully's hypothesis with their observation that a subset of patients with premature coronary artery disease had reduced ability to metabolize homocysteine. Notably, this association was observed in individuals who did not have any of the severe genetic defects that underlie homocystinuria, suggesting that less severe or modest impairment of homocysteine metabolism may contribute to vascular disease risk. Subsequently, the advent of reliable, high-throughput assays for total plasma or serum homocysteine in the 1980s (see the Section on Measurement of Blood Levels) allowed for large-scale epidemiological assessment of associations between homocysteine and vascular diseases, both cross-sectionally and longitudinally. Through the 1990s, an explosion of population and case-control studies established that hyperhomocysteinemia is, indeed, a risk factor for heart attack, stroke, thrombosis, and peripheral atherosclerotic disease. Moreover, the risk associated with hyperhomocysteinemia is independent of other prominent risk factors, such as hypertension, hypercholesterolemia, hyperlipidemia, smoking, male gender, and others. Further indication of the importance of homocysteine with respect to vascular disease is the estimate that the relative risk of coronary artery disease associated with hyperhomocysteinemia is almost equivalent to that associated with hypercholesterolemia. As the evidence mounted, McCully was vindicated and his contribution became widely recognized.

Homocysteine, Cognitive Function, and Dementia

As the relationship between homocysteine and vascular disease became more and more apparent, researchers also addressed the hypothesis that hyperhomocysteinemia may affect cognitive function and the risk of dementia in older adults. This was primarily based not only on the recognized association between homocysteine and cerebrovascular disease, but also on the observation that homocysteine and its metabolite, homocysteic acid, can induce excitotoxicity in neurons.

Throughout the 1990s and into the new century, many cohort studies revealed significant inverse correlations between plasma homocysteine concentration and performance on a variety of cognitive function tests. Moreover, individuals with Alzheimer's disease were found to have higher plasma homocysteine than age- and gender-matched controls, whereas baseline homocysteine levels predicted the risk of incident dementia. Baseline homocysteine levels also predict the rate of brain atrophy in older adults with mild cognitive impairment, and lowering of homocysteine with B-vitamin supplements slows the rate of atrophy.

Homocysteine and Pregnancy Outcomes

Hyperhomocysteinemia has also been suspected as a risk factor for pregnancy complications and birth defects. Elevated plasma homocysteine concentrations have been associated with placental vasculopathy, preeclampsia, and placental infarction, as well as recurrent premature delivery, low-birth weight, and spontaneous abortion. Birth defects associated with hyperhomocysteinemia in the mother include neural tube defects, orofacial clefts, clubfoot, and Down's syndrome. The protective effect of folic acid supplementation and fortification against neural tube defects, and perhaps the other abnormal birth outcomes cited, may be related to reduced homocysteine levels.

Mechanisms

In parallel with epidemiological studies, a significant amount of basic research has focused on the mechanism(s) by which homocysteine may induce atherosclerosis and thrombosis. A definitive answer has proven elusive. Potential mechanisms with significant experimental support include, but are not limited to, the following: (1) modification of the endothelial cell surface, (2) modification of plasma proteins by formation of disulfides, (3) activation of platelets, (4) modification of monocyte functions, (5) increased expression or activity of vascular adhesion molecules, and (6) oxidative damage induced by peroxides formed during disulfide bond formation.

A sixth potential mechanism relates to a known quirk of homocysteine synthesis and metabolism. The equilibrium of the interconversion between SAH and homocysteine (catalyzed by SAH hydrolase, EC 3.3.1.1) actually favors SAH synthesis (Figure 1). *In vivo*, this reaction proceeds toward homocysteine synthesis because of product removal, i.e., the efficient metabolism of homocysteine back to methionine or through cystathionine synthesis. However, when there is a block in homocysteine metabolism, as when it occurs in genetic defects, B-vitamin deficiencies, and other causes delineated previously (see the Section on Hyperhomocysteinemia above), homocysteine accumulates intracellularly. Consequently, SAH also accumulates within cells. The significance of this phenomenon is that SAH is a feedback inhibitor of all SAM-dependent methylation reactions. Therefore, hyperhomocysteinemia may cause or contribute to vascular disease through SAH-mediated inhibition of methylation.

Another area that has received attention is the relationship between homocysteine, nitric oxide, and endothelial function. One of the roles of nitric oxide is as a vasodilator. Homocysteine has been shown to be an inhibitor of nitric oxide synthesis and, therefore, can inhibit vasodilatation. This has led to the hypothesis that hyperhomocysteinemia, by inhibiting nitric oxide synthesis, impairs the ability of the vascular endothelium to maintain homeostasis of vascular tone. This in turn may directly or indirectly increase susceptibility to vascular insults, thereby promoting atherosclerosis and thrombosis.

The search for the definitive pathogenetic mechanism implicating homocysteine as a cause of vascular disease continues, and it is recognized that several mechanisms may contribute synergistically. However, some have questioned whether homocysteine is a cause of vascular disease or simply a consequence.

Cause or Effect?

Although there is considerable evidence, both epidemiological and experimental, that homocysteine is a causative factor in vascular disease, there are data that contradict this conclusion. First, although cross-sectional and case-control studies fairly consistently demonstrate that hyperhomocysteinemia is associated with vascular disease, some prospective studies have found no relationship between baseline homocysteine levels and risk of incident vascular events. Second, several studies have found no relationship between the MTHFR 667C→T polymorphism and venous thrombosis, despite the association of this polymorphism with elevated plasma homocysteine levels.

With these observations in mind, a plausible alternative hypothesis has been put forward to explain the association between hyperhomocysteinemia and vascular disease. One of the organs that can be significantly affected by vascular disease is the kidney. Reduced kidney function caused by atherosclerosis may lead to renal insufficiency and reduced capacity to metabolize homocysteine. In this way, hyperhomocysteinemia may actually result from vascular disease. This hypothesis remains to be tested. The possibility of a vicious cycle, i.e., one in which vascular disease causes homocysteine to become elevated in the blood, which in turn induces further vascular damage, must also be considered.

B-Vitamin Supplementation

In the late 1990s, several large-scale intervention trials were initiated to determine if folic acid supplements with or without vitamin B₁₂ and vitamin B₆, which effectively lower blood homocysteine levels, reduce vascular outcomes and mortality in individuals at increased risk of cardiovascular disease (Table 3). A recent meta-analysis has evaluated the results of these studies. Overall, folic acid with or without vitamin B₁₂ or vitamin B₆ was very effective in lowering homocysteine levels (an average of 25% across all the studies). However, over a median follow-up period of 5 years, no significant reductions in major vascular events, major coronary events, stroke, or vascular mortality were observed. These findings suggest that lowering homocysteine

Table 3 Intervention trials to determine the effect of B-vitamin supplements on homocysteine and the risk of vascular events and mortality

<i>Study</i>	<i>Location</i>	<i>Trial Period</i>	<i>Intervention (in mg)</i>	<i>Outcomes</i>
Cambridge Heart Antioxidant Study 2 (CHAOS-2)	UK	1998–2002	FA (5.0)	Hcy reduced 11%; no effect on major vascular events
Heart Outcomes Prevention Evaluation 2 (HOPE-2)	Canada	2000–06	FA (2.5) B ₁₂ (1.0) B ₆ (50)	Hcy reduced 24%; no effect on death from cardiovascular events, MI, or stroke
Homocysteinemia in Kidney and End Stage Renal Disease (HOST)	USA	2000–07	FA (40) B ₁₂ (2) B ₆ (100)	Hcy reduced 25%; no effect on all cause mortality, MI, or stroke
Norwegian Multi-Center B-Vitamin Intervention Study (NORVIT)	Norway	1999–2006	FA (0.8) B ₁₂ (0.4) B ₆ (40)	Hcy reduced 28%; no effect on MI, stroke, or sudden death due to coronary artery disease
Study of Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH)	UK	1999–2008	FA (2.0) B ₁₂ (1.0)	Hcy reduced 27%; no effect on major coronary events, stroke, noncoronary revascularization, or death due to vascular disease
Vitamins in Stroke Prevention (VISP)	USA	1996–2003	FA (2.5) B ₁₂ (0.4) B ₆ (25)	Hcy reduced 17%; no effect on stroke
Western Norway B-Vitamin Intervention Trial (WENBIT)	Norway	1999–2006	FA (0.8) B ₁₂ (0.4) B ₆ (40)	Hcy reduced 26%; no effect on MI, stroke, or hospitalization for <i>angina pectoris</i>
Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS)	USA	1998–2006	FA (2.5) B ₁₂ (1.0) B ₆ (50)	Hcy reduced 18%; no effect on MI, stroke, coronary revascularization, or cardiovascular mortality
B-Vitamin Treatment Trialists' Collaboration – Meta-analysis	Multiple	–	–	Hcy reduced 25% (mean of all studies); no overall effect on major vascular events, including cardiovascular events, stroke, and revascularization

Abbreviations: FA, folic acid; B₁₂, vitamin B₁₂; B₆, vitamin B₆; Hcy, homocysteine; MI, myocardial infarction.

levels with B vitamins is not an effective strategy for preventing major vascular outcomes. However, it should be noted that the participants in these studies, by design, had preexisting vascular disease, including coronary heart disease, cardiovascular disease, stroke, or renal disease. It is possible that after clinically evident vascular disease is established, the pathogenetic process may be too advanced for B-vitamin supplements to be effective in reducing vascular events. It remains to be determined if B-vitamin intervention at earlier stages in the vascular disease process can provide protection. The uncertain relationship between hyperhomocysteinemia, B vitamins, and vascular disease is summarized in [Figure 2](#). If homocysteine is not a vascular toxin, it may still serve as a marker of both vascular disease and as an indicator of the efficacy of B-vitamin supplementation.

Measurement of Blood Levels

A variety of assays have been developed to quantify blood homocysteine levels, with those employing high-pressure liquid chromatography perhaps the most common. These assays have proven to be relatively accurate and precise (coefficients of variation less than 10%) and are relatively simple and quick to perform. The development of such assays in the 1980s was the technological breakthrough that spurred the exponential increase in homocysteine-related research over the past 20–25 years and the establishment of hyperhomocysteinemia as an independent risk factor for vascular disease.

As described above (in the Section Structure and Forms), homocysteine comes in several forms in the blood, including

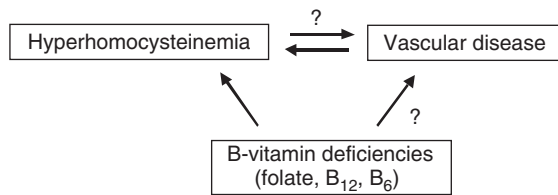


Figure 2 Hyperhomocysteinemia, B vitamins, and vascular disease. There is still some question whether elevated plasma homocysteine is a cause or consequence of vascular disease and whether there are influences of B vitamins on vascular disease risk that are independent of homocysteine.

protein-bound form, mixed disulfides, homocystine, and free-reduced form. Assays for homocysteine usually measure the sum total of all these forms, i.e., total homocysteine. To accomplish this, the first procedure in homocysteine assays is a reduction step to break all disulfide bonds, thus converting all homocysteine to the free-reduced form. The free-reduced form is then quantified by one of the various methods.

Blood sample collection and processing are critical factors in the determination of homocysteine concentrations. Typically, blood samples for homocysteine analysis are collected in tubes containing an anticoagulant (e.g., ethylenediaminetetraacetic acid and heparin). Prompt separation of plasma from the blood cells after centrifugation is required to avoid excess release of intracellular homocysteine into the plasma or removal of homocysteine from the plasma by metabolically active leukocytes after blood withdrawal. Keeping the blood sample cold until centrifugation and separation (ideally within 4 h of blood withdrawal) minimizes this problem. Serum homocysteine concentrations typically exceed plasma concentrations by 20%. This is likely due to the fact that blood collected to isolate serum (i.e., without an anticoagulant) must clot at room temperature for 30–60 min before centrifugation and separation. Therefore, plasma is preferred for measurement of homocysteine. Once separated from the blood cells, the concentration of homocysteine in plasma or serum remains stable for years when stored frozen.

Another important issue in the measurement of homocysteine is the prandial state of the individual. For individuals with adequate B-vitamin status, no genetic abnormalities, and no pathophysiological conditions that affect homocysteine metabolism, plasma homocysteine levels after an overnight fast are similar to levels after a meal (even high-protein meals containing methionine). However, for individuals with low vitamin B₆ status or heterozygous genetic defects in cystathionine β -synthase, postprandial homocysteine levels can be significantly higher than fasting levels. Because of the nutritional or genetic block in the conversion of homocysteine to cystathionine, there is decreased capacity to metabolize the influx of homocysteine synthesized from dietary methionine. This is the basis for the methionine load test for detection of impaired cystathionine β -synthase activity. In this test, baseline blood is drawn after an overnight fast, and then again 4 h

after consumption of a large dose of methionine dissolved in orange juice (100 mg methionine per kilogram body weight). Plasma homocysteine increases to a greater extent in individuals with low vitamin B₆ status or heterozygous genetic defects in cystathionine β -synthase than in individuals without these problems. Importantly, individuals with elevated fasting homocysteine and those with normal fasting levels, but elevated post-methionine load levels, are both at increased risk of vascular disease.

See also: Amino Acids: Chemistry and Classification; Metabolism; Specific Functions. Folic Acid. Riboflavin. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements. Vitamin B₆: Physiology

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Glossary

Hunger The drive to consume, a subjective experience that stimulates and sustains a behavioral event, eating. It has a strong learnt component and can be triggered by physiological need or environmental stimuli. Hunger usually predicts subsequent eating behavior, and is suppressed by that ingestion, an effect that depends on the sensory, cognitive, gastric, gastrointestinal, and metabolic consequences of ingestion and learnt associations with specific foods.

Power of food The appetite for desirable, palatable foods induced by the availability of food, the presence of food, or the taste of food.

Satiation The within-meal processes that bring eating episodes to an end, predominantly driven by cognitive factors and learnt associations, the physical and sensory characteristics of the meal, the bulk of that meal in the stomach, and the rate of gastric emptying.

Satiety The suppression of hunger and eating behavior after a meal. Often referred to as a state sustained by numerous postingestive and postabsorptive mechanisms, triggered as food is digested and nutrients are absorbed and metabolized.

Definition

The term 'hunger' is used in more than one sense by both scientists and the public. One traditional use refers to insufficient food availability, as it may occur in poor regions of the world. Most experts, however, prefer to use the term 'food insecurity' to refer to situations of food scarcity. In the context of this article, hunger describes the drive or the motivational force that urges us to seek and consume food. It is the expression of a biological need to sustain growth and life. Hunger is therefore a purposeful experience that possesses a clear biological function. Therefore, in traditional motivation theory, hunger is a biological drive to satisfy a physical need. In true homeostatic fashion, the drive to consume reduces and that need is satisfied (sated).

There are two components to hunger within nutritional science. Hunger is inferred from directly observable and measurable events. In this way, by inferring increased or high levels of hunger from a long period of food deprivation or an increased willingness to expend effort in order to obtain food, hunger becomes a mediating concept or an intervening variable. However, linked to these directly observable behavioral events are a collection of conscious feelings or sensations that are linked to a desire to obtain and eat food. This is the sense in which lay people understand the term hunger. It is through these objectively observable behavioral events and the vigorous measurement of these subjective feelings, by means of rating scales and other measurement devices, that researchers attempt to capture changes in hunger motivation.

Early investigators used questionnaires in which people were asked to note the presence of physical sensations in a number of bodily areas, together with moods, urges to eat,

and preoccupation with thoughts of food and of the everyday experience of hunger. It was found that the observation, 'I feel hungry,' was typically based on the perception of bodily feelings, which at times are very strong. Gastric sensations, a hollow feeling or stomach rumbling, are frequent indicators of hunger, although people also report sensations in the mouth, throat, and head. These accompany more diffuse feelings of restlessness and excitability as well as an urge to eat. The consumption of food changes both the pattern of physical sensations and the accompanying emotional feelings, with unpleasant and aversive sensations becoming replaced by more pleasant ones. Thus, as classic drive reduction theory would suggest, hunger is associated with unpleasant feelings, and food consumption eases these. Thus, for example, an aching stomach becomes relaxed and the feeling of excitement and irritability is replaced by one of contentment.

Despite this commonality, subsequent study has shown a great deal of variability both within and between individuals. In other words, hunger demands neither the consistent presence of sensations before every act of eating nor that these sensations occur in every person sitting down to eat. However, even with this variability, persons are able to, and frequently do, make judgments regarding their state of hunger, partly through reference to these sensations.

The Measurement of Hunger

The process of measuring hunger is not straightforward. There is an inherent mistrust of subjective reports of appetite. Critics point to the variability in response between individuals and the absence of any objective 'standard' by which internal

experience can be calibrated. This has driven many to use biological proxies (the release or inhibition of particular hormones or activation of certain brain areas) as 'biomarkers' for appetite in the hope that these may be more reliable indicators of behavior. However, no single biomarker has yet been shown to be reliably predictive of subsequent consumption, and issues of validity of biomarkers remain. Hunger must be seen in a wider individual and situation context and cannot rest in the fluctuations of any single biological system. The issues of 'validity' and 'reliability' are more complex than criticism suggests.

The two most common methods for quantifying hunger are fixed-point rating scales and visual analog scales. Fixed-point scales are quick and simple to use, and the data they provide are easy to analyze. Past examples of these scales show that they vary considerably in complexity. In considering the appropriate number of points to be included in this type of scale, the freedom to make a range of possible responses must be balanced against the precision and reliability of the device. Research seems to indicate that scales with an insufficient number of fixed points can be insensitive to subtle changes in subjective experience. In addition, the fixed points are important determinants of the way in which people use the scales and distribute their ratings. One way of overcoming some of these failings is to abolish such points. Thus, visual analog scales are horizontal lines; these lines should be at least 100 mm long (or longer to allow the freedom to make a range of responses), unbroken and unmarked, except for word anchors at each end. The user of the scale is instructed to mark the line at the point that most accurately reflects the intensity of the subjective feeling at that time. The researcher measures the distance to that mark in millimeters from the negative end (no hunger), thus yielding a score of 0–100 (in the case of 100 mm lines). This is done either by hand or automatically if presented by a computer screen (the latter method ensures that they are completed at designated times during a study day). By doing away with all of the verbal labels except the end definitions, visual analog scales retain the advantages of fixed-point scales, while avoiding many of the problems with uneven response distributions.

An important aspect of these methods concerns the interpretation of differences between the fixed points or intervals on a visual analog scale. Thus, for example, it cannot be assumed that the difference between 20 and 30 mm on a hunger scale is perceptually the same as the difference between 80 and 90 mm. Nor can a hunger rating of 80 mm be said to represent a feeling of hunger that is twice the intensity of that rated at 40 mm. The variation of scores on these scales represents the fluctuations in the perception of feeling and not absolute change of some physical commodity. Individuals will use these scales idiosyncratically (each individual may interpret the scale and gauge their response differently) but generally consistently on repeated occasions (they will tend to scale any changes in response within their own parameters, producing a reliable record of alterations in an individual's appetite). One example of this is the problem of 'end effects.' This refers to the reluctance of a minority of subjects to make ratings away from the upper or the lower end points of the scale, despite clear instructions. Despite these limitations, data from such scales are often analyzed using parametric statistical procedures,

such as analysis of variance, and in general, this appears to be a satisfactory approach.

Hunger and Satiety

If hunger is that feeling that drives us to seek food and to consume, then eating eventually relieves hunger, albeit until the next snack or meal. The capacity of a food to reduce the experience of hunger can be termed 'satiating power' or 'satiating efficiency.' This power is the product of the body's handling of the nutritional composition and structure of the food eaten. It follows that some foods will have a greater capacity to maintain suppression over hunger than other foods. This will depend on their sensory impact, physical characteristics, energy density, and macronutrient composition.

The distinction between hunger and satiety is both conceptual and technical. As hunger diminishes, satiety rises. However, it is useful to further separate those events that occur across the course of a meal (prandial) from those between meals (pre- and postprandial). In this way, the prandial process of satiation can be clearly distinguished from the postprandial state of satiety. Satiation can be regarded as the process that develops during eating and that eventually brings a period of eating to an end. Accordingly, satiation can be defined in terms of the measured size of an eating episode (such as its energy, weight, or volume). Hunger declines as satiation develops, and usually reaches its lowest point at the end of a meal. Satiety is defined as the state of inhibition over further eating that follows at the end of a meal and that arises from the consequences of food ingestion. The intensity of satiety can be measured by the duration of time until eating starts once more or by the amount consumed at the next meal. The strength of satiety is also measured by the time that hunger is suppressed, but as satiety ebbs, hunger is restored.

In examining the mechanisms responsible for suppressing hunger, it is clear that they range from those that occur when food is initially sensed to the effects of metabolites on body tissues following the digestion and absorption of food (across the wall of the intestine and into the bloodstream). By definition, satiety is not an instantaneous event but occurs over a considerable time period. Sensory effects are generated through the smell, taste, temperature, and texture of food, and it is likely that these factors have effects on eating in the very short term. Cognitive influences represent the beliefs held about the properties of foods, and these factors may also help inhibit hunger in the short term. These will start to affect hunger even before consumption has commenced and continue to suppress it over the course of the meal.

Postingestive processes such as gastric distension and rate of emptying, the release of hormones such as cholecystokinin, and the stimulation of certain receptors along the gastrointestinal tract increasingly suppress hunger and, as the meal terminates, further inhibit its rebound during early postmeal satiety. The postabsorptive phase of satiety comprises those mechanisms arising from the action of metabolites after absorption into the bloodstream. These include the action of glucose and amino acids, which act directly on the brain after crossing the blood-brain barrier and which influence the

brain indirectly via neural inputs following stimulation of peripheral chemoreceptors. These will continue to suppress hunger late into the postprandial period.

Hunger: Physiological Determinants

According to Rogers, even before food reaches the mouth, potent physiological signals are generated by the mere sight or smell of food, or even learned contextual cues such as location and time of day. These signals, produced in response to exposure to external stimuli, comprise the 'cephalic phase' of appetite. This cephalic response is expressed in several parts of the gastrointestinal tract and acts to anticipate food ingestion. Later, stomach distension and the detection of macronutrients such as fat or protein within the gut are all powerful satiety cues. They bring a meal to an end and, for a time, inhibit further consumption. Eventually, hunger again prevails and food intake follows. The flux between hunger and satiety is episodic and underpins the expression of our eating behavior throughout the day. However, it is not just the absence of episodic satiety cues (e.g., stomach distension and intestinal or absorbed nutrients) that influences the expression of hunger. Reduction in blood glucose levels or in the levels of the circulating adipose tissue hormone leptin indicates a deficit in available energy and in energy reserves. Such events are linked with feelings of hunger. Fluctuation of these factors indicates the metabolism and storage of the body's energy reserves. These are a tonic class of physiological signals that also influence the expression of appetite. Like episodic satiety signals, these tonic signals normally act on inhibitory mechanisms with the hypothalamus (anorexigenic circuits). Their absence elicits an active feeding response. Other tonic factors that indicate the body's energy status, such as adiponectin, cytokines, and gonadal hormones, also appear to act on energy regulator centers within the brain, particularly the hypothalamus, mainly to suppress hunger. Again, their absence serves to unleash appetite.

However, not all physiological signals, episodic or tonic, inhibit hunger. For instance, blood levels of the recently discovered gut hormone ghrelin have been shown to increase before a meal. Subsequent intake has been shown to suppress ghrelin release. Further studies have shown that ghrelin infusions increase food intake. Thus, this is a hormone that acts to promote food intake. Interestingly, ghrelin receptors are found in various hypothalamic locations that form part of the orexigenic circuits promoting food intake. These circuits contain many neuropeptides, such as neuropeptide Y, orexins, melanocortin concentrating hormone, and galanin, which all stimulate food intake. The precise nature of the physiological and neurobiological regulation of appetite is discussed elsewhere in this encyclopedia. Finally, it should be noted that the biological mechanisms critical to the expression of hunger are not independent of psychological ones. Indeed, the sensory and cognitive cues that stimulate hunger produce physiological changes that anticipate the ingestion and metabolism of energy and subsequently aid these processes. This gives rise to the psychological factors critical in the expression of appetite.

Learning and Hunger

As the classical work of Pavlov demonstrates, hunger responses can be easily entrained to specific stimuli. Omnivores, like carnivores, are meal eaters (in contrast to grazers); however, their dietary decisions are more complex. One of the essentials for an omnivore faced with a variety of new and different foods is the capacity to learn. It is not possible for an inborn preference or aversion to guide the choice of every possible food. Therefore, we learn which foods are beneficial (and which are not) by eating them. If the consequence of consumption is normal satiety, the pleasant postingestive signals this generates serve as a positive experience that can lead to learned preferences, as studied by Birch. Alternatively, if consumption leads to an association between that food and negative gastrointestinal consequences such as nausea, then a conditioned aversion to that food (or even other similar tastes and flavors) is likely to develop. The learning process involves the association between the sensory and the postabsorptive characteristics of foods. In this way, the sensory characteristics of foods act as cues and come to predict the impact that foods will later have. Consequently, these cues should suppress hunger according to their relationship with subsequent physiological events. It is possible to demonstrate experimentally how human beings adapt their eating to a food's energy content. A distinctively flavored food that contains 'extra' hidden energy, presented on several occasions, will result in a change in eating and in preference. When deprived of food, subjects' preference for the taste increases with gained experience. If presented when satiated, preference for the taste decreases. This process is also observable in young children, who eat smaller meals following a taste previously associated with a high-energy snack, and larger meals following a taste previously associated with a low-energy snack. Our hunger can thereby be potentially reduced by the learned associations of satiety.

In environments where food sources remain limited and dietary variety is controlled, learned responses may effectively control subjective feelings of appetite. However, when there is distortion, variation, or extreme complexity in the relationship between sensory characteristics and nutritional properties, then the conditioned control of hunger is weakened or lost. In such a scenario, hunger may become less controllable. In many respects, the variety of foods available to us represents a cacophony of different sensory characteristics and has the added complication of ingredients that preserve the sensory qualities while altering their nutritive value. Learned hunger therefore is a relatively less important factor when the food supply contains many food items with identical tastes but differing metabolic properties.

Hunger and Eating Behavior

If hunger is biologically useful and a subjective experience that indicates a depleted nutritional state, then a close correspondence between hunger and eating would be expected. Hunger should be either a necessary or a sufficient condition for eating to occur. However, this is not invariably the case. Instances of people deliberately refraining from eating in spite

of hunger (fasting for moral or political conviction) show hunger not to be a sufficient condition. People can, and regularly do, resist the drive to consume. Examples, from both research and daily experience, of eating a tempting food when otherwise satiated show hunger not to be necessary for eating to take place. In the laboratory, merely changing the food on offer, or increasing its palatability, can encourage eating beyond the limits of normal satiety. Many of us have experienced festive overconsumption or had our interest tweaked by the dessert trolley. However, although the relationship between hunger and eating is not based on biological inevitability, they are not entirely uncoupled and under many circumstances they remain closely linked.

The lack of a one-to-one correspondence between hunger and eating is repeatedly used to question the validity of hunger ratings. But should a high correlation between hunger ratings and subsequent food intake be expected in all circumstances? The previous examples show that in certain circumstances the two can be disengaged. Thus, for example, eating can occur when hunger is low (such as when highly palatable food is offered unexpectedly) and not at other times when hunger is high (when food is unavailable or other activities have priority). The proximity to food cues can have dramatic, instantaneous effects on appetite expression. For some individuals, this responsiveness to food cues overwhelms normal appetite control and the normal rhythm of hunger. For others, eating in the 'absence of hunger' is a component of their natural eating repertoire. It is clear that hunger ratings cannot be used simply as a proxy measure for food intake. Equally, when such factors are taken into account, there is good evidence that self-report ratings of hunger correlate statistically and meaningfully with eating.

In questioning the relationship between hunger and eating, we are also forced to place the action of hunger within a broader context of social and psychological variables that moderate food choice and eating behavior. Eating patterns are maintained by enduring habits, attitudes, and opinions about the value and suitability of foods, and an overall liking for them. These factors, derived from the cultural ethos, largely determine the range of foods that will be consumed and sometimes the timing of consumption. The intensity of hunger experienced may also be determined, in part, by the culturally approved appropriateness of this feeling and by the host of preconceptions brought to the dining table. Fluxes in hunger over time are therefore only one portion of the range of determinants of eating in any given situation.

Disorders of Hunger

The clinical eating disorders anorexia nervosa and bulimia nervosa are commonly believed to encompass major disturbances of hunger. Yet the role that hunger may play is not entirely clear. Contrary to the literal meaning of the term, 'anorexia' is not experienced as a loss of appetite. Rather, clinicians recognize that anorexics may endure intense periods of hunger during their self-restricted eating. For some, their strength in resisting intense episodes of hunger provides a feeling of self-mastery and control that is absent in

other areas of their lives. Research suggests that restricting anorexics (compared with those who binge) have the greatest blunting of hunger response, and that this disturbance in hunger is not a product of other areas of perceptual confusion.

There is evidence that under conditions of total starvation, hunger may become temporarily diminished. This circumstance is extremely rare and obviously relatively brief. Once eating is recommenced, hunger returns rapidly and with extreme intensity. The accounts of the male volunteers who submitted to a 6-month period of semistarvation during World War II (the 'Minnesota Experiment') are a testament to the extreme power of hunger. Referred to as semistarvation neurosis, these men's activities were shaped by their need for food, and their hunger experience was extreme. Nearly two-thirds reported feeling hungry all the time and a similar proportion experienced physical discomfort due to hunger. Participants described a marked increase in what was referred to as 'hunger pain.' For some, this was mildly discomforting and vaguely localized in the abdomen. For others, it was extremely painful. This account is especially useful in reminding us why energy-reduced diets aimed at achieving weight loss are often difficult to maintain and easy to abandon.

Like anorexia, bulimia finds its literal meaning in changed hunger—'ox hunger.' Again, however, the term is imprecise. Close analysis of the precursors of binge episodes shows hunger to be lower than it is before a normal meal. In addition, although the urge to eat may be strong during a binge, the large amount of food consumed implies some defect in satiation rather than in hunger. Moreover, binging is often a well-practiced behavior that develops and changes with time. As with anorexics, it is likely that a stable eating pattern is necessary in order to normalize the experience of hunger, a process that may take a long time to establish.

The question of whether obesity reflects a disorder of hunger is now regarded as largely redundant. Obesity is strictly a disorder of energy balance and partitioning. There is hardly any evidence of heightened levels of hunger contributing to excessive energy input. However, an exception to this is the rare disorder Prader-Willi syndrome. Genetically determined and characterized mainly by intellectual disability, obesity is a well-recognized feature of the syndrome. Emerging research suggests that the excessive levels of food intake are associated with both a delayed reduction in hunger while eating and a more rapid return to premeal states when eating has finished. Clearly, a better understanding of the biological events that accompany such aberrant eating patterns will strengthen understanding of the psychobiological framework that supports hunger.

See also: Appetite: Physiological and Neurobiological Aspects; Psychobiological and Behavioral Aspects. Carbohydrates: Requirements and Dietary Importance. Eating Disorders: Anorexia Nervosa; Bulimia Nervosa. Famine: Causes, Consequences, and Responses. Starvation and Fasting: Biochemical Aspects. Weight Management: Approaches

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HYPERACTIVITY

Nutritional Aspects

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Glossary

Attention deficit hyperactivity disorder (ADHD) A neurobehavioral health condition with a combination of core symptoms of inattention, hyperactivity, and impulsivity that occur to a degree inconsistent with a person's developmental age, are associated with impairment in more than one setting, and are not the result of another mental disorder or medical condition.

Behavior modification Clinical behavior therapy or contingency management programs grounded in learning theory and including principles of classical conditioning, operant conditioning, cognitive-behavioral theory, and social learning theory.

Fatty acids (FAs) The mammalian brain is particularly rich in long-chain polyunsaturated fatty acids (LC-PUFAs) from omega-3 (n-3) and omega-6 (n-6) families, particularly docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (AA, 20:4n-6). These FAs are synthesized by sequential desaturation and elongation of their respective precursors, α -linolenic acid (α -LNA, 18:3n-3) and

linoleic acid (LA, 18:2n-6), known as essential fatty acids (EFAs) because they cannot be synthesized by mammals and are therefore exclusively provided by the diet.

Feingold diet Dr. Benjamin Feingold proposed that at least 50% of children with hyperactivity and learning disabilities improved when placed on diets, which were salicylate, preservative, and additive free.

Oligoallergenic/few foods diet A theory that suggests that some children have atypical allergic reactions, consisting of subtle and behavioral effects to various foods. Treatments have entailed placing a child on a restricted diet and then adding foods one at a time to determine which foods might be related to certain behaviors.

Stimulant medications Medications that act as dopamine and norepinephrine reuptake inhibitors, increasing norepinephrine and dopamine activity primarily in the caudate nucleus and prefrontal cortex. Classes of stimulants most commonly used for ADHD include methylphenidate, dextroamphetamine, and mixed salts of amphetamine.

Introduction

To discuss the issues of hyperactivity and diet, it is first important to understand the issues related to the diagnosis of hyperactivity or what is now called the Attention Deficit Hyperactivity Disorder (ADHD). Because most of the recommendations for dietary changes have been for children who have been diagnosed with ADHD, this article will first review the historic and current changes in the diagnosis of ADHD and then review the diets and supplements that have been recommended for treatment and the evidence as to their efficacy.

Diagnostic Issues

Hyperactivity or, as it is now known, ADHD, is a condition that has been recognized for many years. ADHD has been quite extensively researched, and three aspects have changed relatively little over time: (1) the diagnosis, (2) efficacious treatments, and (3) the controversy surrounding the condition

and its treatment. Its symptoms were first described by a German physician, Heinrich Hoffman, in a children's book he wrote in 1848. The symptoms were represented by two children, Harry who looks in the air (inattention) and Fidgety Phil (hyperactivity). In 1902, George Still presented a lecture in England about 20 children who were aggressive, defiant, excessively emotional, and lacking inhibitory volition who were also noted to have impaired attention and overactivity. A more etiological conceptualization of the condition did not occur until after World War I.

Symptoms of hyperactivity and inattention were suspected to be caused by the influenza epidemic occurring after World War I when post encephalitic behavioral manifestations in children included extreme examples of hyperactivity and inattention, resulting in the concept that these symptoms were due to organic brain damage. The concept of inattention and hyperactivity being part of a spectrum with less intense manifestations secondary to subtle injuries became known as the syndrome of Minimal Brain Damage in the 1960s. However, the lack of clear evidence for brain damage eventually resulted in a shift to a more descriptive labeling of the

disorder. This was reflected in the 1970s when the United States psychiatric classification system (Diagnostic Statistical Manual of Mental Disorders, or DSM) defined the Hyperkinetic Reaction of Childhood. The same disorder was similarly described in Britain as reflected in the World Health Organization (WHO) classification. However, the conditions described differed in that the British disorder included more severe symptomatology requiring that the symptoms be present in all settings.

In 1980, the USA characterization of inattention and hyperactivity was changed in several ways. It was conceptually defined by three symptom dimensions – inattention, impulsivity, and hyperactivity – with inattention playing a more prominent role. In addition, to address the heterogeneity within the disorder, two subtypes (Attention Deficit Disorder with and Attention Deficit Disorder without Hyperactivity) were defined. Again, different from the British criteria, the symptoms were only required to be present in one setting such as school. Keeping to the concept that the major contribution to the symptoms were related to innate characteristics in the child rather than to environmental influences, the symptoms were required to have been present before the age of 7 years and to have lasted for at least six months. The British system continued to use the title Hyperkinetic Syndrome of Childhood and to include the pervasive nature of the symptoms.

The most recent changes in criteria for DSM-IV, Text Revision, and WHO have moved the definitions closer to agreement. Considering the most recent studies, there is evidence to support two dimensions. In DSM-IV, TR, the first dimension, inattention, is characterized by the ‘often’ occurrence of at least six of nine of the inattentive behaviors presented in [Table 1](#). The second dimension consists of both hyperactivity and impulsivity and is characterized by the ‘often’ occurrence of at least six of nine of the hyperactive or impulsive behaviors presented in [Table 1](#). The WHO definitions

are similar but do not attempt to quantify the specific behaviors and do not include impulsivity in the hyperactivity dimension ([Table 1](#)).

In DSM, the two dimensions define three subtypes. These are: predominantly inattentive type (meeting criteria on the inattentive dimension only), predominantly hyperactive/impulsive type (meeting criteria on the hyperactive/impulsive dimension only), and combined type (meeting criteria on both dimensions). In addition, there are other general criteria including the onset of symptoms before seven years of age, the presence of symptoms for at least six months, the presence of symptoms in two or more settings (e.g., home, school, or work) causing some impairment, and evidence that the symptoms cause significant clinical impairment in social, academic, or occupational functioning. The WHO condition has been renamed Disturbances of Activity and Attention. The DSM system is currently undergoing revision to DSM-V but the changes probably will not occur until 2013.

Treatments Other than Diet

In considering dietary interventions, it is first important to identify the known efficacious treatments for individuals with ADHD, including medications and behavior modification, because considerations about dietary interventions must be viewed within the context of these other interventions. Both efficacious treatments provide symptomatic relief, but do not cure the condition, and a positive or negative response to treatment is itself not diagnostic for the condition. Stimulants, the primary efficacious medications, have been particularly popular in the USA because they are safe, effective, and low cost. In addition three nonstimulant medications have more recently also demonstrated efficacy. Up until the mid 1990s, the stimulant medications consisted of immediate-release preparations, including racemic methylphenidate (Ritalin), dexamethylphenidate (Focalin), dextroamphetamine (Dexedrine), mixed salts of amphetamine (Adderall), and pemoline (Cylert), now off the market. In the last 15 years, several different longer-acting stimulant preparations have been approved in a number of countries, including 8 h preparations of methylphenidate (Ritalin LA, Metadate CD, and Focalin XR), 12 h preparations of methylphenidate (Concerta and Daytrana patch), and 10–12 h preparations of amphetamines (Adderall XR and Vyvanse). Additionally, nonstimulant medications including atomoxetine (Strattera), a selective norepinephrine uptake inhibitor, and extended-release guanfacine (Intuniv) and extended-release clonidine (Kapvay), centrally acting alpha-2 antagonists are now available. A review of over 3000 studies has shown that stimulants improve the core behaviors of inattention, impulsivity, and hyperactivity for the duration of action of the medication as well as provide temporary improvement of associated features including aggression, social interaction, and academic productivity. The margin of safety is very high, and the side effects on appetite, sleep, and, infrequently, tics or bizarre behavior are all reversible when the medication is stopped. In some children growth can be slowed initially for the first several years. Although often abused by adults, the stimulants are rarely abused by children because they usually do not find taking the

Table 1 DSM-IV Behaviors for ADHD

Inattention

- careless mistakes
- difficulty sustaining attention
- seems not to listen
- fails to finish tasks
- difficulty organizing
- avoids tasks requiring sustained attention
- loses things
- easily distracted
- forgetful

Hyperactivity

- fidgeting
- unable to stay seated
- moving excessively (restless)
- difficulty engaging in leisure activities quietly
- “on the go”
- talking excessively

Impulsivity

- blurting answers before questions completed
- difficulty awaiting turn
- interrupting/intruding on others

medication pleasurable. Nonstimulant medications do not have abuse potential.

Effective behavioral interventions consist of direct contingency, a combination of strategies, including behavioral parent training, classroom management programs for teachers, and peer-relationship interventions for children. A recent large meta-analysis by Fabiano found statistically significant improvement with behavior modification programs with overall moderate to large effect sizes. Like medication, these interventions are not specific to ADHD and have no proven long term benefit when used in isolation. Other approaches such as traditional psychotherapy and play therapy have not been found to be effective in ADHD. The evidence that the use of medication or behavioral interventions alone has long term benefits has not been documented, and it remains unclear whether the combination is beneficial in the long term. These findings are not surprising in that the benefits of either therapy are dependent on continuing the treatments, and maintaining either medication or behavioral therapy for extended periods of time can be difficult.

Dietary Interventions

The concept that specific dietary components may adversely or positively affect behavior have been based on two premises, restricting offending agents or supplementing deficiencies. These are presented in **Table 2**.

The idea that food might have an adverse effect on child behavior was first raised formally in 1922 by Shannon. This concept was further elaborated in 1947 by Randolph in his description of the Tension Fatigue Syndrome, a behavioral extension of the vomiting reaction to milk proteins, and was also promoted by Speer. Their theory suggested that some children have atypical allergic reactions, consisting of subtle and behavioral effects to various foods. Their treatment entailed placing a child on a restricted diet and then adding foods one at a time to determine which foods caused an adverse reaction. This has been subsequently referred to as the oligoallergenic, or few foods diet, by more recent clinical/research groups, although the use of the term allergenic perhaps gives the erroneous impression that food allergies are at play.

A specific focus on sugar as a nutrient adversely affecting behavior occurred in the 1970s first with a study by Langseth and Dowd. Among 271 hyperactive children, a large number were found who, during glucose tolerance tests, had patterns of blood glucose levels similar to the pattern seen in adults with functional reactive hypoglycemia. Similar results have also been found in aggressive criminal offenders. A subsequent study showed that the patterns that Langseth and Dowd found can be normal variations in childhood, but the Langseth and Dowd study was followed by two correlational

studies, which suggested an association between sugar intake and hyperactivity. The hyperactive children who consumed more sugar displayed more hyperactive and aggressive behavior.

Another restriction dietary intervention suggested to improve behavior was proposed by Dr Benjamin Feingold in 1975. He reported that at least 50% of children with hyperactivity and learning disabilities improved when placed on diets, which were salicylate, preservative, and additive free.

Over time, the restriction dietary interventions were combined so that proposed dietary restrictions tended to incorporate recommendations from all three. However, it is useful to examine the scientific evidence for each of these dietary interventions separately.

The other dietary intervention category endorses supplementation with nutrients and minerals, particularly fatty acids (FAs) and zinc. Polyunsaturated fatty acids (PUFAs) are known to be essential for brain development and function so the theory proposes that a deficiency may contribute to a range of developmental disorders, including ADHD. The rationale for zinc supplementation is based on its role as a cofactor for many enzymes involved with neural metabolism, including an indirect effect on dopamine metabolism, with reports of zinc deficiency in children with ADHD in comparison with controls. It has been suggested that response to stimulants may depend on adequate zinc stores.

Objective Standards

To discuss the evidence for the efficacy of dietary interventions in treating ADHD, it is first important to review the criteria required to determine efficacy. The major point to emphasize is that it is virtually impossible to prove the null hypothesis, that is that no relationship exists between dietary constituents and behavior or cognitive function given the wide within and between child variability in behavior. Therefore, a realistic approach needs to be similar to that taken by the US Food and Drug Administration for the criteria it requires to license a new medication. Basically, pharmaceutical companies are required to demonstrate that a new medication is both efficacious and does not cause significant harm. It is not the role of the FDA to disprove the efficacy of a drug treatment. With dietary interventions, they should not be recommended as a primary intervention for behavioral problems until there is clear evidence of their efficacy.

For psychotropic medications, Sprague and Werry have described the important criteria required to objectively evaluate efficacy. These are presented in **Table 3**.

It is useful to use these criteria to evaluate the scientific merit of any studies on interventions affecting behavior. It is also important to examine the pattern of results of multiple studies from different research groups. Ideally, where other efficacious therapies are available (e.g., medication and behavior modification for children with Attention Deficit Hyperactivity Disorder), the proposed therapy should be compared to those existing therapies. This latter examination has been undertaken to a limited extent with any of the dietary interventions.

Table 2 Types of dietary intervention for ADHD

1. Oligoallergenic Diet
2. Sugar Restriction
3. Feingold Diet
4. Fatty Acids and Other Supplements

Table 3 Objective study criteria*Objective Study Criteria*

- Uniformity of Subjects
- Standard Doses
- Objective Verifiable Dependent Measures
- Control Group
- Placebo
- Double Blind

Study Designs

The gold standard in intervention studies is the randomized controlled trial (RCT). This design consists of randomly dividing the children to be studied into two separate groups. One of the groups receives the intervention while the other group receives a diet made to look like an intervention diet but does not contain or restrict those dietary elements that are the focus of the study (a placebo diet.) It is important that neither the families nor the researchers who are implementing or measuring the results know which children are receiving the intervention diet and which are receiving the placebo diet. The reason for the design is because some individuals who think they are receiving a diet to improve their behavior will improve just based on the power of suggestion. The RCT tries to reduce the possibility of that bias occurring.

RCT studies tend to be more difficult and expensive to implement in studying dietary interventions. What are more practical to implement are two designs, which can be more easily employed to study the effects of nutrients on behavior. The studies are referred to as within subject designed studies, or crossover studies. The most commonly employed design is the challenge study. This first places the children on the diet to be studied for a period of time and then challenges them with a food containing the offending agent (e.g., sucrose or tartrazine) or a food that does not contain the offending agent but looks and tastes identical to the offending agent referred to as a placebo. This is the most commonly employed design because it is the easier and less expensive type to complete. The other design develops diets, which appear similar, but the diets differ in what they contain (e.g., sugar or artificial sweeteners). In both designs the children, their families and the researchers need to be blinded about which diet or challenge food the children receive at any given time. In these studies, the children are used as their own controls. They are able to receive both diets or challenges in a sequence because the diets are not believed to result in permanent changes lasting once the diet is stopped.

The measures used to assess the effects (dependent measures) are then completed within the few hours after a challenge, or repeatedly while the children are on diets. Although parents, clinicians and teachers are utilized as observers (completing behavior rating scales), ideally multiple measures are employed including some that are by independent observers or include objective assessments (e.g., performance on a continuous performance test, measuring activity level etc.). Finally, there need to be multiple studies performed by different groups of researchers, and a clear pattern of effects should emerge.

Oligoallergenic Diet

Although this is the oldest of the dietary interventions, few controlled studies meeting the objective criteria outlined above have been undertaken. Five investigations have studied the effects of placing children on restricted diets. These studies all included additives and simple sugars as part of their dietary restrictions. The studies found beneficial effects from placing children on restricted diets compared to a placebo diet, or they found worsening behaviors in children on the restricted diets when they were challenged with offending foods compared to placebo challenges. In all but one of the studies, the only successfully completed dependent measure was behavior rating scales completed by the parents. Although these are important measures and are collected in most studies, the raters are not independent of the children's behaviors. One study had multiple measures, but only those of the parents and physician found a significant difference between the offending agent and placebo challenges. More extensive research by additional research groups and additional independent measures are required to document the efficacy of this intervention. Because the initial diet is extremely restrictive, care must be taken to make sure that the diet is adequately balanced and contains adequate amounts of essential micronutrients and other components of the diet, including energy. Consultation with a registered dietician is recommended before embarking on this or any other restricted diet.

Sugar Restriction

Sugar restriction usually refers to limiting the amount of sucrose in the diet. Although most of the studies evaluated sucrose restriction, some also examined fructose or glucose. The artificial sweetener aspartame was often employed as a placebo, but several studies used saccharin or both aspartame and saccharin. The type of placebo used did not seem to affect the results.

Sugar restriction has been studied as a treatment for children since 1982. There have been a total of 23 appropriate objective studies contained within 16 reports employing a wide variety of types of children including children with Attention Deficit Hyperactivity Disorder, and children with aggression as well as normal children, and varying in age from preschool age children to adolescents. All of the studies with two exceptions were challenge crossover studies where children were challenged with drinks containing either sugar (sucrose in most studies) or a placebo artificial sweetener. The other two studies consisted of giving the children diets that were high in sucrose content or low in sucrose and sweetened with aspartame or saccharin. A meta-analysis in 1995 of the 23 studies did not find any significant behavioral or cognitive effects from sugar restriction. There were not enough studies to reach a definitive conclusion, and there was insufficient statistical power to detect small effects or to detect effects on a small subset of children. To date there is not enough evidence to warrant the recommendation to restrict a child's sugar intake for the purpose of improving the child's behavior or cognitive functioning.

Feingold Diet

The Feingold Diet restricts foods with dyes, preservatives, and salicylate compounds. The studies of this diet were reviewed in 1986. Those studies generally studied children who had what is today called ADHD. Most of the studies were ones where the children were kept on an additive-free diet and then were challenged with a food containing an additive or a non-additive-containing food as placebo. Two studies employed additive-containing and additive-free diets. A problem in comparing studies was the variation in type and dose of additives employed. There were a total of 13 controlled studies. The summation of the findings found little, if any, effect. At the best there was some suggestion that a small percentage of children (1%) were adversely affected by additives. One study found that 24 of 34 children referred for hyperactivity (no formal diagnosis was established) who responded in an open clinical trial to an additive free diet, responded adversely to challenges with varying doses of tartrazine compared to placebo whereas all except two of 20 in a comparison group did not. The dependent measures were two behavior rating scales completed by the parents. There appeared to be a dose response which would be contrary to a usual allergic response. This is a much higher rate of response than found in any previous study including those employing tartrazine.

Since 2004, two large community-based randomized controlled trials have found a correlation in hyperactivity level with the amount of artificial food colors and sodium benzoate preservative in the diets of 3-year-old children. In one study, the correlation was only detectable by parent report and not by clinic assessment. The other study found a significant correlation in hyperactivity of 3-year-olds based on parent and teacher behavior reports. However, this effect was not replicated in a population of 9-year-old children in that study. Additionally, neither of these studies focused on children with formal diagnoses of ADHD.

Further study is required to substantiate these results because they run contrary to most of the previous research. Overall, the evidence to date does not confirm the efficacy of the Feingold Diet to warrant its promotion as a treatment for most children with behavioral problems. In addition, if the diet is strictly maintained including foods containing salicylate compounds, the diet may be deficient in vitamin C.

FA Supplementation

Supplementation with FAs or dietary alterations that increase FA intake have been suggested to be effective in decreasing the symptoms of ADHD and other neurobehavioral disorders since at least 1987. However, relatively few studies have evaluated these effects in a randomized controlled manner. On review, seven randomized controlled trials were found assessing the effect of dietary supplementation with FAs. Two studies found statistically significant improvements in ADHD symptoms, but the other five studies found no statistically significant improvements in the patients' core ADHD core symptoms.

All seven studies assessing FA supplementation effects used appropriate randomization techniques, but several had major

limitations. The two studies supporting the positive effect of FA supplementation each had major limitations. One study compared patients taking PUFA supplements alone with those taking PUFA supplements plus a multivitamin and with those taking a placebo pill. Both groups of subjects taking PUFA supplements showed significant improvement in Conners Parent Rating Scale scores of ADHD symptoms compared with placebo, but there were no significant changes in Conners Teacher Rating Scale scores. Another study evaluated 117 children who met diagnostic criteria for Developmental Coordination Disorder with 102 also meeting criteria for ADHD based on the Conners Teacher Rating Scale (CTRS) but without a formal ADHD diagnosis. The study found statistically significant improvements of ADHD symptoms in those taking omega-3 fish oil supplementation compared with placebo, but these findings were based only on CTRS scores with no initial or follow-up parent questionnaires used for assessment. In a third trial, supplementation with omega-3 EFA showed statistically significant improvement in continuous performance testing that correlated with blood lipid alterations, but there was no significant effect noted on the parental Abbreviated Conners Rating Scale, no data was obtained from teachers, and the dropout rate of the study was fairly high. A fourth study found no statistically significant difference in children consuming a diet high in omega-3 fatty acids compared with a placebo diet, but the study only had 40 participants with only 32 meeting DSM criteria for an ADHD diagnosis. None of the studies found reports of significant side effects, although two studies mentioned subjects that complained of minor abdominal discomfort and nosebleeds. Overall, some of these studies suggest possible improvement in ADHD symptoms attributed to FA supplementation, but a recommendation of FA supplementation as an adequate alternative treatment for ADHD cannot be supported at this time. There is a need for larger and preferably multisite studies utilizing both FA and placebo supplements with adequate randomization and double blinding finding consistent significant effects.

Zinc Supplementation

Zinc supplementation has been suggested as a treatment for a presumed or relative deficiency in the bodily level of zinc in children with ADHD and it is suggested that deficiencies in zinc cause the ADHD behaviors. Four randomized placebo-controlled trials have been conducted evaluating the changes in ADHD symptoms after supplementation with zinc or comparing zinc supplementation as an adjunct to stimulant treatment. Three of these studies were conducted in the Middle East with a report of significant decrease in hyperactive and impulsive symptoms in one large study conducted. Another trial conducted in Turkey found a decrease in ADHD symptoms but the effect size was small and there were no formal diagnoses of ADHD in the sample. A third study in the Middle East had a small sample size ($n=44$) but found significant improvement in parent and teacher ratings of ADHD symptoms in children treated with zinc plus methylphenidate compared with placebo plus methylphenidate. In a USA study comparing zinc as monotherapy with zinc as an adjunct to

amphetamine, no significant differences were found between the two groups.

It has been postulated that the significant effects found in the studies in the Middle East may have been a result of a high prevalence of zinc deficiency reported in those populations. Trials that measure zinc status as well as treatment effect of supplementation would be beneficial. At this time, the overall results remain ambiguous, and a recommendation of zinc supplementation as adequate monotherapy or adjunct to stimulant therapy for ADHD cannot be supported at this time.

The Challenge of Dietary Interventions

With all the dietary interventions, maintaining compliance may be difficult. Children who have behavioral problems to begin with are less likely to be compliant, and it can require a major effort detracting from efforts to control other areas of behavior. Diets are also problematic because they require the children to eat foods different from their peers and to sometimes eat foods that are less palatable than their usual diets. In children who are already singled out as different, this can harm self-esteem. Significant dietary changes can also involve increased preparation time. Additionally, the oligoallergenic/few foods diet requires close monitoring from a nutrition standpoint as it is initially fairly restrictive, and the Feingold diet may be deficient in Vitamin C if strictly maintained. Furthermore, adverse reactions of supplements have to be considered, and it must be acknowledged that the US FDA regulates dietary supplements as foods, rather than as drugs, which means that studies in humans to demonstrate safety and effectiveness are not required before the supplements are marketed. Also, the FDA does not require that supplement labels be accurate so it is difficult to monitor for contamination with other herbs, pesticides, and environmental pollutants. Care has to be taken to weigh the theoretical benefits of diets with as yet objectively unproved effects against the potential harm and difficulties in administering them.

Despite the lack of data suggesting efficacy, parents may still decide to embark on a restricted diet, and it is preferable that medical and nutritional follow-up be continued and that the duration of dietary restriction be agreed on in advance. Regardless of the chosen intervention, parents should proceed in a consistent, systematic manner. Ideally there should be close monitoring of the child's level of functioning and core behaviors of ADHD with frequent parent-clinician conversations and review of standardized questionnaires to assess changes in these core behaviors. A review of dietary intake by a registered dietician is recommended. Physicians should be able to discuss parental concerns and offer feedback regarding the risks and benefits of dietary interventions compared with medication and behavioral interventions.

Summary

ADHD is a mental disorder whose diagnosis is based on a child manifesting the symptoms of inattention, hyperactivity, and impulsivity to the extent that the symptoms impair the child's ability to function. The main beneficial treatments are medication and behavioral therapy. Thus far, the long term benefits of either intervention alone or combined are less clear. Dietary interventions have included diets that restrict 'allergenic' foods starting with a generally restricted diet and adding foods that do not worsen the child's behavior, a diet that restricts food additives and preservatives referred to as the Feingold diet, and diets that restrict sugar. Additionally, dietary supplements, including zinc and FAs, have been considered. To date, none of these dietary interventions have been proven to be efficacious.

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HYPERLIPIDEMIA

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Overview

Prevention and Management

Overview

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Normal Lipid Metabolism

Lipids are a heterogeneous group of substances soluble in organic solvents but insoluble in water. They are largely intracellular but circulate in blood as lipoprotein particles. There are four general functions for lipids:

1. Structural components of membranes
2. Storage forms of metabolic fuel
3. Transport forms of metabolic fuel
4. Protective functions as an outer coating of the organism.

Lipids consist of cholesterol and its derivatives, fatty acids, triacylglycerols, phospholipids, and apolipoproteins. The lipoprotein particle has a core of neutral lipids (cholesterol esters and triacylglycerol) and a surface coat of polar lipids (unesterified cholesterol and phospholipids) and apolipoproteins. They are classified in terms of density. The following are the main lipoproteins:

1. Chylomicrons,
2. Very low-density lipoprotein (VLDL)
3. Intermediate-density lipoprotein (IDL)
4. Low-density lipoprotein (LDL)
5. High-density lipoprotein (HDL).

Synthesis of lipoproteins occurs in the intestine or liver. They are then modified by enzymes and taken up by cell

surface receptors in processes largely regulated by the apolipoproteins. A series of receptors, transporters, and enzymes are important in lipoprotein metabolism and function, as detailed later. The physicochemical characteristics of the main lipoprotein classes are shown in **Table 1**.

Interest in lipids lies in circulating lipid concentrations and their relationship to atherosclerosis, particularly coronary heart disease (CHD), stroke, and peripheral vascular disease.

Cholesterol

Cholesterol is a sterol with the structure shown in **Figure 1**. Daily cholesterol intake is 0.5–1.0 g, half of which is absorbed. On a low-cholesterol diet ($<300 \text{ mg day}^{-1}$) the body synthesizes approximately $800 \text{ mg of cholesterol day}^{-1}$, mainly in the liver and, to a lesser extent, in the intestine.

The rate-limiting step in synthesis is highly sensitive to cellular levels of cholesterol, themselves sensitive to circulating levels of cholesterol. This feedback regulation occurs through changes in the amount and activity of an enzyme called 3-hydroxy-3-methylglutaryl CoA reductase (HMG-CoA reductase), which catalyzes the formation of mevalonate, the rate-limiting step in cholesterol biosynthesis. The rate of synthesis of HMG-CoA reductase mRNA is controlled by the sterol regulatory element binding protein (SREBP). SREBP in its inactive state is attached to the endoplasmic reticulum or

Table 1 Physicochemical characteristics of the major lipoprotein classes

Lipoprotein	Density (g ml^{-1})	Molecular weight ($\text{Da} \times 10^6$)	Diameter (nm)	Triacylglycerol (% lipid)	Cholesterol (% lipid)	Phospholipid (% lipid)	Source
Chylomicrons	0.95	>400	75–1200	80–95	2–7	3–9	Intestine
VLDL	0.95–1.006	10–80	30–80	55–80	5–15	10–20	Liver
IDL	1.006–1.019	5–10	25–35	20–50	20–40	15–25	Catabolism of VLDL
LDL	1.019–1.063	2.3	18–25	5–15	40–50	20–25	Catabolism of IDL
HDL	1.063–1.21	1.7–3.6	5–12	5–10	15–25	20–30	Liver, intestine

nuclear membrane, but when cholesterol levels decline the amino-terminal domain is released from its association with the membrane by proteolytic cleavage; it migrates to the nucleus and binds to the sterol regulatory element on the 5' side of the reductase gene to enhance transcription. As cholesterol levels increase, the proteolytic release of SREBP is blocked, SREBP in the nucleus is rapidly degraded, and cholesterol synthesis is switched off.

Cholesterol is found in the body largely as free cholesterol in membranes, but in the plasma it is two-thirds esterified, mainly as cholesterol linoleate and cholesterol oleate. Free cholesterol in plasma exchanges freely with cholesterol in membranes. The major route of cholesterol excretion is through the bile, directly as cholesterol or after conversion to bile salts, some of which are reabsorbed from the terminal ileum in the enterohepatic circulation.

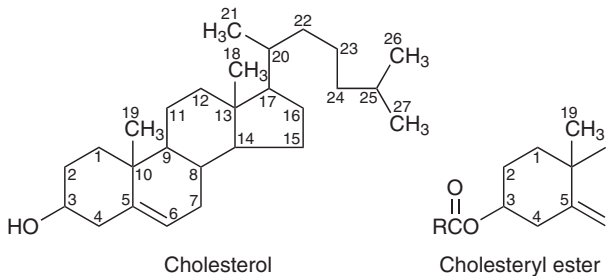


Figure 1 Structure of cholesterol and cholesteryl ester.

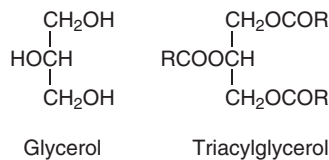


Figure 2 Structure of glycerol and triacylglycerol. 'R' denotes the position of a fatty acid within the triacylglycerol.

Triacylglycerol

Triacylglycerols are glycerol molecules esterified with three fatty acid molecules (**Figure 2**). Diacylglycerols and monoacylglycerols have two and one fatty acid molecules, respectively. Triacylglycerols constitute the main energy storage form in mammals and are the main storage form of fatty acids.

Fatty Acids

Fatty acids can be present as triacylglycerol, as part of lipoprotein particles, and as free fatty acids (bound to albumin). Common fatty acids and their sources are listed in **Table 2**.

Fatty acids are straight-chain compounds of differing lengths connecting a hydrocarbon group to a hydroxyl group. With only single bonds in the straight chain, the fatty acid is saturated; with one or more additional double bonds, the fatty acid is unsaturated. Fatty acids with only one double bond are said to be monounsaturated (e.g., oleic acid, $C_{18:1}$), whereas fatty acids with two or more double bonds are said to be polyunsaturated (e.g., arachidonic acid, $C_{20:4}$). The presence of a double bond allows there to be two isomers, depending on whether the hydrogen atoms attached to the carbon atoms on either side of the double bond lie on the same side (*cis*) or opposing sides (*trans*). *Cis* isomers are the only naturally occurring isomers and form kinks in the fatty acid chain. *Trans* isomers occur as part of food processing and maintain the straight direction of fatty acid chains. The common saturated fatty acids are palmitic ($C_{16:0}$) and stearic ($C_{18:0}$) acids.

Diets rich in omega-3 polyunsaturated fatty acids (n-3 PUFAs), such as α -linoleic acid, eicosapentaenoic acid, and docosahexaenoic acid, are associated with less CHD, and conjugated linoleic acids have beneficial effects against atherosclerosis. The n-3 PUFAs function mainly by changing membrane lipid composition, cellular metabolism, signal transduction, and regulation of gene expression. It is postulated that receptors exist for fatty acids or their metabolites that are able to regulate gene expression and affect metabolic or signalling pathways associated with CHD. Three nuclear receptors are thought to be fatty acid receptors that respond to

Table 2 Fatty acids and their sources

Fatty acid	Structure	Source	Melting point ($^{\circ}C$)
Saturated			
Lauric	$C_{12:0}$	Coconut oil and palm kernel oil	44
Palmitic	$C_{16:0}$	Palm oil, milk, butter, cocoa, butter, beef, pork, and lamb	63
Stearic	$C_{18:0}$		69
Behenic	$C_{22:0}$	Some seed oils and especially peanut	80
Lignoceric	$C_{24:0}$		84
Unsaturated			
Oleic	$C_{18:1}$	Olive oil and most commonly occurring fatty acid	11
Linoleic	$C_{18:2}$	Corn oil, soya bean oil, sunflower oil, and sunflower seed oil	-5
Linolenic	$C_{18:3}$	Linseed oil	-11
Arachidonic	$C_{20:4}$	Fish oils	-50
Eicosapentaenoic	$C_{20:5}$	Cod, salmon, pilchard, mussel, and oyster	-54
Docosahexenoic	$C_{22:6}$		

Source: Reproduced from Durrington PN (2004) *Hyperlipidaemia: Diagnosis and Management*. London: Hodder Arnold.

dietary and endogenous ligands: peroxisome proliferator activated receptors, retinoid X receptors, and liver X receptors.

Phospholipids

The common phospholipids in plasma are derived from glycerol and consist of triacylglycerol containing phosphate and a nitrogenous base (glycerophospholipids). The phosphate group is usually attached at position 3 of the glycerol molecule, and the nitrogenous base is usually an amino acid or an alcohol. The phosphatidyl cholines (lecithins) are the most common phospholipid and are found in plasma and in cell membranes. Lecithin-cholesterol acyl transferase (LCAT) catalyzes the transfer of a fatty acyl group at position 2 on glycerol to cholesterol to produce cholesteryl ester and leaves monoacyl glycerophosphate (lysolecithin). Another class of phospholipids, the cephalins, includes phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol.

Phospholipids are able to bridge nonpolar lipids and water and act to allow lipids to mix with water in an emulsion. The nonpolar hydrocarbon end of the phospholipid is attracted to lipid, whereas the polar phosphate group is attracted to water. In a lipid droplet, the inner oily center is surrounded by phospholipid, which has its outer phosphate group attracted to the surrounding water environment, to form a micelle.

Apolipoproteins A, B, C, and E

The lipoprotein particle (VLDL, LDL, and HDL) is composed of lipid and protein molecules. Among the protein molecules are a group of proteins found at the surface of the lipoprotein particle called apolipoproteins. Their function is integral to the metabolism of lipoproteins. They interact with phospholipids to solubilize cholesterol esters and triacylglycerol, regulate the reaction of enzymes (LCAT, lipoprotein lipase, and hepatic lipase (HL)) with lipid, and bind with cell surface receptors to determine the metabolism of lipoproteins.

Apolipoprotein A

This is the main protein of HDL and has two forms, apoA-I and apoA-II. ApoA-I is the main protein component in HDL, and the production and catabolism of apoA-I determine the plasma concentration of HDL cholesterol. It acts as an activator of LCAT, which is responsible for esterification of free cholesterol in plasma, and allows the binding of HDL to many cell surfaces. ApoA-II is a structural component of HDL.

Apolipoprotein A-I Milano

ApoA-I Milano is a specific form of apoA-I seen in some Italian families, which appears to protect against the development of atherosclerosis.

Apolipoprotein B

ApoB-100 is the main protein component of LDL and is synthesized in the liver. It is also found in chylomicrons and VLDL. ApoB-48 is synthesized from the intestine and is the amino-terminal half of apoB-100 synthesized from the same gene. ApoB-100 is the receptor ligand for the LDL receptor (LDLR).

Apolipoprotein C

ApoC is composed of three separate apolipoproteins. ApoC-I is mainly found in VLDL and also in chylomicrons and HDL. ApoC-II is present in a circulating reservoir of HDL, transferring to chylomicrons and VLDL, where it acts as an activator of lipoprotein lipase, allowing the lipolysis of triacylglycerols from circulating triacylglycerol-rich lipoproteins. ApoC-III is the most abundant form of apoC and may act as a modulator of lipoprotein lipase.

Apolipoprotein E

ApoE is a glycoprotein with several isoforms designated as apoE-2, -E-3, and -E-4. ApoE-3 is the most common isoform. It is present in VLDL, IDL, and HDL (mainly HDL₂). ApoE facilitates chylomicron remnant metabolism through the chylomicron remnant and VLDL receptors of the liver. ApoE-3 and -E-4 bind avidly with hepatic receptors, whereas apoE-2 is poorly bound. Patients with only apoE-2 isoform clear chylomicron remnants and IDL slowly, and apoE-2 is associated with dysbetalipoproteinemia (type III hyperlipoproteinemia). ApoE also facilitates metabolism through the LDLR (particularly the apoE-4 isoform). A large number of tissues express mRNA for apoE, including the brain, although the reason for this is unclear.

Apolipoprotein (a)

Apo(a) joined together with one LDL particle, which contains apoB, constitutes a lipoprotein called Lp(a). Interest in Lp(a) arose because apo(a) shows close sequence homology with plasminogen, suggesting that a high level of Lp(a) would impair thrombolysis. Lp(a) is an independent risk factor for developing vascular disease, with levels above a cutoff value of 300 mg l⁻¹ placing individuals at risk, especially if combined with other risk factors.

Lipoproteins

The main function of the lipoproteins is to transport lipids from one organ to another. Their main characteristics are shown in [Table 1](#).

Chylomicrons

These are the largest lipoproteins, consisting mainly of triacylglycerol with apoB-48 and apoA, -C, and -E. Triacylglycerol is hydrolyzed with endothelial-bound lipoprotein lipase, changing the chylomicron into a chylomicron remnant rich in cholesteryl ester. These remnants are removed from the circulation by interaction with the remnant receptors mainly present on hepatocytes. Peak chylomicronemia occurs 3–6 h after a meal, with a half-life of less than 1 h, and is cleared from the circulation after a 12-h fast.

Very Low-Density Lipoproteins

These triacylglycerol-rich lipoproteins are secreted mainly by the liver, with apoB-100 and apoE on their surface, whereas some VLDLs are synthesized by the gut. They are transformed into mature VLDLs by accumulating cholesterol ester, apoC, and apoE from HDLs. They then either interact with lipoprotein lipase to convert into IDLs, which can be taken up by

the liver, or convert to LDLs by interacting with hepatic triacylglyceride lipase.

VLDL particles vary in size. Small VLDL is converted into LDL, via IDL, to a greater extent than large VLDL, which is converted to a form of IDL that appears to be removed from the plasma before conversion to LDL.

Intermediate-Density Lipoproteins

IDLs are intermediate particles formed from the conversion of VLDL to LDL. Also known as VLDL remnants, some are removed directly from plasma, whereas some convert into LDL.

Low-Density Lipoproteins

LDL is the major cholesterol-carrying particle in the plasma. The core is cholesterol ester and has one apolipoprotein, apoB-100, per LDL particle. There are different sizes of LDL. Approximately one-third of the intravascular pool is catabolized per day and three-fourths of the circulating LDL is cleared through the liver, mainly through the LDLR. Small, dense LDL is more common in some dyslipidemias and may be more easily oxidized than larger LDL. Normal LDL does not cause foam cell formation, but lipid peroxidation of LDL makes the LDL a ligand for certain receptors (the scavenger receptor and perhaps a specific receptor for oxidized LDL) and results in the formation of cholesterol-laden foam cells. In addition, oxidized LDL in the cell wall stimulates the production of cytokines and growth factors, resulting in monocyte recruitment and the proliferation of smooth muscle cells. This mechanism underlies one model of atherogenesis.

High-Density Lipoproteins

Nascent HDL is secreted by the liver and gut. It acquires unesterified cholesterol in the circulation, catalyzed by LCAT to cholesteryl ester. HDL can pass cholesteryl ester to VLDL in exchange for triacylglycerol, facilitated by cholesterol ester transfer protein (CETP), or HDL can be taken up by the liver directly. The idea that HDL protects against CHD comes from epidemiological studies. A 0.026 mmol l⁻¹ increase in plasma HDL cholesterol decreases CHD risk by 2% in men and 3% in women.

Enzymes and Transfer Proteins

Acylcoenzyme A

Cholesterol acyltransferase (ACAT; EC 2.3.1.26) ACAT-1 and ACAT-2 are membrane-bound proteins responsible for cholesterol ester formation, metabolizing excess cholesterol within cells to cholesterol ester, which is allosterically activated by cholesterol.

Adenosine-Binding Cassette Transporter

In peripheral tissues, adenosine-binding cassette transporter (ABCA-1) protein facilitates transfer of intracellular cholesterol out of cells to lipid-poor apoA-1 or pre- β HDL particles. When it is deficient or inactive, cholesterol accumulates in peripheral tissues as in Tangier disease or familial HDL deficiency.

Cholesterol Ester Transfer Protein

CETP mediates the exchange of cholesteryl ester from HDL with triacylglycerol from VLDL or chylomicrons.

Fatty Acid Binding Protein

Fatty acid binding proteins (FABPs) play a role in the solubilization of long-chain fatty acids (LCFAs) and their CoA-esters to various intracellular organelles. FABPs serve as intracellular receptors of LCFAs and are involved in ligand-dependent transactivation of peroxisome proliferator-activated receptors (PPARs) in trafficking LCFAs to the nucleus.

Hepatic Lipase (EC 3.1.1.3)

HL is an endothelial-bound enzyme that removes triacylglycerol from lipoproteins in the metabolism of chylomicrons, VLDL, and HDL. HL hydrolyzes HDL triacylglycerol and phospholipids to form HDL₃ from HDL₂, contributing to the process of HDL regeneration in the reverse cholesterol transfer process.

Lecithin-Cholesterol Acyltransferase (EC 2.3.1.43)

LCAT mediates the esterification of cholesterol by transferring a fatty acid from lecithin to cholesterol to form cholesteryl ester.

Lipoprotein Lipase (EC 3.1.1.34)

Lipoprotein lipase and HL are endothelial-bound enzymes that remove triacylglycerol from lipoproteins. Lipoprotein lipase is activated by apoC-II and is involved in catabolism of chylomicrons and VLDL. Endothelial lipase, lipoprotein lipase, and HL belong to the same gene family.

Microsomal Triglyceride Transfer Protein

Microsomal triglyceride transfer protein is present in enterocytes and hepatocytes, and it is responsible for adding neutral lipid to apoB to protect it from ubiquitinylation and degradation.

Phospholipid Transfer Protein

Phospholipid transfer protein transfers phospholipids from other lipoproteins to HDL, contributing to the functionality of HDL.

Sterol Regulatory Element Binding Protein

SREBP is a protein that binds with part of the LDLR promoter to increase cholesterol synthesis.

Receptors

A large number of lipoprotein receptors have been identified. Some of the more important receptors are discussed here. Lipoprotein uptake at the cell membrane may be nonreceptor-mediated, perhaps by pinocytosis, where 'binding' is of low affinity but is not saturable.

LDL Receptor

The LDLR is a transmembrane glycoprotein present on most cell surfaces, encoded on chromosome 19. Free cholesterol,

building up in the cell through the receptor, reduces both cell synthesis of cholesterol and cell uptake of more LDL cholesterol.

LDLR-Related Protein

The LDLR-related protein (LRP) is a multifunctional receptor (binding VLDL/chylomicron remnants and other nonlipid ligands such as bacterial toxins) present in nearly all tissues. It has a high affinity for apoE and a low affinity for apoB-100.

VLDL Receptor

This receptor binds VLDL, β -VLDL, and IDL. It recognizes apoE and is located mainly in adipose tissue and muscle.

Scavenger Receptors

These receptors are found on macrophages and hepatic endothelium. They bind and degrade chemically modified LDL, such as oxidized or acetylated LDL. They are not downregulated by intracellular cholesterol accumulation. Hepatocellular uptake of HDL and its cholesteryl ester content is facilitated by a scavenger receptor and a HDL receptor.

Other Remnant Receptors

The lipolysis-stimulated receptor found on fibroblasts recognizes surface apoE and takes up VLDL, chylomicrons, and LDL. Two membrane-binding proteins (MBP 200 and MBP 235) have been described on macrophages and appear to bind VLDL. Remnants from both chylomicrons and VLDL (after hydrolysis of more than 70% of their triacylglycerol content) appear to be removed by both the LDL and the LRP receptors.

Peroxisome Proliferator-Activated Receptors

PPARs are a family of intranuclear receptors, including PPAR α and PPAR δ , that regulate a variety of genes involved in lipid metabolism, thrombosis, and inflammation.

Exogenous (Dietary) Lipid Pathways

Ingestion of food containing fat (triacylglycerol) and cholesterol results in absorption into the enterocyte of fatty acids, monoacylglycerols, free cholesterol, and lysolecithin. In the enterocyte, reesterification of fatty acids into triacylglycerol and cholesterol into cholesteryl ester occurs to form chylomicrons, to which is added a surface layer of apoB-48, -A-I, -A-II, and -A-IV, phospholipid, and free cholesterol. This allows secretion of the chylomicron into the intestinal lymphatics. ApoB-48 is required for secretion of the chylomicron. ApoB-48 is a truncated form of apoB-100, synthesized in the liver but missing the LDLR-binding domain of apoB-100. The action of the apoB-editing enzyme in enterocytes changes a nucleotide base in apoB mRNA to a stop codon. There is one apoB-48 per intestinal triglyceride-rich particle.

Chylomicrons in the circulation take up apoC from HDL (releasing it back to HDL later) and acquire apoE. ApoC-II allows the chylomicron to activate lipoprotein lipase on capillary endothelial cells of muscle and fat. This allows hydrolysis of triacylglycerol, releasing glycerol and fatty acids to be taken up by local tissue. Surface phospholipids, free cholesterol, and apoC transfer to HDL as the particle shrinks. This small chylomicron is called a chylomicron remnant and is

catabolized through the LDLR and other remnant receptors on the liver. This transport of dietary lipid from the intestinal to the peripheral tissues is shown in **Figure 3**.

Endogenous Lipid Pathways

The liver is the main source of endogenous lipid (**Figure 4**). In particular, the liver secretes the triacylglycerol-rich lipoprotein VLDL. Triacylglycerol, which is formed from fatty acids either newly synthesized or taken up from plasma, together with free cholesterol, synthesized from acetate or delivered to the liver in chylomicron remnants, join with apoB and phospholipids to form VLDL. ApoC and apoE are added in the circulation. Triacylglycerol is progressively removed from VLDL in the same way as occurs with chylomicrons. Free cholesterol transfers to HDL and is esterified with LCAT and transferred back to VLDL, using a protein called cholesteryl ester transfer protein (CETP), in exchange for triacylglycerol transfer from VLDL to HDL. In this way, VLDL becomes smaller and transforms to become IDL, although some small VLDLs may be removed directly. IDL is further changed through interaction with HL to LDL. In this way, most VLDL is transformed to LDL.

Reverse Cholesterol Transport

Lipids are transported to the peripheries from the gut and the liver. They return to the liver via HDL in a process known as reverse cholesterol transport (**Figure 5**). HDL particles arise in the liver and gut from a coalescence of apoA-I and phospholipid to form cholesterol-deficient bilayered discs in the form of HDL₃. Circulating HDL particles, particularly a subset of HDL₃ called pre- β HDL or lipid-poor apoA-I, come into contact with cells, and ABCA-1 acts to move free cholesterol from the cell surface and out of the cells. This cholesterol is converted by LCAT to cholesteryl ester and moves into the core of the HDL, forming mature cholesterol-rich HDL. After accumulating cholesterol, the HDL starts to accept other apolipoproteins and becomes HDL₂. In turn, HDL₂ appears to pass cholesteryl ester to triglyceride-rich lipoproteins such as chylomicrons, chylomicron remnants, and VLDL under the influence of CETP. The cholesterol then finds its way back to the liver in the form of chylomicron remnants, IDL, and LDL. Some of the HDL₂ particles may lose cholesterol directly to the liver and some may be taken up directly by the liver.

Consequences of Hyperlipidemia

Clear evidence exists that as serum cholesterol rises, the risk of CHD rises, and as serum cholesterol falls, the risk of developing CHD falls. The epidemiological evidence comes from within-country studies, between-country studies, and migration studies. Support comes from animal studies and there is evidence of the beneficial effects of reducing serum cholesterol in both primary and secondary prevention of ischemic heart disease.

The within-country studies include the Multiple Risk Factor Trial Intervention (MRFIT) study, which followed 360 000 middle-aged men screened and followed up for CHD

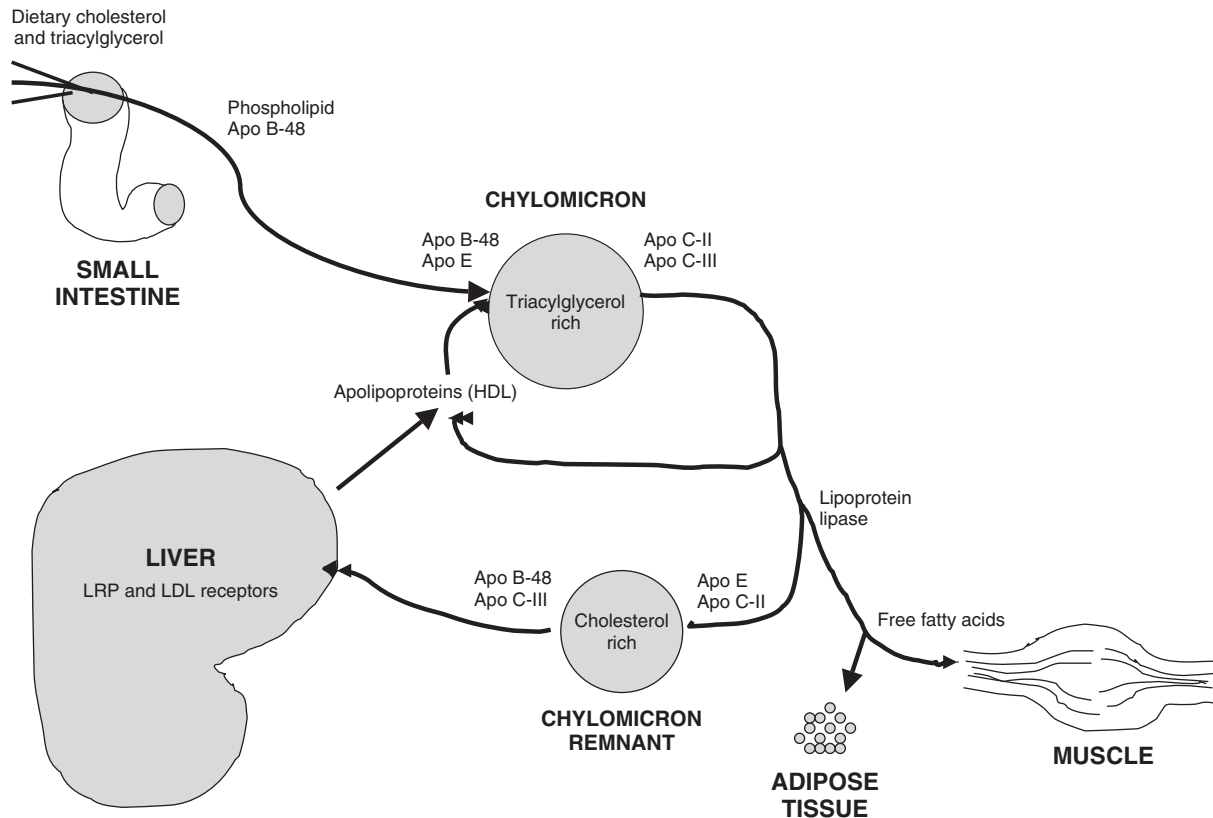


Figure 3 Exogenous (dietary) lipid pathway. This shows the transport of dietary lipid from intestine to peripheral tissues and liver. Movement of apolipoprotein between HDL and chylomicrons is shown. Low-density lipoprotein (LDL); receptor-related protein (LRP).

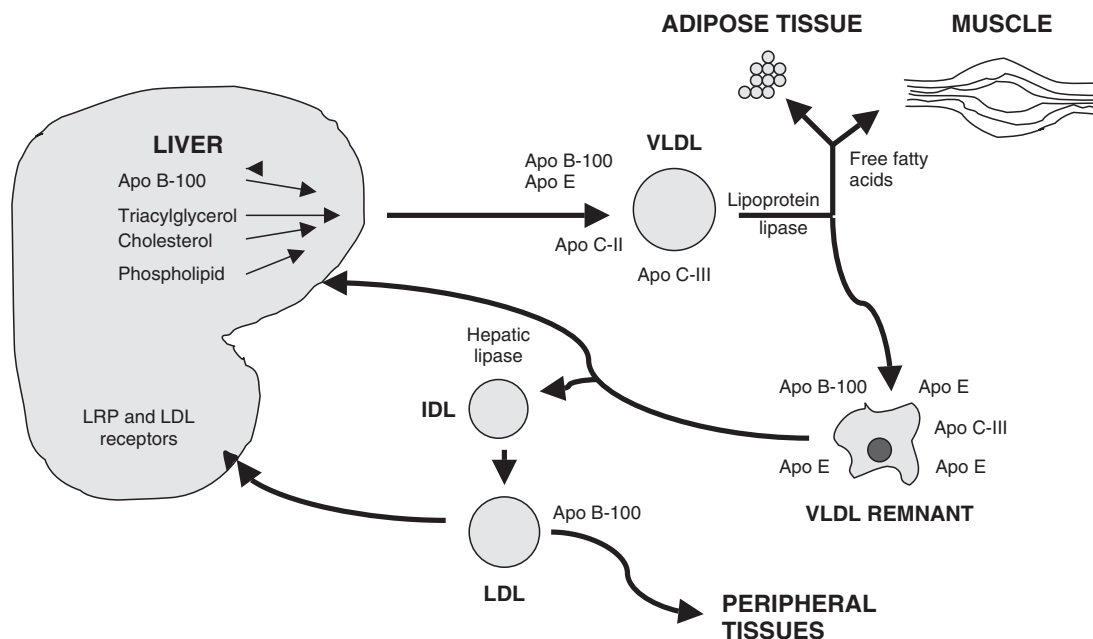


Figure 4 Endogenous lipid pathway. This shows the formation of VLDL lipid particles (VLDL₁ and VLDL₂) in the liver with the interconversion, through the action of lipoprotein lipase, to VLDL remnant and through IDL to LDL. Lipids are taken up from LDL both peripherally and in the liver. Low-density lipoprotein (LDL); receptor-related protein (LRP).

cholesterol and reductions in CHD risk. A 1% increase in HDL was equivalent to a 3% decrease in CHD risk.

Studies such as the Cholesterol Lowering Atherosclerosis Study, in which patients were allocated to drug therapy or placebo, used coronary angiography to follow the effect of drugs on disease. A small reduction in cholesterol results in a disproportionately larger reduction in cardiovascular events.

These studies show that it is possible to arrest progress of the disease and, in some cases, bring about regression of atherosclerosis. The extent to which this happens seems to depend on the underlying disease and the degree of cholesterol lowering.

Atherosclerosis has a complex and multifactorial etiology characterized by inflammation. Clinical markers of inflammation include C-reactive protein, modified LDL, homocysteine, lipoprotein (a), and fibrinogen, which are emerging risk factors and may give prognostic information for patient management. Folate may be beneficial by reducing plasma homocysteine, enhancing endothelial nitric oxide, and showing antiinflammatory properties. Other antiinflammatory agents, such as IL-10, may be of benefit.

Classification of Hyperlipidemia

There are a number of classification systems available. In 1967, Fredrickson, Levy, and Lees introduced the first

classification as a method of reporting that lipoproteins were raised. The World Health Organization adopted this classification (Table 3).

In 1987, the European Atherosclerosis Society recommended a five-group classification of primary hyperlipidemia (Table 4), and the National Cholesterol Education Program Adult Treatment Panel III published guidelines in 2002 for normal and elevated lipid levels (Table 5).

Clinically, the most important step is to determine if the lipid abnormality is primary or secondary to another condition. Table 6 shows the lipid changes seen in some common conditions. In practice, it is often easiest to classify lipid abnormalities into three categories: raised total cholesterol, raised triacylglycerol, and mixed hyperlipidemia.

It is becoming clear that certain lipoprotein patterns are particularly atherogenic. Elevated IDL with increased small, dense LDL particles and low HDL is one such pattern. Classifications based on these patterns may emerge.

Causes of Hypercholesterolemia

Serum cholesterol at birth does not exceed 2.5 mmol l^{-1} and is rarely above 4.0 mmol l^{-1} in children. The values for adults are given in Table 5. A raised cholesterol level, with little or no elevation of triacylglycerol, is usually a result of raised LDL level. Occasionally, a raised HDL level is responsible for high cholesterol, as seen in the familial condition of primary hyper-

Table 3 Fredrickson/WHO classification of hyperlipoproteinemia

Type	Lipids increased	Lipoprotein increased
I	Triacylglycerol	Chylomicrons
II-a	Cholesterol	LDL
II-b	Cholesterol and triacylglycerol	LDL and VLDL
III	Cholesterol and triacylglycerol	Chylomicron remnants and IDL
IV	Triacylglycerol	VLDL
V	Cholesterol and triacylglycerol	Chylomicrons and VLDL

Table 6 Lipid changes in some common conditions

Condition	Total cholesterol	HDL cholesterol	Triacylglycerol
Diabetes mellitus	Normal or ↑	↓	↑
Hypothyroidism	↑	↑	Can be ↑
Chronic renal failure	Normal or ↑	↓	↑
Nephrotic syndrome	↑	Often ↓	Often ↑
Cholestasis ^a	↑	↓	Can be ↑

^aAn abnormal lipoprotein called LpX is present.

Table 4 European Atherosclerosis Society classification of hyperlipoproteinemia

Group	Total cholesterol (mmol l^{-1})	Triacylglycerols (mmol l^{-1})
Normal	<5.2	<2.3
A (Mild hypercholesterolemia)	5.2–6.5	And <2.3
B (Moderate hypercholesterolemia)	6.5–7.8	And <2.3
C (Isolated hypertriglyceridemia)	<5.2	And 2.3–5.6
D (Combined hyperlipidemia)	5.2–7.8	And 2.3–5.6
E (Severe hypercholesterolemia and/or hypertriglyceridemia)	>7.8	And/or >5.6

Table 5 Adult Treatment Panel III levels for blood lipids

Classification	Total cholesterol (mmol l^{-1})	LDL cholesterol (mmol l^{-1})	Triacylglycerols (mmol l^{-1})
Normal	<5.2	<2.59	<1.7
Above optimal	–	2.6–3.3	–
Borderline high	5.2–6.2	3.4–4.1	1.8–2.2
High	>6.2	4.2–4.8	2.3–5.6
Very high	–	>4.9	>5.6

α -lipoproteinemia. Secondary causes given in Table 6 include hypothyroidism, nephrotic syndrome, some cases of diabetes mellitus, and cholestasis. Primary causes include polygenic familial hypercholesterolemia (FH), in which several gene abnormalities together with environmental effects serve to raise serum cholesterol. Several genetic loci contribute to increased plasma LDL levels, but there are five specific monogenic disorders that increase LDL: FH (LDLR gene), familial ligand-defective apoB-100 (apoB gene), autosomal recessive hypercholesterolemia gene, sitosterolemia (*ABCG5* or *ABCG8* genes), and cholesterol 7 α -hydroxylase deficiency (*CYP7A1* gene).

Much less common, but more clearly defined, are the two autosomal conditions of familial combined hyperlipidemia (FCH) and monogenic FH. In FCH, there appears to be an increase in apoB production and thus an increase in serum LDL. Serum VLDL levels are raised in one-third of these subjects with an associated triacylglycerol increase, one-third show increases in LDL, and one-third show increases in LDL and VLDL. Monogenic FH is caused by a defect in the LDLR. The consequent reduced LDL uptake by cells, particularly in the liver, results in raised LDL and cholesterol levels. There are 683 mutations in the LDLR gene. Of these, 58.9% are missense mutations, 21.1% are minor rearrangements, 13.5% are major rearrangements, and 6.6% are splice site mutations. Majority of mutations are found in two functional domains of the LDLR, the ligand binding domain (42%), and the epidermal growth factor precursor-like domain (47%).

Predominant hypertriglyceridemia may result from raised VLDL or chylomicron levels. Secondary causes include excess alcohol ingestion, obesity and excess carbohydrate intake, diabetes mellitus, renal failure, and pancreatitis. Primary hypertriglyceridemia can be a result of familial combined hypertriglyceridemia, familial endogenous hypertriglyceridemia, or hyperchylomicronemia.

Familial endogenous hypertriglyceridemia results from increased hepatic triacylglycerol production with increased VLDL production. It is associated with obesity, glucose intolerance, and hyperuricemia. Hyperchylomicronemia is a result of inherited or acquired impairment of lipoprotein lipase activity.

Reduced insulin levels in diabetes mellitus impair the activity of lipoprotein lipase, and hyperchylomicronemia can occur. Inherited deficiency of the lipase enzyme is rarely seen, as is deficiency of the apolipoprotein (apoC-II) required to activate the enzyme.

Mixed hyperlipidemia is often a secondary condition. Primary causes include FCH and type III hyperlipidemia (dys- β -lipoproteinemia or broad β disease). Type III hyperlipidemia is associated with the apoE 2/2 phenotype, resulting in impaired recognition of apoE by hepatic receptors and accumulation of IDL.

Dyslipoproteinemia is a central feature of the metabolic syndrome, which is associated with accelerated atherosclerosis. Visceral obesity, dyslipidemia, insulin resistance, hypertension, and a proinflammatory and prothrombotic state are the main characteristics of this condition. It has been defined by the World Health Organization and the National Cholesterol Education Programme. The worldwide increase in levels

of obesity in the developed world may presage an increase in CHD.

Dietary Effects

Principles of Treatment

Treatment of hyperlipidemia is part of the management of CHD risk. This encompasses lifestyle changes, such as stopping smoking, increasing exercise, and modifying diet, as well as management of hypertension. Diet is the cornerstone of treating hyperlipidemia, best delivered by qualified dietitians, involving the whole family.

The main aims of diet are to correct excess calorie intake and to reduce the cholesterol and saturated fat content. Patients with hyperlipidemia can expect to see benefits from diet after 6 weeks and are reviewed every 4 months.

Diet can reduce total cholesterol 8–12%, with 60–80% of this change attributed to reductions in saturated fatty acid intake. The remaining change comes from reduced dietary cholesterol and changes in the intake of fiber and mono-unsaturated and PUFAs. Dietary modification may not be successful in some primary hyperlipidemias. The Diet and Reinfarction Trial and the Mediterranean Diet Study in post-myocardial infarction survivors showed that dietary modification, not necessarily accompanied by plasma cholesterol lowering, can improve short-term prognosis.

Fat

Most of the saturated fats in the diet come from just four fatty acids: lauric acid ($C_{12:0}$), myristic acid ($C_{14:0}$), palmitic acid ($C_{16:0}$), and stearic acid ($C_{18:0}$). The first three fatty acids reduce LDLR activity, raising LDL and total cholesterol by approximately 0.25 mmol per l per 10 g of saturated fat ingested. Watts and coworkers showed that total dietary fat (mainly saturated) increases hepatic VLDL-apoB secretion, so decreasing total fat intake should decrease hepatic apoB secretion.

Monounsaturates are being recommended more often. The most common is oleic acid ($C_{18:1}$), found in the Mediterranean diet as olive oil. Animal fats are rich in monounsaturates but are also rich in saturated fats. The *trans* isomers of monounsaturates may raise total and LDL cholesterol and are best avoided.

In both type I and type V hyperlipidemia, the dietary management is to reduce fat intake to 20–40 g day⁻¹. Medium-chain triacylglycerols are used and fish oils can be tried, but the mainstay of therapy is reduced fat intake. Dietary β -sitosterol can block cholesterol absorption to a limited extent but is not used therapeutically.

Carbohydrate and Calories

Obesity is a common cause of hypertriglyceridemia due to raised VLDL levels in the obese subject. This may be because of an increase in insulin resistance resulting from obesity with concomitant hyperinsulinemia and elevation in hepatic VLDL synthesis. Some hypertriglyceridemic patients experience a further increase in triacylglycerol levels with an increase in carbohydrate intake, known as carbohydrate induction. This situation is accompanied by an increase in serum insulin

levels. With weight reduction, the hypertriglyceridemia reduces and HDL cholesterol increases after 24 months.

Mild alcohol ingestion increases HDL cholesterol. Excess alcohol ingestion can precipitate hypertriglyceridemia of a type IV phenotype due to increased hepatic synthesis and secretion that, in subjects who cannot clear triacylglycerols efficiently, can progress to a type V phenotype. Serum LDL levels are usually low in alcoholics, although in some individuals they can be elevated.

Protein

Changes in dietary protein intake have minimal effects on lipid levels. Vegetarians have lower serum lipids than non-vegetarians, but it is not clear how much of this is the result of a change from animal to vegetable protein.

Table 7 AHA dietary recommendations

Nutrient	Recommendations (% of total calories)	
	AHA step 1	AHA step 2
Total fat	<30	<30
Fatty acids	—	—
Saturated fat	<10	<7
Polyunsaturated fatty acid	<10	<10
Monounsaturated fatty acids	10–15	10–15
Carbohydrates	50–60	50–60
Protein	10–20	10–20
Cholesterol (mg day ⁻¹)	<300	<200
Reduce total calories to achieve and maintain desirable weight		

Source: Reproduced from Denke MA (1994) Diet and lifestyle modification and its relationship to atherosclerosis. *Medical Clinics of North America: Lipid Disorders* 78: 197–223.

Fiber

Soluble fiber such as oat bran and guar lower cholesterol levels, perhaps by reducing bile acid absorption.

Recommendations

The National Food Survey 1999 showed that the total amount of fat in the British diet decreased from 93 g day⁻¹ in the 1980s to 75 g day⁻¹ in 1998 and so fat now contributes approximately 40% of calories, of which 15–20% comes from saturated fat. Cholesterol intake in the diet is approximately 500 mg day⁻¹. The American Heart Association (AHA) has recommended a two-step approach to dietary change, outlined in [Table 7](#), and European recommendations for the diet of the population are shown in [Table 8](#). The central approach of dietary therapy is to reduce cholesterol-raising fatty foods, reduce cholesterol intake, and achieve a desirable body weight. The AHA step 1 diet can reduce total cholesterol by 0.5–1.0 mmol l⁻¹ and the step 2 diet can provide a further 0.2–0.4 mmol l⁻¹ reduction. Saturated fat in the diet is best replaced by increasing complex carbohydrates, with modest increases in monounsaturated and ω -6 PUFA. Increased fish oil intake giving additional ω -3 fatty acids will reduce triacylglycerol levels (but increase LDL cholesterol in certain patients).

Although a low-fat, high-carbohydrate, and energy-deficient diet may be used for weight reduction in obese subjects, increasing evidence suggests that increased carbohydrate may not be desirable. Recently, a low-carbohydrate, high-protein, and high-fat diet (the Atkins diet) has become popular. Although current studies are promising, the long-term effects of this diet are unknown and it is not currently recommended. Fresh fruit, vegetables, and fiber are encouraged.

Table 8 Intermediate and ultimate nutrient goals for Europe

	Intermediate goals		Ultimate goal
	General population	Cardiovascular high-risk group	
Percentage of total energy ^a derived from			
Complex carbohydrates ^b	> 40	> 45	45–55
Protein	12–13	12–13	12–13
Sugar	10	10	10
Total fat	35	30	20–30
Saturated fat	15	10	10
P:S ratio ^c	≥ 0.5	≥ 1.0	≥ 1.0
Cholesterol (mg day ⁻¹)	< 300	< 300	< 300
Fiber (g day ⁻¹)	30	> 30	> 30
Salt (g day ⁻¹)	7–8	5	5

^aAll values given refer to alcohol-free total energy intake.

^bThe complex carbohydrate data are implications of the other recommendations.

^cThe ratio of polyunsaturated to saturated fatty acids

Source: Reproduced from Pyörälä K, De Backer G, Graham I, Poole-Wilson P, and Wood D (1994) Prevention of coronary heart disease in clinical practice. Recommendations of the Task Force of the European Society of Cardiology, European Atherosclerosis Society and the European Society of Hypertension. *European Heart Journal* 15: 1300–1331, with permission from OUP.

See also: Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Dietary Fiber: Role in Nutritional Management of Disease. Fats and Oils. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids; Metabolism. Fiber: Physiological and Functional Effects. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Trans-Fatty Acids: Health Effects, Recommendations, and Regulations. Vitamin E: Metabolism and Requirements; Physiology and Health Effects

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Prevention and Management

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Glossary

β -glucan One type of dietary soluble fiber. β -glucan is a polysaccharide of glucose molecules linked by β -glycosidic bonds. β -glycosidic bonds are resistant to digestion in the human gastrointestinal track.

Cholesterol A sterols molecule. Cholesterol is critical for the formation of cell membranes, lipoproteins, and synthesis of steroid hormones, bile acids and vitamin D.

Fatty acid geometric isomers Unsaturated fatty acid isomers that differ only in the conformation of at least one double bond (*cis* or *trans*).

Fatty acid positional isomers Unsaturated fatty acid isomers that differ only in the location (position) of at least one double bond (e.g., omega-3 and omega-6 linolenic acid).

Phytosterols Includes compounds referred to as plant sterols. This group of compounds is structurally similar to cholesterol, differing in their sterol side chain. The phytosterols have weak estrogenic activity. Saturated forms of plant sterols are referred to as plant stanols.

***trans*-Fatty acids** Polyunsaturated fatty acid in which at least one double bond is in the *trans*, as opposed to the more common *cis*, configuration.

There is a wide range of dietary factors purported to alter the risk of developing cardiovascular disease (CVD). Some were identified early in the twentieth century, whereas others have been recognized more recently. These emerging data have resulted in shifts in certain guidance over time. None of the changes are without controversy. Some of this controversy is more likely attributable to biological variation among individuals (e.g., genetics and gender), interaction among putative dietary factors (e.g., macronutrients), and differences in biological (e.g., body weight) and lifestyle (e.g., physical activity) factors than actual differences on whether they contribute to CVD outcomes. This article will present current trends in dietary approaches for the prevention and management of CVD.

Assessing CVD Risk

It is logistically difficult, if not impossible, to directly assess the effect of dietary interventions on CVD outcomes because the natural course of the disease is measured in years or decades rather than days or months. Hence, most dietary interventions aimed at reducing CVD risk are evaluated on the basis of surrogate disease biomarkers. As the number of dietary variables potentially associated with CVD risk has increased over the past few decades, so has the number of potential biomarkers of CVD used to assess the efficacy of dietary interventions. The independence and relative importance of each biomarker is yet to be adjudicated. Potentially, different biomarkers may be of greater predictive value for particular dietary modifications.

Traditionally, total cholesterol, high-density lipoprotein cholesterol (HDL-C), and triacylglycerol (triglyceride) concentrations were used to evaluate the efficacy of a dietary intervention for CVD risk. Total cholesterol, HDL-C, and triglyceride were measured directly, whereas low-density lipoprotein cholesterol (LDL-C) concentrations were calculated using the Friedewald formula ($\text{LDL-C} = \text{total cholesterol} - \text{HDL-C} - (\text{triglyceride}/5)$) unless the triglyceride concentrations were higher than 400 mg dl^{-1} . Reliable automated direct assays for

LDL-C are now available and obviate the need for this calculation. Plasma factors such as lipoprotein(a) (Lp(a)), C-reactive protein (CRP) and other markers of inflammation, LDL particle size, hematologic factors, apoprotein concentrations, genotypes, insulin and glucose concentrations, plant sterol and intermediates of cholesterol biosynthesis concentrations, HDL subspecies concentrations, efflux capacities, and remnant-like particle concentrations are also potentially informative in estimating the effect of dietary intervention on CVD risk. Adequate data are not available at present to suggest that any dietary interventions result in an alteration in any of the above-mentioned factors that is strong enough to modify CVD risk estimates. For this reason, current dietary guidance to decrease CVD risk is made on the basis of plasma lipid and lipoprotein concentrations. Some factors, such as CRP, are taken into consideration for individuals thought to be at an intermediate risk to advise them on risk management. As new data emerge, the relative importance of these variables and potentiality additional variables will be clarified.

Dietary Lipids: Approaches for the Prevention and Management of CVD

Amount of Dietary Fat

For most individuals consuming Western diets, dietary fat serves as a major energy source. One gram of fat contributes 9 cal, a little more than twice that is contributed by a protein or a carbohydrate (4 cal g^{-1}) and somewhat more than that contributed by alcohol (7 cal g^{-1}). When focusing on the importance of dietary fat amount on CVD prevention and management, there are two independent factors for consideration; plasma lipoprotein profiles and body weight. It is important to consider the later factor due to the secondary effects excess body weight has on plasma lipid concentrations and factors associated with the metabolic syndromes (blood pressure, waist circumference (intra abdominal/visceral fat), dyslipidemia, and blood glucose concentrations).

With respect to the effect of dietary fat amount (as a percent of energy) on plasma lipoprotein profiles, the focus is on triglyceride and HDL-C concentrations, and the total cholesterol to HDL-C ratio. Relatively consistent evidence indicate that when body weight is maintained at a constant level, a decrease in the percent of energy contributed by dietary fat, with a concomitant increase in dietary carbohydrate, results in an increase in triglyceride concentrations, decrease in HDL-C concentrations, and less favorable (higher) total cholesterol to HDL-C ratio. Both low HDL-C and high triglyceride concentrations have been associated with increased risk for CVD, and frequently coexist. Lower fat diets are of particular concern/importance for individuals with compromised glucose homeostasis because they are more likely to have dyslipidemia (low HDL-C and high triglyceride concentrations).

Available historical data suggest a null or weak association between the amount of dietary fat and body weight. Recent data from a long-term weight loss intervention indicate that the amount of dietary fat (percent of energy) had little effect on the success of the participants to decrease their body weight (Figure 1) or plasma lipids or measures of glucose homeostasis. There were similar rates of weight loss and regain over a wide range of macronutrient profiles. The major determinate of body weight loss, rather than the proportion of dietary fat or other macronutrients, was adherence to the diet as assessed by number of group sessions attended during the intervention period (Figure 2). Other evidence suggests that dietary fiber may be a mitigating factor in promoting weight loss. That is, substituting fruits, vegetables, and whole grains for fat rather than fat-free cookies, cakes, and snack foods is more efficacious in promoting weight loss within the context of

low-fat diets. The area of dietary fat and body weight is clearly complex.

Type of Dietary Fat

The relationship between dietary fat type and atherosclerosis, the major form of CVD, was first identified at the turn of the twentieth century. A series of meticulous studies undertaken on humans during the 1960s demonstrated that changes in individual dietary fatty acid altered plasma total cholesterol concentrations. As analytical techniques became more sophisticated, the focus on biomarkers for CVD risk shifted from total cholesterol to LDL-C and HDL-C. Although many studies have confirmed these early observations, inconsistencies among more recent works are not rare. The occurrence of these inconsistencies are likely contributed by factors such as multiple variable differences among experimental diets, including absolute amount of dietary fatty acid, length of intervention period, and background diet before the start of the study period onto which the dietary variable(s) was superimposed. Additional variations among studies include differences among study participants in age, sex, genetics, and efficiency of cholesterol absorption and initial plasma lipid concentrations.

Saturated Fatty Acids

Early evidence demonstrated that diets relatively higher in saturated fatty acids (SFAs) increased plasma total cholesterol concentrations compared with diets relatively higher in

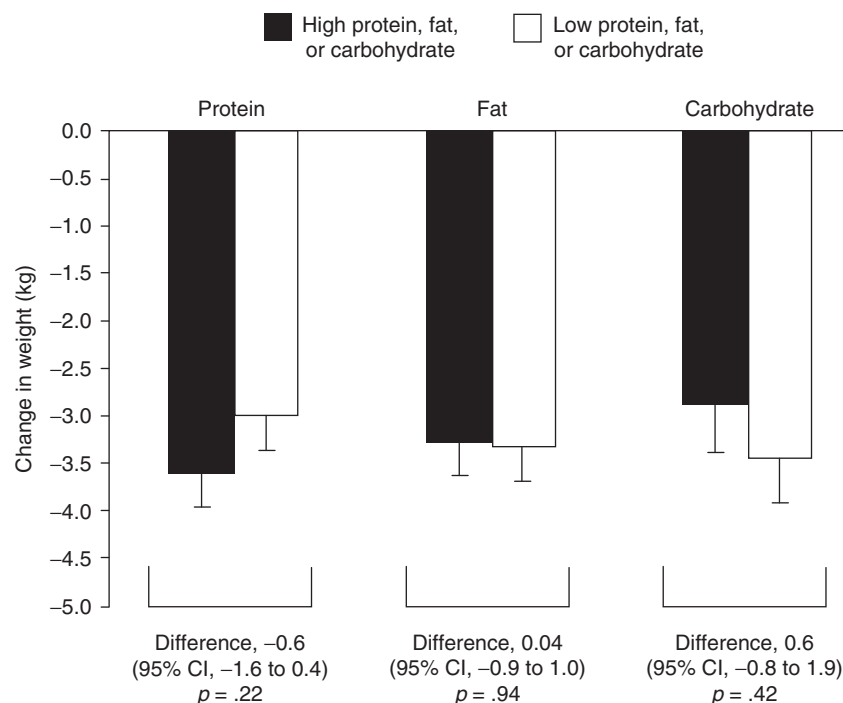


Figure 1 Mean change in body weight and waist circumference from baseline to 2 years according to dietary macronutrient content. Abbreviation: CI, confidence interval. Reproduced from Sacks FM, Bray GA, Carey VJ, *et al.* (2009) Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *New England Journal of Medicine* 360(9): 859–873.

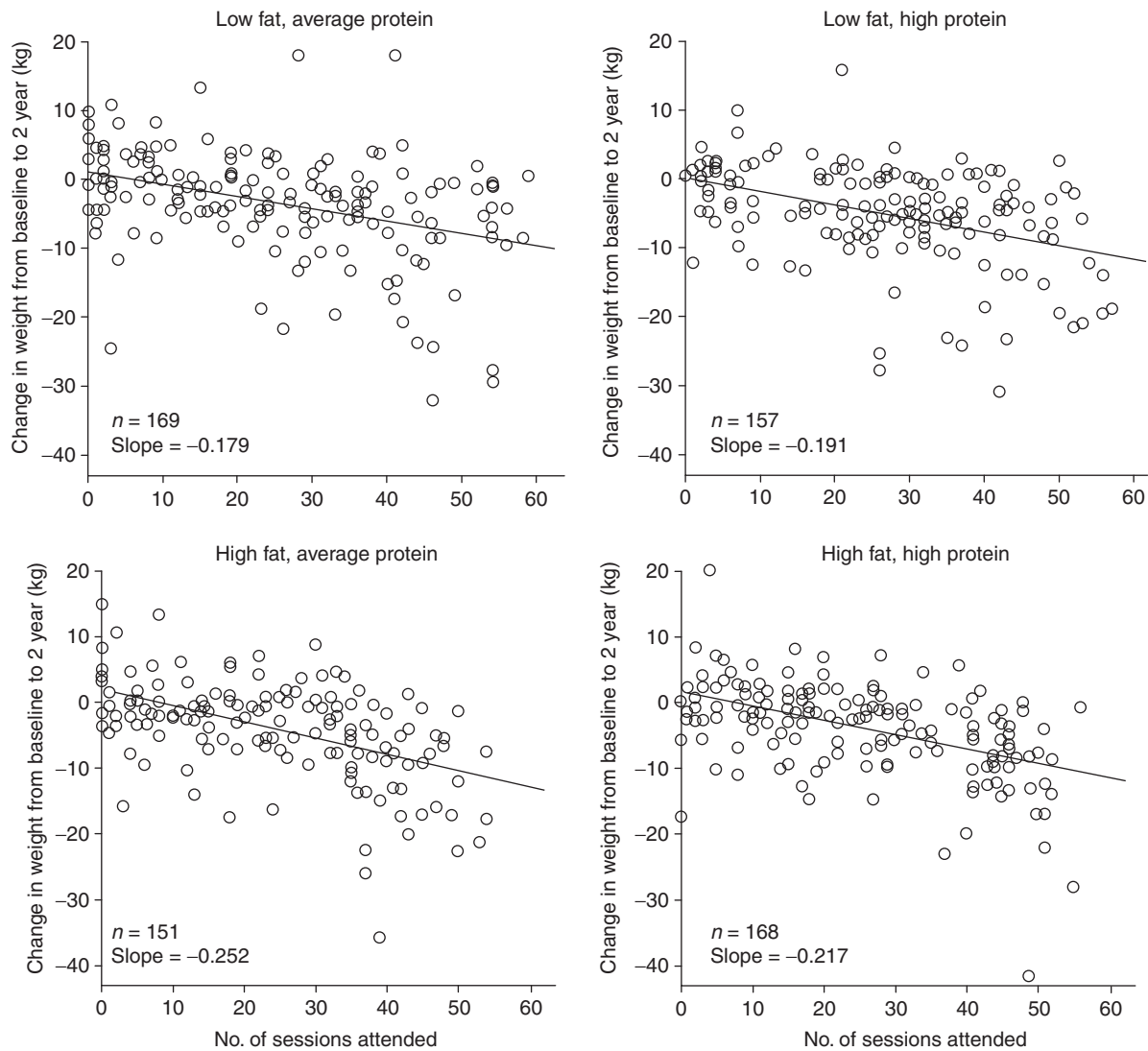


Figure 2 Change in body weight from baseline to 2 years according to attendance at counseling sessions for weight loss. Reproduced from Sacks FM, Bray GA, Carey VJ, *et al.* (2009) Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *New England Journal of Medicine* 360(9): 859–873.

unsaturated fatty acids, and that not all SFA had identical effects. Subsequent work confirmed the hypercholesterolemic effect of SFA, demonstrated that SFA intake results in an increase in both LDL-C and HDL-C concentrations, the absolute magnitude is greater for LDL-C than HDL-C, and reaffirmed that individual SFA had different effects on the plasma parameters. Short-chain fatty acids (6:0–10:0) and stearic acid (18:0) appear to have little or no effect in LDL-C and HDL-C concentrations, whereas SFA with intermediate chain lengths, such as lauric (12:0), myristic (14:0), and palmitic (16:0) acids, appear to be the most potent in increasing plasma cholesterol concentrations (Table 1). Limited data suggest that a high proportion of stearic acid (18:0) is converted to oleic acid (18:1) and, therefore, has a relatively neutral effect on plasma cholesterol concentrations. The underlying mechanism by which fatty acids with 10 or fewer carbon atoms have different effects from those with 12–16 carbons on plasma cholesterol concentrations may be related

to the mode of absorption (portal vein rather than lymphatic system) and preferential hepatic oxidation of these fatty acids. A recent pooling study has shown that when dietary SFA is displaced by polyunsaturated fatty acids (PUFAs), CVD risk is lowered (Figure 3). When dietary SFA is displaced by carbohydrate (resulting in lower fat diets), this advantage is lost.

SFA tend to be solid at room temperature. Notable exceptions include the tropical oils, such as palm, palm kernel, and coconut. Tropical oils are liquid at room temperature because they have high concentrations of short-chain SFA.

Approaches to reduce dietary SFA include replacing fatty cuts of meat with leaner cuts, trimming excess fat and skin before and after cooking, decreasing portion size, substituting nonfat and low-fat (1% fat) dairy products for their full-fat counterparts, and avoiding foods made with tropical oils. Judicious attention to ingredient listings and nutrient labels can facilitate the goal of reducing SFA intakes.

Table 1 Major dietary fatty acids

Code	Common name	Formula
Saturated		
12:0	Lauric acid	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$
14:0	Myristic acid	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$
16:0	Palmitic acid	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$
18:0	Stearic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$
Monounsaturated		
16:1 <i>n</i> -7 <i>cis</i>	Palmitoleic acid	$\text{CH}_3(\text{CH}_2)_5\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_7\text{COOH}$
18:1 <i>n</i> -9 <i>cis</i>	Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_7\text{COOH}$
18:1 <i>n</i> -9 <i>trans</i>	Elaidic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=(\text{t})\text{CH}(\text{CH}_2)_7\text{COOH}$
Polyunsaturated		
18:2 <i>n</i> -6, 9 all <i>cis</i>	Linoleic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_7\text{COOH}$
18:3 <i>n</i> -3, 6, 9 all <i>cis</i>	α -Linolenic acid	$\text{CH}_3\text{CH}_2\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_7\text{COOH}$
18:3 <i>n</i> -6, 9, 12 all <i>cis</i>	γ -Linolenic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_4\text{COOH}$
20:4 <i>n</i> -6, 9, 12, 15 all <i>cis</i>	Arachidonic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CHCH}_2\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_3\text{COOH}$
20:5 <i>n</i> -3, 6, 9, 12, 15 all <i>cis</i>	Eicosapentaenoic acid	$\text{CH}_3(\text{CH}_2)\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_5(\text{CH}_2)_3\text{COOH}$
22:6 <i>n</i> -3, 6, 9, 12, 15, 18 all <i>cis</i>	Docosahexaenoic acid	$\text{CH}_3(\text{CH}_2)\text{CH}=(\text{c})\text{CH}(\text{CH}_2)_6(\text{CH}_2)_2\text{COOH}$

Unsaturated Fatty Acids

Unsaturated fatty acids are fatty acids that contain one or more double bonds in the acyl chain. As their name implies, monounsaturated fatty acids (MUFAs) have one double bond and PUFA have two or more double bonds. Most double bonds in fatty acids occurring in food are in the *cis* configuration, that is, the hydrogen atoms associated with the carbons forming the double bond are oriented on the same side of the acyl chain. Alternatively, some double bonds occur in the *trans* configuration, that is, the hydrogen atoms associated with the carbons forming the double bond are on the opposite side of the acyl chain. This difference in double bond conformation has implications for bond angle (see the section on 'trans-Fatty Acids'). This part of the discussion of unsaturated fatty acids will be restricted to those containing *cis*-double bonds.

Relative to SFA, both MUFA and PUFA lower both LDL-C and HDL-C concentrations. The absolute magnitude of the change is greater for LDL-C than HDL-C. Most data suggest that MUFA has a somewhat smaller effect than PUFA in lowering both LDL-C and HDL-C concentrations (Figure 4). Nonetheless, the change in the total cholesterol/HDL-C ratio (decrease) is similar. Because of changes in plasma LDL-C and HDL-C concentrations caused when unsaturated fat displaces SFA from the diet, such a shift should be encouraged in the prevention and management of CVD.

MUFA

The major MUFA in the diet is oleic acid (18:1) (Table 1). Common vegetable oils high in MUFA include canola (rape-seed) and olive oils. Fat from meats are also relatively high in MUFA; but unlike vegetable oils, they also contain relatively high levels of SFA and therefore cannot be recommended as a good source of dietary MUFA.

PUFA

There is a wider range of PUFA than MUFA in the diet. Dietary PUFA vary on the basis of chain length, degree of saturation (number of double bonds), and position of the double bond(s) (positional isomers). Two positional isomers of interest with respect to diet and CVD risk are omega *n*-6 and *n*-3 (Table 1). The distinction is made on the basis of the location of the first double bond from the methyl end of the fatty acyl chain (as opposed to the carboxyl end). If the first double bond is six carbons from the methyl end, then the fatty acid is classified as *n*-6 fatty acid. If the first double bond is three carbons from the methyl end, then the fatty acid is classified as *n*-3 fatty acid.

Vegetable oils high in PUFA include soybean, corn, sunflower, and safflower oils. The major *n*-6 PUFA in the diet is linoleic acid (18:2*n*-6). However, other *n*-6 PUFA, such as γ -linolenic acid (18:3*n*-6) and arachidonic acid (20:4*n*-6), occur in smaller amounts. These fatty acids are important for a wide range of metabolic functions.

n-3 Fatty Acids

Quantitatively, the major *n*-3 PUFA in the diet is α -linolenic acid (18:3*n*-3). The major dietary source of this fatty acid is soybean and canola oils. Two metabolically important *n*-3 PUFA that occur in lower amounts in the diet are eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3). These fatty acids are sometimes referred to as very long-chain *n*-3 fatty acids (Table 1). The major dietary source of these fatty acids is marine oils found in seafood. Higher intakes of fish or very long-chain *n*-3 fatty acids has been associated with decreased risk of coronary heart disease (Figure 5). The beneficial effects of EPA and DHA has been attributed to a variety of factors, including lower rates of ventricular fibrillation resulting in a decrease of sudden death, as well as lower triglyceride concentrations, platelet

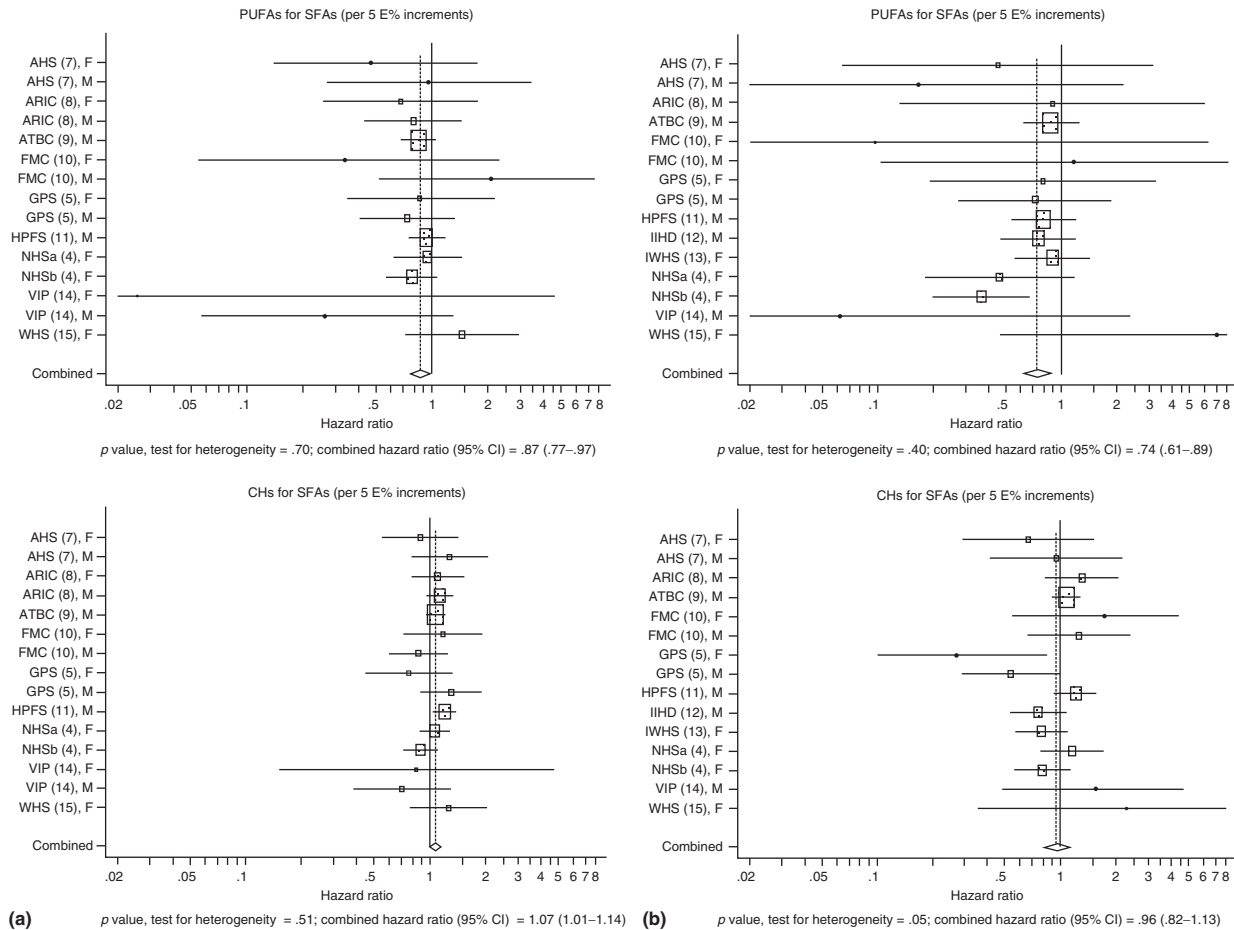


Figure 3 Study-specific and combined hazard ratios and 95% *cis* for (a) coronary events and (b) coronary deaths in the pooling project of cohort studies on diet and coronary disease. The squares and horizontal lines represent the study-specific hazard ratios and 95% *cis*, respectively. The area of the squares reflects the study-specific weight (inverse of the variance). The diamonds represent the combined hazard ratios and 95% CI. **Abbreviations:** AHS, Adventist Health Study; ARIC, Atherosclerosis Risk in Communities Study; ATBC, α -Tocopherol and β -Carotene Cancer Prevention Study; FMC, Finnish Mobile Clinic Health Study; GPS, Glostrup Population Study; HPFS, Health Professionals Follow-Up Study; IIHD, Israeli Ischemic Heart Disease Study; IWHS, Iowa Women's Health Study; NHSa, Nurses' Health Study 1980; NHSb, Nurses' Health Study 1986; VIP, Västerbotten Intervention Program; WHS, Women's Health Study; CH, carbohydrate; CI, confidence interval. Reproduced from Jakobsen MU, O'Reilly EJ, Heitmann BL, *et al.* (2009) Major types of dietary fat and risk of coronary disease: A pooled analysis of 11 cohort studies. *American Journal of Clinical Nutrition* 89(5): 1425–1432.

aggregation, and blood pressure. Similar effects of α -linolenic acid have not been consistently reported. Although humans have the capacity to convert α -linolenic acid to EPA and DHA, the efficiency is low, estimated at approximately 3–5%.

trans-Fatty Acids

trans-Fatty acids, by definition, contain at least one double bond in the *trans* configuration (Figure 6). They can either be MUFA or PUFA. Dietary *Trans*-fatty acids occur naturally in meat and dairy products as a result of anaerobic bacterial fermentation in ruminant animals and subsequent absorption and deposition in milk and muscle. *trans*-Fatty acids are also introduced into the foods when they are prepared with partially hydrogenated fat. Partial hydrogenation results in a number of changes in the fatty acyl chains, such as conversion of *cis* to *trans* double bonds, saturation of double bonds, and

migration of double bonds along the acyl chain. All these effects result in multiple geometric and positional isomers. Vegetable oils are partially hydrogenated to increase viscosity (change a liquid oil into a semiliquid or solid) and to extend shelf life (decrease susceptibility to oxidation). The major source of dietary *trans*-fatty acids worldwide is from partially hydrogenated fat, primarily in products, such as commercially fried foods and baked goods. However, over the past decade, there has been a shift away from the use of partially hydrogenated fats by the food industry. In some cases, this change was spurred by consumer demand, and in some cases, by legislation. It is expected that this trend away from partially hydrogenated fats will continue.

The shifts away from the use of partially hydrogenated fat can be traced to the early 1990s, when the first reports emerged on the relationship between *trans*-fatty acid intake and lipoprotein concentrations and subsequent CVD risk. Similar to SFA, *trans*-fatty acids increase LDL-C concentrations; however,

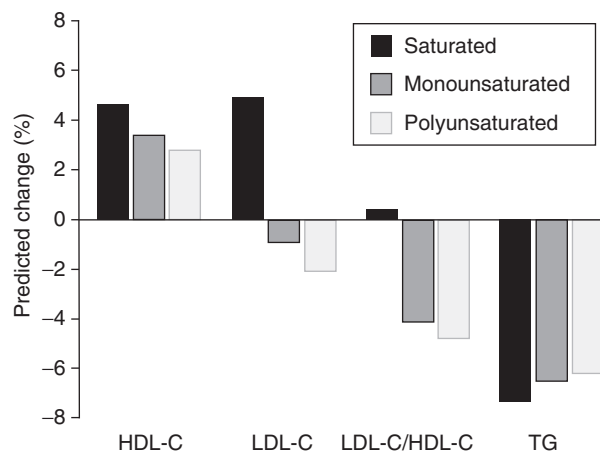


Figure 4 Predicted changes based on replacement of 5% of energy as carbohydrates with specific fatty acids under isocaloric conditions. Reproduced from Hu FB and Willett WC (2002) Optimal diets for prevention of coronary heart disease. *Journal of the American Medical Association* 288(20): 2569–2578.

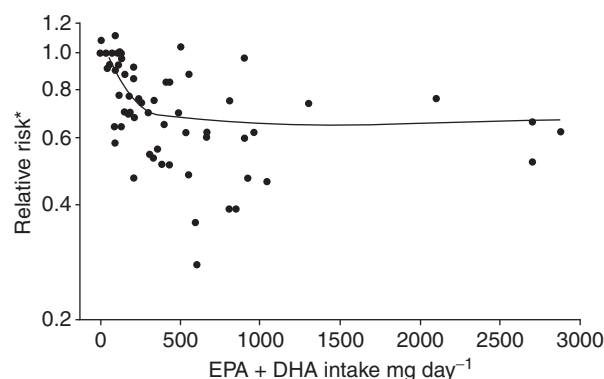


Figure 5 Relationship between intake of fish or fish oil and relative risks of coronary heart disease death in prospective cohort studies and randomized clinical trials. Reproduced from Mozaffarian D and Rimm EB (2006) Fish intake, contaminants, and human health: Evaluating the risks and the benefits. *Journal of the American Medical Association* 296(15): 1885–1899.

in contrast to SFA, they do not raise HDL-C concentrations. The changes result in a higher, less favorable effect on total:HDL-C or LDL-C:HDL-C ratios, with respect to CVD risk (Figure 7). A trend toward higher triglyceride concentrations has frequently been reported. Some researches have also suggested that *trans*-fatty acids may increase Lp(a) concentrations. Concentrations of Lp(a) are positively correlated with the risk of developing CVD. However, at present, it appears that the magnitude of increase in Lp(a) concentrations reported is not within the biological range predicted to alter CVD risk.

Estimates from 14 west European countries report *trans*-fatty acid intakes ranging from 0.8% (Greece) to 1.9% (Iceland) of energy in women and 0.5% (Greece and Italy) to 2.1% (Iceland) of energy in men. Data collected in USA and

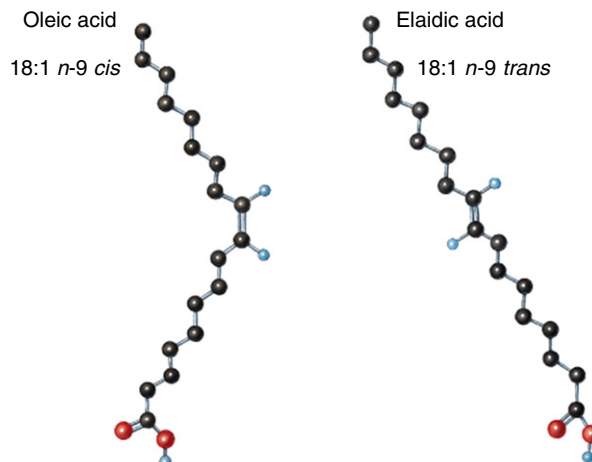


Figure 6 Geometric isomers of 18:1 n -9 (oleic and elaidic acids). Reproduced from Ascherio A, Katan MB, Zock PL, Stampfer MJ, and Willett WC (1999) *trans*-Fatty acids and coronary heart disease. *New England Journal of Medicine* 340(25): 1994–1998.

Canada suggest average *trans*-fatty acid intakes ranging from 1% to 2.5% of energy. By way of contrast, estimates of SFA intake range from 10% to 19% of energy. With the shift away from the use of partially hydrogenated fat, intakes of *trans*-fatty acids are likely to decrease with time.

More recent interest has focused on potential differences in response to dietary *trans*-fatty acids from rudiment and partially hydrogenated fat. Although in some cases small differences have been identified, most evidence suggests that there is a little difference between the two dietary sources of *trans*-fatty acids with respect to plasma lipid and lipoprotein response.

Dietary Cholesterol

The observation that dietary cholesterol increased plasma total cholesterol concentrations and was associated with the development of arteriosclerosis was originally made early in the twentieth century from studying rabbits. In humans, a positive correlation has been repeatedly observed between dietary cholesterol and both plasma cholesterol concentrations and CVD risk; although relative to SFA, the effect is modest. Whether the increase in plasma cholesterol concentrations induced by dietary cholesterol is linear or curvilinear, or whether there is a break point or threshold/ceiling relationship beyond which individuals are no longer responsive, remains to be determined. Currently, the mean dietary cholesterol in most Western countries is well below 300 mg day⁻¹. At that level of intake, the effect of SFA would be predicted to far exceed the effect of dietary cholesterol on elevating LDL-C concentrations.

Dietary cholesterol is present only in foods of animal origin. Therefore, restricting dietary SFA is likely to result in a decrease in dietary cholesterol. A notable exception is the egg, which provides a concentrated source of dietary cholesterol but not SFA.

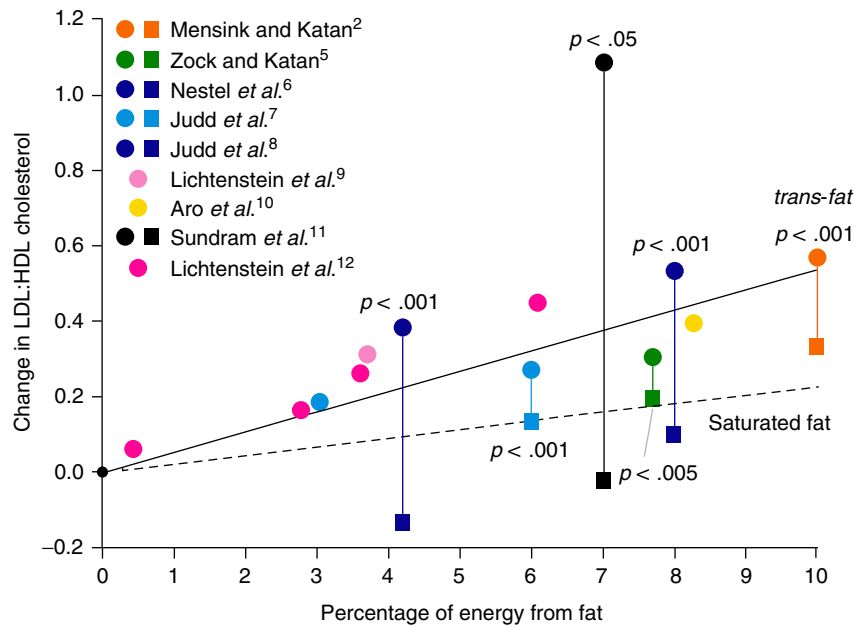


Figure 7 Change in LDL:HDL cholesterol ratio in response to *trans*-fatty acids (solid line) and saturated fatty acids (dashed line). Reproduced from Ascherio A, Katan MB, Zock PL, Stampfer MJ, and Willett WC (1999) *trans*-Fatty acids and coronary heart disease. *New England Journal of Medicine* 340(25): 1994–1998.

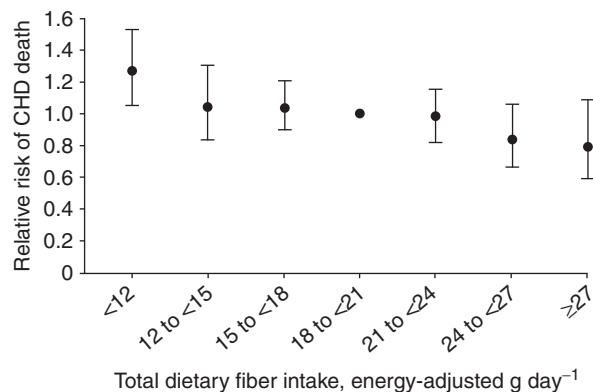


Figure 8 Relative risk of death from coronary heart disease by category of total dietary fiber intake. Reproduced from Pereira MA, O'Reilly E, Augustsson K, *et al.* (2004) Dietary fiber and risk of coronary heart disease: A pooled analysis of cohort studies. *Archives of Internal Medicine* 164(4): 370–376.

Other Dietary Considerations for the Prevention and Management of CVD

Fiber

Dietary soluble fiber, primarily β -glucan, has been reported to have a modest independent effect on decreasing LDL-C concentrations. Data from a pooled analysis of 11 cohort studies reported for a 6–10-year period of time concluded that every 10 g day⁻¹ increment of energy-adjusted total dietary fiber was associated with a 14% lower relative risk of all coronary events and a 27% lower relative risk of coronary death (Figure 8). Most evidence suggests that soluble fiber exerts its hypocholesterolemic effect by binding bile acids and cholesterol

in the intestine, resulting in an increased fecal loss and altered colonic metabolism of bile acids. The fermentation of fiber polysaccharides in the colon yields short-chain fatty acids. Some evidence suggests that these compounds may have hypocholesterolemic effects via alterations in hepatic metabolism.

At present, there is no evidence that insoluble fiber has a significant effect on plasma lipid concentrations. Nevertheless, data from observational studies consistently report a significant negative association between cereal fiber (rich in insoluble fiber) and CVD outcomes but not with vegetable and fruit fiber (rich in soluble fiber).

Plant Sterols (Phytosterols)

The term sterol represents a group of compounds that are essential constituents of cell membranes in animals and plants. Cholesterol is the major sterol of mammalian cells. Phytosterols, such as β -sitosterol, campesterol, and stigmasterol, are the major sterols of plant cells (Figure 9). In humans, plant sterols cannot be synthesized, are poorly absorbed, and interfere with cholesterol absorption. It is this latter property that has been exploited for use of these compounds as LDL-C-lowering agents. Maximal LDL-C lowering attributable to plant sterols occurs at a dose of approximately 2 g day⁻¹ (Figure 10). Although a relatively wide range of responses have been reported, most work suggests a maximal expected LDL-C lowering of approximately 10% in hypercholesterolemic subjects. This response can be in addition to other approaches to lower LDL-C concentrations, such as the use of statins. Plant sterol-enriched margarines, orange juice, yogurts, and other foods are currently available in some countries. In some cases, the saturated form of the plant sterol, plant stanols, are added to the products (Figure 9). With

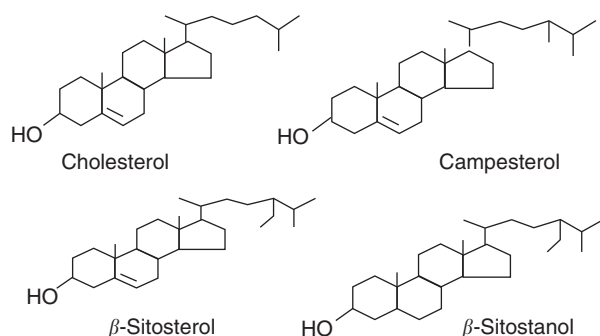


Figure 9 Structures of cholesterol, and the phytosterols campesterol, β -sitosterol, and sitostanol. Reproduced from Katan MB, Grundy SM, Jones P, Law M, Miettinen T, and Paoletti R (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clinic Proceedings* 78(8): 965–978.

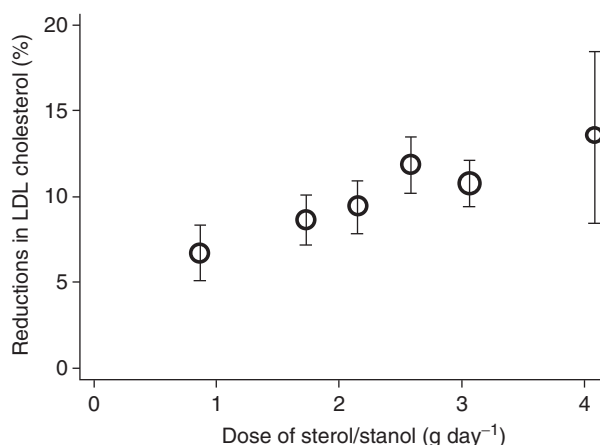


Figure 10 Summary estimates from randomized placebo-controlled trials of the percentage reductions in LDL-C according to dose. The area of each circle is proportional to the total number of persons in the trials in each dose range. Reproduced from Katan MB, Grundy SM, Jones P, Law M, Miettinen T, and Paoletti R (2003) Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. *Mayo Clinic Proceedings* 78(8): 965–978.

respect to LDL-C lowering, the efficacy of plant sterols and stanols are similar. It is important to note that if patients are counseled to incorporate a plant sterol-enriched food into their diet, it is important to stress that the food should displace, rather than supplement, habitual intakes. It is also important to stress that the maximal effective dose is approximately 2 g day⁻¹, and no benefit can be anticipated from intakes above that amount.

Vitamin Supplements

In the past, considerable interest had been generated for the potential benefit of supplementation with vitamin C, β -carotene, vitamin E, or folate in reducing CVD risk. Support for this approach came from a number of different venues. Some epidemiological observations suggested that antioxidant vitamins from either diet or supplements were associated with

decreased CVD rates. *In vitro* work demonstrated the potential of antioxidant vitamins to decrease the susceptibility of the LDL particles to oxidation after exposure to a pro-oxidant. Subsequent cell culture studies demonstrated that oxidized LDL is taken up more avidly than native LDL, thereby promoting cholesterol accumulation in blood vessel walls.

Observational studies reported a positive association between plasma homocysteine concentrations and subsequent CVD risk. Elevated homocysteine concentrations are related to compromised folate status. Folate supplementation reduces homocysteine concentrations. Despite the biologically plausible link, randomized controlled intervention trials have failed to demonstrate a benefit of folate supplementation and CVD outcomes.

At present, the data do not support a recommendation to use antioxidant vitamins or folate supplementation for the prevention or management of CVD. Current interest is now on vitamin D and CVD outcomes. In contrast to prior work on vitamins and CVD outcomes where the focus was on supplemental amounts of the above-mentioned nutrients that are known to prevent deficiency states, the potential relationship between vitamin D and CVD is focused on nutrient insufficiency. The outcome of ongoing randomized control trials is needed to test the hypothesis of a cause and effect relationship.

Conclusions

The relationship between diet, lipoprotein profiles and CVD risk is clearly established. The current recommendation is to displace SFA and *trans*-fatty acids with unsaturated fatty acids, particularly PUFA. Additional recommendations include restricting dietary cholesterol; consuming at least two fish meals per week to ensure adequate *n*-3 fatty acid intake; and consuming fiber rich diets, including those found in whole grains, fruits, and vegetables. Daily intake of plant sterols (phytosterols) can result in an additional lowering of plasma LDL-C concentrations. At this time, the data do not support the use of nutrient supplements for the prevention or treatment of CVD. Attainment or maintenance of a healthy body weight should be emphasized along with engagement in regular physical activity. These recommendations are the culmination of more than a century of work. They have evolved slowly and continue to be fine tuned as new findings emerge. There is no doubt that further modifications will occur. It is important for nutrition scientists to implement current recommendations aimed at optimizing plasma lipoprotein profiles and favorably affecting newer surrogate markers of CVD risk, and to reassess these recommendations as new findings emerge.

See also: Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Coronary Heart Disease: Lipid Theory; Prevention. Dietary Fiber: Physiological Effects and Health Outcomes. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Fatty acids: Health Effects of Saturated Fatty Acids; Metabolism; Hyperlipidemia: Overview. *Trans*-Fatty Acids: Health Effects, Recommendations, and Regulations

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HYPERTENSION

Dietary Factors

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Worldwide, elevated blood pressure is an extraordinarily common and important risk factor for cardiovascular and kidney diseases. As blood pressure (BP) rises, so does the risk of these diseases (Figure 1). The relationship is strong, consistent, continuous, independent, and etiologically relevant. Accordingly, the adverse consequences of elevated BP are not just restricted to individuals with hypertension (a systolic BP (SBP) ≥ 140 mm Hg or a diastolic BP ≥ 90 mm Hg). Those with prehypertension, namely, a SBP of 120–139 mm Hg or diastolic BP of 80–89 mm Hg, have a high probability of developing hypertension and carry an excess risk of cardiovascular disease compared to those with a normal BP (SBP < 120 mm Hg and diastolic BP < 90 mm Hg). In fact, almost one-third of BP-related deaths from coronary heart disease occur in individuals with BP in the nonhypertensive range. It has been estimated that

54% of strokes and 47% of coronary heart disease events can be attributed to elevated BP.

In Western countries and most economically developing countries, SBP rises with age in both children and adults. As a consequence, the lifetime risk of developing hypertension is extremely high, approximately 90% among US adults older than the age of 50 years. However, the rise in BP with age is not inevitable. There are numerous isolated populations in which the rise in BP is blunted or even flat. These populations are typically characterized by extremely low intakes of salt, relatively high intakes of potassium, and a lean body habitus.

Lifestyle modification, which includes dietary changes and increased physical activity, has important roles in both nonhypertensive and hypertensive individuals. In nonhypertensive individuals, including those with prehypertension,

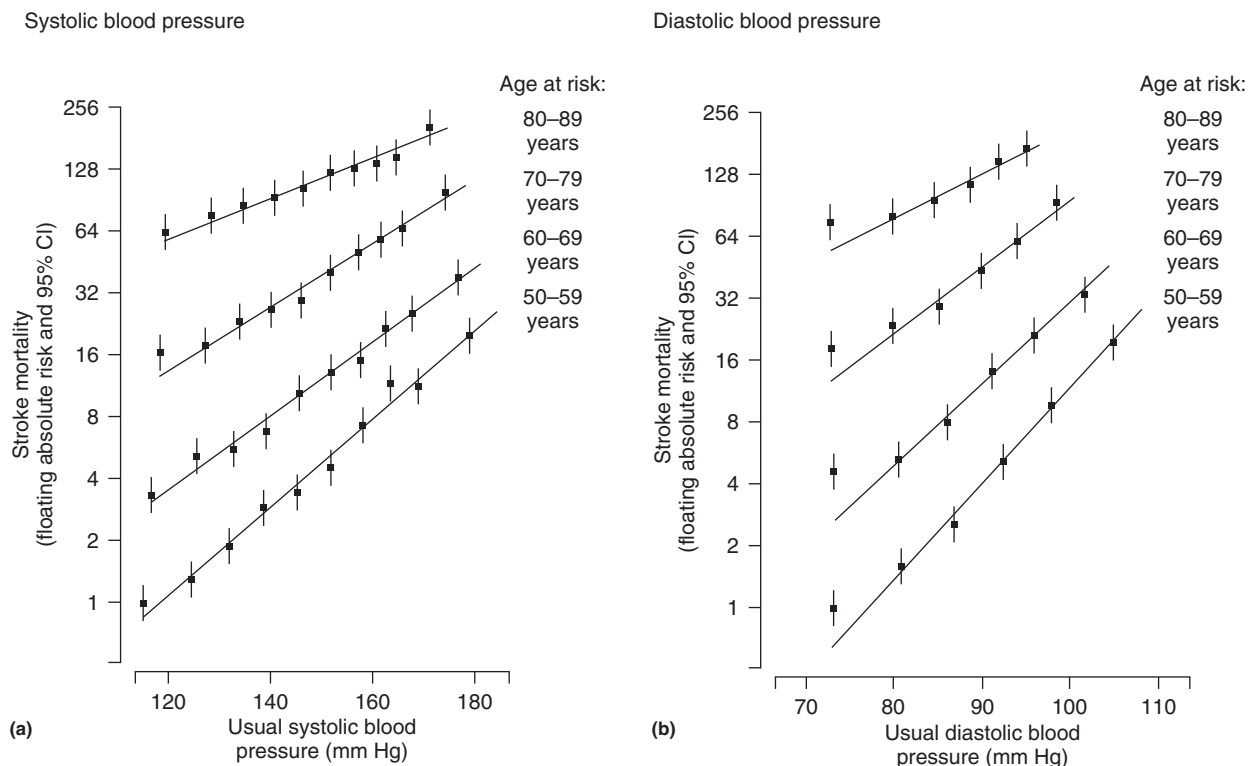


Figure 1 Stroke mortality rate by decade of age versus SBP (a) and diastolic BP (b): meta-analysis of 61 prospective studies with 2.7 million person-years. Reproduced from Lewington S, Clarke R, Qizilbash N, Peto R, and Collins R (2002) Age-specific relevance of usual blood pressure to vascular mortality: A meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet* 360: 1903–1913.

lifestyle modifications have the potential to prevent hypertension, reduce BP, and thereby lower the risk of BP-related cardiovascular disease. Even an apparently small reduction in BP, if applied to an entire population, could have an enormous beneficial impact. It has been estimated that a 3 mm Hg reduction in SBP could lead to an 8% reduction in stroke mortality and a 5% reduction in mortality from coronary heart disease (Figure 2). In hypertensive individuals, lifestyle modifications can serve as initial treatment before the start of drug therapy and as an adjunct to medication in people already on antihypertensive drug therapy. In hypertensive individuals with medication-controlled BP, lifestyle therapies can facilitate drug step-down and potentially drug withdrawal in individuals who sustain lifestyle changes.

Dietary Factors That Lower BP

Weight Loss

On average, as weight increases, BP also increases. The importance of this relationship is reinforced by the high and increasing prevalence of overweight and obesity throughout the world. With rare exception, clinical trials have documented that weight loss lowers BP. Importantly, reductions in BP occur before and without attainment of a desirable body weight. In one meta-analysis that aggregated results across 25 trials, mean SBP and diastolic BP reductions from an average weight loss of 5.1 kg were 4.4 and 3.6 mm Hg, respectively. Greater weight loss leads to greater BP reduction. Still, the long-term effects of weight loss on BP are unclear, with some studies suggesting that BP reductions attenuate over time.

Additional trials have documented that modest weight loss can prevent hypertension by approximately 20% among overweight, prehypertensive individuals, and can facilitate medication step-down and drug withdrawal. Lifestyle intervention trials have uniformly achieved short-term weight loss, primarily through a reduction in total calorie intake. In some instances, substantial weight loss has also been sustained over 3 or more years.

In aggregate, available evidence strongly supports weight reduction, ideally attainment of a body mass index less than

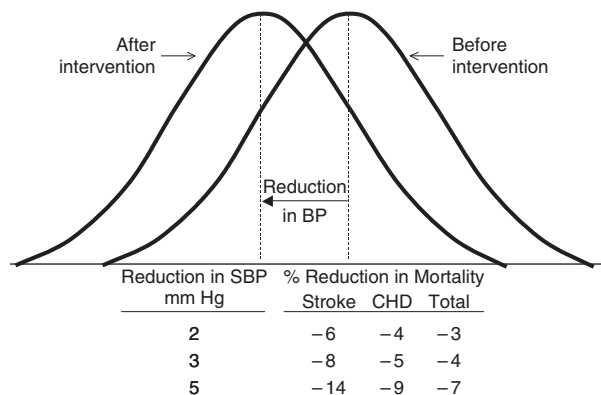


Figure 2 Estimated effects of population-wide shifts in SBP on mortality. Reproduced from Stamler R (1991) Implication of the intersalt study. *Hypertension* 17: 1-16-1-20, with permission from LWW.

25 kg m⁻², as an effective approach to prevent and treat hypertension. Weight reduction can also prevent diabetes and control lipids. Hence, the beneficial effects of weight reduction in preventing cardiovascular-renal disease should be substantial. Finally, in view of the well-recognized challenges of maintaining weight loss, efforts to prevent weight gain among those with a normal body weight are critical.

Reduced Salt Intake

On average, as dietary salt (sodium chloride) intake rises, there is rise in BP. (In view of the format of published data and of dietary recommendations, data are presented as g/day (mmol/day) of sodium rather than g/day of salt.) Till date, more than 50 randomized trials have tested the effects of salt on BP, including several dose-response trials. Approximately 10 meta-analyses have aggregated data across these trials. In a recent meta-analysis that focused on moderate reductions in salt intake, a reduced sodium intake of 1.8 g day⁻¹ (77 mmol day⁻¹) led to average SBP/diastolic BP reductions of 5.2/3.7 mm Hg in hypertensives and 1.3/1.1 mm Hg in nonhypertensives.

One of the most important dose-response trials is the Dietary Approaches to Stop Hypertension (DASH)-Sodium trial, which tested the effects of three different salt intakes separately in two distinct diets – the DASH diet and a control diet more typical of what Americans eat. As displayed in Figure 3, the rise in BP with higher salt intake was evident in both diets. The BP response to salt intake was nonlinear. Specifically, decreasing salt intake caused a greater lowering of BP when the starting sodium intake was less than 2.3 g day⁻¹ (100 mmol day⁻¹) than when it was above this level.

The BP response to changes in salt intake is heterogeneous. Despite the use of the terms 'salt sensitive' and 'salt resistant' to classify individuals in research studies, the change in BP in

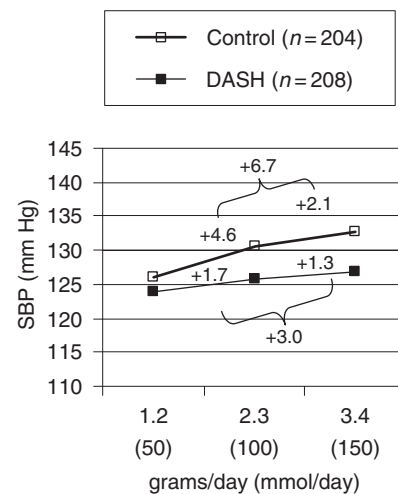


Figure 3 Mean SBP change in the DASH-Sodium trial from salt reduction in two diets and from the DASH diet at three salt levels. Reproduced with permission from Sacks FM, Svetkey LP, Vollmer WM, et al. (2001) A clinical trial of the effects on BP of reduced dietary sodium and the DASH dietary pattern (The DASH-Sodium Trial). *New England Journal of Medicine* 344: 3-10.

response to a change in salt intake is not binary. Instead, the change in BP from a reduced salt intake has a continuous distribution, with individuals having greater or lesser degrees of BP reduction. Genetic factors influence the response to salt reduction. Concomitant diet also modifies the effects of salt on BP. The rise in BP for a given increase in salt intake is blunted in the setting of either the DASH diet or a high potassium intake (Figure 3). In general, the effects of salt on BP tend to be greater in blacks, middle-aged and older people, and individuals with hypertension, diabetics, or chronic kidney disease. Although it is possible to identify groups that tend to be salt sensitive, it is impossible, given currently available diagnostic tools, to identify individuals who are salt sensitive.

In addition to lowering BP, clinical trials have documented that a reduced salt intake can prevent hypertension by approximately 20% (with or without concomitant weight loss) and can lower BP in the setting of antihypertensive medication. Evidence from observational studies suggests that a reduced salt intake can blunt the age-related rise in SBP (Figure 4). A reduced salt intake may also reduce the risk of left ventricular hypertrophy, osteoporosis, and gastric cancer. Still, the evidence base does have limitations, largely because of the difficulty in measuring sodium intake. For this reason, results from observational studies have been inconsistent and occasionally paradoxical.

Still, the effects of salt on health have been debated. Some have argued that the increases in plasma renin activity and perhaps insulin resistance that occur as a result of a reduced salt intake mitigate the beneficial effects of salt reduction on BP. However, in contrast to BP, the clinical relevance of increased plasma renin activity is uncertain, especially because antihypertensive medications that raise plasma renin levels actually lower cardiovascular disease risk. It has also been argued that a reduced salt intake has little or no effect on BP in many individuals and that other aspects of diet (e.g., increased potassium intake or adoption of a mineral-rich diet) mitigate the harmful effects of salt on BP. Although one cannot guarantee that all individuals will achieve a lower BP from salt reduction, the fraction of individuals who will benefit is substantial.

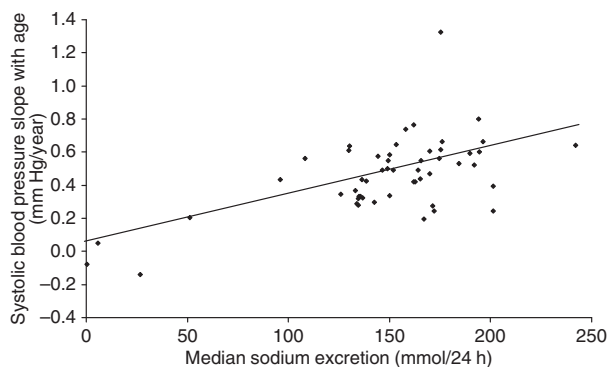


Figure 4 Slope of SBP increase with age plotted by median sodium excretion in 52 communities worldwide: Results from intersalt. Reproduced with permission from Rose G, Stamler J, Stamler R, et al. (1988) Intersalt: An international study of electrolyte excretion and BP. Results for 24 h urinary sodium and potassium excretion. *British Medical Journal* 297: 319–328.

In view of the progressive dose–response relationship between salt intake and BP, it is difficult to set specific levels for dietary recommendations. An Institute of Medicine committee set 1.5 g day⁻¹ (65 mmol day⁻¹) of sodium as an adequate intake level and 2.3 g day⁻¹ (100 mmol day⁻¹) as an upper limit. Western-type diets that provide 1.5 g day⁻¹ (65 mmol day⁻¹) have been shown to provide adequate levels of other nutrients. This level of salt intake also allows for excess sweat salt loss among unacclimatized individuals who become physically active or who become exposed to high temperatures. In the United States, the recommended upper limit for adults is 2.3 g day⁻¹ (100 mmol day⁻¹) of sodium with a more stringent recommended intake level of 1.5 g day⁻¹ (65 mmol day⁻¹) in blacks, hypertensive, and middle- and older-aged individuals.

In most Western counties, average intake of sodium is high, greatly exceeding 2.3 g day⁻¹ (100 mmol day⁻¹). In the United States, the median intake of sodium from foods, not including salt added at the table, varies by age and, according to a recent survey, ranges from 3.1 to 4.7 g day⁻¹ (135–204 mmol day⁻¹) in adult men and 2.3–3.1 g day⁻¹ (100–135 mmol day⁻¹) in adult women. Worldwide, there is greater variation in sodium intake, ranging from an estimated mean intake of 0.02 g day⁻¹ (1.0 mmol day⁻¹) in Yanomamo Indians to more than 10.3 g day⁻¹ (450 mmol day⁻¹) in northern Japanese.

In aggregate, available data strongly support current population-wide recommendations to lower salt intake. To reduce salt intake, consumers should choose foods low in salt and limit the amount of salt added to food. However, even motivated individuals find it difficult to reduce salt intake because more than 75% of consumed salt comes from processed foods (Figure 5). Hence, any meaningful strategy to reduce salt intake must involve the efforts of food manufacturers, who should reduce the amount of salt added during food processing.

Increased Potassium Intake

High levels of potassium intake are associated with reduced BP. Observational data have been reasonably consistent in documenting this inverse relationship, whereas data from individual trials have been less consistent. However, three meta-analyses of these trials have each documented a significant inverse relationship between potassium intake and BP. In one meta-analysis, average net SBP/diastolic BP reductions from

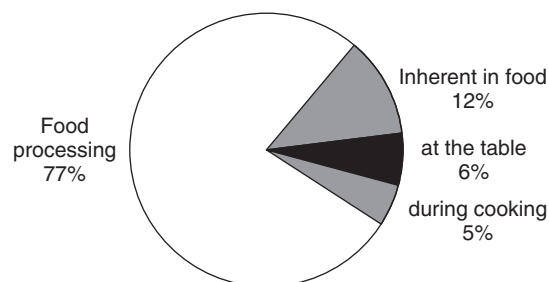


Figure 5 Sources of dietary sodium. Data from Mattes RD and Donnelly D (1991) Relative contributions of dietary sodium sources. *Journal of the American College of Nutrition* 10: 383–393.

increased potassium intake were 4.4/2.4 mm Hg. Available studies have documented greater BP reductions from potassium in African Americans compared to non-African Americans and in hypertensive compared to nonhypertensive individuals. A high potassium intake has been shown to blunt the rise in BP in response to increased salt intake. Potassium has greater BP lowering in the context of a higher salt intake and lesser BP reduction in the setting of a lower salt intake. Conversely, the BP reduction from a reduced salt intake is greatest when potassium intake is low. These data are consistent with subadditive effects of reduced salt intake and increased potassium intake on BP.

Most trials that tested the effects of potassium on BP used pill supplements, typically potassium chloride. However, in foods, the conjugate anions associated with potassium are mainly citrate and other bicarbonate precursors. The latter is important because other potential benefits of foods rich in potassium (i.e., reduced risk of kidney stones and reduced bone turnover) likely result from effects of the conjugate anion. Because a high dietary intake of potassium can be achieved through diet rather than pills and because potassium derived from foods also comes with a variety of other nutrients, the preferred strategy to increase potassium intake is to consume foods, such as fruits and vegetables, rather than supplements.

On the basis of available data, an Institute of Medicine committee set an Adequate Intake for potassium of 4.7 g day⁻¹ (120 mmol day⁻¹) for adults. This level of dietary intake should maintain lower BP levels, reduce the adverse effects of salt on BP, reduce the risk of kidney stones, and possibly decrease bone loss. Currently, dietary intake of potassium is considerably lower than this level. In recent surveys, the median intake of potassium by adults in the United States was approximately 2.9–3.2 g day⁻¹ (74–82 mmol day⁻¹) for men and 2.1–2.3 g day⁻¹ (54–59 mmol day⁻¹) for women. Because African Americans have a relatively low intake of potassium and a high prevalence of elevated BP and salt sensitivity, this subgroup of the population would especially benefit from an increased potassium intake.

In the generally healthy population with normal kidney function, a potassium intake from foods higher than 4.7 g day⁻¹ (120 mmol day⁻¹) poses no potential for increased risk because excess potassium is readily excreted in the urine. However, in individuals whose urinary potassium excretion is impaired, a potassium intake of less than 4.7 g day⁻¹ (120 mmol day⁻¹) is appropriate because of adverse cardiac effects (arrhythmias) from hyperkalemia. Common drugs that impair potassium excretion are angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and potassium-sparing diuretics. Medical conditions associated with impaired potassium excretion include diabetes, chronic renal insufficiency, end stage renal disease, severe heart failure, and adrenal insufficiency. Elderly individuals are at increased risk of hyperkalemia because they often have one or more of these conditions or take one or more of the medications that impair potassium excretion.

Moderation of Alcohol Intake

The relationship between alcohol intake and BP is direct and progressive, particularly at an alcohol intake above approximately two drinks per day (~1 oz. or ~28 g of

ethanol per day). A meta-analysis of 15 trials reported that decreased consumption of alcohol (median reduction in self-reported alcohol consumption of 76%) lowered SBP and diastolic BP by 3.3 and 2.0 mm Hg, respectively. In non-hypertensives and hypertensives, BP reductions were similar. In aggregate, evidence supports moderation of alcohol intake (among those who drink) as an effective approach to lower BP. It is recommended that alcohol consumption be limited to no more than 1 oz. (30 ml) of ethanol (e.g., 24 oz. (720 ml) beer, 10 oz. (300 ml) wine, or 2 oz. (60 ml) 100-proof whiskey) per day in most men and to no more than 0.5 oz. (15 ml) ethanol per day in women and lighter weight people.

Whole Dietary Patterns

Vegetarian Diets

Vegetarian diets have been associated with low BP. In observational studies, vegetarians also experience a markedly lower, age-related rise in BP. Aspects of a vegetarian lifestyle that might affect BP include nondietary factors (e.g., physical activity), established dietary risk factors (e.g., salt, potassium, weight, and alcohol), and other aspects of a vegetarian diet (e.g., high fiber and no meat). To a very limited extent, observational studies have controlled for the well-established determinants of BP. Hence, it is unclear whether BP reductions result from established dietary risk factors that affect BP or from other aspects of a vegetarian diet.

DASH-Style Dietary Patterns

The DASH trial tested whether modification of whole dietary patterns might affect BP. In this trial, participants were randomized to eat one of three diets: (1) a control diet; (2) a diet rich in 'fruits and vegetables' but otherwise similar to control; or (3) the DASH diet. The DASH diet emphasizes fruits, vegetables, and low-fat dairy products, includes whole grains, poultry, fish, and nuts; and is reduced in fats, red meat, sweets, and sugar-containing beverages. Accordingly, it is rich in potassium, magnesium, calcium, and fiber and reduced in total fat, fat, and cholesterol; it is also slightly increased in protein.

Among all participants, the DASH diet significantly lowered mean SBP by 5.5 mm Hg and mean diastolic BP by 3.0 mm Hg. The fruits and vegetables diet also reduced BP but to a lesser extent – approximately half of the effect of the DASH diet. The effect was relatively rapid; the full effect was apparent after 2 weeks (**Figure 6**). In subgroup analyses, the DASH diet significantly lowered BP in all major subgroups (men, women, African Americans, non-African Americans, hypertensives, and nonhypertensives). However, the effects of the DASH diet were especially prominent in African Americans, who experienced net SBP/diastolic BP reductions of 6.9/3.7 mm Hg, and hypertensive individuals, who experienced net BP reductions of 11.6/5.3 mm Hg.

Subsequently, the OmniHeart trial compared three variants of the DASH diet (a diet rich in carbohydrate (58% of calories), a second version rich in protein (approximately half from plant sources), and a third diet rich in unsaturated fat (predominantly monounsaturated fat)). In several respects, each diet was similar to the DASH diet – each was reduced in saturated fat, cholesterol, and sodium, and rich in fruit,

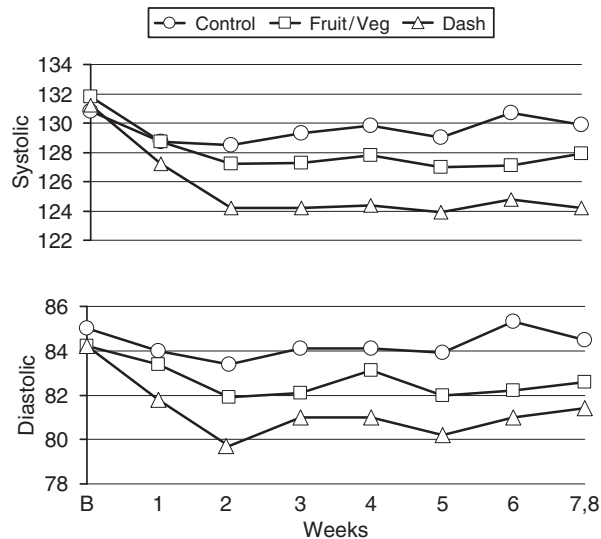


Figure 6 BP by week during the DASH feeding study in three diets (control diet, fruits and vegetables diet, and DASH diet). Reproduced with permission from Appel LJ, Moore TJ, Obarzanek E, *et al.* (1997) The effect of dietary patterns on BP: Results from the Dietary Approaches to Stop Hypertension (DASH) clinical trial. *New England Journal of Medicine* 336: 1117–1124.

vegetables, fiber, and potassium at recommended levels. Although each diet lowered SBP, substituting some of the carbohydrate (approximately 10% of total kcal) with either protein (approximately half from plant sources) or with unsaturated fat (mostly monounsaturated fat) further lowered BP.

Results from the DASH and OmniHeart trials have important clinical and public health implications. The effect of the DASH diet in hypertensive individuals was similar in magnitude to that of drug monotherapy. From a public health perspective, the DASH diet could potentially shift the population distribution of BP downward, thereby reducing the risk of BP-related cardiovascular disease (Figure 2).

Fish Oil Supplementation

High-dose, ω -3 polyunsaturated fatty acid (commonly termed 'fish oil') supplements can lower BP in hypertensive individuals. In a meta-analysis of trials, average SBP and diastolic BP reductions in hypertensive individuals were 5.5 and 3.5 mm Hg, respectively. The effect of fish oil appears to be dose dependent, with BP reductions only occurring at relatively high doses, namely 3 g day^{-1} or more. In nonhypertensive individuals, BP reductions were nonsignificant and small. Side effects, including belching and a fishy taste, are common. In view of the side effect profile and the high dose required to lower BP, fish oil supplements are not routinely recommended.

Dietary Factors with Limited or Uncertain Effect on BP

Fiber

Evidence from observational studies and several clinical trials suggests that increased fiber intake may reduce BP.

A meta-analysis documented that supplemental fiber (average increase of 14 g day^{-1}) was associated with net systolic/diastolic reductions of 1.6/2.0 mm Hg, respectively. Still, high-quality epidemiological studies and clinical trials are needed before one can recommend increased fiber intake as a means to lower BP.

Calcium and Magnesium

Evidence that increased calcium intake might lower BP comes from a variety of sources, including animal studies, observational studies, clinical trials, and meta-analyses. Meta-analyses of trials documented modest reductions in SBP and diastolic BP of 0.89–1.44 and 0.18–0.84 mm Hg, respectively, with calcium supplementation ($400\text{--}2000 \text{ mg day}^{-1}$). There is also evidence that calcium intake may affect the BP response to salt. Overall, data are insufficient to recommend supplemental calcium alone as a means to lower BP.

The body of evidence implicating magnesium as a major determinant of BP is inconsistent. In observational studies, often cross-sectional in design, a common finding is an inverse association of dietary magnesium with BP. However, in pooled analyses of clinical trials, there is no clear effect of magnesium intake on BP. Hence, data are insufficient to recommend increased magnesium intake alone as a means to lower BP.

Fats (Other Than Fish Oil) and Cholesterol

Numerous studies, including both observational studies and clinical trials, have examined the effects of fat intake on BP. Overall, there is no apparent effect of saturated fat and n -6 polyunsaturated fat intake on BP. Although a few trials suggest that an increased intake of monounsaturated fat may lower BP, evidence is insufficient to make recommendations. Likewise, few studies have examined the effect of dietary cholesterol intake on BP. Hence, although modification of dietary fat and cholesterol intake can be recommended as a means to prevent and treat hyperlipidemia and dyslipidemia, evidence is insufficient to recommend these changes alone as a means to lower BP.

Protein Intake

A large and generally consistent body of evidence from observational studies has documented that higher protein intake, particularly protein from plant-based sources, is associated with lower BP. In contrast to the large volume of evidence from observational studies, comparatively few trials have examined the effects of protein intake on BP. Recent trials have tested the effects of soy-based interventions on BP. In several but not all of these trials, soy supplementation reduced BP. As previously discussed, the OmniHeart trial documented that increased protein intake from mixed sources, predominantly plants, lowers BP.

Vitamin C

Laboratory studies, depletion–repletion studies, and epidemiological studies suggest that increased vitamin C intake

or status is associated with lower BP. Several trials, many with methodological limitations, have also addressed this issue. Overall, it remains unclear whether an increased intake of vitamin C lowers BP.

Gene–Diet Interactions

A rapidly increasing body of evidence indicates that genetic factors affect BP levels and the BP response to dietary changes. Most of the evidence relates to genetic factors that affect the BP response to salt. Several genotypes that influence BP have been identified. Most of these genotypes influence the renin–angiotensin–aldosterone axis or renal salt handling.

Special Populations

Children

Elevated BP begins well before adulthood, during the first two decades of life and perhaps earlier during gestation. In addition to the age-related rise in BP observed in children, numerous studies have documented that BP tracks with age from childhood into the adult years. Hence, efforts to reduce BP and to prevent the age-related rise in BP in childhood are prudent.

With the exception of sodium, few trials have tested the effects of dietary factors as a mean to lower BP in children and adolescents. In a meta-analysis of 10 trials conducted in children, sodium reduction significantly lowered BP. There is some direct evidence from studies conducted in children that the dietary determinants of BP in children and adults are similar. In this setting, the effect of diet on BP in children and adolescents is, in large part, extrapolated from studies of adults. Such extrapolations are reasonable because elevated BP is a chronic condition resulting from the insidious rise in BP throughout childhood and adulthood.

Pregnant Women

Hypertension during pregnancy is a constellation of diverse clinical conditions, some of which can be extremely serious. Of substantial concern are preeclampsia and eclampsia. Both are multisystem disorders that are manifest by the onset of hypertension and proteinuria during the second half of pregnancy. Convulsions occur in the setting of eclampsia but not preeclampsia. The cause of these disorders is unknown. Several dietary interventions, including sodium reduction, fish oil supplementation, and calcium supplementation, have been tested as a means to prevent preeclampsia, but none is considered effective. Although a meta-analysis of small trials suggested that calcium supplementation has some benefit in high-risk women, a large trial of calcium supplementation documented no benefit, either overall or in high-risk subgroups.

Older People

Because of the age-related rise in SBP and because of the high prevalence of BP-related cardiovascular disease in middle-aged

and older people, dietary strategies should be especially beneficial as adults age. It is well documented that older people can make and sustain dietary changes, specifically weight loss and dietary salt reduction. Furthermore, salt sensitivity increases as individuals age. Lastly, because of the high attributable risk associated with elevated BP in older people, the beneficial effects of dietary changes on BP should translate into substantial reductions in cardiovascular risk in this age group.

Populations Defined by Race/Ethnicity or Geography

Worldwide, there is substantial variation in BP among populations. In certain primitive societies, such as the Yanomamo Indians in Brazil, BP does not rise with age, and hypertension is absent. In rural Africa and southern China, the prevalence of hypertension is less than 20%. Among urbanized populations, the prevalence of hypertension is high, especially among African Americans, a population in which the prevalence of hypertension approaches 40%. Other groups, such as Australian Aborigines, Eastern Europeans, and Russians, also have a high prevalence of hypertension.

Understanding the causes of geographic variation is difficult. However, migration studies provide strong evidence that modifiable environmental factors (e.g., diet and physical activity) rather than genetic factors or geographic factors account for this variation. Furthermore, as noted previously, trials have documented that compared to non-African Americans, African Americans achieve greater BP reduction from several nonpharmacological therapies, specifically a reduced salt intake, increased potassium intake, and the DASH diet. The potential benefits of these dietary therapies is amplified because the US survey data indicate that African Americans consume less potassium than non-African Americans. On average, salt intake is high and similar in African Americans and non-African Americans. Hence, changes in diet should provide a means to reduce racial and perhaps geographic disparities in BP.

Conclusion

In view of the continuing epidemic of BP-related cardiovascular disease, efforts to reduce BP in both nonhypertensive and hypertensive individuals are warranted. Such efforts will require individuals to change behavior and society to make substantial environmental changes. The current challenge to health care providers, researchers, government officials, and the general public is to develop and implement effective clinical and public health strategies that lead to sustained dietary changes among individuals and more broadly among populations.

See also: Alcohol: Absorption, Metabolism and Physiological Effects. Ascorbic Acid (Vitamin C): Deficiency States. Calcium. Magnesium. Obesity: Complications. Older People: Physiological Changes. Potassium. Pregnancy: Energy Requirements and Metabolic Adaptations. Sodium: Physiology

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HYPOGLYCEMIA

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Introduction

Hypoglycemia is defined as a blood glucose concentration of 2.5 mmol l^{-1} (plasma glucose concentration of 3.0 mmol l^{-1}) or less. Its definition is necessarily arbitrary and owes its importance to the fact that hypoglycemia (literally low blood glucose) of this severity produces brain dysfunction by depriving its neurons of glucose. It must be distinguished from the symptoms to which it may or may not give rise.

Hypoglycemia is not a disease but a manifestation of it. It has, however, come to have a totally different meaning, amongst certain sections of the population, that has very little to do with blood glucose concentration but a lot to do with their feelings of well being, discomfort, and attitudes to life but, above all, with the role of diet in the achievement and maintenance of good health. And although no discussion of the dietary treatment of hypoglycemia can be meaningful without reference to this concept – referred to, for want of a better term, as nonhypoglycemia – hypoglycemia will, throughout this article, be used only to describe a condition associated with a measured low blood glucose concentration.

Brain Function and Hypoglycemia

The brain malfunction and symptoms to which hypoglycemia gives rise will be referred to as neuroglycopenia to distinguish them from a low blood glucose concentration as measured.

The brain is often thought of as being incapable of using metabolites other than glucose as a source of energy. This is untrue. It has been established for more than 35 years that the brain is able, under certain circumstances including prolonged fasting, to utilize the 'ketone bodies' β -hydroxybutyrate and acetoacetate derived from partial oxidation of fatty acids and produced exclusively in the liver. Under these circumstances the need for glucose and its supply through gluconeogenesis is drastically reduced. The survival value of this ability is immense as it permits fat stores rather than structural muscle and other tissue proteins to be utilized for maintenance of vital processes under these stressful conditions. Only when fat stores have become completely exhausted and plasma ketone levels fail to rise does the brain's demand for glucose exceed the ability of gluconeogenesis to provide it. It is at this point that hypoglycemia intervenes and portends death from starvation or inanition (see Starvation).

The Blood Glucose Concentration

Failure to appreciate the differences between arterial and venous blood glucose is a major cause of the confusion that has surrounded the recognition and diagnosis of hypoglycemia and

been responsible for nonhypoglycemia becoming a common diagnosis amongst those whom Singer and coworkers refer to as the folk sector, which includes many health writers.

In the fasting subject the concentration of glucose in arterial and venous blood is virtually identical but following ingestion of a carbohydrate rich meal it may differ by as much as 2.5 mmol l^{-1} . Because it is arterial blood glucose that determines glucose supply to the brain, regulates the secretion of insulin and other hormones, and is itself homeostatically controlled, it is necessary to define hypoglycemia in terms of glucose in arterial (or more realistically free flowing capillary) than in venous blood.

Mechanism of Hypoglycemia

The Glucose Pool in Fasting Subjects

Glucose is confined within the body to the extracellular or interstitial fluid where it is referred to as the glucose pool; detailed discussion of its regulation is outside the scope of this article except to stress that its size is reflected by the concentration of glucose in the blood. This remains remarkably constant despite huge changes in the rates of delivery and utilization of glucose, by meals and exercise (and fasting), respectively, and is described as glucose homeostasis (Figure 1). The main but far from sole regulator is insulin.

Insulin Release in Response to Eating and Fasting

After a carbohydrate-containing meal, glucose derived from food enters the portal vein. From here it is conveyed to the liver where about one-third of it is extracted and converted to glycogen. What remains unabsorbed passes into the systemic circulation, producing small and variable rises in arterial, capillary, and, initially, venous blood glucose concentrations. The modest rise in arterial blood glucose concentration perfusing the pancreas, augmented by nervous stimuli and insulinotrophic hormones, most notably glucagon-like polypeptide 1 (GLP-1) and gastric inhibitory hormone, sometimes called Glucose Dependent Insulinotrophic Peptide (GIP), and collectively called incretins. These are released from the gut in response to meals containing carbohydrate and fats and lead to the secretion of insulin in greater amounts than is occasioned simply by the rise in blood glucose concentration.

Evidence for a 'cephalic phase' of insulin secretion in humans is scanty and conflicting. Most observers have found a minimal, if any, response to the prospect of eating, or the reality of drinking, a noncalorigenic sweet drink. Others have reported insulin secretion especially in the 10 min or so after

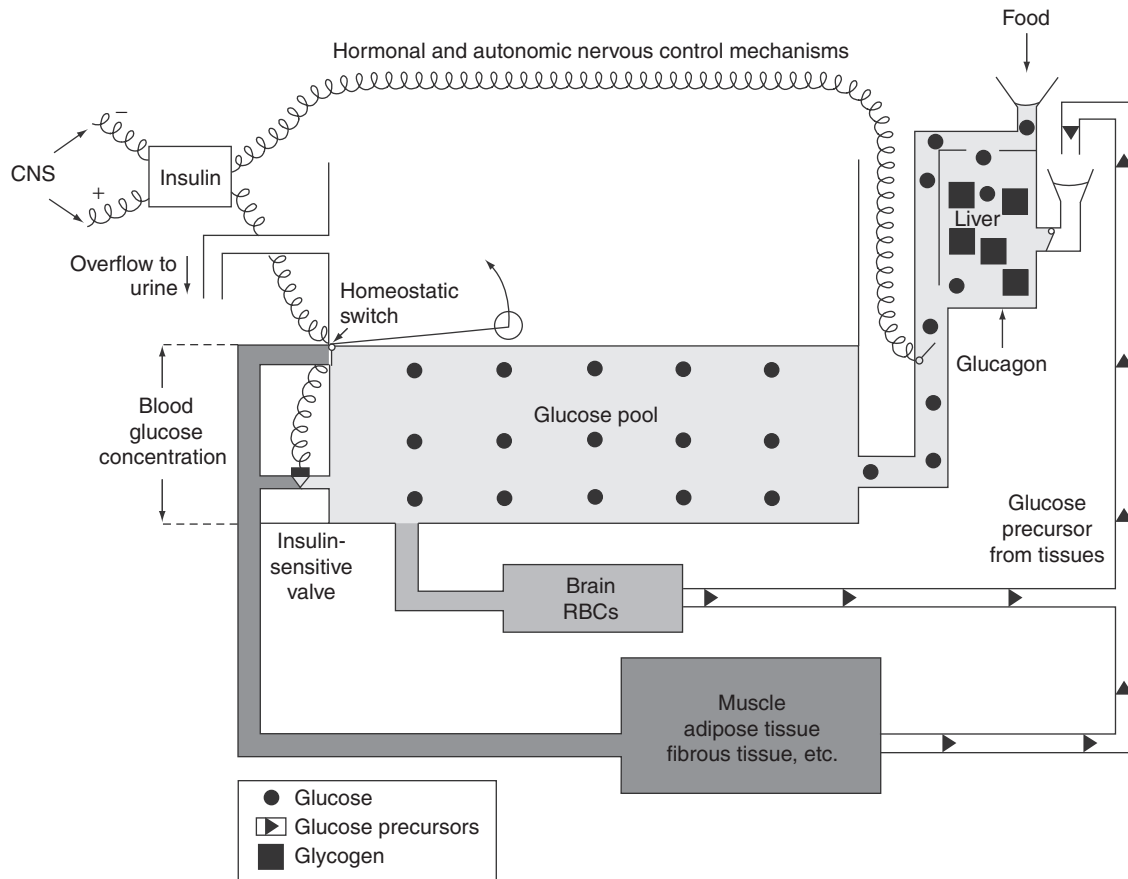


Figure 1 Schematic representation of homeostatic control of blood glucose level and mechanism of hypoglycemia. Hypoglycemia results whenever inflow of glucose from the gut or liver fails to meet the outflow of glucose from the glucose pool, which consists of glucose dissolved in the extracellular water only. Imbalance arises from: (1) excessive outflow into the tissues owing to insulin (or very rarely IGF-II) overproduction or activity; or (2) in the fasting state, an inability of the liver to liberate or produce glucose at a rate sufficient to meet the noninsulin-dependent, and obligatory, requirements of the brain and red blood cells for glucose.

eating an appetizing meal and before the blood glucose concentration has risen. They attribute it to vagal activation.

In the postprandial period, as the blood glucose concentration falls toward its homeostatically controlled level, insulin secretion declines to a level that is just sufficient to suppress unbridled lipolysis. Absence of this constitutive insulin secretion in patients with type 1 diabetes is the cause of diabetic ketoacidosis.

The Role of the Liver in Glucose Homeostasis

The liver, under the influence of insulin reaching it in high concentration in the portal vein after ingestion of a meal, switches from being a net exporter to net importer of glucose from the glucose pool. Any insulin not extracted and degraded by the liver passes through the heart and lungs to reach peripheral tissues, notably muscle, adipose tissue, and skin, where, providing the concentration of insulin in blood is sufficiently high, it promotes glucose uptake.

Except in disease, the glucose pool, amounting to just 5–15 g, rarely expands by more than 100% even after ingestion of a meal providing up to 300 g of carbohydrate as

starch or glucose; nor does it shrink to less than 4 g, corresponding to a blood glucose concentration of $\sim 3.5 \text{ mmol l}^{-1}$, even after many days of fasting.

Entry of glucose into the glucose pool is limited by the rate at which it can be absorbed from the intestine. This is normally in the region of $25\text{--}50 \text{ g h}^{-1}$. In people with normal glucose tolerance, venous blood glucose levels generally return to overnight fasting values within 2–3 h of eating a meal regardless of how much carbohydrate it contains. Arterial blood glucose levels take somewhat longer to return to preingestion levels but they too are always within the normal fasting range by 3–4 h, even though the evidence provided by measurement of the gut hormone Glucose Dependent Insulinotropic Peptide (GIP), indicates that absorption of large meals continues for much longer. Absorption of a 200 g liquid glucose meal by normal healthy subjects, for example, is still incomplete 5 h later even though both their venous and arterial blood glucose levels have long since returned to normal.

The outflow of glucose into the tissues depends upon many factors; the two most important are the plasma insulin concentration and the blood concentration itself. Under maximum insulin stimulation – and at ‘normal’ blood glucose levels – glucose disappears from the glucose pool at a rate of

up to 40–50 g h⁻¹ but these conditions are rarely encountered except experimentally or in cases of gross insulin overdose.

Onset of insulin action is almost instantaneous and persists for as long as insulin remains bound to insulin receptors. This is generally slightly longer than insulin levels in the blood themselves remain elevated. In other words glucose continues to enter insulin dependent cells for up to 30 min after plasma insulin levels have returned to 'fasting' levels. During this time the glucose pool may shrink sufficiently to produce hypoglycemia unless replenished by glucose continuing to enter from the intestine (or experimentally/therapeutically by intravenous infusion) or from the liver, once it has switched from the glycogenic to glycogenolytic mode.

Small, and always temporary, imbalances between the rate at which insulin action declines and glucose enters the glucose pool can occur in healthy subjects after ingestion of a large dose of glucose in solution on an empty stomach, but is rare following the ingestion of an ordinary mixed meal.

A slight delay in stimulating insulin release in response to a meal is the earliest and most characteristic abnormality observed in patients with typical type 2 diabetes mellitus who may secrete more insulin in total than people of comparable age, though not of body mass index. They are, however, generally insulin resistant, which explains why, despite the larger amounts of insulin secreted in response to meals in the early stages of their illness, they do not suffer from meal-induced hypoglycemia.

Hypoglycemic Syndromes

Brain Malfunction from Hypoglycemia

The brain ordinarily requires a regular and plentiful supply of glucose, which gets to it from the blood by active transport utilizing the glucose transporter protein GLUT1. Reduction of supply to below critical limits causes the brain to malfunction and this manifests itself subjectively as symptoms and objectively as activation of the autonomic nervous system and cognitive nervous deficit. The blood glucose level at which autonomic activation and cognitive impairment occurs varies. Symptoms are unusual at blood glucose levels above 3.0 mmol l⁻¹ except in diabetic and elderly subjects in whom they may occur at higher levels. Objective evidence of cerebral impairment can however often be discerned by an investigator at blood glucose levels around 3.5–4 mmol l⁻¹.

Causes of neuroglycopenia other than hypoglycemia, i.e., normoglycemic neuroglycopenia, are currently thought to be rare but include congenital or acquired reduction in GLUT1 and poorly controlled diabetes. The possibility that such defects are more common than currently supposed and are responsible for some cases of 'nonhypoglycemia' cannot be completely dismissed at the present time, and would help explain why, under research conditions, some people diagnosed with this condition appear to develop symptoms at higher blood glucose levels than control subjects.

Neuroglycopenic Syndromes

Four more or less distinct neuroglycopenic syndromes (one of which is so rare that it will not be considered further here) can

be recognized. They are not mutually exclusive, nor do they depend upon the cause of the hypoglycemia.

Acute Neuroglycopenia (Adrenergic Symptoms)

This syndrome comprises a collection of vague symptoms such as feelings of alternating hot and cold, feeling unwell, anxiety, panic, inner trembling, unnatural feelings, blurring of vision, and palpitations, any or all of which may be accompanied by objective signs of facial flushing, sweating, tachycardia, and unsteadiness of gait. There is no particular order in which these features occur, nor are they constant. Nevertheless, patients on insulin or sulphonylurea therapy for diabetes, in whom they are common, rely upon them to warn of more severe neuroglycopenic impairment culminating in loss of consciousness. These patients can be taught to abort progression of symptoms by eating carbohydrate hence them often being referred to as warning symptoms.

Many of the features of acute neuroglycopenia resemble those produced by adrenaline and consequently are often referred to as adrenergic.

Sub-acute Neuroglycopenia or Hypoglycemic Unawareness

This syndrome is more insidious and may go completely unrecognized unless or until the patient loses consciousness. Often, however, there is loss of spontaneous activity, impairment of cognitive function, and the onset of somnolence that is more discernible to the bystander than to the patient and which, when it occurs *de novo* in a previously typical insulin-treated diabetic, is often referred to as hypoglycemia unawareness. Although suffering from sub-acute neuroglycopenia patients may perform quite complicated actions, such as driving a motorcar, of which they are total unaware when restored to consciousness. In other words they may behave as an automaton.

Acute can proceed to sub-acute neuroglycopenia and both can progress to stupor or coma unless relieved by food or injection of glucagon. Even when this is not done, however, full recovery, under the influence of endogenous counter-regulatory mechanisms, is almost invariable and is the reason why treatment with insulin is so safe despite the dangers of hypoglycemia.

Chronic Neuroglycopenia

The third syndrome is exceedingly rare. It occurs only when the blood glucose concentration remains low, either owing to the presence of an insulin-secreting tumor of the pancreas or overzealous treatment of diabetes with insulin for weeks or months on end. It is characterized by mental dysfunction resembling clinical depression, schizophrenia, or dementia, the symptoms of which are not relieved by restoring the blood glucose level to normal. Partial recovery may, however, take place over the ensuing months or years if the cause of the hypoglycemia is remedied.

This condition might be confused with 'nonhypoglycemia' were it not for the fact that the blood glucose concentration is invariably low (<3.0 mmol l⁻¹) while the patient is fasting,

does not rise normally in response to food, and evidence of underlying disease can always be found.

Diagnosis

Causes of Hypoglycemia

There are something in the region of 100 causes of hypoglycemia but all, apart from exogenous (or iatrogenic) insulin overdose, are uncommon. Some of the most important causes of recurrent hypoglycemia are listed and briefly described in **Table 1**. Simultaneous occurrence of symptoms, a measured low blood glucose concentration, and relief from intravenous glucose are a *sine qua non* for diagnosis. Differentiation is seldom simple and always rests heavily upon the results of laboratory data of which measurements of plasma insulin, proinsulin and C-peptide are the most important.

Endocrinological and other anatomico-pathological causes of hypoglycemia will not be considered further. Nor will iatrogenic or toxic causes, of which alcohol-induced fasting hypoglycemia is easily the most common. Instead, attention will be given to those conditions (including 'non-hypoglycemia') that have a mainly or exclusively dietary etiology and which respond partially or completely to dietary measures.

Spontaneous Reactive Hypoglycemia

Within a year of the discovery of insulin, and the symptoms to which hypoglycemia can give rise, Seale Harris, an American physician, had proposed that spontaneous overproduction of endogenous insulin might produce a similar condition. Confirmation of this hypothesis soon followed. The seminal work of Whipple on the diagnosis of insulinoma and of Conn on diet-induced postprandial reactive hypoglycemia, both in 1936, distinguished between fast-induced (fasting) hypoglycemia and that which occurred only in response to feeding. The latter, reactive or postprandial hypoglycemia, could be reproduced by oral administration of large doses of glucose in solution and this became the standard criterion for its diagnosis – the 5 h glucose tolerance or load test.

Glucose Load Test

The observation that in a substantial percentage of normal healthy people glucose taken in solution on an empty stomach produces a rebound fall in venous blood glucose levels to below fasting levels was made very soon after blood glucose measurements became possible and before the discovery of insulin. It attracted little attention at the time being considered to have only curiosity value and little pathological significance.

The situation changed dramatically during the early 1950s and, subsequently, particularly in the US, with the appearance of books written for lay consumption attributing a vast array of common symptoms to hypoglycemia, whether the blood glucose concentration was low at the time or not. Belief in the importance and prevalence of hypoglycemia grew amongst fashionable medical practitioners and the general public alike

to such an extent that, by the early 1970s, alarm bells began to ring amongst consumer action groups and the scientific medical community.

With the passage of time the original, well-defined syndrome of postprandial reactive hypoglycemia had become so distorted, and the criteria for its diagnosis so blurred, that anyone with vague symptoms could be, and often was, described as suffering from hypoglycemia, without anyone bothering to measure their blood glucose concentration.

Not until a consensus Statement on Post Prandial or Reactive Hypoglycemia was issued by the Third International Symposium on Hypoglycemia and generally recognized by medical practitioners throughout the world did scientific criteria for the diagnosis of reactive hypoglycemia gain universal acceptance and its purported incidence declined dramatically.

Definition

It is now accepted that some people exhibit, in the course of their everyday life, symptoms similar to those caused by acute neuroglycopenia and may, if accompanied by a capillary or arterialized venous blood glucose concentration of $3.0\text{--}2.5\text{ mmol l}^{-1}$ or less, justify description as being of postprandial reactive hypoglycemic origin. Reactive hypoglycemia may itself be a consequence of anyone of a large number of well-recognized but generally uncommon organic conditions, such as an islet cell tumor, that also produce fast-induced hypoglycemia. It is, therefore, only after all of these have been excluded by appropriate laboratory investigations that a diagnosis of functional or dietary reactive hypoglycemia is justified.

Specifically, the prolonged oral glucose load (tolerance) test is now deemed inappropriate for the diagnosis of postprandial or reactive hypoglycemia because the incidence of false-positive results with this test is so high as to make it meaningless, especially if, as is so often the case, venous rather than arterial blood is sampled.

The Postprandial Syndrome

Typically, the patient is a normal-weight woman of 20–50 years whose main complaint is of vague feelings of distress occurring predominantly mid-morning, about 11.00 a.m.–12.00 noon, but occasionally mid afternoon or evening and never before breakfast. In between attacks, characterized by feeling of faintness, anxiety, nervousness, irritability, inner trembling, rapid heartbeat, headache, and sweatiness, either alone or in combination, they may be completely well. More often they describe themselves as suffering from increased tiredness, lacking in zest for life, and apathetic much, or all, of the time: symptoms often associated with depression or chronic alcohol abuse.

Patients seldom notice any fixed relationship to food unless, as so often happens nowadays, they have diagnosed themselves, on the basis of articles they may have read, as suffering from hypoglycemia. Almost without exception they reject the possibility that their symptoms might have a contributory, or even large, psychogenic element.

Table 1 Diagnostic criteria and treatment of the main types of hypoglycemia

<i>Description</i>	<i>Mechanism</i>	<i>Diagnostic criteria</i>	<i>Dietary considerations</i>
Fasting hypoglycemia			
Insulin-secreting tumor (insulinoma) and nesidioblastosis	Abnormal B-cells with failure to suppress insulin secretion in response to hypoglycemia	Inappropriate high plasma insulin ($> 18 \text{ pmol l}^{-1}$) and C-peptide ($> 200 \text{ pmol l}^{-1}$) concentrations in presence of hypoglycemia ($\text{BG} < 2.5 \text{ mmol l}^{-1}$). Suppressed β -hydroxybutyrate levels ($< 2700 \text{ } \mu\text{mol l}^{-1}$) after 72 h fast	High carbohydrate intake orally or intravenously until curative surgical ablation, or effective hyperglycemic therapy with diazoxide plus chlorothiazide or with octreotide, can be instituted.
Nonislet cell tumor hypoglycemia (NICTH)	Abnormal tumor cells secreting big IGF-II	Low plasma insulin and C-peptide levels: low plasma IGF-I, normal or raised IGF-II levels: abnormal IGF-I: IGF-II ratio. Suppressed β -hydroxybutyrate levels ($< 500 \text{ } \mu\text{mol l}^{-1}$)	High carbohydrate intake orally or intravenously until curative surgical ablation of effective hyperglycemic therapy with growth hormone and prednisone can be instituted.
Endocrine disease, for example, Hypopituitarism, Addison's disease	Reduced availability of diabetogenic or hypoglycemia counter-regulatory hormones	Clinical features of primary disease with subnormal levels of appropriate counter-regulatory hormones, for example, cortisol, growth hormone. Appropriately raised β -hydroxybutyrate levels ($> 500 \text{ } \mu\text{mol l}^{-1}$) during hypoglycemia.	High carbohydrate intake orally or intravenously until effective hormone replacement therapy has been established
Glycogen storage disease	Inability to release glucose from liver during fasting	Usually present in childhood: low blood glucose, high β -hydroxybutyrate levels, low insulin and C-peptide: high lactate: impaired or absent glucose response to glucagon	Avoid fasting: a constant intake of slowly absorbed carbohydrate may be required day and night in infants
Disorders of mitochondrial β -oxidation	Defective utilization of fat as fuel in tissues: compensatory increase in glucose utilization	Occurs in infancy: low glucose, low insulin and C-peptide, high FFA, normal lactate, low β -hydroxybutyrate, increased urinary organic acids. Hypocarnitinemia in some cases	Avoid fasting: frequent high carbohydrate low fat feeding.
Fasting alcohol-induced hypoglycemia	Alcohol impaired hepatic gluconeogenesis	Low blood glucose, raised blood alcohol, lactate and usually β -hydroxybutyrate: low plasma insulin and C-peptide	Avoid drinking alcohol although fasting or on a low energy diet.
Idiopathic ketotic hypoglycemia of childhood	Varied: but always owing to exhaustion of hepatic glycogen stores faster than cerebral adaptation to ketosis can occur	Low blood glucose: high plasma fatty acids and β -hydroxybutyrate: low insulin and C-peptide	High carbohydrate feeding; avoidance of prolonged abstinence from food particularly during intercurrent illness, especially infections
Stimulative hypoglycemia			
Inborn errors of metabolism, for example, hereditary fructose intolerance, galactosemia	Impaired release of glucose from liver in response to hepatotoxicity of food constituent	hypoglycemia evoked by ingestion of foods containing appropriate noxious stimulus: galactose in galactosemia: fructose in hereditary fructose intolerance and fructose 1–6 biphosphatase deficiency	Avoid foods containing provocative sugars eg fructose, galactose as appropriate.

(Continued)

Table 1 Continued

<i>Description</i>	<i>Mechanism</i>	<i>Diagnostic criteria</i>	<i>Dietary considerations</i>
Autoimmune insulin syndrome	Delayed release of insulin from antibody binding after all of meal has been absorbed	Profound hypoglycemia from 3–12 h after last eating; total plasma insulin high; C-peptide high, normal or low; proinsulin normal or high. Antibodies to insulin present. Common in Japan, infrequent elsewhere	Frequent small mixed meals, low in rapidly absorbed carbohydrate; rich in dietary fiber.
Postgastrectomy and rapid gastric emptying; bariatric surgery	Accelerated deposition of nutrients in duodenum and increased release of insulinotropic hormones, for example, GIP, GLP-1.	Normal blood glucose during fasting: hypoglycemia only follows 1–3 h after eating. History of gastrectomy or objective evidence of rapid gastric emptying. Exaggerated insulinemic response to food.	Frequent small mixed meals rich in dietary fiber. May benefit from treatment with acarbose or miglitol (α -glucosidase inhibitors)
Idiopathic reactive or functional hypoglycemia	Unknown: probably heterogeneous including increased insulin sensitivity, lowered cerebral threshold to neuroglycopenia	hypoglycemia 3–5 h after eating. Normal blood glucose during fasting: low capillary (arterial) blood glucose during spontaneous symptomatic neuroglycopenic episodes (<3 mmol l ⁻¹). All other objective tests of glucose homeostasis normal (including GIP and GLP-1 responses to food). Exclude noninsulinoma pancreatogenic hypoglycemia by intra-arterial calcium test.	Frequent small mixed meals low in absorbed carbohydrates: rich in soluble dietary fiber. May benefit from treatment with acarbose or miglitol (α -glucosidase inhibitors)

Symptoms wax and wane during middle life but often remit completely for years or may never recur. They are not progressive and never cause severe neurological dysfunction such as coma, psychosis, or dementia. Hypoglycemia cannot be demonstrated during spontaneous symptomatic episodes in most people with the postprandial syndrome and some other explanation should be sought for them. Psychotherapy is often helpful.

Differential Diagnosis

Studies using continuous interstitial glucose monitoring confirm finger-prick blood sampling undertaken during spontaneous symptomatic episodes that only a very small proportion of sufferers from the postprandial syndrome have hypoglycemia at the relevant time. Of those who do, a substantial proportion have an identifiable cause for it. The commonest, in the West, is partial gastrectomy and bariatric surgery for the treatment of obesity: in the Far East, for example, Japan, it is the autoimmune insulin syndrome (AIS) which also occurs in Europe and the US but is rare. Other rare causes include insulinoma, the newly described condition of noninsulinoma pancreatogenic hypoglycemia, and abnormalities of GLP-1 secretion.

In some people reactive hypoglycemia occurs only in response to a specific dietary indiscretion, for example, ingestion of moderate amounts of gin (alcohol) and tonic (sugar and quinine) on an empty stomach or fructose in people with rare inborn errors of metabolism. A hard core of subjects remains for whom no satisfactory pathogenic mechanism can be identified. Only in them is it justified to describe them as suffering from (idiopathic or functional) reactive hypoglycemia (Figure 2).

Dietary Management

Treatment of Attacks

Because of their short duration and modest severity, acute spontaneous neuroglycopenic episodes owing to reactive hypoglycemia require no specific treatment beyond ingestion of a rapidly assimilable form of carbohydrate (e.g., a lump of sugar), exactly as for iatrogenic hypoglycemia.

There is no evidence that this ever produces rebound hypoglycemia and should it do so the grounds for making the diagnosis should be reviewed.

Prevention

Dietary prevention of reactive hypoglycemia, whether of the idiopathic, alimentary variety, or secondary to some other disease, is based on the premise that it is caused by imbalance between the timing and amount of insulin secreted in response to the ingestion of a meal and disposal of the glucose derived from it. Evidence for this supposition is small and disputed but provides the best explanation for the apparent breakdown in glucose homeostasis in patients with idiopathic reactive hypoglycemia.

Frequent small meals containing only modest amounts of sugars (glucose and sucrose) and refined starches but rich in poorly absorbed complex carbohydrates and containing dietary fiber have replaced the diets rich in proteins (and fats) previously advocated, but evidence of their unique efficacy is lacking. Avoidance of drinks rich in sucrose or glucose, especially with alcohol, may be helpful in subjects who are highly susceptible to this combination. There is no evidence that confectionery eaten in moderation is uniquely detrimental, though excessive use should be discouraged on general health grounds.

The long-term outcomes of such dietary advice in patients in whom strict criteria for diagnosis were adopted are not available and most published studies on the subject have drawn attention to the need for supplementary pharmacological methods in order to achieve a satisfactory therapeutic outcome.

Pharmaceutical agents that have been used include guar, acarbose, and miglitol, all of which slow glucose absorption and decrease the insulinemic response to food, whereas others including phenytoin and propranolol do not. Paradoxically, diazoxide, which inhibits insulin secretion by direct action, has not been found effective except in patients with proven endogenous hyperinsulinism owing to insulinoma or islet hyperplasia.

Nonhypoglycemia

No account of dietetic treatment of hypoglycemia would be complete without a brief description of nonhypoglycemia, which has been described as a controversial illness and epidemic in the US. Clinically, the illness is indistinguishable from (idiopathic) reactive hypoglycemia, except that the blood glucose level is never pathologically low during symptomatic episodes. Moreover, although transient turns are often a major feature of the illness, only rarely, if ever, does the patient consider their health, between turns, as normal.

The attribution of these patients' illness to hypoglycemia had its origins in the early 1950s with the appearance, in the US, of a book by Drs Abrahams and Pezet entitled 'Body, Mind and Sugar.' Other American practitioners, notably John Tintera, founder of the Hypoglycemia Foundation Inc., Stephen Gyland, Harry Saltzer and, others, including the medical writer Carlton Fredericks, publicized the concept. This led to hypoglycemia being held, by a large section of the public, responsible for such diverse diseases as coronary artery disease, allergy, asthma, rheumatic fever, susceptibility to viral infections, epilepsy, gastric ulcer, alcoholism, suicide, and even homicide, as well as for a whole galaxy of symptoms in their own right. Hypoglycemia was treated as though it were a disease entity and asserted by its advocates to be "one of the most common illnesses in the United States" and that because of it "thousands of Americans have forgotten, or perhaps never known, what it is like to feel completely healthy." Diagnosis of nonhypoglycemia generally depends upon the results of the discredited 6 h oral glucose tolerance test, using venous blood, although some have dispensed even with this discredited formality in favor of just purely clinical criteria.

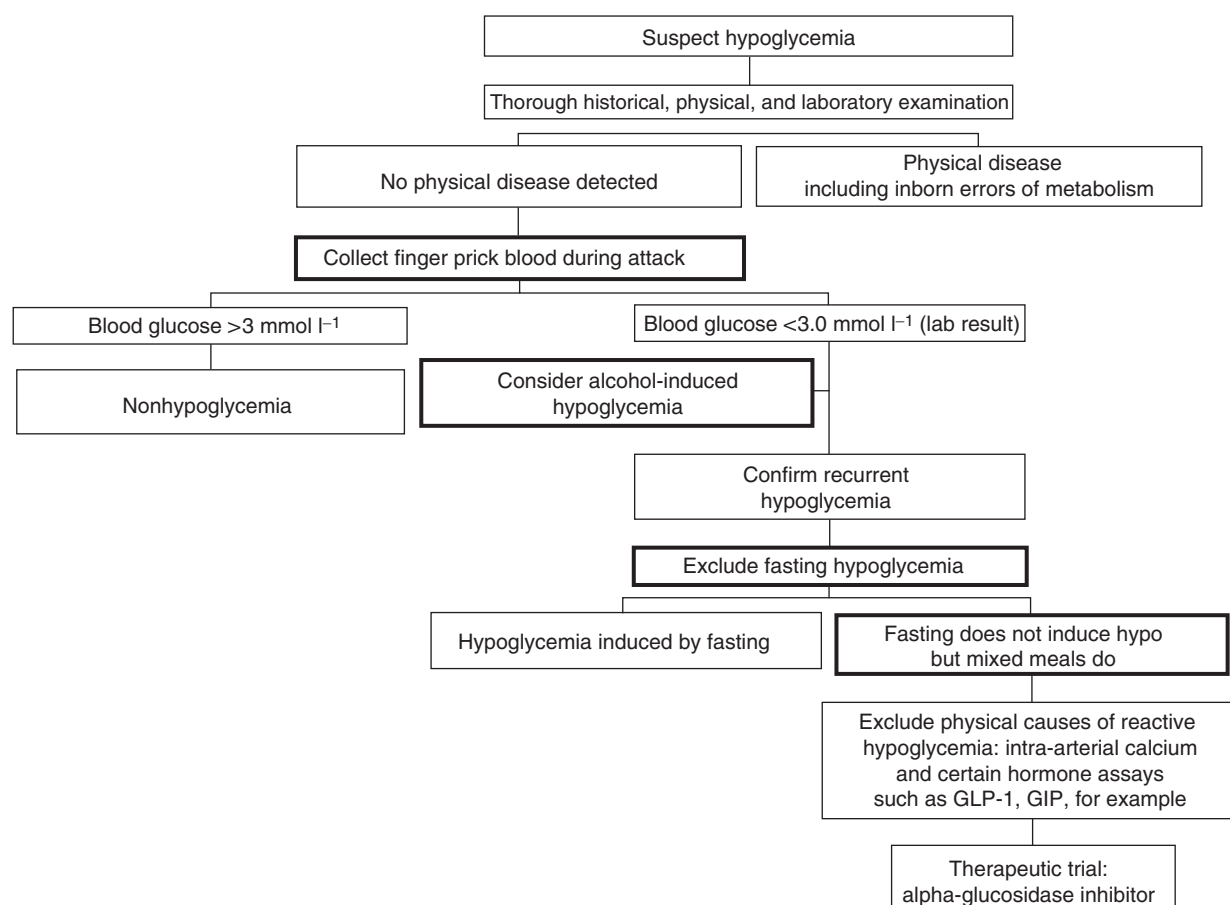


Figure 2 Investigation of reactive hypoglycemia. Steps in the diagnosis of reactive hypoglycemia of unknown etiology.

The appearance in the *New England Journal of Medicine* of an article in 1974 entitled "Nonhypoglycemia is an epidemic condition" first drew international attention to its existence. It had previously been almost unknown outside the US and Australia, though known to a few fashionable medical practitioners in Britain and elsewhere.

Many patients with nonhypoglycemia undoubtedly derive some benefit, probably through a powerful placebo effect, from severely restrictive dietary regimes. Although differing in details most of the diets emphasize the purported specifically detrimental effects of sugar (sucrose), salt, alcohol, and caffeine.

Although the cause of illness in people with nonhypoglycemia remains unknown, it is unlikely to be the same in all cases. In some it is chronic alcoholism and in a tiny number of others it is owing to caffeine intoxication, which can be confirmed by a dietary history and, above all, by measurement of plasma caffeine levels. Such patients do benefit specifically from reducing their intake of caffeinated beverages, though not necessarily from avoiding them completely. Ironically, and probably significantly, caffeine restores hypoglycemia awareness to diabetic patients on insulin who have become insensitive to it. The possibility exists, therefore, that a combination of reasonable or normal caffeine intake occurring in combination with the normal rebound in arterial blood glucose to just below fasting levels that sometimes

occurs 3–5 h after a meal in someone with an unusually low threshold for neuroglycopenia, might precipitate symptoms. This explanation must, however, be considered no more than speculative.

On the other hand such diverse illnesses as hyperventilation, panic attacks, drug abuse, and genuine food intolerances are all established as capable of producing the nonhypoglycemia syndrome and should always be considered in the differential diagnosis.

Exercise-Induced Hypoglycemia

Previously only associated with marathon running, hypoglycemia is now recognized to be comparatively common in inadequately trained individuals undertaking strenuous exercise. Consumption of rapidly absorbed carbohydrate before taking exercise may encourage its appearance although consumption of slowly absorbed, low glycemic index foods may prevent it as does appropriate training.

Hepatic and Renal Failure

Considering the importance of the liver and kidneys in the maintenance of blood glucose levels hypoglycemia is rare in both liver and kidney disease. In liver disease hypoglycemia is virtually confined to patients with acute toxic hepatic necrosis,

whether owing to overwhelming viral infection or specific hepatotoxins such as poisonous mushrooms, unripe akee fruit, or paracetamol in excess. Its appearance always portends an extremely poor prognosis as it does in all critically ill patients regardless of their pathology.

The association of hypoglycemia with primary cancer of the liver is comparatively common and owing to over-expression and secretion of aberrant, or big IGF-II, and is not, as was once supposed, owing to nonspecific destruction of hepatic tissue. Hypoglycemia is also a very rare complication of tumors arising anywhere in the body that secrete an abnormal form of IGF-II and must be distinguished from hypoglycemia produced by insulin-secreting tumors.

Kidney failure is one of the most common causes of hypoglycemia in nondiabetic hospital inpatients but does not carry quite as grave a prognostic significance as in patients with liver disease. It generally responds to appropriate dietary and other supportive treatments for end-stage kidney disease.

Inborn Errors of Metabolism

Hypoglycemia is a manifestation of many inborn errors of metabolism (see [Table 1](#)) especially in children but also occasionally in adults. It is particularly important in some varieties of liver glycogen storage diseases, especially types I and III, and in disorders of fatty acid metabolism in which it is often the presenting symptom.

Type I liver glycogen storage disease is caused by a defect in glucose-6-phosphatase activity and produces a severe form of fasting hypoglycemia. Fortunately, it responds to dietary therapy in the form of continuous feeding with slowly absorbed starch solution through a nasal or gastrostomy tube, especially during the night when the body normally has to resort to glycogenolysis to maintain the supply of glucose to the brain. Hypoglycemia in untreated type I patients produces hypoinsulinemia and high to very high plasma ketone levels. Children with abnormalities of fatty acid metabolism, on the other hand, are characterized by hypoglycemia and hypoketonemia. As with children with liver glycogen disease, treatment is to ensure that they are constantly supplied with carbohydrates and are never fasting for more than a very short period.

Starvation

Although average fasting blood glucose levels are lower in victims of famine than in well-fed populations, overt hypoglycemia is rare. Even in patients suffering from Kwashiorkor, hypoglycemia is uncommon and is usually associated with infection, hypothermia, and coma. Patients with anorexia nervosa develop hypoglycemia only as an agonal phenomenon and its appearance generally portends imminent death. The characteristic clinical biochemistry findings are of low plasma insulin, proinsulin, C-peptide, and IGF-1 levels, grossly depressed plasma nonesterified fatty acids (NEFA) and β -hydroxybutyrate, and elevated growth hormone and cortisol levels. Relief of hypoglycemia by re-feeding is the only measure carrying any chance of preventing death, but it is rarely successful.

Hypoglycemia in the Elderly Sick

The high incidence of hypoglycemia in sick elderly patients – especially those with infections – has become apparent from the use of routine blood glucose measurements. The cause is seldom attributable to any of the well-recognized causes of hypoglycemia found in younger fitter people. It is probably owing to chronic malnutrition that is so common in the elderly sick, compounded by coincident disease but which is not of itself sufficiently severe to produce hypoglycemia.

Sepsis

Infections of all sorts – usually bacterial – not necessarily severe enough to produce a full blown Systemic Inflammatory Response Syndrome (SIRS) – is increasingly being recognized as a cause of hypoglycemia, uncovered serendipitously by a blood glucose measurement, and generally portends a fatal outcome. Treatment is that of the primary condition as well as of the hypoglycemia.

See also: Aging. Cancer: Epidemiology and Associations Between Diet and Cancer; Epidemiology of Gastrointestinal Cancers other than Colorectal Cancers. Diabetes Mellitus: Classification and Chemical Pathology; Dietary Management; Etiology and Epidemiology. Famine: Causes, Consequences, and Responses. Fatty Acids: Metabolism. Glucose: Chemistry and Dietary Sources; Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level. Liver Disorders: Nutritional Management. Starvation and Fasting: Biochemical Aspects

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(a site devoted to hypoglycemia mainly due to non-iatrogenic causes).
- <http://www.hypodiab.com/>
(a site devoted to iatrogenic hypoglycemia).

Edited by
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Encyclopedia of Human Nutrition

Third Edition



ENCYCLOPEDIA OF HUMAN NUTRITION

THIRD EDITION

ENCYCLOPEDIA OF HUMAN NUTRITION

THIRD EDITION

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VOLUME 3



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Dr. Caballero is Professor of International Health at the Bloomberg School of Public Health and Professor of Pediatrics at the School of Medicine, Johns Hopkins University, Baltimore, MD, USA. He has over 20 years of experience as a scholar, researcher, and leader in the area of child health and nutrition. He obtained his MD from the University of Buenos Aires, Argentina and his PhD (in neuroendocrine regulation) from MIT, Cambridge, MA, USA. He started his faculty career at Harvard Medical School, and moved to Johns Hopkins in 1990 to found the Center for Human Nutrition.

Dr. Caballero is a recognized expert on the nutritional needs of children and adults, and on nutrient requirements in undernourished populations. For the past 10 years, he has focused on the problem of childhood obesity in the US and in developing countries, and explored the impact of dietary transition and globalization on health indicators. He is an active participant in key scientific committees advising the US government on issues of diet and health, including the Dietary Reference Intakes (DRI) Committee; the Expert Panel on Macronutrient Requirements; and the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences. He was a member of the Dietary Guidelines for Americans Advisory Committee, and is currently a member of the Scientific Advisory Board of the Food and Drug Administration (FDA) and of the International Life Sciences Institute (ILSI).

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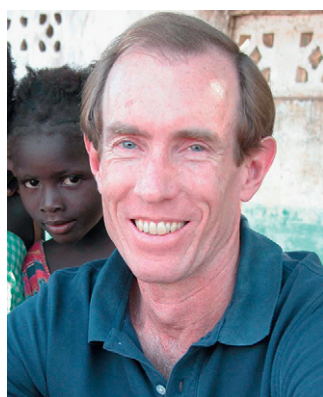
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PREFACE

By the turn of the twentieth century, nutrition science had completed a slow but remarkable historical transition, from a discipline focused on preventing nutrient deficiencies (and hence focused on identifying minimum nutrient needs) to one aimed at reducing disease risk and optimizing health, seeking to define an elusive optimal diet. But progress in our knowledge has not yet caught-up with that transition in focus, and our understanding of how diet constituents affect long-term disease risks is still not on a par with our knowledge of essential nutrients, their metabolism, and required intake levels. One reason is that the experiments needed to unravel diet–health interrelationships are more complex, costly, and in some cases unfeasible, compared with the classical studies that identified vitamins and other essential nutrients. Another reason is that, although the discovery of essential nutrients was based on a strong, unifying scientific paradigm (the concept of a compound essential for human life but which humans are unable to make), there is no single or unifying paradigm from which to explore diet–health relationships. In addition, our ability to timely process and integrate scientific discoveries is now continuously challenged by the massive volume of information of the digital era.

In that context, the need to provide accurate, succinct, and up-to-date information on a wide range of topics is more important than ever, and is the aim of this Encyclopedia. Currently, nutrition research and practice is fundamentally a multidisciplinary endeavor, so we aim to offer scientific information to a wide audience of researchers and professionals. In addition, the information revolution of the internet has

changed the consumer from a passive recipient of advice to an active participant in decisions involving health and related issues. Thus, although this work is not specifically targeted to the general public, we hope that the educated readers with a minimum scientific background should also be able to obtain from this book useful (and reliable) information on their topic of interest.

This third edition builds on the success of the previous one. We have included new articles or made extensive updates when needed, while keeping the proven core of established knowledge. The comprehensive index and extensive cross-referencing will allow readers to quickly identify specific topics, and to move deeper into related areas if desired.

We have a great debt of gratitude to the hundreds of authors who contributed to the large body of knowledge represented here. In turn, authors benefited from the valuable feedback of our distinguished Editorial Advisory Board. Of course, as editors we are ultimately responsible for the content, particularly for any errors. Finally, both the print and electronic version have the unmistakable production quality of the Major Reference Works division of Elsevier, and this is the result of the unrelenting enthusiasm and hard work of our editorial team.

We hope this work will be a valuable addition to the knowledge base of any person interested in the critical area of nutrition, diet, and human health.

Benjamin Caballero
Editor-in-Chief

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GUIDE TO USE OF THE ENCYCLOPEDIA

Structure of the Encyclopedia

The Encyclopedia is arranged as a series of entries in alphabetical order. Some entries comprise a single article, whilst entries on more diverse subjects consist of several articles that deal with various aspects of the topic. In the latter case the articles are arranged in a logical sequence within an entry.

To help you realize the full potential of the material in the Encyclopedia we have provided three features to help you find the topic of your choice.

Contents Lists

Your first point of reference will probably be the contents list. The complete contents list appearing in each volume will provide you with the volume number and page number of the entry. On the opening page of an entry a mini-contents list is provided so that the full details of the articles within the entry are immediately available.

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Example

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INBORN ERRORS OF METABOLISM

Contents

Classification and Biochemical Aspects
Nutritional Management of Phenylketonuria

Classification and Biochemical Aspects

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Abbreviations

ASL	argininosuccinate lyase	LCHAD	long chain 3-hydroxy acyl CoA dehydrogenase
ASS	argininosuccinate synthetase	MCAD	medium chain acyl CoA dehydrogenase
BCAA	branched chain amino acids	MS/MS	tandem mass spectrometry
BH ₄	tetrahydrobiopterin	MSUD	maple syrup urine disease
BIA	bacterial inhibition assay	NAG	<i>N</i> -acetylglutamine
Cbl	cobalamin	NH ₃	ammonia
CPS	carbamoyl synthetase	OTC	ornithine transcarbamylase
CPT I, II	carnitine palmityl transferase	PAH	phenylalanine hydroxylase
GALE	UDP galactose 4-epimerase	PKU	phenylketonuria
GALK	galactokinase	SCAD	short chain acyl CoA dehydrogenase
GALT	galactose-1-phosphate uridyl transferase	TCA	tricarboxylic acid cycle
HCS	holocarboxylase synthetase	VLCAD	very long chain acyl CoA dehydrogenase

Introduction

Garrod described the first inborn error of metabolism in 1902 when he associated the symptoms that had been observed in patients with Alkaptonuria as being due to an inherited enzyme deficiency. Since that time more than 400 disorders have been described that are due to an enzyme deficiency in the catabolic pathways of protein, fatty acids, and carbohydrates. The resulting accumulation of the toxic intermediates, and in some cases, the depletion of a necessary end product, causes a variety of metabolic derangements, often with significant neurological sequelae. The severity and the age of onset of symptoms usually, although not always, depend on the amount of residual enzyme activity.

Genetic disorders are inherited by various modes. The most common pattern is autosomal recessive, where each parent carries a recessive mutation (also referred to as being heterozygous for the disorder) in a specific gene in one of the autosomal chromosomes, i.e., not one of the sex-determining chromosomes, X or Y. As there are four possible combinations of the chromosomes carrying the abnormal gene that can occur at fertilization, the offspring has 25% chance of inheriting two mutations (one from each parent) and therefore being affected (also referred to as being homozygous for the disorder) with the disorder, a 50% chance of inheriting one mutation from one or other parent, thus being an unaffected carrier (heterozygous) and a 25% chance of inheriting two normal genes.

In autosomal dominant inheritance, a dominant mutation in one of the autosomal chromosomes is inherited in 50% of the offspring, irrespective of sex. The affected parent is usually symptomatic, although the onset of symptoms may not be apparent until later in life (such as in Huntington's disease), or the spectrum of severity may be variable in different generations, so the disease may not always be recognized. Approximately 30% of dominant mutations arise spontaneously and are therefore not inherited.

In X-linked inheritance, the mutation occurs on the X chromosome. Females have two X chromosomes (one of which is always inactivated, usually randomly), and males an X and a Y. At fertilization, the mother contributes one X and the father either an X or a Y chromosome. If there is a mutation on the inherited X chromosome and the offspring receives a Y chromosome from the father, the mutation will be expressed. If the offspring receives a normal X chromosome from the mother, and an X from the father, the offspring will be a normal female. If the abnormal X is received with a Y from the father, the son will be affected. There is thus a 50% chance of sons from a carrier mother being affected and a 50% chance of daughters being carriers. In rare circumstances, X inactivation is nonrandom or skewed, so that the female carrying the abnormal gene can be symptomatic. X inactivation is also often referred to as lyonization, named after Dr Mary Lyon who first described the phenomenon. In X-linked disorders, approximately 30% of mutations arise spontaneously, so not inherited from the mother.

The vast majority of genetic disorders are inherited in an autosomal recessive fashion.

While the individual inborn errors of metabolism are rare, based on recent results of expanded newborn screening programs (in which more than 30 disorders can be detected), the overall incidence is approximately 1/5000 live births worldwide. The incidence of disorders may vary in different populations because of the 'founder effect,' where a specific mutation arises and is maintained in subsequent generations, or where there is a higher incidence of consanguinity.

With a few exceptions, infants are normal at birth because the placenta efficiently eliminates the toxic metabolites.

Newborn Screening

Mass population screening of newborns was introduced in the 1960s, initially for phenylketonuria (PKU), after the development of the bacterial inhibition assay (BIA) for phenylalanine by Robert Guthrie. This simple method, popularly referred to as the Guthrie Test, is still the mainstay of screening for PKU in much of the world. Essentially, the method entails the addition of a solution of *B. subtilis* into an agar well, into which is added a standardized punched sample from the newborn screening filter paper from which the blood is then eluted. High levels of phenylalanine inhibit growth of the bacteria, and the laboratory technician can easily visually identify this 'no-growth' zone as abnormal. Quantification is necessary, using a follow-up method such as high performance liquid chromatography (HPLC). BIA has been adapted for screening for elevated levels of leucine (for maple syrup urine disease (MSUD)) and for methionine (for homocystinuria).

The most significant advance in newborn screening since its inception has been the adaptation of tandem mass spectrometry (MS/MS). With this technology, multiple compounds can be identified (both amino acids and acylcarnitine species) from the same dried blood filter paper sample after a simple preparation. More than 30 different inborn errors of metabolism can now be identified. The major drawback, however, is the relative expense of the equipment and lack of long-term data on the outcome of infants detected and treated presymptotically. Further modification of MS/MS will enable future screening for many more inborn errors of metabolism, as well as increased efficiency by adding already screened disorders to the same platform. Methods have been developed for screening for several lysosomal storage disorders. New screening methods are also under development.

Disorders of Protein Metabolism

Amino Acid Disorders

Amino acidopathies are due to an enzyme deficiency early in the catabolic pathway of one or more amino acids that results in the accumulation of the amino acid(s), detected by plasma amino acid analysis of serum or plasma. Symptoms may be due to the chronic accumulation of toxic amino acid(s) or due to acute metabolic decompensation, for which aggressive intervention is necessary to prevent death or severe morbidity. Treatment is dietary restriction of the toxic amino acid by limiting the intake of whole protein and supplementing with special modular amino acid formulas to provide the appropriate nutrients for normal growth and development. All disorders are inherited in an autosomal recessive fashion.

The classic example is PKU. In PKU, a deficiency of the phenylalanine hydroxylase (PAH) enzyme (**Figure 1**) results in

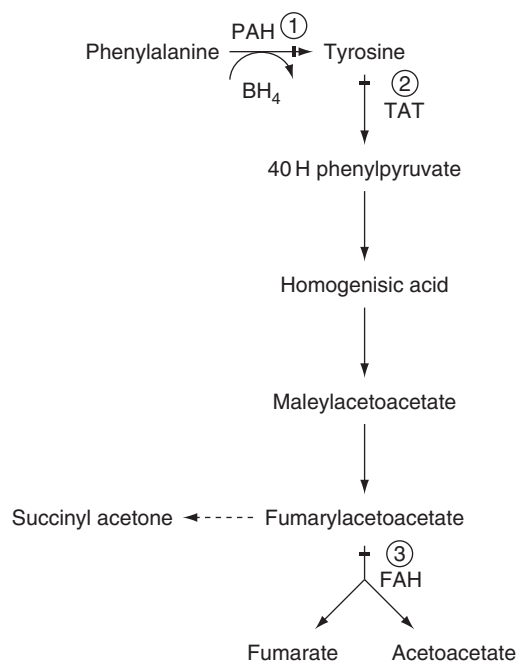


Figure 1

a high level of phenylalanine, which, if not treated with dietary restriction of phenylalanine in the early newborn period, causes severe, irreversible mental retardation. The diagnosis is confirmed by finding a phenylalanine level $> 1200 \mu\text{mol l}^{-1}$ in an infant on unrestricted protein intake. The incidence of PKU is approximately 1/20 000 in Caucasians. Although PKU is pan ethnic, the incidence varies in certain populations. The level of phenylalanine can vary in individual patients because of the amount of residual enzyme activity, which in turn depends on the specific mutations. There are currently more than 400 known mutations. Most patients are compound heterozygotes (i.e., have one copy each of two different mutations). Before the introduction of newborn screening, PKU was the commonest cause for inherited mental retardation. Early recognition of the presymptomatic infant allows for institution of dietary treatment with a phenylalanine-restricted diet, with the best outcomes when recommended phenylalanine levels are attained by 2 weeks of age.

Untreated patients develop progressive severe mental retardation, often with seizures and Parkinson disease-like neurological symptoms. The primary pathogenesis is due to the toxic effect of phenylalanine on the central nervous system; secondary symptoms may be due to a deficiency of tyrosine, which is an important precursor for the synthesis of some neurotransmitters. These symptoms include anxiety and depression.

Treatment is discussed in detail in the following article.

Benign or mild hyperphenylalaninemia is due to allelic variants of PAH, which result in greater residual enzyme activity. On an unrestricted diet, levels are typically in the range $120\text{--}360 \mu\text{mol l}^{-1}$, and no dietary treatment is necessary.

Moderate elevation of phenylalanine is also present with defects of tetrahydrobiopterin (BH_4), the cofactor for PAH. BH_4 is also the cofactor for other enzymes, tryptophan hydroxylase and tyrosine hydroxylase. These amino acids are important precursors for the neurotransmitters 5-hydroxytryptophan and dopamine. A deficiency causes a neurological syndrome characterized by hypotonia, seizures, and movement disorder (dystonia).

MSUD has an incidence approximately 1/185 000 births. It is due to a deficiency of the branched chain ketoacid dehydrogenase enzyme and the resulting accumulation of the branched chain amino acids (BCAAs), leucine, isoleucine, and valine, which are detected on plasma amino acid analysis. Elevation of alloisoleucine (a derivative of isoleucine) is pathognomonic. In classic MSUD, symptoms typically occur in the first week of life and, if untreated, rapidly progress to cerebral edema, coma, and death. Toxicity is due primarily to high levels of leucine. The characteristic maple syrup (or burnt sugar) odor is due to presence of sotolone, a metabolite of isoleucine or alloisoleucine. It is only detectable when the BCAAs are significantly elevated; the ester is concentrated in the urine and in the earwax of affected patients.

Variant Forms of MSUD also Occur

Intermediate MSUD typically presents in infancy with developmental delay; seizures may occur. Moderate levels of the BCAAs (including alloisoleucine) are present.

Intermittent MSUD is associated with intermittent symptoms during acute infections or periods of prolonged fasting. Typical symptoms include ataxia, vomiting, and seizures. Acute severe decompensation may occur, similar to the classic form of MSUD. The BCAAs are only elevated during the episode of acute symptoms. Other disorders are listed in [Table 1](#).

Urea cycle defects are due to enzyme deficiencies associated with the elimination of waste nitrogen produced by the normal catabolism of protein. There are six enzymatic steps involved in this process ([Figure 2](#)): a deficiency in any of the first five enzymes causes accumulation of nitrogen, in the form of ammonia (NH_3) and increased levels of the amino acids glutamine and glycine. Another very rare disorder has recently been characterized, due to a defect of *N*-acetylglutamate synthase (NAGS). *N*-acetylglutamate is the essential cofactor for the first enzyme in the urea cycle, carbamylphosphate synthase (CPS).

Symptoms occur typically in the newborn period, except for arginase deficiency, but milder late-onset variants have been well described. Symptoms include lethargy, poor feeding,

Table 1

<i>Disorder (deficient enzyme)</i>	<i>Elevated analyte</i>	<i>Clinical features</i>	<i>Treatment</i>
Tyrosinemia type I (fumarylacetoacetase)	Tyrosine SA	Cirrhosis Liver failure Failure to thrive Renal tubular acidosis Rickets Hepatocellular carcinoma (late)	NTBC (inhibits SA production) Tyrosine restriction
Tyrosinemia type II (tyrosine aminotransferase)	Tyrosine ($\uparrow \uparrow$)	Keratoconjunctivitis Palmar keratosis Mental retardation	Tyrosine restriction
Homocystinuria (cystathionine β synthase)	Methionine Total homocysteine Free homocysteine + Mixed disulfides	Mental retardation Thromboembolism Lens dislocation Osteoporosis	Vitamin B ₆ (50% respond) Methionine restriction
Nonketotic hyperglycinemia (glycine cleavage enzyme deficiency)	Glycine ($\uparrow \uparrow$) (plasma and CSF)	Seizures developmental delay	Sodium benzoate (decreases glycine)

CSF, cerebrospinal fluid; SA, succinylacetone; NTBC, 2-(2-nitro-4-trifluoro-methylbenzoyl)-1,3-cyclohexanedione.

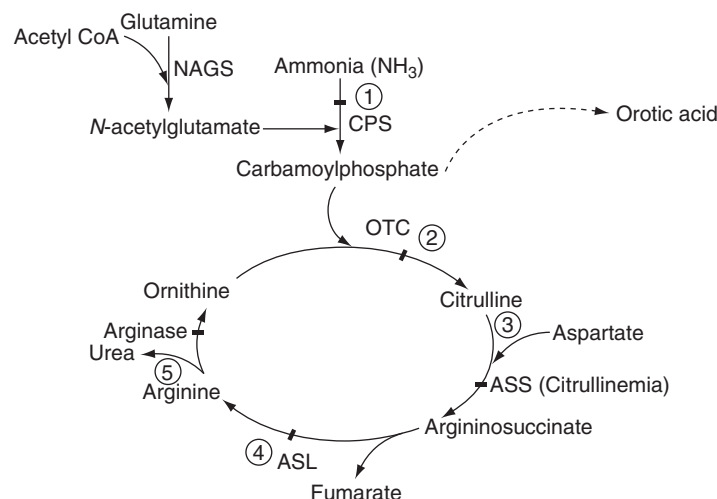


Figure 2

vomiting, tachypnea, and progressive encephalopathy. Routine biochemical testing shows respiratory alkalosis and hyperammonemia. The liver transaminases are usually elevated. Hypoglycemia is not typical.

Plasma amino acid and urine organic acid analysis is necessary to make a presumptive diagnosis. In ASS citrulline is elevated, in ASL argininosuccinic acid and citrulline are elevated and in arginase deficiency, arginine is elevated. In OTC, however, the citrulline is very low or absent. In each of these disorders, orotic acid is also present (found on urine organic acid analysis), produced via the pyrimidine cycle from excessive carbamoylphosphate that accumulates due to each enzyme defect. In CPS and NAGS, however, the amino acid and orotic acid levels are normal, so the diagnosis is essentially one of exclusion in a patient who presents with the typical symptoms and severe hyperammonemia in which no other cause is determined. Confirmation of the diagnosis requires a liver biopsy for enzyme analysis for CPS, NAGS, and OTC. Skin fibroblasts can be assayed for ASS and ASL and red blood cells for arginase deficiency; mutation analysis may be possible in some cases.

OTC is the most common urea cycle defect. It is inherited in an X-linked disorder (all other disorders are autosomal recessive). Male infants usually present in the first few days of life with severe life-threatening hyperammonemia. Newborn screening may be abnormal because of absent citrulline, although severe symptoms and irreversible brain damage may occur before results are available. Females carrying the X-chromosome mutation may be asymptomatic, but in some cases, symptoms can occur. They can present with variable symptoms ranging from acute hyperammonemia to recurrent episodes of nausea, vomiting, and headache. The severity of these symptoms depends on the degree of lyonization (the inactivation of one of the X chromosomes) and the resultant residual enzyme activity. Some women may remain asymptomatic and a diagnosis can only be made after the birth of a symptomatic son.

Patients with ASL deficiency also have progressive cirrhosis of the liver, possibly due to the direct toxic effect of the

argininosuccinic acid. In arginase deficiency, hyperammonemia is rare (most of the urea has already been eliminated) but arginine itself is toxic to the central nervous system causing progressive spastic quadriplegia and developmental delay; seizures are common.

The toxicity of these disorders is primarily due to the accumulation of ammonia (NH_3) and glutamine, which is increased because of the transfer of excess ammonium ions (transamination). Acute, severe hyperammonemia in the newborn period is catastrophic and often fatal. Survivors have variable neurological deficits.

Acute treatment of hyperammonemia due to a urea cycle defect is elimination of dietary protein, elimination of ammonia (by hemodialysis or peritoneal dialysis), high concentration of dextrose to reverse catabolism, arginine (except in arginase deficiency) to regenerate the cycle, and the nitrogen scavenging drugs, sodium benzoate (which conjugates with glycine to form hippurate) and sodium phenylacetate (which conjugates with glutamine to form sodium phenylbutyrate). Early reintroduction of limited dietary protein is necessary to provide substrate for anabolism and to prevent further catabolism. This should consist of whole protein and a special formula to provide enough essential amino acids to ensure normal weight gain, without producing excessive amounts of nitrogen for ammonia production. Chronic treatment includes similar dietary protein restriction, arginine and an oral form of nitrogen scavenging medication, sodium phenylbutyrate. A derivative of the amino acid, L-glutamic acid, N-carbamylglutamate (NCG), has recently been approved for treatment of NAGS, by providing an alternate activator of CPS. For arginase deficiency, dietary protein restriction and formula are usually adequate.

Organic acidemias are due to enzyme deficiencies further along the catabolic pathway, usually of several amino acids, resulting in the accumulation of the toxic products of intermediary metabolism (organic acids). In some cases, there is a functional defect of the enzyme due to a deficiency of the enzyme cofactor, rather than of the enzyme itself. Examples of

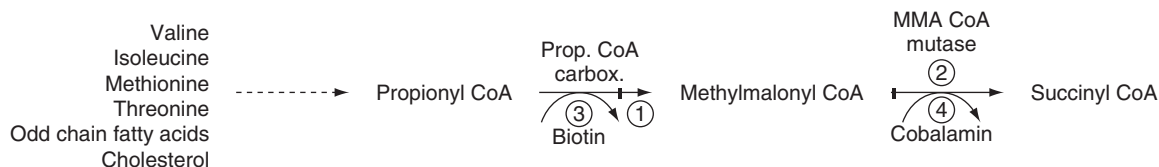


Figure 3

this are biotinidase deficiency and defects of cobalamin (vitamin B₁₂) metabolism.

Accumulation of large amounts of organic acids causes severe metabolic acidosis and ketosis. Hyperammonemia is often present, due to secondary inhibition of the urea cycle. Hypoglycemia may be variably present, due to secondary inhibition of fatty acid oxidation. Symptoms are often present in the newborn period; recurrent episodes of metabolic decompensation can occur because of excessive protein intake or because of catabolism (and therefore an increased load of amino acids endogenously released from muscle) associated with acute infections or prolonged periods of fasting. Morbidity and mortality are due to acute acidosis and the associated neurologic sequelae.

The diagnosis is made by finding high levels of the characteristic organic acids in urine. Newer analytic methods, such as MS/MS can detect even small elevations of characteristic plasma acylcarnitine and urine acylglycine conjugates of the intermediary metabolites. Confirmation is by enzyme analysis, usually in skin fibroblasts; DNA mutation analysis is available for many disorders. Treatment typically entails limitation of dietary protein and supplementation with a specific metabolic formula that is depleted in the amino acids in the specific catabolic pathway.

Propionic acidemia is a typical organic acidemia. It is due to an isolated defect of the enzyme, propionyl CoA carboxylase in the catabolic pathways of the amino acids isoleucine, valine, methionine, and threonine as well as cholesterol and odd chain fatty acids (Figure 3). The resulting accumulation of the intermediary metabolites, 3-hydroxypropionic acid, methylcitric acid, propionylglycine, and tiglylglycine, can cause severe metabolic acidosis, ketosis, coma, and death. Other associated symptoms can be hyperammonemia, hypoglycemia, and pancytopenia, due to bone marrow suppression by the accumulated toxic organic acids.

Symptoms can occur within days of birth in the classic disease or later in infancy or childhood in the milder variant forms. The later-onset form may be associated with persistent vomiting, failure-to-thrive, and developmental delay, but often without severe episodes of metabolic acidosis. Dystonia may occur due to infarction of the basal ganglia.

Cofactor Deficiencies

Biotin is an essential cofactor for the four carboxylase enzymes, propionyl CoA carboxylase, methylcrotonyl CoA carboxylase, pyruvate CoA carboxylase, and acetyl CoA carboxylase. It is endogenously derived from lysine and also present in its protein-bound form in small amounts in many foods. Holocarboxylase synthetase (HCS), which forms the inactive parent apoenzyme, is also biotin dependent. Enzyme activation requires free biotin, which is released by the action

of biotinidase; this enzyme also plays an essential role in the recycling of biotin for further use. A deficiency of biotinidase, therefore, results in depletion of biotin and a functional defect of the carboxylases. Symptoms include hypotonia, lethargy, vomiting, and ataxia. Recurrent metabolic acidosis may occur. Alopecia and a generalized erythematous rash are common. The symptoms are more severe in HCS deficiency. The characteristic pattern of organic acids is present in both disorders. The diagnosis is distinguished by measuring biotinidase activity in plasma or carboxylase enzyme activity in leukocytes or fibroblasts. Treatment with pharmacologic doses of biotin is effective.

Multiple defects of cobalamin (vitamin B₁₂) metabolism can occur, starting with the transport of vitamin B₁₂ into the cell (defects of the transporter proteins, Transcobalamin I and II) or subsequent intracellular utilization of the different biologically active forms. These disorders are classified as complementation groups, depending on whether the defect is in adenosylcobalamin (Cbl A and B), methylcobalamin (Cbl G and E), or both (Cbl C and D).

Adenosylcobalamin is the cofactor for methylmalonyl CoA mutase; a defect results in a milder form of methylmalonic acidemia than found with a defect of the enzyme itself. Methylcobalamin is the cofactor for methionine synthase, which results in low methionine and homocystinuria (distinct from classic homocystinuria due to a defect of cystathionine β synthase). A defect of both adenosyl and methylcobalamin causes both methylmalonic acidemia and homocystinuria (Table 2).

Symptoms vary with the complementation group, but can include metabolic acidosis, hypotonia, developmental delay, muscular degeneration, and megaloblastic anemia.

Treatment with hydroxocobalamin corrects some of the biochemical derangements, especially in Cbl A and B. Addition of betaine, a remethylating agent, may decrease homocysteine levels in CblC. Treatment is less successful in the other groups.

A syndrome similar to Cbl C has been described in the breastfeeding infants of strict vegetarian (vegan) mothers and in mothers with pernicious anemia, who are vitamin B₁₂ deficient.

Disorders of Fatty Acid Oxidation

These disorders have only been recognized since the early 1980s, but as a group representing the most common inborn errors of metabolism. Fat provides a significant source of energy as glucose and ketone bodies during times of metabolic stress (such as febrile illness) or with prolonged fasting. Free fatty acids, released from the adipose tissue, are

Table 2

Disorder (deficient enzyme)	Elevated analyte(s)	Clinical features	Treatment
Methylmalonic acidemia (methylmalonyl CoA mutase)	Methylmalonic acid	Metabolic acidosis Hyperammonemia Failure to thrive Vomiting	Protein restriction Carnitine
Isovaleric acidemia (isovaleryl CoA dehydrogenase)	Isovalerylglycine	Metabolic acidosis Vomiting	Protein restriction Glycine
Glutaric aciduria type I (glutaryl CoA dehydrogenase)	3-Hydroxyglutaric acid Glutaric acid	Metabolic acidosis Vomiting Macrocephaly Developmental delay	Protein restriction Carnitine
3-Methylcrotonyl glycinuria (3-methylcrotonyl CoA carboxylase)	3-Hydroxyisovaleric acid 3-Methylcrotonylglycine	Metabolic acidosis Hypoglycemia Hyperammonemia Seizures (Some patients asymptomatic)	Protein restriction Carnitine
Mitochondrial acetoacetyl CoA thiolase deficiency	2-Methyl-3-hydroxybutyrate acid 2-Methylacetoacetic acid Tiglylglycine	Metabolic acidosis Vomiting	Protein restriction Carnitine

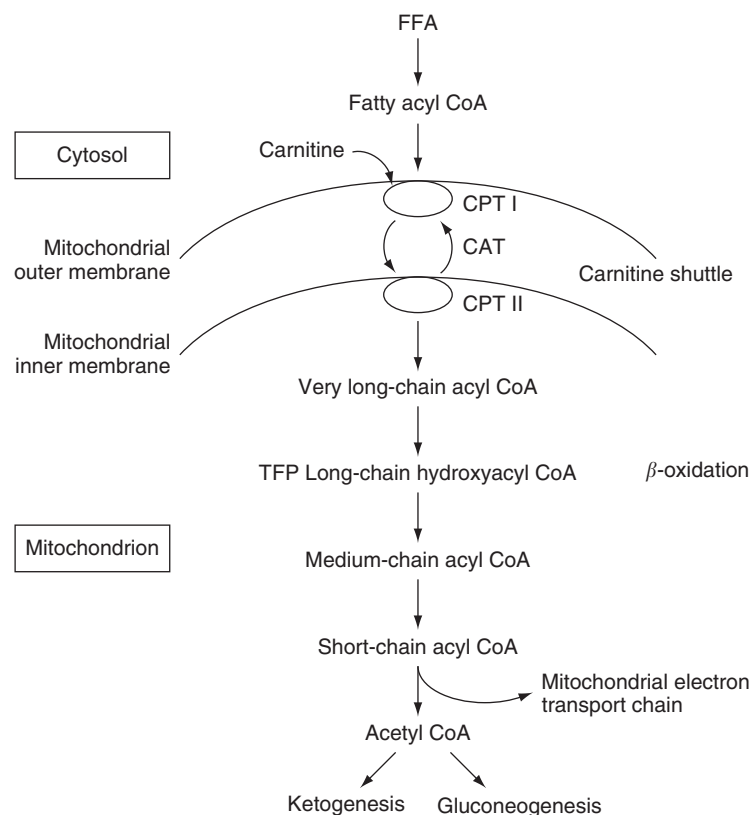


Figure 4

transported into the mitochondria via the carnitine shuttle system, where they then undergo β -oxidation (Figure 4), the progressive cleavage from an 18-carbon length very long-chain fatty acid to the 2-carbon length acetoacetyl CoA, the substrate

for glucose (via the TCA cycle) and ketones. A deficiency of any of the enzymes in this pathway can cause symptoms primarily of hypoketotic hypoglycemia and hepatic encephalopathy, with hyperammonemia (due to secondary inhibition

of the urea cycle) and sudden death. Many cases of what would previously have been diagnosed as Reye syndrome are now known to be due to fatty acid oxidation defects. Symptoms can occur at any time, from the newborn period to adulthood.

Carnitine has a dual role – as well as its critical role in the transport of free fatty acids into mitochondria, it also conjugates with the fatty acyl CoA intermediates that accumulate proximal to an enzyme block, forming acylcarnitine species that can be excreted by the kidneys. They can also be measured in plasma for diagnostic purposes and in the newborn screening dried blood spot. Increased utilization of carnitine due to an enzyme defect causes a secondary depletion, further impairing fatty acid oxidation.

Long-chain fatty acid oxidation defects (CPT I and II, VLCAD, TFP, and LCHAD) may present in the newborn period or later in infancy with severe hypoketotic hypoglycemia, cardiomyopathy and hepatic encephalopathy, due to deposition of fat in the heart and liver. Rhabdomyolysis (lysis of muscle cells) is common. Pigmentary degeneration of the retina may be present in LCHAD, thought to be due to impaired endogenous production of DHA, which is necessary for normal retinal function. Milder variant forms of CPT II and VLCAD may present in adolescence or adulthood with muscle cramping and rhabdomyolysis, which may be severe enough to cause acute renal failure due to the deposition of the muscle pigment, myoglobin, in the renal tubules.

Treatment for these disorders, which can reverse the cardiac and liver disease, includes frequent feeding and avoidance of fasting, but also limitation of dietary fat and supplementation with medium-chain triglycerides (MCT), which bypass the metabolic block. There is no clear consensus on the amount of MCT needed; the general recommendation is to provide 20–40% of total calories from fat, with about half of these calories coming from MCT. Special formulas can provide the MCT requirements, but some are deficient in some essential fatty acids, such as linoleic and linolenic acids and DHA. Addition of oil, such as canola, provides most of the essential fatty acids. DHA is not currently commercially available, but fish oil may provide an alternative source. Uncooked cornstarch can be used to provide an alternate source of complex carbohydrate (especially for overnight fasting) after the age of about 9 months. Normal pancreatic amylase activity is necessary, and may not be adequate before this age.

Treatment for the medium- and short-chain defects (MCAD and SCAD) is simpler, involving avoidance of fasting and early intervention during acute illness to prevent hypoglycemia. Carnitine supplementation is frequently used to prevent secondary depletion. Dietary fat recommendations are approximately 30% of total calories, or a 'heart healthy' diet.

Disorders of Carbohydrate Metabolism

Galactosemia

Galactose is derived primarily from dietary lactose, which is the major disaccharide in dairy products, human breast milk and many fruits and vegetables. There is also a small contribution from endogenous production. There are three known enzyme deficiencies in the pathway that oxidizes galactose to glucose (Figure 5); all are autosomal recessive genetic disorders.

Classic galactosemia is due to almost complete absence of galactose-1-phosphate uridyl transferase (GALT) activity. Symptoms generally occur in the first few weeks of life, with poor weight gain, lethargy, hypotonia, and liver disease (hyperbilirubinemia, coagulopathy, and hepatomegaly) and renal tubular acidosis. Hypoglycemia can occur. *E. coli* sepsis may also be a complication; elevated galactose is thought to impair leukocyte bactericidal activity, allowing the bacteria to more easily invade the red blood cells with subsequent dissemination. Mental retardation is a long-term complication.

The underlying pathogenesis of galactosemia is not fully understood; despite compliance with the lactose-restricted diet, speech delay is almost universal; some patients have learning disorders; many female patients have ovarian failure, though successful pregnancies have been reported.

Treatment is restriction of lactose in the diet, primarily by elimination of dairy products and other foods known to be high in galactose.

Variant forms of galactosemia occur due to mutations in the GALT gene that result in greater residual enzyme activity. The commonest variant is the Duarte Variant, in which there is usually one copy of a classic galactosemia mutation (e.g., Q188R) and one copy of the variant N314D. This combination results in approximately 25% residual enzyme activity. There is varying opinion on whether or not dietary treatment is necessary; some programs consider that the residual enzyme activity is adequate to prevent the pathologic sequelae, others elect to treat with lactose restriction for the first year of life. There are no long-term outcome data to support either approach.

Galactokinase (GALK) deficiency causes excessive accumulation of galactitol, which is oxidized from galactose by an alternative pathway. High levels of galactitol cause cataract formation, which is the only symptom of this disorder. Lactose restriction is necessary.

Epimerase deficiency (GALE) is very rare. There are two isoforms of the enzyme, one isolated in red blood cells, and one in the liver. The most common disorder is due to an isolated deficiency of the RBC isoform, which will be detected incidentally by newborn screening programs that measure total

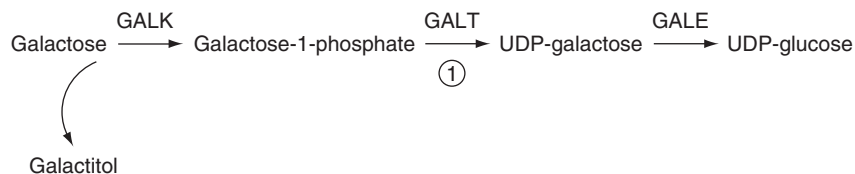


Figure 5

galactose. There are no clinical symptoms and no treatment is necessary. A defect of both isoforms will cause symptoms similar to classic galactosemia and should be treated similarly.

Glycogen Storage Disorders

Glycogen is a complex carbohydrate stored primarily in liver and muscle. Liver glycogen provides glucose to maintain blood sugar levels in between normal feedings; defects of the liver enzymes for glycogen degradation lead to hypoglycemia and/or liver disease because of excessive accumulation of glycogen. Muscle glycogen is an important substrate for energy production for normal muscle function so symptoms are mainly cramping with exercise.

Glycogen Storage Disease Type I (GSD I) (Figure 6), the most common disorder, is due to a deficiency of glucose-1-phosphatase in liver, kidney, and intestinal mucosa. Symptoms typically occur in infancy when frequency of feeding decreases. Profound hypoglycemia can occur; progressive hepatomegaly and liver dysfunction is due to storage of glycogen. Other metabolic derangements include lactic acid-

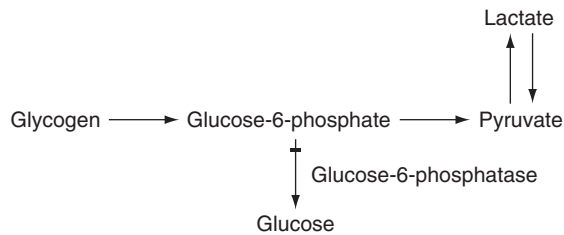


Figure 6

emia, due to increased pyruvate production; increased fatty acid synthesis causes hypertriglyceridemia and hypercholesterolemia (causing xanthomas); hyperuricemia (causing gout and renal calculi) is due to decreased renal excretion (lactate is preferentially excreted) as well as increased uric acid production due to phosphate depletion. Other long-term complications include progressive renal disease (proteinuria) and hepatocellular carcinoma. Treatment is frequent meals and continuous nocturnal feeding (in infants) and supplemental uncooked cornstarch to provide exogenous glucose. Other disorders are summarized in Table 3.

Disorders of Fructose Metabolism

There are three disorders of fructose metabolism, all inherited in an autosomal recessive fashion. Fructose is widely distributed in the diet as the primary sugar in fruits, vegetables, and honey. It is also derived from sucrose and sorbitol, which are also found in a large variety of products, including infant formulas and intravenous fluids. The toxic effect of fructose is due to inhibition of gluconeogenesis by high levels of fructose-1-phosphate and subsequent depletion of inorganic phosphate and thus, ATP.

Essential fructosuria is a benign disorder due to a defect of the enzyme, fructosekinase. Patients have increased urinary excretion of fructose, which is usually an incidental finding on routine testing for reducing substances.

Hereditary fructose intolerance (HFI) is due to a deficiency in aldolase B, which splits fructose-1-phosphate into glyceraldehyde and dihydroxyacetone. Symptoms only occur after exposure to fructose, usually from dietary ingestion, although

Table 3

Disorder	Deficient enzyme	Primary affected tissue	Symptoms	Treatment
GSD 0	Glycogen synthase	Liver	Hypoglycemia	Uncooked cornstarch, frequent feeds
GSD I	Glucose-6-phosphatase	Liver, muscle	Hypoglycemia, hepatomegaly, growth retardation, proteinuria, lactic acidemia, hyperlipidemia, hyperuricemia (gout), hepatocellular carcinoma	Uncooked cornstarch, frequent feeds
GSD II (Pompe disease)	Acid maltase (α glucosidase)	Lysosomes of muscle (skeletal and cardiac)	Cardiomyopathy, skeletal myopathy, cardiorespiratory failure	Enzyme replacement (in clinical trial)
GSD III	Debranching enzyme (amylo-1, 6-glucosidase)	Liver, muscle	Hypoglycemia (mild), hepatomegaly, myopathy, hyperlipidemia	Uncooked cornstarch, frequent feeds
GSD IV (amylopectinosis)	Branching enzyme	Liver	Hepatomegaly, cirrhosis, liver failure, myopathy	Liver transplant, Uncooked cornstarch
GSD V (McArdle disease)	Myophosphorylase	Muscle	Muscle cramping (with exercise)	Oral glucose, high-protein diet
GSD VI (Hers disease)	Liver phosphorylase	Liver	Hepatomegaly, hypoglycemia, myopathy	Frequent feeds
GSD VII (Tarui disease)	Phosphofructokinase	Muscle	Fatigue exercise intolerance, cramping	Avoidance of strenuous exercise
GSD IX	Phosphorylase kinase	Liver, muscle	Hepatomegaly, growth retardation	Frequent feeds

GSD, glycogen storage disorder.

they are more severe after intravenous infusion. These symptoms include gastrointestinal discomfort, vomiting, and hypoglycemia. Chronic exposure causes failure to thrive, liver disease, and renal tubular acidosis. Affected patients are often misdiagnosed as having behavioral problems or an eating disorder. Treatment is elimination of fructose from the diet.

Fructose-1,6-bisphosphatase is a defect of gluconeogenesis and is not dependent on exposure to fructose. Symptoms of recurrent episodes of vomiting, lactic acidosis, tachypnea, seizures and apnea, occur when dietary glucose and glycogen stores are depleted, such as during periods of fasting or with febrile illnesses. Approximately 50% of patients are symptomatic in the newborn period. Treatment is prevention of fasting and supplementation with uncooked cornstarch to provide a source of complex carbohydrate. Acute episodes respond to intravenous infusions of dextrose.

Disorders of Micronutrient Metabolism

Disorders of Copper Metabolism

Copper plays an essential role in normal cell metabolism as a cofactor for several critical enzyme pathways. Normal homeostasis is regulated through a balance of gut absorption and biliary excretion. There are two major inherited disorders of copper metabolism, Wilson disease and Menkes disease.

Wilson disease is an autosomal recessive disorder with an incidence of approximately 1/30 000 (higher in China, Japan, and Sardinia), due to a deficiency of hepatic ATPase. Symptoms usually start in childhood, but there is a wide range of clinical features. Copper that cannot be excreted in the bile is initially deposited in the liver, with reduced incorporation into the carrier protein ceruloplasmin, causing chronic liver dysfunction (cirrhosis), and in some cases, acute liver failure. Increasing levels of copper are also deposited in other tissues, especially the cornea (Kayser-Fleischer rings) and the central nervous system, which is usually later in life, manifesting as a movement disorder and poor coordination. Difficulties with speech and swallowing can also occur. Psychiatric symptoms are also common, often beginning in adolescence.

The diagnosis is made by finding decreased serum ceruloplasmin with increased urine excretion of copper; copper deposition may also be seen on liver biopsy. Confirmation is by mutation analysis.

Treatment is aimed at increasing the excretion of the stored copper with chelating agents, such as D-penicillamine, and a copper-depleted diet. Liver transplantation may be an option.

Menkes disease is an X-linked disorder due to a deficiency of the membrane transporter, ATP 7A, resulting in intracellular copper accumulation and lack of transport to critical tissues, such as the CNS, kidneys, and also to connective tissue. It usually presents in the first few months of life with typical facial features and unusual kinky hair (pili torti), seizures, poor weight gain, and loss of developmental milestones. Death usually occurs by approximately 3 years of age. Diagnosis is made by finding decreased serum copper and ceruloplasmin and mutation analysis. Treatment with copper chloride injections before 10 days of age may slow the progression of the disease in some patients.

A milder form of the disease (due to higher residual enzyme activity) is Occipital Horn Syndrome. Patients have characteristic occipital calcifications (occipital horns), borderline cognitive delay, a distinctive facial appearance, and connective tissue abnormalities (e.g., joint laxity). Treatment is not currently available.

Disorders of Iron Metabolism

Hemochromatosis

There are four different subtypes of hereditary hemochromatosis, due to excessive absorption of iron from the gastrointestinal system, leading to toxic accumulation of iron in various tissues.

Hereditary hemochromatosis is the most common disorder, inherited in autosomal recessive fashion, due to its defect in the HFE gene. The carrier frequency is approximately 1:10 in Caucasians, with a disease frequency of approximately 1/400. The typical presentation is in adults by age 40–60 years, with increased skin pigmentation, chronic liver disease (cirrhosis), portal hypertension, and diabetes mellitus (also called 'bronzed diabetes'), due to excessive iron deposition in skin, liver, and pancreas, respectively. Deposition also occurs in the heart and other endocrine tissues, including the pituitary, leading to hypogonadism in men. Hepatocellular carcinoma is a complication in approximately 30% of patients. There appears to be a male preponderance for end-stage organ failure. Homozygosity for the common mutation, C282Y appears to be associated with greater iron overload, but there is clear no correlation with disease severity. The diagnosis is suggested by increased serum iron and ferritin studies, with confirmation by mutation analysis. Current treatment is removal of excess iron by regular phlebotomy.

Other rare forms of inherited hemochromatosis are juvenile hemochromatosis, which is due to mutations in a different gene, presents earlier and is more severe. Hemochromatosis type 3, due to a defect in the transferrin receptor-2 gene, is similar to HFE-related hemochromatosis. Neonatal hemochromatosis is very severe, with prenatal evidence of iron deposition, but the etiology is not known.

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Nutritional Management of Phenylketonuria

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Abbreviations

ARA	arachadonic acid
BH4	tetrahydrobiopterin
DHA	docosahexaenoic acid
LC-PUFA	long-chain polyunsaturated fatty acid
LNAA	large neutral amino acid

PKU	phenylketonuria
PUFA	polyunsaturated fatty acid
RBP	retinol-binding protein
RDA	recommended daily allowance
RDI	recommended daily intake

Introduction

Phenylketonuria (PKU) is a disorder of amino acid metabolism caused by a deficiency in the enzyme phenylalanine hydroxylase, which converts the essential amino acid phenylalanine to tyrosine. High levels of phenylalanine are toxic to the central nervous system (CNS), resulting in severe irreversible mental retardation. Details of the biochemistry are discussed elsewhere in the encyclopedia.

PKU is often considered a paradigm for the nutritional therapy for metabolic disorders. It was the first inborn error of metabolism identified by newborn screening, thus allowing for early dietary treatment. Early treatment was successful in preventing the mental retardation associated with untreated PKU. Since the advent of successful dietary treatment of PKU four decades ago, the field has expanded greatly, but the principle of treating PKU remains the same – to control the intake of the amino acid that is not metabolized normally. This principle applies to all amino acidopathies, but PKU is used here as an example.

Dietary treatment is started as soon as the diagnosis is confirmed in a newborn. Outcomes are best when the diet is implemented and the phenylalanine levels are within the recommended guidelines by 2 weeks of age. Diet is now recommended to be lifelong. Adult and adolescent patients who have resumed an unrestricted diet, although intellectually normal, have been shown to have an increased incidence of neuropsychiatric illness such as increased anxiety and depression. Others report poor concentration, headaches, and sleep disturbance.

The pathophysiology of PKU is not well understood, although recent focus has been on the role of amino acids in the brain. Phenylalanine competes with other large neutral amino acids for transport across the blood–brain barrier, and it is theorized that high levels of brain phenylalanine and low levels of other amino acids, specifically tyrosine and tryptophan, may impede neurotransmitter synthesis in the brain and be responsible for the symptoms associated with untreated PKU. Although the ideal brain level of phenylalanine has not been established, treatment guidelines have been established for blood levels at various ages, as these guidelines differ slightly in different countries. In the United States, recommendations have been developed by an expert

panel convened under the direction of the National Institutes of Health (NIH) and the American Academy of Pediatrics (Table 1).

The goal of nutritional therapy, therefore, is to keep blood phenylalanine controlled while providing a nutritionally sound diet. This necessitates the use of a special medical food (most often as a formula) that provides amino acids other than phenylalanine. A medical food is required because the phenylalanine restriction required to maintain blood levels within the desired range is so severe that the amount of natural protein allowed in the diet would not support normal growth and development. Several medical foods are currently available. When PKU was first treated, only one medical food was commercially available – a protein hydrolysate from which most of the phenylalanine had been removed. Now, medical foods for PKU use synthetic L-amino acids (other than phenylalanine) as the protein source and are phenylalanine-free. The medical foods vary in the amount of amino acids that they contain; in addition, most of them also provide carbohydrates, fats, vitamins, and minerals, but others do not. The amount of medical food prescribed is intended to meet protein needs at various ages in the life cycle, which is shown in Table 2.

Introduction of Dietary Therapy

Infant formulas for PKU come in a powdered form and are mixed with water and taken as a substitute for regular infant formula or breast milk. In some clinics, only phenylalanine-free formula is given for a few days so that blood

Table 1 Treatment goals for PKU

Age (years)	Phenylalanine level ($\mu\text{mol l}^{-1}$)
0–12	120–360
12–adult	120–900 (120–600 preferred in adolescents)
Maternal PKU	120–360

Source: Adapted from NIH Consensus Development Conference Statement (2001) Phenylketonuria, screening and management. *Pediatrics* 108(4): 972–982.

Table 2 Recommended daily nutrient intakes (ranges) for infants, children, and adults with PKU

Age	Nutrient				
	PHE	TYR	Protein	Energy	Fluid
Infants	(mg kg ⁻¹)	(mg kg ⁻¹)	(g kg ⁻¹)	(kcal kg ⁻¹)	(ml kg ⁻¹)
0 to <3 months	25–70	300–350	3.50–3.00	120 (145–95)	160–135
3 to <6 months	20–45	300–350	3.50–3.00	120 (145–95)	160–130
9 to <12 months	15–35	250–300	3.00–2.50	110 (135–80)	145–125
7 to <9 months	10–35	250–300	3.00–2.50	105 (135–80)	135–120
Girls and boys	(mg kg ⁻¹)	(g day ⁻¹)	(g day ⁻¹)	(kcal day ⁻¹)	(ml day ⁻¹)
1 to <4 years	200–400	1.72–3.00	≥30	1300 (900–1800)	900–1800
4 to <7 years	210–450	2.25–3.50	≥35	1700 (1300–2300)	1300–2300
7 to <11 years	220–500	2.55–4.00	≥40	2400 (1650–3300)	1650–3300
Women					
11 to <15 years	140–750	3.45–5.00	≥50	2200 (1500–3000)	1500–3000
15 to <19 years	230–700	3.45–5.00	≥55	2100 (1200–3000)	1200–3000
≥19 years	220–700	3.75–5.00	≥60	2100 (1400–2500)	2100–2500
Men					
11 to <15 years	225–900	3.38–5.50	≥55	2700 (2000–3700)	2000–3700
15 to <19 years	295–1100	4.42–6.50	≥65	2800 (2100–3900)	2100–3900
≥19 years	290–1200	4.35–6.50	≥70	2900 (2000–3300)	2000–3300

PHE, phenylalanine; TYR, tyrosine.

Source: Reproduced with permission from Acosta PB and Yanicelli S (2001) *Nutrition Support Protocols*, 4th edn. Columbus, OH: Abbott Laboratories.

phenylalanine will quickly decrease to an acceptable level. A prescribed amount of breast milk or standard infant formula, however, should be shortly introduced into the diet. Whole protein is needed to meet phenylalanine requirements and prevent phenylalanine deficiency, which will lead to muscle protein catabolism and inadequate weight gain. For formula-fed infants, both standard infant formulas and PKU medical foods are used in prescribed amounts and are bottle-fed. Breast-feeding of an infant with PKU is possible and, as with all infants, should be encouraged whenever possible. Mature breast milk contains approximately 46 mg per 100 cc of phenylalanine compared to approximately 59 mg per 100 cc in cows' milk protein-based formula and approximately 88 mg per 100 cc in soy-based formulas. Breast-fed infants, therefore, may initially have slightly lower plasma phenylalanine levels. If a mother chooses to continue breast-feeding, she is advised about the proper ratio of breast milk to PKU medical food to feed her infant. The key to either method is frequent monitoring of blood phenylalanine and adjusting the diet based on phenylalanine intake, weight gain, and blood levels. Guidelines for the frequency of monitoring were also recommended by the NIH Consensus Panel (Table 3). The method used for monitoring varies depending on the resources available at individual PKU clinics – either frequent visits to the clinic for blood drawing or filter paper samples (as used for newborn screening) that can be collected at home and then mailed to the clinic or, in some cases, to the newborn screening program, for analysis. Because of the time delay in the latter method, it is more suitable for use after the initial stabilization period.

When an infant with PKU is 4–6 months old, solid food is introduced. Because nearly all food contains some phenylalanine, it must be measured and counted. Lists of the phenylalanine content of foods are available and are essential

to diet management. The phenylalanine content of foods is listed in milligrams; in some clinics, an exchange system is used where one exchange is equal to a given amount of phenylalanine (often 15 mg per exchange, but in some cases 20 or 50 mg). Because the total amount of phenylalanine taken daily remains the same (adjusted for weight gain), adjustments are made in the amount of regular infant formula or breast milk given to the infant once solid foods are started. This process continues until all of the phenylalanine requirement is provided as food. In general, infants with PKU begin with fruit and small amounts of infant cereal. As the infant's appetite increases, other foods are added, but the choices are limited to fruits, vegetables, and in some cases, small portions of bread and cereal products. For some individuals with PKU, the phenylalanine restriction is severe enough to preclude any regular grain products. Instead, specialty low-protein foods are available, often through mail order. A whole array of low-protein breads, cereals, crackers, bagels, pasta, cakes, cookies, and even low-protein cheeses and peanut butter are critical to proper diet management. These foods provide the much-needed variety and calories to the diet. High-protein foods such as meat, fish, poultry, dairy, nuts, eggs, and legumes are not allowed on a PKU diet. Thus, the phenylalanine-free medical food continues to be the main source of protein for life.

A wide variety of medical foods are now available for children and adults with PKU in order to meet different tastes and caloric needs. Some of the medical foods for children, teens, and adults are packaged in pouches or sachets for convenience, and several foods are available in bar, capsule, or tablet form to promote ease of use. Nevertheless, many individuals with PKU struggle with this aspect of the diet. If the full amount of medical food is not taken, nutritional intake is inadequate and may lead to catabolism of lean

Table 3 Monitoring for PKU

Age (years)	Frequency of testing for phenylalanine
0–1	Weekly
1–12	Twice monthly
12–adult	Monthly
Maternal PKU	Twice weekly

Source: Adapted from NIH Consensus Development Conference Statement (2001) Phenylketonuria, screening and management. *Pediatrics* 108(4): 972–982.

body mass, which, in turn, leads to poor control of blood phenylalanine.

Once established, the amount of dietary phenylalanine an individual is allowed remains the same, except for periods of rapid growth, when more phenylalanine may be necessary. A typical phenylalanine intake for a child with severe PKU is 250 mg day⁻¹ and that for a child with moderate PKU is 400 mg day⁻¹. Thus, in addition to getting the proper amount of medical food, the crux of the diet is to provide the prescribed amount of phenylalanine while making the diet taste and appear as appetizing and socially acceptable as possible. Families require a good deal of support in doing this. Internet-based support groups, newsletters, regional networks, family gatherings, as well as camps for children with PKU provide a link for families and a forum for exchange of practical information and emotional support. PKU clinic personnel are another source of support and reliable information on medical advances in treating PKU.

All patients with PKU should have their blood phenylalanine and other amino acids monitored regularly as long as they remain on diet; they should also be seen on a regular basis for a physical examination, especially for assessment of growth parameters in children and adolescents, and review of the dietary intake since the previous visit. Extensive dietary counseling is an ongoing process. It is also recommended that adult patients, who are not following phenylalanine-restricted diets with prescribed medical foods, should be seen at least yearly for nutritional assessment, as they often tend to self-limit their protein intake and may have inadequate diets.

Adequacy of Nutritional Therapy

Carefully executed diet therapy for individuals with PKU is widely considered to be safe as well as efficacious in preventing mental and neurological impairment. However, it cannot be assumed that largely synthetic diets supplemented with individual vitamins, minerals, and trace elements will confer the same benefits as diets composed of whole foods. Synthetic diets may have an inherent inability to supply all essential nutrients. In addition, patients who are non-compliant or partially compliant with their intake of medical food are at increased nutritional risk. Formerly treated patients who are 'off diet' tend to select high-carbohydrate diets and continue their habit of avoiding high-protein foods such as meat, milk, and eggs. Micronutrients formerly supplied by the

medical food, such as vitamin B₁₂, zinc, and iron, may not be replaced in adequate amounts on such a self-selected diet.

Growth

A strict PKU diet supplies 80–90% of its prescribed protein via a phenylalanine-free medical food. Most of the nitrogen in medical foods is supplied via essential amino acids. Meals that supply most of the protein as L-amino acids result in more rapid absorption and oxidation than observed after consumption of whole protein meals. L-Amino acids also may not be as efficiently absorbed as whole protein. Owing to these reasons, protein requirements for patients with PKU are considered to be greater than the World Health Organization (WHO) guidelines and the recommended daily intakes (RDIs). Normal growth and protein status has been observed in infants consuming at least 3 g of protein per kilogram per day. Long-term inadequate protein intake will result in impaired growth in infants and children, low plasma prealbumin concentrations, radiological bone changes (osteopenia), and reduced phenylalanine tolerance. Because phenylalanine is an essential amino acid, it is crucial to prevent its deficiency. Phenylalanine deficiency will result in catabolism of body protein stores and subsequent elevation of blood phenylalanine levels, anemia, and mental retardation as well as the above symptoms accompanying overall inadequate protein intake.

Fatty Acids

Diet-treated children and adults with PKU consume very small amounts of animal fats, including long-chain polyunsaturated fatty acids (LC-PUFAs) such as docosahexaenoic acid (DHA) and arachadonic acid (ARA). Infants are likely to receive more LC-PUFAs than older patients as a controlled amount of breast milk or standard cows' milk/soy formula is used to supply their phenylalanine requirements. Standard formulas in Europe and the United States are now supplemented with DHA and ARA. However, infant metabolic formula products (supplying phenylalanine-free protein) may or may not be supplemented, and child and adult formulations in the United States are not routinely supplemented. Many of the medical foods designed for children and adults with PKU are fat-free and, therefore, are not sources of precursors (linoleic acid and alpha linolenic acid). The diets of children with PKU provide similar energy, higher carbohydrate, and lower lipid (with higher unsaturated/saturated ratio) and cholesterol content than controls. Plasma lipid and erythrocyte levels of treated PKU infants contain lower concentrations of ARA and DHA than controls when LC-PUFAs are not supplied. In adults, usually only DHA levels are significantly dependent on LC-PUFA intake. LC-PUFAs are a structural component of all cell membranes. Alpha linolenic acid-derived compounds are essential for proper development of the CNS and retina. Linoleic acid-derived compounds play a role in promoting normal growth, skin, and reproduction.

Some studies indicate that healthy breast-fed infants have better visual and cognitive development than unsupplemented formula-fed infants. In theory, patients receiving adequate amounts of the essential fatty acid precursors, linoleic acid and

alpha linolenic acid, would be able to synthesize LC-PUFAs via elongation and desaturation reactions. It is unclear whether the amount of LC-PUFAs synthesized would be adequate for optimal function. Conversion rates are low and result in lower LC-PUFA levels than those seen in omnivores consuming a LC-PUFA-containing diet. DHA and ARA may be conditionally essential nutrients. Trials of LC-PUFA supplementation of PKU patients are under way to explore whether DHA depletion may be responsible for subtle neurological deficits. A study of 36 children supplemented with 15 mg DHA per kilogram body weight showed improvement in visual processing, motor function, and coordination though they had been receiving adequate alpha linolenic acid. This study controlled for variations in the phenylalanine level.

One hypothesis proposed to explain suboptimal growth and development of the CNS in untreated PKU and poorly controlled maternal PKU is that phenylalanine metabolites impair elongation and desaturation reactions. There is no firm evidence that this is the reason for the markedly low DHA levels in plasma and erythrocyte phospholipids seen in PKU patients.

Iron, Zinc, Vitamin A, and Selenium

Some diet-treated patients with PKU have exhibited altered iron, zinc, vitamin A, and selenium status. With the exception of selenium, aberrations have been demonstrated even when patients consumed close to or greater than the RDI levels of the vitamin/mineral in question. The mechanisms of these changes are unclear and may be multifactorial. The actual impact of these changes on the health of the individual patients is unknown.

Low serum ferritin but appropriate hemoglobin and mean erythrocyte volumes have been noted even in patients consuming close to three times the recommended daily allowance (RDA) for iron. Iron absorption or bioavailability may be inhibited by the presence of calcium and phosphorous salts, diets high in polyunsaturated fatty acids (PUFAs), and dietary fiber. The presence of alterations in the PUFA composition of gut cell membranes could affect iron absorption. In vitamin A-deficient rats, anemia occurred, which was not remedied by the administration of oral iron. This suggests that vitamin A deficiency in PKU patients could result in anemia unresponsive to iron therapy. The iron status of diet-treated patients should be serially monitored.

Low serum zinc has occurred in infants and children receiving greater than or equal to 70% of the RDA for zinc. Low serum zinc occurred more often in patients receiving casein hydrolysates than in patients receiving L-amino acids alone. Serum zinc may not be an accurate marker for assessment of zinc deficiency. Zinc absorption, in general, is inhibited by a PUFA-rich diet, fiber, phosphorous, and large amounts of iron. Competitive inhibition between calcium and zinc also occurs.

Low plasma retinol levels have been observed in infants and young children despite consumption of up to three times the RDA for vitamin A. Retinol is transported on retinol-binding protein (RBP); zinc is needed for the synthesis of RBP. Prealbumin is a carrier for RBP. RBP levels have been normal and zinc levels have been normal in nearly all patients with low retinol levels. Low prealbumin levels or abnormal release of

RBP from prealbumin may be responsible for the low serum retinol levels. In fact, a number of children run low prealbumin levels despite receiving adequate protein and energy intakes.

Until recently, selenium was not routinely added to PKU formulas. Selenium was formerly supplied to patients via contamination of foods grown in selenium-containing soil. Low serum, whole blood, urine, and hair levels of selenium have been observed in some patients with PKU on strict diet therapy. Low activity of the selenium-containing enzyme glutathione peroxidase also occurs. Clinical symptoms of selenium deficiency in the patients studied have not been reported.

Bone Mineral Density

Osteopenia is prevalent in diet-treated persons with PKU from early life. Reduced bone mineral density and bone mass have been detected in up to approximately 50% of patients screened by various methods. These methods have included dual-energy X-ray absorptiometry (DEXA), peripheral quantitative computed tomography (pQCT), and single-photon absorptiometry (SPA). The defect seems to be characterized by a reduction in the velocity of bone mineralization, especially after the age of 8 years. Osteoporosis is an important cause of morbidity and mortality in older adults in the general population. Reduction in bone mass increases the risk of fracture. A reduction of one standard deviation in spine bone mass is associated with a bone fracture rate of 2.0–2.5. Some authors have reported an increased fracture rate in children older than 8 years with PKU.

The pathogenesis of osteopenia in PKU is under study. Discrepant associations have been reported between osteopenia and blood phenylalanine levels, serum vitamin and mineral levels, protein, vitamin, and mineral intakes, serum markers of bone formation and PTH, and ratios of urinary minerals: creatinine. One theory is that impaired mineralization is a direct effect of the lifelong disease process of PKU. The total and the bone-specific fraction of alkaline phosphatase are reduced in some patients. This reduction may affect osteoblast activity and impact bone formation and turnover. High levels of phenylalanine in blood have not been consistently correlated with osteopenia. High levels of phenylalanine and phenylalanine metabolites in blood would result in their increased urinary excretion. Chelating of minerals with phenylalanine and phenylalanine derivatives could theoretically result in significant mineral losses.

Osteopenia may be an accumulated result of lifelong diet treatment or poor diet compliance at vulnerable stages of bone development. Compliant patients tend to have low variation in their lifelong intake of whole protein, as controlled amounts of whole protein are required to maintain good metabolic control. Compliant patients tend to have similar trends in overall intakes. Lack of adequate trace elements, whole protein, vitamins, and minerals may be culprits. Impaired absorption of the synthetic diet or the type of medical food used (hydrolysate vs. elemental formulation) may exert an independent effect. Inadequate intakes of calcium and phosphorous are known risk factors for the development of osteoporosis in nonaffected persons. Tailoring medical foods to specifically deliver the amounts of calcium and phosphorous recommended in the new RDIs may help to prevent osteopenia.

Trials of Clairol (1-25 (OH)₂ D) supplementation in estrogenic patients with PKU are in progress. Clairol has been chosen as most patients are already receiving expected sun exposure from participating in normal outdoor activities, and their intakes of dietary vitamin D generally meet or exceed the RDA. Clairol has been shown to be a useful treatment; treated patients require close monitoring of urinary calcium excretion and blood calcium levels.

Maternal PKU

For women with PKU who intend to become pregnant, following a strict phenylalanine-restricted diet and controlling blood phenylalanine to 120–360 mol l⁻¹ is critical to offspring health. Women with PKU who have high blood phenylalanine levels are at high risk of having children with microcephaly, mental retardation, low birth weight and, congenital heart anomalies. In an International Study of Maternal PKU, women who were in good metabolic control by 10 weeks' gestation had babies with good birth outcomes and development. In women in poor control, the degree of microcephaly and mental retardation was proportional to the level of blood phenylalanine. Congenital heart disease, however, was not directly related to the degree of metabolic control, suggesting that etiology is multifactorial. The recommendation is for women to be on diet and in good control before conceiving in order to prevent damage to the fetus. Nevertheless, many women come to medical attention during pregnancy, indicating the need for better strategies for keeping women on diet for life and for helping them return to diet before pregnancy. Although blood phenylalanine during pregnancy was the best predictor of outcome in maternal PKU in the Collaborative Study, other nutritional factors, including sufficient energy, protein, vitamin B₁₂, and fat also played an important role.

Alternative Therapies

Tetrahydrobiopterin

Tetrahydrobiopterin (BH₄) is the cofactor for phenylalanine hydroxylase. Some mutations for PAH are considered to be relatively milder than others; some studies have shown that certain of these mutations result in enzyme activity that may be improved by the addition of BH₄ to the diet. In these cases, the degree of phenylalanine restriction needed to maintain good control could be liberalized, although not eliminated altogether.

Large Neutral Amino Acid Supplementation

The large neutral amino acids (LNAA), phenylalanine, tyrosine, tryptophan, and the branched-chain amino acids share the

same L-amino acid transport system across the blood-brain barrier. High levels of phenylalanine in the blood, therefore, impede the transport of these other amino acids into the CNS. Tyrosine and tryptophan are important neurotransmitter precursors; relative deficiency or imbalance of which may contribute to the neuropsychiatric symptoms seen in some adult PKU patients who have resumed an unrestricted diet. Treatment with supplemental LNAAs (in tablet form) theoretically will increase the competition with phenylalanine for transport into the CNS. A net reduction in phenylalanine and an increase in CNS tyrosine and tryptophan may result in improvement in symptoms. Long-term outcome data are not yet available. This treatment is not suitable for children or women in the child-bearing years that might be contemplating pregnancy.

See also: Brain and Nervous System: Biology, Metabolism, and Nutritional Requirements. Breast Feeding. Osteoporosis: Nutritional Factors. Selenium. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. Vitamin K. Zinc: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements

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Nutritional Management in Adults

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Metabolic and Nutritional Changes in Patients with Infection

An increased blood glucose concentration is the most common abnormality in the infected hospitalized patient. This section discusses the metabolic abnormalities in glucose, protein, and fat metabolism as well as abnormalities in specific nutrients in this population. Specific nutritional treatment plans are presented. In addition, the host response to injury and why patients may not be able to become anabolic with conventional nutritional support are discussed. The acute phase response typifies the host's response to infection. Mechanisms to blunt the catabolic state are important because the extent of muscle wasting and weight loss is inversely correlated with long-term survival. The potential uses of conventional nutritional support and newer nutritional adjunctive techniques utilized for patients are discussed.

Glucose Utilization in Injury and Infection

In nearly all studies of glucose metabolism in patients with infection, injury, or cancer, there is a significant reduction in glucose utilization. This occurs even when the insulin concentrations are in the physiological range. This effect is not overcome even with administration of supraphysiological insulin concentrations. In sepsis, the insulin resistance associated with injury is due to defective insulin-mediated activation of the glycogen storage pathway. By approximately 7 h after the onset of injury, there is a reduction in glucose utilization via the nonoxidative pathway. This injury response persists until the source of injury, infection, or tumor is removed.

Hepatic Glucose Metabolism

During infection, the liver increases glucose production to defend against hypoglycemia. In fact, the increase in hepatic glucose production is the major reason why patients with infection have an elevated blood glucose concentration. For example, patients with active malaria can have an increase in fasting glucose concentration due to an increase in gluconeogenesis and overall glucose production. Approximately 75% of cancer patients, like patients with infection, also have an elevated rate of glucose production. Cancer patients also have a mild form of injury, as demonstrated with mild elevation in AM serum cortisol concentration and rate of hepatic glucose production. In 18 studies, hepatic glucose production

for normal volunteers ranges between 1.6 and 3.0 mg kg⁻¹ min⁻¹, with an average of 2.1 mg kg⁻¹ min⁻¹. Glucose production for cancer patients without weight loss ranges from 1.7 to 5.1 mg kg⁻¹ min⁻¹, with a mean of 2.75 mg kg⁻¹ min⁻¹. This is a 30% increase in the fasting rate of hepatic glucose production. For cancer patients with weight loss, glucose production ranges from 2.3 to 3.3 mg kg⁻¹ min⁻¹, with a mean of 2.96 mg kg⁻¹ min⁻¹. This represents a 41% increase in the rate of hepatic glucose production. Not all cancer types have an elevation in hepatic glucose production. For example, head and neck cancer patients may not have an elevation in fasting hepatic glucose production, but it is commonly elevated in lung cancer patients, probably because they have an increased injury response.

In cancer patients, the etiology for the elevated rate of fasting hepatic glucose production is not known. Early studies tested whether excessive growth hormone (GH) release in cancer patients might be responsible. However, there was no direct correlation between GH secretion pattern and hepatic glucose production. Furthermore, the administration of GH to cancer patients for a 3-day period failed to increase the rate of glucose production. Koea and Shaw suggested that the rate is related to the bulk of the tumor, and others have suggested that it is related to cytokines or other factors. Earlier studies on normal volunteers demonstrated that the loss of the first-phase insulin response causes a delay in the normal inhibition of glucose production. Although the latter effect may explain postprandial hyperglycemia, which is especially seen in patients with type 2 diabetes mellitus, it is an unlikely explanation for fasting hepatic glucose production.

Gluconeogenesis is elevated in head and neck cancer patients and also in lung cancer patients. Gluconeogenesis accounts for approximately 50% of the overall glucose production after an overnight fast. It was demonstrated that glucose carbon recycling was elevated in five of seven published studies. Glucose carbon recycling is an indicator of increased gluconeogenesis. The ability to measure gluconeogenesis was not possible in humans until the 1990s, when a method using [U-13C] glucose and isotopomer analysis was developed. The Cori cycle is increased in cancer patients and has been estimated to account for 300 kcal of energy loss per day. In 70% of published studies, cancer patients have a significant elevation in the rate of gluconeogenesis compared to normal weight-matched controls. Gluconeogenesis was directly related to the morning blood cortisol concentration in both the normal volunteers ($r=0.913$, $p<.01$) and the cancer patients ($r=0.595$, $p<.05$). In the septic host, the increase in glucose production is likely due to an elevation of multiple counter-regulatory

hormones (cortisol, GH, catecholamines, and glucagon) and cytokines (interleukin-1 (IL-1), tumor necrosis factor- α (TNF- α), etc.).

It is important to note that unlike diabetic patients with an elevated blood glucose concentration, cancer patients with an elevated glucose production rate frequently have a normal blood glucose concentration. Fasting glucose concentrations may be 110–120 mg dl⁻¹, which may be overlooked as a subtle indicator of an elevated glucose production rate. The increased rate may contribute to an increased energy cost. Data indicate that the resting energy expenditure (REE) is elevated in lung cancer patients and those with other types of cancer compared to weight-matched controls. As expected, energy expenditure is increased in most critically ill patients a few days after admission. However, the precise measurement of energy expenditure is difficult in this setting. Early in the course of critically ill patients, one should focus on excellent blood glucose control. A total caloric intake of 20–25 kcal kg⁻¹ day⁻¹ should be provided to the nonthermal injured adult patient. Protein intake should be 1.5 g per kg body weight per day. This has been increased to 2.0 g per kg body weight per day in adults with acute kidney injury.

Unlike the normal fasting blood glucose that is seen in cancer patients, patients with injury or infection most commonly have an increase in blood glucose. This has been associated with a large increase in hospital mortality (Table 1). Hyperglycemia as a marker of intensive care unit (ICU) mortality may be greater in surgical patient compared to medical ICU patients. In a prospective randomized clinical trial in which intravenous insulin was provided to surgical patients, preventing the increase in blood glucose associated with injury and infection, there was significantly reduced mortality.

Unfortunately, the benefits seen early on with IV insulin in surgical patients have not been confirmed in hospitalized patients without surgery. In the largest and most recent study, a large group of ICU patients were randomly treated with IV insulin to obtain a fasting blood glucose between 80 and 110 or keep the blood glucose between 140 and 180. Hospital mortality was significantly increased in the group of patients given the IV insulin who had a blood glucose goal between 80 and 110 mg dl⁻¹ (Nice Sugar NEJM 2009). The primary outcome was 90-day mortality for these ICU patients, which was increased from 24.9% in the control arm to 27.5% in the aggressive IV insulin arm ($p < .05$). This would translate to

increased 26 deaths seen in 1000 ICU patients treated with aggressive IV insulin. Part of the difference may have occurred due to the fact that in the surgical study by Van den Berghe, all the patients were force fed with TPN or enteral feeding. In the other studies tight control was compared to moderate control (blood glucose under 200 mg dl⁻¹), which may have not truly tested the hypothesis that hyperglycemia (blood glucose > 199 mg dl⁻¹) is harmful in patients with sepsis. Such a study has yet to be completed to clarify the best goals for patients with hyperglycemia.

Although surgical patients appear to benefit from a reduced glucose concentration after surgery, the same benefit has not been confirmed in medical patients. Patients with known diabetes and elevated blood glucose have a very low mortality risk and may not need to be given aggressive insulin treatment when they have moderate elevations in their blood glucose concentration outside the setting of diabetic keto-acidosis or nonketotic hyperosmolar condition. In comparison, the mortality risk is very high in patients with new onset hyperglycemia. It is in this author's opinion that patients with new onset hyperglycemia may benefit from semiaggressive treatment of their elevated blood glucose resulting in blood glucose concentrations between 120 and 150 mg dl⁻¹.

Protein Metabolism

Sepsis is associated with an increase in skeletal muscle catabolism and a reduction in the rate of skeletal protein synthesis. Both contribute to a large loss of lean body mass during injury and infection. Skeletal protein breakdown occurs more in the fast-twitch or white muscle fibers than in the red fibers. In addition to sepsis, injury and cancer are also associated with muscle wasting and malnutrition. The etiology is multifactorial, including poor dietary intake, insulin resistance, elevated REE, and other unknown factors. Muscle wasting is due to a combination of increased skeletal muscle protein catabolism and reduced skeletal muscle protein synthesis. For example, in an experimental model of cancer cachexia, protein synthesis was reduced in rats with several tumor types and it occurred at small tumor burdens. In humans with renal cell cancer, the rate of muscle protein synthesis was reduced. In this cancer host, the loss of skeletal muscle appears to be due in part to reduced protein synthesis and in part to a normal

Table 1 Mean blood glucose concentrations and hospital mortality

Patients	Controls		IV insulin		Reference
	Glucose (mg dl ⁻¹)	Mortality (%)	Glucose (mg dl ⁻¹)	Mortality (%)	
1600 Mixed ICU	152	20.9	131	14.8*	Krinsley (2004)
1548 C-T surgery	153	10.9	103	7.2*	Van den Berghe et al. (2001)
139 DM with acute MI	162	26.1 ^a	153	18.6*	Malmeberg (1995)
620 DM with acute MI	162	43.9 ^b	148	33.3*	Malmeberg (1999)
3554 DM with C-T surgery	213	5.3	177	2.4*	Furnary (2003)
6104 Mixed ICU	144 ± 23	24.9	115 ± 18	27.5	NICE-SUGAR (2009)
Mean ± SEM	168 ± 11	21.4 ± 6.7	142 ± 12	15.3 ± 5.3	

^aOne-year mortality.

^bThree-year mortality.

* $p < .01$ vs. mortality at baseline.

rate of protein catabolism. This can occur even in the face of an adequate dietary intake.

Whole body protein metabolism can be measured in many ways. The most common isotope is that of the essential amino acid leucine. In the majority of studies on cancer, injury, and infected patients, the rate of plasma amino acid appearance or turnover is elevated. This rate of plasma appearance is a reflection of multiple sites of protein metabolism. The most important are the skeletal muscle, liver, and gastrointestinal (GI) mucosa. Other sites also play an important role, as does the tumor. Studies have demonstrated that the rate of plasma amino acid appearance is related to the bulk of the tumor mass. Measurements of protein metabolism in tumor tissue have demonstrated that the tissue has a very high fractional protein synthesis rate of 50–90% day⁻¹. This is similar to that of the liver, and it contrasts with a rate of 1–3% for the skeletal muscle. However, because the body is composed mostly of skeletal muscle, its overall contribution to whole body amino acid metabolism is large and it contributes to a significant proportion of plasma amino acid appearance rates. Data suggest that the increase in the protein catabolism in humans is via the effect of cytokines (IL-1, IL-6, and TNF) and glucocorticoids, which are known to stimulate the ubiquitin-proteasome pathway of skeletal muscle protein catabolism. Earlier work demonstrates that TNF administration reduces skeletal muscle amino acid content by 20%, but it has no effect on skeletal muscle protein synthesis. The loss of amino acids without stimulation of protein synthesis suggests that TNF stimulates protein catabolism via a loss of amino acids from inside the skeletal muscle. This effect of TNF wanes after 6 h because animals studied at 60 h have a 30% increase in the rate of protein synthesis and a normal skeletal muscle amino acid content. The increased rate of protein synthesis probably reflects the recovery of the depleted amino acid pool due to earlier administration of TNF. The increased intracellular concentration of amino acids in the skeletal muscle may stimulate synthesis. The direct effect of TNF 60 h after a single administration is not likely because it has a short half-life. Chronic administration results in a reduction in whole body protein synthesis and a net loss of skeletal muscle protein but an increase in liver protein synthesis. An increase in the thyroid hormone tri-iodothyronine also plays an important role in promoting protein breakdown in both the ubiquitin-proteasome pathway and the lysosomal pathway. However, under most conditions, patients with malignancy have either a normal or a reduced tri-iodothyronine concentration. Similar processes are responsible for the loss of protein seen in infection.

Data suggest that humans make and break down approximately 300 g of protein per day, which is exchanged and re-used. This is mediated by the flow of amino acids into and out of cells. Because the amino acid pool is small (only 60 g), the turnover is large. An average person ingests approximately 70 g of protein per day and loses approximately 70 g per day in the form of nitrogen (approximately 11 g of nitrogen, of which 80% is found in the urine as urea). The cellular proteins, including muscle and extracellular proteins, are approximately 10 400 g. These proteins are broken down and reused at various rates. The key to a small intake of amino acids in the diet is the reutilization of amino acids locally inside the cell and the

maintenance of the plasma amino acid pool. The amino acid pool is only 0.6% of the whole body amino acid content, but it plays a vital role in the maintenance of protein synthesis.

Cancer patients who have an elevated plasma amino acid appearance rate survive and those with a normal rate have a worse survival. In one study, stage D colorectal cancer patients who were able to sustain an increased whole body protein metabolism over a 3-month period, as measured by amino acid kinetics, survived and those who had a normal or reduced rate died. Although fasting plasma glucose concentrations were greater in the survivors (100 ± 2 vs. 92 ± 3 mg dl⁻¹), there was no difference in glucose production rate, age, and body weight. Carcinoembryonic Antigen (CEA) concentrations were higher in the patients who died, which suggests that they had a larger tumor burden. There may be subgroups of patients who are able to mount an acute phase response, which may improve survival. It is not known why some patients mount an increased amino acid appearance rate with cancer, and further research is needed to confirm that it may predict survival. Historically, an elevated plasma amino acid appearance rate was believed to represent protein wasting, but recent data suggest that an elevated rate of whole body protein metabolism may not reflect maladaptive processes but rather a healthy response to the tumor. An adequate acute phase response to tumor may reflect a greater fight against cancer. The absence of a response may be unfortunate, as data from patients with colorectal carcinoma suggest. Unfortunately, there are no similar data from infected patients for this comparison.

Lipid Metabolism

Energy in the body is stored mainly in body fat, which is depleted during the wasting process. This process is normally increased during fasting without tumor or injury. When the patient has a tumor, there is a metabolic response to the injury that also promotes lipid mobilization. Several authors have implicated a lipid mobilization factor as being responsible for this process, which is believed to occur in both infection and cancer. Data suggest that this factor may also be responsible for the depletion of liver glycogen in cancer cachexia. This (These) factor(s) increases (increase) lipolysis and plasma triglyceride concentrations. The former effect may be due to an increase in the hormone-sensitive lipase and the latter effect due to inhibition of lipoprotein lipase activity. However, the exact factor(s) that is (are) responsible for these effects is (are) not known.

Cancer patients with weight loss have an increase in whole body lipid turnover measured by radioactively labeled fatty acids. However, when weight loss is prevented, there is no increase in the rate of lipolysis. Similarly, the rates of lipid oxidation are normal in cancer patients compared to weight-matched controls. In more severe injury, as seen in sepsis, the rate of lipolysis is increased.

Hormonal Response to Injury, Infection, and Cancer

Infection, cancer, or any injury to the body results in an increase in counter-regulatory hormones as well as insulin

concentration. As a result of cancer, sepsis, or injury, many patients develop the syndrome of insulin resistance even though they had no history of diabetes before cancer. In cancer patients, when the overall injury can be small, many studies have failed to demonstrate an elevation in counter-regulatory hormones. Mild elevations in cortisol concentrations may contribute to the increased protein catabolism and increased gluconeogenesis. When serum insulin is measured with a sensitive assay, cancer patients demonstrate a small but significant elevation in serum insulin concentration. This is consistent with the observation that these patients have insulin resistance. Cancer patients, like diabetics, have a reduced glucose utilization and loss of the first-phase insulin response, and many have an increased fasting hepatic glucose production rate. As mentioned previously, underweight cancer patients frequently have increased fatty acid oxidation and plasma fatty acid appearance rates. Triglyceride hydrolysis involves much more than fat oxidation, so albumin-bound fatty acids are used partially for energy but many are utilized for re-esterification or substrate cycling back to triglyceride.

The rise in serum cortisol as the host's response to the tumor is one of many factors that are responsible for the development of insulin resistance. Insulin resistance is easy to diagnose because the patient's fasting glucose will be elevated. An elevated fasting glucose level of approximately 110 mg dl⁻¹ is a good marker of insulin resistance. This is not likely seen in mild injury alone unless the patient has a predisposition to the development of diabetes mellitus. Although insulin resistance is present, the presence of frank diabetes (blood glucose level > 126 mg dl⁻¹ or > 7 mm) is not common in cancer or mild injury. It is more common in patients with severe infection or injury. Although most of the counter-regulatory hormones are usually normal, serum cortisol and glucagon can be mildly elevated. Newer glucagon assays measure the normal value as 35–45 ng ml⁻¹, so a significant increase in injury can be detected, which was difficult to do with the older glucagon assays developed by Unger. Recent data from pancreatic cancer patients have shown elevated glucagon concentrations, which may be contributing to the development of diabetes. Earlier work found that GH secretion was increased in cancer patients by 24-h analysis and by random sampling. However, after careful study, the increase in GH does not appear to have a major influence on hepatic glucose metabolism. Although there may be a small effect on glycogen breakdown, the major effect is likely via inhibition of glucose utilization in the skeletal muscle.

The sick euthyroid state, in which total tri-iodothyronine (T3) concentrations are reduced in severely injured and infected patients, is common. This is likely a normal response to conserve energy in the injured person as the body's ability to convert the stored form of a thyroid hormone (thyroxine (T4)) into the active form of thyroid hormone, T3, becomes impaired. T4 is converted to an inactive thyroid hormone known as reverse-T3 hormone (rT3). This event may have evolved as a necessary energy-saving response during a severe injury or illness to reduce the known contribution of T3 to REE. The low T3 syndrome is an adaptive way to reduce the normal day-to-day effect of T3 on REE. This process can occur in the aggressive cancers, for which the patient's response is similar to that of an injury response.

In septic and injured patients, all counter-regulatory hormones are routinely elevated, contributing to an increase in protein catabolism, glucose production, gluconeogenesis, and glycogen breakdown and a major reduction in glucose utilization and anabolism.

Acute Phase Response

The development of injury, infection, or cancer cachexia elicits an acute phase response. This is one of the most basic responses of the body to defend itself against injury. Phylogenetically, this response could be considered the most primitive response of the body. This stereotypical response is similar for injury from an accident, burn, infection, foreign objects, and, in some cases, from a tumor. Unfortunately, this response does not occur for all tumors until the tumor burden becomes large. There are some tumors that routinely present with moderate injury responses like that seen with infection, such as in lung cancer, lymphoma, and leukemias. The host develops a response that includes reductions in serum iron and zinc concentrations, increased serum copper, ceruloplasmin, vitamin B₁₂ and ferritin levels, alterations in amino acid distribution and metabolism, an increase in acute phase globulin synthesis, and gluconeogenesis. Although not common, fever can occur, and a negative nitrogen balance results.

Similar to infections, many tumors can elicit a sequence of events that include changes in cytokine levels as well as several classical hormone levels. For example, a malignant process in the lung will attract monocytes that will be transformed into macrophages at the tissue site of tumor. These macrophages will secrete proteins known as cytokines and other peptides that can attract other white blood cells and initiate an inflammatory response common to many types of injury. Cytokines include TNF- α and IL-1–IL-20. TNF and other cytokines circulate to the liver, inhibit albumin syntheses, and stimulate the synthesis of acute phase proteins. Acute phase proteins include C-reactive protein, which promotes phagocytosis, modulates the cellular immune response, and inhibits the migration of white blood cells into the tissues; α 1-antichymotrypsin, which minimized tissue damage due to phagocytosis and reduces intravascular coagulation; and α 2-macroglobulin, which forms complexes with proteases and removes them from circulation, maintains antibody production, and promotes granulopoiesis and other acute phase proteins. Unfortunately, the majority of tumors do not elicit a large acute phase response. This limited response may result in a decreased inflammatory and tumoricidal effect.

Urine Urea Nitrogen Loss as a Marker of Catabolism

As part of the host response to injury, infection, or tumor, patients frequently lose protein in the urine in the form of nitrogen. For example, 16 g of urea nitrogen in the urine per day represents a 1-lb (0.454-kg) loss of lean body mass, such as muscle tissue. In some aggressive cancers, urea nitrogen loss can be as high as 24 g day⁻¹. The loss of 1 g of urinary urea nitrogen is equal to 6.25 g of dry protein. A total of 6.25 g of dry protein is equal to approximately 1 oz (28.35 g) of lean

body mass. A loss of 16 g of urinary urea is equal to the loss of 1 lb (0.454 kg) of skeletal muscle or lean body mass per day. Specific areas of lean body mass loss that may result in a functional impairment of the respiratory muscles include the diaphragm, heart muscle, and GI mucosa. The loss of lean body mass in these areas can contribute to the development of respiratory failure, heart failure, and diarrhea, respectively. The rapid development of malnutrition can occur in patients with infection due to large losses of lean body mass per day.

Vitamin Deficiencies

Reduced serum concentrations of several vitamins, including vitamins C and E, have been reported in patients with sepsis. In one study, the administration of additional vitamins E and C resulted in a significant reduction in 28-day mortality (67.5 vs. 45.7). Clearly, cancer patients with a poor intake can have deficiencies of many vitamins. For example, cancer patients have been noted to have significant reductions in plasma levels of many vitamins, especially folate, vitamin A, and vitamin C.

Vitamin C and Vitamin A

Patients with a premalignant lesion called leukoplakia also have reductions in plasma levels of retinol (vitamin A), β -carotene, and vitamin C. A study of healthy elderly demonstrated that approximately 20% had a reduced vitamin C level ($<0.5 \text{ mg dl}^{-1}$) and 10% had a reduced serum vitamin A level ($<33 \mu\text{g dl}^{-1}$). The replacement of multiple vitamins and minerals with 80 mg of vitamin C and 15 000 IU of vitamin A per day for 1 year resulted in a significant reduction in the number of days associated with infection-related illnesses (48 ± 7 – $23 \pm 5 \text{ day year}^{-1}$). The multiple vitamin and mineral supplement improved the lymphocyte response to phytohemagglutinin and the natural killer cell activity. In another study, the administration of a multivitamin for 1 year demonstrated a 41% reduction in infectious illnesses. In addition, there was a 63% reduction in infection-related absenteeism compared to that of placebo-treated individuals. The administration of MVI to pregnant HIV mothers also reduced HIV progression and mortality (24.7% vs. 31.1% mortality, $p < .05$).

Vitamin deficiency states are difficult to diagnose. Plasma levels of vitamins are not the best way to assess deficiency. Vitamin C decreases during injury. Although plasma vitamin C concentrations reflect whole body stores, the measurement of plasma vitamin A (retinol) is not the best marker of an actual deficiency state. Liver vitamin A measurements may be a better marker. Patients who die of cancer and subsequent infections have an 18% incidence of moderate liver deficiency of vitamin A at autopsy. Serum vitamin A (retinol) levels are low in up to 92% of patients with serious infections. This depletion of liver stores of vitamin A may be due to excessive loss of retinol in the urine in patients with sepsis. In contrast to what is noted in patients with cancer or serious infections, trauma patients who die within 7 days of hospitalization have only a 2% incidence of severe liver vitamin A deficiency. Vitamin A can be

Table 2 Drug-induced nutrient deficiencies

<i>Drug</i>	<i>Nutrient(s) affected</i>
Steroids	Vitamin A, potassium
Phenothiazines	Vitamin B ₂
Tricyclic antidepressants	Vitamin B ₂
Hydralazine	Vitamin B ₆
Isoniazid	Vitamin B ₆ , niacin
Penicillamine	Vitamin B ₆
Metformin	Vitamin B ₁₂
Proton pump inhibitor	Vitamin B ₁₂
Ammonium chloride	Vitamin C
Aspirin	Vitamin C
Phenobarbital and phenytoin	Vitamin C, vitamin D
Tetracycline	Vitamin C
Coumadin	Vitamin K
Estrogen and progesterone compounds	Folic acid, vitamin B ₆
Aminoglycoside	Magnesium, zinc
Platinum	Magnesium, zinc
Diphenylhydantoin	Niacin
Antacid	Phosphorus, phosphates, Vitamin B ₁₂
Diuretics	Sodium, potassium, magnesium, zinc
Laxatives	Sodium, potassium, magnesium
Cholestyramine	Triglycerides, fat-soluble vitamins

provided by supplementation dietary intake, parenteral intake, or intramuscular vitamin A administration. In addition to the changes in folate, vitamin A, and vitamin C mentioned previously, excessive losses of several vitamins have been observed in patients receiving medications that interfere with normal utilization or elimination (Table 2).

Mineral Deficiencies

Multiple elevated cytokines are likely responsible for the commonly observed reduction in serum mineral concentrations. This is known as part of the cytokine-mediated inflammatory response. In addition, in patients with injury, infection, or cancer, the reduced mineral content may also occur secondary to poor oral intake, increased requirements, and excessive urinary and stool losses.

Magnesium

Total body stores are 2028 g of magnesium. Communications with several experts on magnesium and current work on the antiarrhythmic actions of magnesium suggest that the commonly used normal values for serum magnesium levels should be increased from 1.7 – 2.3 mg dl^{-1} to 2.0 – 2.6 mg dl^{-1} . Large losses can occur in conditions such as diarrhea, in which the stool may have up to 12 meq of magnesium per liter and the urine may have up to 25 meq day^{-1} . Large urinary losses can occur in cancer patients given aminoglycosides, diuretics, and ketoconazole. Furthermore, large losses can occur in some of the intestinal fluids (Table 3) in cancer and other operative patients who develop GI fistulas.

Table 3 Electrolyte contents of body fluids

Body fluid	Electrolyte and mineral concentration (meq l ⁻¹)					
	Sodium	Potassium	Chloride	Bicarbonate	Magnesium	Zinc (mg)
Bile	145	5	100	15–60	1–2	–
Colonic fluids	50	30–70	15–40	30	6–12	17
Diarrheal fluids	50	35	40	45	1–13	17
Duodenum	130	5–10	90	10	1–2	12
Ileal fluids	140	10–20	100	20–30	6–12	17
Pancreatic juice	140	5	75	70–115	0.5	–
Saliva	10	20–30	15	50	0.6	–
Stomach fluids	100	10	120	0	0.9	–
Urine	60–120	30–70	60–120	–	5	0.1–0.5
Urine post-Lasix	15 × normal	2 × normal	–	–	20 × normal	–

Zinc

Total body stores are only 2 or 3 g of zinc. Zinc concentration in the blood decreases as an early response to cytokines. This is commonly seen in many different types of injury as well as in cancer patients. There are minor tissue stores of zinc in skin, bone, and intestine. Zinc is redistributed to liver, bone marrow, thymus, and the site of injury or inflammation. This redistribution is mediated by IL-1 and the other cytokines secreted from macrophages. In hospitalized cancer patients, a reduced serum zinc concentration ($<70 \mu\text{g dl}^{-1}$) is not uncommon. The administration of approximately 50 mg of zinc per day is associated with a normalization of the zinc level after 3 weeks of feeding. Fifteen percent of healthy elderly have been found to have reduced serum zinc levels ($<67 \mu\text{g dl}^{-1}$). The replacement of a multivitamin with 14 mg of zinc per day for 1 year resulted in a significant reduction in the number of days associated with infection-related illnesses ($48 \pm 7 - 23 \pm 5 \text{ day year}^{-1}$). This vitamin and mineral supplementation improved the lymphocyte response to phytohemagglutinin and the natural killer cell activity. There was no change in the placebo-treated group.

Zinc supplementation in hospitalized patients may help with normal immune response for minor infection and wound healing. Zinc is needed for cell mitosis and cell proliferation. It has also been demonstrated to improve wound healing in patients provided 600 mg of zinc sulfate (136 mg of elemental zinc) orally per day who had a serum zinc level on admission of less than $100 \mu\text{g dl}^{-1}$. In this double-blind study, the healing rate increased more than twofold in those randomized to receive zinc supplementation. In addition, large losses of zinc can occur via intestinal losses (Table 3). It is important to note that intestinal fluids can contain up to 17 mg of zinc per liter, so the replacement rate of zinc should take into account the abnormal sources of zinc loss as well as the routine nutritional requirements.

Copper

Total body stores are very small at 60–80 mg. Serum copper status is normal or increased compared to that of serum zinc, and cytokines are also believed to be responsible for these changes. The benefits of or rationale for these increased concentrations are not known.

Iron

Total body stores are 3.5–4.5 g of iron. An increase in cytokines also contributes to the observed decrease in serum iron concentration. This is a mediated response to cancer, injury, or infection. The exact mechanism is not known, but iron is stored in the Kupffer cells of the liver until the injury wanes. This is probably a beneficial effect because many microbes use iron as a source of energy. Iron administration should be restricted in patients who have a serious infection because it has been shown to cause harm with fungal, parasitic, malarial, or other types of low-grade or quiescent infections.

Summary

Vitamins and minerals act as cofactors for essential processes in health and in illness. The requirements for the healthy person have been well established and are published as the recommended daily requirements (Tables 4 and 5). The exact needs for the infected, injured, or cancer patient are not well documented and evaluations are in progress. Reduced levels of vitamin C, vitamin A, copper, manganese, and zinc have been observed, and all of these are associated with poor wound healing. Wound dehiscence is eight times more common with decreased vitamin C levels. This is probably due to the fact that vitamin C enhances capillary formation, decreases capillary fragility, is a necessary component of complement, and is key to the hydroxylation of proline and lysine in collagen synthesis. Vitamin A enhances collagen synthesis and crosslinking of new collagen, enhances epithelialization, and antagonizes the inhibitory effects of glucocorticoids on cell membranes. Manganese is a cofactor in the glycosylation of hydroxylysine in procollagen. Copper acts a cofactor in the polymerization of the collagen molecule and as a cofactor in the formation of collagen crosslinks.

Nutritional Assessment and Predictors of Hospital Outcome

Markers of Nutritional Assessment

Conventional nutritional assessment in injured, infected, or cancer patients is of major clinical value. Body weight and

Table 4 Adult daily vitamin nutritional intake (RDI 2010)

Nutrient	Oral	Intravenous	Special requirements (diagnosis)
Vitamin A	4667–6000 IU day ⁻¹ (700–900 µg day ⁻¹)	3300 IU day ⁻¹ (495 mg)	5000 + IU day ⁻¹ (serious infections)
Vitamin B (biotin)	30 µg day ⁻¹	60 µg day ⁻¹	
Vitamin B (folic acid)	1.1–1.2 mg day ⁻¹	0.4 mg day ⁻¹	5 mg day ⁻¹ (ICU patients/thrombocytopenia)
Vitamin B (niacin)	14–16 mg day ⁻¹	40 mg day ⁻¹	
Vitamin B ₁ (thiamin)	1.1–1.2 mg day ⁻¹	3 mg day ⁻¹	50 mg day ⁻¹ (alcoholics/Wernike–Korsakoff)
Vitamin B ₂ (riboflavin)	1.1–1.3 mg day ⁻¹	3.6 mg day ⁻¹	
Vitamin B ₆ (pyridoxine)	1.3–1.7 mg day ⁻¹	4 mg day ⁻¹	
Vitamin B ₁₂	2.4 µg day ⁻¹	5 µg day ⁻¹	
Vitamin C	70–95 mg day ⁻¹	100 mg day ⁻¹	
Vitamin D	600–800 IU day ⁻¹	200 IU day ⁻¹ (5 µg)	
Vitamin E	15 mg day ⁻¹	10 mg	
Vitamin K	90–120 µg day ⁻¹	^a	
Pantothenic acid	5 mg day ⁻¹	15 mg day ⁻¹	

^aVitamin K is routinely given as 10 mg SQ on admission and then every Monday.

Table 5 Daily nutritional requirements (RDI 2010)

Nutrient	Adult daily nutritional requirements		
	Oral	IV	Special requirements (diagnosis)
Macronutrients			
Protein	1.5–2.0 g kg ^{−1}	1.5–2.0 g kg ^{−1}	2–3 g kg ^{−1} (thermal injury, acute renal injury)
Glucose	20–25 kcal kg ^{−1}	20–25 kcal kg ^{−1}	3000 kcal goal in alcoholic liver disease patients
Lipid	4% of kcal	4% of kcal	Can administer up to 60% of calories to prevent hyperglycemia
Micronutrients			
Sodium	1.2–1.5 g	60–150 meq	
Potassium	4.7 g	40–80 meq	
Chloride	1.8–2.3 g	40–100 meq	
Acetate	10–40 meq	10–40 meq	
Phosphorus	700 mg	10–60 mmol	
Calcium	1000–1200 mg	5–20 meq	100 meq or more severe hypocalcemia and hungry bone syndrome
Magnesium	320–420 mg day ^{−1}	10–20 meq	50–100 meq (cardiac arrhythmias, diarrhea)
Zinc	8–11 mg	8–11 mg	10–100 mg (diarrhea, fistula, wounds)
Copper	900 µg	1–1.5 mg	
Chromium	20–35 µg	10–15 µg	40 µg (diarrhea, gastrointestinal losses)
Molybdenum	45 µg	100–200 µg	
Manganese	1.6–2.3 mg	150–800 µg	
Iodine	150 µg day ^{−1}		
Iron	8–18 mg day ^{−1}		
Fluoride	3–4 mg day ^{−1}		
Selenium	55 µg	40–120 µg	120–200 µg (thermal injury, wounds)

history of weight loss are some of the best indicators of survival in patients with infection or cancer. For example, if the patient with lung cancer has a 10% weight loss the survival compared to no-weight loss over the prior 6 months is much shorter. Furthermore, a 30% weight loss predicts a very poor prognosis as compared to a 20% weight loss. The American Society of Parenteral and Enteral Nutrition (ASPEN) has recently (2009) detailed the requirements for mild, moderate, and severe protein calorie malnutrition (PCM). Mild PCM is a weight loss of 10% over the prior 3–6 months. Moderate PCM is a 20% weight loss and severe is a 30% or greater weight loss over the prior 3–6 month period. Similarly, a person without weight loss who is at 90% of ideal body weight (IBW; approximately BMI of 21.3 for a 6-ft (1.8 m) person or 23 for a 5-ft (1.5 m) person) has mild PCM, 80% of IBW (approximately BMI of 18.8 for a 6 ft (1.8 m) person and 20.5 for a 5 ft

(1.5 m) person) has moderate PCM, and 70% of IBW (approximately BMI 16.5 for a 6 ft (1.8 m) person or 17.8 for a 5-ft (1.5 m) person) has severe PCM. Serum albumin is no longer used in the classification of malnutrition. Serum albumin is a negative acute phase protein and drops in severity and duration of the injury.

Serum albumin concentration upon admission is probably one of the best predictors of hospital survival (Table 2). In the past, serum albumin concentration was commonly used as an indicator of nutritional status. Its level provides the clinician with an index of visceral and somatic protein stores for most medical illnesses. A level less than 3.0 was considered malnutrition, and was also called hypoalbuminemic malnutrition or protein malnutrition. Currently, the use of albumin should be used only with regard to severity of illness, be it injury, infection, liver disease, or renal disease. The reason why

albumin is not a marker of malnutrition is exemplified by the isolated starved state such as anorexia nervosa, which is known as a case of pure starvation also known as marasmus. In these patients the serum albumin is normal at 4.5 g dl^{-1} . The serum albumin level remains normal in these patients until they become infected and injured, and then it drops dramatically, predicting a very high mortality rate.

During the injury response due to infection, injury, cancer, surgery etc., the serum albumin can drop. It drops approximately 1 g dl^{-1} with each injury. Therefore, if the serum albumin drops from 4.5 to 3.5 the patient likely has undergone a single injury, urinary tract infection (UTI), pneumonia, etc. If the serum albumin drops from 4.5 to 2.5 then there are two injury (infections, cancer) processes ongoing or the injury process has been ongoing for several weeks. Whereas the drop in serum albumin is acute and due to cytokines turning off mRNA synthesis (TNF- α), its recovery takes a long time. Serum albumin has a 21-day half-life, and this can reflect processes that have been ongoing for a few weeks. The benefit of serum albumin is that it quantifies the body response to the severity and duration of the injury. The further it declines, the more severe the injury response or response to more than one injury (cancer, infection, trauma, inflammation, etc.).

Predictors of Clinical Outcome

The best marker of injury is serum albumin concentration. It is an excellent predictor of survival in patients with cancer and other types of illnesses (Table 6). More than 20 studies have shown that a serum albumin level below normal can be used

to predict disease outcomes in many groups of patients. One of the first studies in this area was a Veterans Administration study in which 30-day mortality rates were evaluated for a total of 2060 consecutive medical and surgical admissions. Investigators found that 24.7% of the patient population had a low albumin level defined as 3.4 g dl^{-1} or lower. The 30-day mortality rate for hypoalbuminemia patients was 24.6% compared to 1.7% for patients with a normal albumin level. These investigators demonstrated an excellent correlation between serum albumin levels and 30-day mortality rates. A 1-g decrease in serum albumin levels ($3.5\text{--}2.5 \text{ g dl}^{-1}$) translated into a 33% increase in mortality. Patients with an average albumin level of 1.8 g dl^{-1} had a mortality rate of 65%. It is interesting to note that of 15 hypoalbuminemia patients in this study who were provided with total parenteral nutrition, only 1 died (7% mortality). This suggests that additional nutritional support in patients with a very low serum albumin may provide substrate (protein, vitamins, etc.) that may help patients recover from their illness. Although the use of serum albumin is currently considered not to be a good nutritional assessment tool, there is little else available with such a stable and long half-life that shows recent injury and or infection drops per-potion. Other short lived proteins can also be helpful for more acute changes in the injury and recover process such as prealbumin, transferrin, TIBC, and retinol binding protein.

Weight loss or being underweight, just like having diabetes, is associated with a greater risk for infection, especially fungal infections. In one study, the most important risk factor for the development of candidemia was PCM. In addition to weight loss and diabetes, the reduced serum albumin level is an

Table 6 Serum albumin and mortality

Patient population	Mortality				
	With normal albumin		With low albumin (%)	Increased risk (-fold)	Albumin cutoff level (g dl^{-1})
	n	%			
VA hospital	2060	1.7	24.7	14.7	3.5
Medical and surgical patients	500	1.3	7.9	6.1	3.5
Hodgkins	586	1.0	10	10.0	3.5
Lung CA	59	49	85	1.7	3.4
VA hospital	152	3.3	25.8	7.8	3.5
Surgical patients	243	4.7	23	4.9	3.5
Malnutrition	92	8.0	40	5.0	3.5
Surgery (colorectal)	83	3.0	28	9.3	3.5
ETOH hepatitis	352	2.0	19.8	9.9	3.5
Pneumonia	38	0	100	–	3.0
Cirrhosis	139	32	52	1.6	2.9
ICU patients	55	10	76	7.6	3.0
Cardiovascular disease	7735	0.4	2.3	5.7	4.8
Trauma	34	15.4	28.6	1.9	3.5
Sepsis	199	0.7	15.9	22.7	2.9
Pneumonia	456	2.1	8.3	4.0	3.5
Multiple myeloma	23	25	50	2.0	3.0
CABG/cardiac valve surgery	5156	0.2	0.9	5.7	2.5
Preoperative (VA hospital)	54 215	2.0	10.3	5.1	3.5
Beth Israel Hospital	15 511	4.0	14.0	3.5	3.4
Hemodialysis	13 473	8.0	16.6	2.1	4.0
Average \pm SEM	4275	7.8 ± 2.8	31 ± 7	15 ± 2 -fold risk	3.5 ± 0.1
Total no. of patients	101 178				

additional risk factor for nosocomial infections. The greater the protein malnutrition, and the more severe the injury (low albumin) the greater the risk for nosocomial infections.

New onset hyperglycemia in nondiabetic patients (fasting blood glucose >125 mg dl⁻¹ or nonfasting >199 mg dl⁻¹) increase hospital mortality by 4–20 fold. Although having an elevated blood glucose associated with diabetes appears to have risk, the risk associated with not having diabetes and an elevated blood glucose is dramatic.

ABC Score: An Acute Predictor of Hospital Survival in Patients with Infection, Injury, or Inflammation:

As mentioned above the amount of weight loss upon presentation to hospital predicts hospital outcome. In addition, the serum albumin concentration also predicts hospital mortality. Lastly, calorie intake in the hospital predicts hospital outcome. To simplify the estimate of mortality risk in the hospital an ABC score for hospital outcome was developed. The score consists of adding points to mortality risk based on serum albumin, weight loss, and calorie intake (see Table 7). All values can be collected over a single day. If the patients are put NPO they should receive no points for calorie intake but if they chose not to eat then the amount eaten is calculated into their ABC score.

Add up risk to obtain hospital mortality risk.

For example, 30 points represents a 30% mortality rate risk, 60 points a 60% mortality rate risk, and 100 points a 100% mortality rate risk. These estimates are risk and intervention with making the correct diagnosis and treating the nutritional deficiency may reduce these risks.

In summary, weight loss or being underweight predicts risk for a poor hospital outcome. Probably the most helpful by itself is the severity of injury, which can be estimated with the use of a serum albumin concentration. In addition, calorie intake also provides an estimate of mortality risk (Hiesmayr 2009). PCM and severity of metabolic injury as reflected by serum albumin concentration provide the clinician with a tool to help predict recovery or mortality. Albumin levels should be monitored at regular intervals (every 3 or 4 days) for hospitalized patients. Once hypoalbuminemia is documented, it is not an ideal indicator of nutritional rehabilitation because it returns to normal slowly (21-day half-life) and lags behind

other indices of nutritional status, such as transferrin (7-day half-life), prealbumin (1-day half-life), or retinol binding protein (4-h half-life). Prealbumin is also a negative acute phase protein and reflects the severity of injury. Owing to its short half-life it is a better protein to follow in the hospital because it returns towards normal after the injury is reduced. Albumin replacement does not reverse the metabolic process that the hypoalbuminemia state represents and it should not be routinely given. The reduced level of protein reserves in the patient and the severity of the metabolic injury or cancer are the two most important determinants of serum albumin level.

Nutritional Diagnoses Commonly Seen in Hospitalized Patients

The diagnosis of malnutrition is made by taking a good history and obtaining a physical exam. It is important to ask the patient if he or she has been able to maintain his or her appetite and body weight during the past several months. A history of a recent hospitalization is also important to note due to the common development of protein malnutrition during hospital stay. The physical exam involves inspection of the muscle mass, especially noting a loss of 'temporalis' muscle, 'squaring off' of the deltoid muscle, and loss of the thigh muscles. Obtaining a measured body weight should be standard on all admissions, and this weight should be followed on a daily basis.

Up to 50% of hospitalized surgical and medical patients have a reduced serum albumin, which is a marker of severe injury. Weight loss is seen in approximately 30% of patients in the hospital. The severity of weight loss is a marker of a poor outcome. In addition, a reduced serum albumin, transferrin, prealbumin, or retinol binding protein level also marks the patient as having a severe injury response. Albumin levels are most commonly used to help quantitate the severity of injury that the cancer or infection is causing. A reduced serum albumin <3.0 g dl⁻¹ in one study was associated with a 4-fold increase in dying and a 2.5-fold increased risk of developing a nosocomial infection and sepsis. As indicated in Table 6, a low serum albumin level predicts a significant increase in mortality across many diseases.

Loss of Lean Body Mass

The use of body weight as an index of muscle mass in the cancer patient is very difficult due to the possible fluid shifts that occur in the extracellular compartment. Body weight can be divided into three compartments: extracellular mass, lean body mass, and fat mass. Extracellular mass is known to increase in malnutrition and as a result of hypoalbuminemia. An increase in extracellular fluid occurs more commonly in the malnourished patient. A large portion of the fluid shift noted in cancer patients is due to a reduction in the plasma colloid oncotic pressure. Lean body mass is the mixture of skeletal muscle, plasma proteins, skin, skeleton, and visceral organs. The skin and skeleton account for 50% of the lean body mass. Currently, there are no convenient markers to

Table 7 ABC score for hospital mortality (albumin, body weight, and calorie intake)

Reduced serum albumin	<3.0 g dl ⁻¹ Minimal	10
	<2.5 g dl ⁻¹ Mild	20
	<2.0 g dl ⁻¹ Moderate	30
	<1.5 g dl ⁻¹ Severe	40
	<1.0 g dl ⁻¹ Dangerous	50
Weight loss	$>10\%$ Mild or BMI <22	10
	$>20\%$ Moderate or BMI <20	20
	$>30\%$ Severe or BMI <18	30
Calorie intake	$<50\%$ of goal (<1000 cal)	3
	$<25\%$ of goal (<500 cal)	6
	Zero calorie intake	10

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determine the loss of nitrogen from either skin or skeleton. The plasma proteins account for only 2% of the lean body mass, but albumin measurement can reflect the overall status of the lean body mass. The viscera accounts for 12% of the lean body mass, and decreases in some visceral sizes (gut atrophy and cardiac atrophy) are noted in cancer patients. Unfortunately, there is no convenient marker of loss of lean body mass that originates from the visceral organs. However, urine creatinine is a marker of skeletal muscle mass. The skeletal muscle accounts for 35% of the lean body mass, and it provides the major storage area for amino acids needed during illness. The standard way to assess the size of the skeletal mass is to determine the creatinine height index by collecting 24-h urine and comparing the value to normal values of creatinine excretion for age, sex, and height. A simplified way is to collect 24-h urine and divide the total amount by the IBW based on the patient's height. The normal value for an adult male is 23 mg kg^{-1} of IBW, and that for a female is 18 mg kg^{-1} . A value of 10% less than normal would be consistent with a 10% loss in the muscle mass for unit height. A value of 20% less than the lower range of normal would classify patients as having mild muscle loss. A 20–40% loss would classify them as having a moderate loss, and a 40% or greater reduction in the creatinine per weight would document severe muscle loss. The most accurate estimate is to obtain urine creatinine over a 3-day period and to repeat at intervals to document the loss of muscle mass over an extended period of time. Dietary creatine or creatinine intake has only a minor influence (<20%) on urinary creatinine in the normal eating individual. Changes in dietary intake may influence the accuracy of the collection, but repeating the values over 3 days will help average variations in dietary intake. Mild impairment of renal function has little effect on creatinine excretion and does not exclude the creatinine height index as a marker of muscle mass. However, when there is total renal failure, the use of the creatinine height index is not accurate because the urinary creatinine is reduced. In addition to urinary creatinine measurement, the serum creatinine is another marker of muscle loss as seen in many cancer patients with normal renal function. A 20% weight loss can be associated with a reduction in the serum creatinine concentration. For example, a 70 kg person has a normal creatinine of 0.8 mg dl^{-1} . A serum creatinine of 0.4 reflects a loss of approximately 50% of muscle mass. The loss of more than 50% of the muscle mass or an IBW of 60% or less has an extremely high risk of mortality due to severe PCM. Aggressive nutritional support should be provided to prevent infection and demise.

Elevated Resting Energy Expenditure

REE is directly linked to the size of the lean body mass. REE is difficult to determine accurately in volunteers because the method of indirect calorimetry has variations when the same individual is restudied. Several studies have demonstrated an elevated rate of energy expenditure when compared to controls of similar weight. The use of D₂O₁₈ (doubly labeled water) has helped in the estimate of energy expenditure and will improve our understanding of energy expenditure in the future.

Nutritional Feeding of the Patient: Enteral versus Parenteral

Vitamins and Minerals

The standard oral and intravenous vitamin intake and what is currently being given at Harbor-UCLA Medical Center and UCLA Medical Center are listed in Table 4. Also included are the few exceptions to the routine intravenous amounts for both Tables 4 and 5. The mineral and trace element requirements are listed in Table 5. These vitamin, mineral, and trace mineral recommendations are for hospitalized cancer patients and noncancer patients who are hospitalized. They should not have oliguric renal failure or cholestatic liver disease. In acute oliguric renal failure, vitamins A and D should be reduced or eliminated from the enteral or parenteral solutions. Potassium, phosphorus, magnesium, zinc, and selenium should be reduced or eliminated. Iron and chromium are known to accumulate in renal failure and should be removed from the parenteral or enteral formulations. In cholestatic liver disease, the trace elements copper and manganese are excreted via the biliary tree in the bile and should be reduced or eliminated to prevent toxicity. In comparison, large amounts of electrolytes and minerals can be lost in GI fluids and in urine (Table 3). It is essential to replace the estimated amounts lost on a daily basis in the parenteral nutrition.

Enteral versus Parenteral Feeding

In all situations, if the gut is functional, then it should be used as the route of calorie administration. Gut atrophy predisposes bacterial and fungal colonization and subsequent invasion associated with bacteremia. Sepsis due to microbial or toxin translocation into the portal system is a frequent source of fever evaluations that do not indicate an obvious source of infection. Utilization of the GI tract can reduce the incidence of bacterial translocation.

Enteral Products

Enteral nutrition is best taken by mouth if the patient can ingest the required amount. If the patient cannot, then either supplements or full tube feeding is the method of choice. Protein in the peptide form is better absorbed than the free amino acid form due to specific transporters in the small intestines for amino acids, dipeptides, and tripeptides. Feeding tube placement is best in the small bowel up to the ligament of Treitz. This can be obtained best by the direct use of fluoroscopy or may be obtained by the passage of the feeding tube into the small bowel by a corkscrew technique, in which the distal tip of the feeding tube is bent at an approximately 30° angle with the wire stylet in place. Upon placement into the stomach, the tube is rotated so that the tip may pass via the pylorus into the small bowel. The infusion of enteral products into the small bowel will reduce the incidence of aspiration because the infusion is below the pylorus. Intubated patients have a low risk for aspiration due to the endotracheal cuff, so

placement of a feeding tube into the small bowel is less essential.

Supplementation of enteral products with higher than standard amounts of the amino acid arginine has been done to enhance immune function. Published data on its beneficial effect in surgical patients have demonstrated some benefit; however, data from nonsurgical patients suggest harm. Immunonutrition should not be given to patients with severe infection, especially patients with pneumonia.

Branched-chain amino acid-enriched enteral products are available and have been shown to improve mental function and mortality in patients with hepatic encephalopathy. Albumin synthesis is also stimulated by branched-chain-enriched amino acid solutions. However, additional branched-chain amino acids did not improve morbidity or mortality in trauma or septic patients randomized to receive branched-chain-enriched amino acids compared to conventional feeding.

Glutamine-enriched enteral formulas are very common. There are many enteral products used in hospitalized patients and for home enteral nutritional support. These can be found at several enteral nutrition pharmaceutical Web sites.

The choice of lipid composition in enteral products is a field that is rapidly evolving, and this is an important decision to be made by the clinician depending on the type of disease being treated. The use of omega-3-enriched fatty acids in the enteral product (fish oil-enriched) has been associated with an ability to modify the inflammatory response that may be related to the increased arachidonic acid metabolism and a decrease in the omega-6 pathway fatty acid metabolism. Unfortunately, most commercially available enteral products that have omega-3 fatty acids also have other additives, such as arginine, glutamine, and nucleotides, so that the benefits attributed to the use of an omega-3-enriched fatty acid enteral diet await future clinical studies. Recent data would suggest that the addition of omega 6 and gamma linolenic acid (GLA) approximately 5% of the calories each has reduced duration of time in the ICU and ICU mortality (McClave 2009). Further studies will be needed to confirm these benefits.

Energy Intake for Patients with Malnutrition

The diagnosis of moderate PCM can be made when the patients have a 20% weight loss during the preceding 3–6 months, or they have a reduced IBW (<80% for height based on IBW, or a BMI of 18.8). Mild PCM is made when the patients have lost 10% of body weight or have no weight loss but are at 90% of IBW (BMI of 21.3). Severe PCM is when the patient has 30% weight loss or no weight loss but have a weight that is at or below 70% of IBW or a BMI of 16.5. Remember that mortality is excessive with severe PCM (30% weight loss or 70% of IBW or BMI 16.5).

There are currently only three studies that support the importance of energy intake in malnourished patients. Elderly hospitalized patients who consume less than 50% of their estimated maintenance caloric requirement have an 8-fold increase in hospital mortality (11.8% vs. 1.5%). This suggests that an intake of less than 1000 kcal may not be helpful. In a prospective study providing approximately 400 additional calories as 'sip feeds,' reduced mortality was seen in severely

malnourished (body mass index <5th percentile or approximately 21), medically ill elderly patients. In this study, patients were randomized to receive 120 ml of enteral supplements provided by the registered nurse three times per day or provided no additional sip feeds. Patients who received the sip feeds had a significantly better energy intake (1409 kcal) than nonsupplemented patients (1090 kcal), and they had an increased overall weight gain compared with a loss in the controls. Patients in the underweight group who received intervention had a significant reduction in mortality compared to controls (15% vs. 35%, $p < .05$). The less underweight or normals did not demonstrate the same benefit. In the third study, patients with less than 25% of recommended calorie intake (<600 kcal) had a 3.7-fold increased rate of nosocomial bloodstream infections. Candida and coagulase-negative *Staphylococcus* accounted for 63% of the nosocomial infections, with candida accounting for 29%. The energy intake is a surrogate marker of protein intake in the critically ill. Usually the protein content of food and enteral products given to critically ill patients is 20%. There are also data that hypocaloric feeding in the critically ill where protein intake is provided at the required level can improve outcome in certain circumstances. Protein intake, in this author's opinion, is more important than total calorie intake. This is especially true if the calorie intake results in new onset hyperglycemia, which can be seen in many critically ill patients who have a history of prediabetes (A1c of 5.7–6.4%).

See also: Amino Acids: Chemistry and Classification. Ascorbic Acid (Vitamin C): Deficiency States. Biochemical Indices. Cancer: Dietary Management; Epidemiology of Gastrointestinal Cancers Other than Colorectal Cancers; Epidemiology of Lung Cancer. Carbohydrates: Regulation of Metabolism. Cholesterol: Sources, Absorption, Function, and Metabolism. Copper. Cytokines: Nutritional Aspects. Dental Disease: Etiology and Epidemiology. Diabetes Mellitus: Classification and Chemical Pathology. Energy Metabolism. Glucose: Chemistry and Dietary Sources; Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level. Iodine: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements. Iron: Physiology, Dietary Sources, and Requirements. Magnesium. Malnutrition: Secondary, Diagnosis and Management. Nutritional Assessment: Anthropometry; Clinical Examination. Nutritional Support: Adults, Enteral. Parenteral Nutrition. Protein Deficiency. Vitamin A: Deficiency and Interventions. Vitamin B₆: Physiology

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IODINE

Contents

Deficiency Disorders and Prevention Programs Physiology, Dietary Sources, and Requirements

Deficiency Disorders and Prevention Programs

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Glossary

Cretinism A congenital condition caused by a deficiency of thyroid hormone during prenatal development and characterized in childhood by dwarfed stature and mental retardation.

Goiter A noncancerous enlargement of the thyroid gland, visible as a swelling at the front of the neck, that is often associated with iodine deficiency.

Hypothyroidism A physiological state characterized by insufficient production and/or action of thyroid hormone,

which can result in a decreased basal metabolic rate, causing weight gain and fatigue.

Iodine deficiency disorders The collective term for the multiple adverse effects on growth and development in animals and humans caused by iodine deficiency and the resulting inadequate thyroid hormone production.

Thyroglobulin A thyroid protein that is the precursor to iodine-containing hormones and is typically present in the colloid of thyroid gland follicles.

Dietary Sources, Absorption, and Metabolism

Iodine (as iodide) is widely but unevenly distributed in the Earth's environment. In many regions, leaching from glaciation, flooding, and erosion have depleted surface soils of iodide, and most iodide is found in the oceans. Iodide ions in seawater are oxidized to elemental iodine, which volatilizes into the atmosphere and is returned to the soil by rain, completing the cycle. However, iodine cycles in many regions are slow and incomplete, leaving soils and drinking water iodine depleted. Crops grown in these soils will be low in iodine, and humans and animals consuming food grown in these soils become iodine deficient. Iodine-deficient soils are common in mountainous areas (e.g., the Alps, Andes, Atlas, and Himalaya ranges) and areas of frequent flooding, especially in South and Southeast Asia (for example, the Ganges River plain of northeastern India). Many inland areas, including central Asia and Africa, and central and eastern Europe are iodine deficient. Iodine deficiency in populations residing in these areas will persist until iodine enters the food chain through addition of iodine to foods (e.g., iodization of salt) or dietary diversification introduces foods produced outside the iodine-deficient area.

The native iodine content of most foods and beverages is low. In general, commonly consumed foods provide 3–80 µg per serving. Foods of marine origin have higher iodine content because marine plants and animals concentrate iodine from

seawater. Major dietary sources of iodine in the US are bread and milk. In Switzerland, based on direct food analysis, mean intake of dietary iodine is ~140 µg day⁻¹, mainly from bread and dairy products. Much of the iodine content of dairy products is added during processing, for example, through the use of iodine-containing disinfectants. In many countries, use of iodized salt in households for cooking and at the table provides additional iodine. Dietary supplements often contain iodine. Based on data from the US Third National Health and Nutrition Examination Survey, 12% of men and 15% of nonpregnant women took a supplement that contained iodine, and the median intake of iodine from supplements was ~140 µg day⁻¹ for adults.

Iodine is ingested in several chemical forms. Iodide is rapidly and nearly completely absorbed in the stomach and duodenum. Iodate, widely used in salt iodization, is reduced in the gut and absorbed as iodide. In healthy adults, the absorption of iodide is >90%. Iodine is cleared from the circulation mainly by the thyroid and kidney, and although renal iodine clearance is fairly constant, thyroid clearance varies with iodine intake. In conditions of adequate iodine supply, ≤10% of absorbed iodine is taken up by the thyroid. In chronic iodine deficiency, this fraction can exceed 80%. During lactation, the mammary gland concentrates iodine and secretes it into breast milk to provide for the newborn.

The body of a healthy adult contains up to 20 mg of iodine, of which 70–80% is in the thyroid. In chronic iodine deficiency, the iodine content of the thyroid may fall to

<20 µg. In iodine-sufficient areas, the adult thyroid traps about 60 µg of iodine/day, either from dietary iodine or from iodine released during thyroid hormone turnover, to balance losses and maintain thyroid hormone synthesis. Thyroglobulin (Tg), a large glycoprotein (molecular weight 660 000), is the carrier of iodine in the follicles of the thyroid. Thyrocytes produce and secrete the two thyroid hormones from Tg, thyroxine (T4) (the major form) and triiodothyronine (T3). In the circulation, thyroid hormones are bound noncovalently to carrier proteins, mainly thyroxine-binding globulin. In target tissues, including liver, kidney, heart, muscle, pituitary, and the developing brain, T4 is converted to T3. T3 is the main physiologically active form of thyroid hormone and binds to nuclear receptors.

Thyroid hormone regulates a variety of physiologic processes, including reproductive function, growth and development, as well as the basal metabolic rate. During pregnancy, thyroid hormone crosses the placenta to the fetus early in the first trimester, before the fetal thyroid is functioning. In the developing brain, it influences cell growth and migration. It also promotes growth and maturation of peripheral tissues and the skeleton. Thyroid hormone increases energy metabolism in most tissues. It also increases the basal metabolic rate.

Both T4 and T3 are degraded through a complex series of pathways, and their turnover is relatively slow: the half-life of T4 is ~5 days and for T3, 1.5–3 days. The released iodine enters the plasma iodine pool and can be taken up again by the thyroid or excreted by the kidney. More than 90% of ingested iodine is ultimately excreted in the urine, with only a small amount appearing in the feces.

Iodine Deficiency Disorders

Iodine deficiency has multiple adverse effects on growth and development in animals and humans. These are collectively termed the iodine deficiency disorders (IDD) (Table 1), and are one of the most important and common human diseases. They result from inadequate thyroid hormone production due to lack of sufficient iodine.

Table 1 Iodine deficiency disorders, by age group

Age groups	Health consequences of iodine deficiency
All ages	Goiter Increased susceptibility of the thyroid gland to nuclear radiation
Fetus	Abortion Stillbirth Congenital anomalies Perinatal mortality
Neonate	Infant mortality Endemic cretinism
Child and adolescent	Impaired mental function Delayed physical development
Adults	Reduced work productivity Toxic nodular goiter; iodine-induced hyperthyroidism Hypothyroidism in moderate-to-severe iodine deficiency

Thyroid enlargement (goiter) is the classic sign of iodine deficiency. It is a physiologic adaptation to chronic iodine deficiency. As iodine intake falls, the ratio of T4 to T3 produced by the gland decreases, secretion of TSH increases in an effort to maximize uptake of available iodine, and thyroid-stimulating hormone (TSH) stimulates thyroid hypertrophy and hyperplasia. Large goiters may be cosmetically unattractive, can obstruct the trachea and esophagus, and may damage the recurrent laryngeal nerves and cause hoarseness.

Although goiter is the most visible effect of iodine deficiency, the most serious adverse effect is damage to reproduction and fetal development. Severe iodine deficiency during pregnancy is associated with a greater incidence of stillbirths, abortions, and congenital abnormalities. The fetal brain is particularly vulnerable to iodine deficiency. Normal levels of thyroid hormones are required for neuronal migration and myelination of the central nervous system. The most severe form of neurological damage from fetal hypothyroidism is termed cretinism. It is characterized by gross mental retardation along with varying degrees of short stature, deaf mutism, and spasticity. Up to 10% of populations with severe iodine deficiency may be cretinous. Iodine prophylaxis has completely eliminated the appearance of new cases of cretinism in previously iodine-deficient Switzerland and other countries.

Although new cases of cretinism are now rare, iodine deficiency still affects up to 30% of the global population (see below), and can impair cognitive development. A meta-analysis of 18 studies concluded that moderate-to-severe iodine deficiency reduces mean IQ scores by 13.5 points. Iodine deficiency is thus considered one of the most common causes of preventable mental retardation worldwide. Even in areas of mild-to-moderate iodine deficiency, cognitive impairment in school-aged children is at least partially reversible by administration of iodine. Overall, iodine deficiency produces widespread adverse effects in a population, including decreased educability and productivity, resulting in impaired social and economic development.

Only a few countries, including Switzerland, the Scandinavian countries, Australia, the US, and Canada, were completely iodine sufficient before 1990, due to iodized salt programs and adventitious iodine added during processing of foods. Since then, widespread introduction of iodized salt has produced dramatic reductions in iodine deficiency. The World Health Organization (WHO) recently estimated the worldwide prevalence of iodine deficiency. Just over 2 billion individuals have inadequate iodine nutrition, of whom 266 million are school-aged children (Table 2); the global prevalence of iodine deficiency in school-aged children is 31.5%.

Iodine Requirements

The US Food and Nutrition Board of the National Academy of Sciences has set an Adequate Intake (AI) for iodine in infancy and a Recommended Dietary Allowance (RDA) for children, adults, and pregnant and lactating women (Table 3). The WHO has established recommended nutrient intakes for iodine (Table 3).

Table 2 Prevalence of iodine deficiency, as total number (millions) and percentages, in general population (all age-groups) and in school-aged children (6–12 years) and the percentage of households with access to iodized salt

WHO regions ^a	Population with urinary iodine < 100 µg l ^{-1b}		Percentage of households with access to iodized salt ^c
	General population	School-aged children	
Africa	312.9 (41.5%)	57.7 (40.8%)	66.6
America	98.6 (11.0%)	11.6 (10.6%)	86.8
Eastern Mediterranean	259.3 (47.2%)	43.3 (48.8%)	47.3
Europe	459.7 (52.0%)	38.7 (52.4%)	49.2
Southeast Asia	503.6 (30.0%)	73.1 (30.3%)	61.0
Western Pacific	374.7 (21.2%)	41.6 (22.7%)	89.5
Total	2000.0 (30.6%)	263.7 (31.5%)	70.0

^a193 WHO Member States.^bBased on population estimates for 2006 (United Nations, Population Division, World Population Prospects: the 2004 revision).^cThese figures do not include data for non-UNICEF countries (e.g., the US and Western Europe).**Table 3** Recommendations for iodine intake (µg day⁻¹) by age or population group

Age or population group	US Institute of Medicine		Age or population group	World Health Organization
	EAR	AI or RDA		
Infants 0–12 months	–	110–130	Children 0–5 years	90
Children 1–8 years	65	90	Children 6–12 years	120
Children 9–13 years	73	120		
Adults ≥ 14 years	95	150	Adults > 12 years	150
Pregnancy	160	220	Pregnancy	250
Lactation	209	290	Lactation	250

Abbreviations: AI, adequate intake; EAR, estimated average requirement; RDA, recommended daily allowance; RNI, recommended nutrient intake.

Assessment of Iodine Status

Several methods are available for assessment of iodine nutrition. The most commonly used are measurement of thyroid size, concentration of urinary iodine (UI), and serum or dried blood spot thyroglobulin. As discussed below, UI is a sensitive indicator of recent iodine intake (days) and serum Tg shows an intermediate response (weeks to months), whereas changes in the goiter rate reflect long-term iodine nutrition (months to years).

Two methods are available for measuring goiter: neck inspection and palpation, and thyroid ultrasonography. Goiter surveys are usually done in school-aged children. By palpation, a thyroid is considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the subject being examined. In areas of mild-to-moderate iodine deficiency, where goiters are small, measurement of thyroid size by ultrasonography is a more objective and precise method, and is preferable to palpation. Portable ultrasound equipment can be used in the field, and goiter classified according to international reference criteria for iodine-sufficient children by age, gender, and body surface area. The total goiter rate is used to define severity using the following criteria: <5%, iodine sufficiency; 5.0–19.9%, mild deficiency; 20.0–29.9%, moderate deficiency; and >30%, severe deficiency.

Because >90% of ingested iodine is excreted in the urine, UI is an excellent indicator of recent iodine intake. Most methods of measuring UI are based on the Sandell-Kolthoff reaction, in which iodide catalyzes the reduction of yellow ceric ammonium sulfate to the colorless cerous form, in the presence of arsenious acid. For populations, because it is impractical to collect 24-h samples in field studies, UI can be measured in spot urine specimens from a representative sample of the target group, and expressed as the median, in µg l⁻¹ (Table 4). Spot UI measurements from populations are often misinterpreted; it is a common mistake to assume that all subjects with a spot UI <100 µg l⁻¹ are iodine deficient, but even in iodine sufficient regions, individual spot UI concentrations are highly variable from day-to-day.

Tg is synthesized only in the thyroid, and is the most abundant intrathyroidal protein. In iodine sufficiency, small amounts of Tg are secreted into the circulation, and serum Tg is normally <10 µg l⁻¹. In areas of endemic goiter, serum Tg increases due to greater thyroid cell mass and TSH stimulation. Serum Tg is well correlated with the severity of iodine deficiency as measured by UI, and is a sensitive indicator of iodine repletion. Tg can also be assayed on dried blood spots taken by a finger prick, simplifying collection and transport. A reference range for Tg on dried blood spots in school-aged children has recently been published, and Tg is now recommended to assess iodine status.

Table 4 Epidemiological criteria from the World Health Organization for assessment of iodine nutrition in a population based on median urinary iodine concentrations

Urinary iodine concentrations ($\mu\text{g l}^{-1}$)	Iodine intake	Iodine nutrition
School-aged children		
<20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Optimum
200–299	More than adequate	Risk of iodine-induced hyperthyroidism in susceptible groups
>300	Excessive	Risk of adverse health consequences (iodine-induced hyperthyroidism, autoimmune thyroid disease)
Pregnant women		
<150	Insufficient	
150–249	Adequate	
250–499	More than adequate	
$\geq 500^a$	Excessive	
Lactating women^b		
<100	Insufficient	
≥ 100	Adequate	
Children less than 2 years of age		
<100	Insufficient	
≥ 100	Adequate	

^aThe term excessive means in excess of the amount needed to prevent and control iodine deficiency.

^bIn lactating women, the numbers for median urinary iodine are lower than the iodine requirements, because of the iodine excreted in breast milk.

Prophylaxis and Treatment of Iodine Deficiency

There are two methods commonly used to correct iodine deficiency in a population: iodized oil and iodized salt. In nearly all regions affected by iodine deficiency, the most effective way to control iodine deficiency is through salt iodization. All salt for human consumption, including salt used in the food industry, should be continuously iodized. Iodine can be added to salt in the form of potassium iodide (KI) or potassium iodate (KIO_3). Because KIO_3 has higher stability in the presence of salt impurities, humidity, and porous packaging, it is the recommended form. Iodine is usually added at a level of 20–40 mg iodine/kg salt, depending on local salt intake.

However, in industrialized countries, because about 90% of salt consumption is from purchased processed foods, if only household salt is iodized it will not supply adequate iodine. Thus, it is critical that the food industry uses iodized salt. The current push to reduce salt consumption to prevent chronic diseases and the policy of salt iodization to eliminate iodine deficiency do not conflict: iodization methods can fortify salt to provide recommended iodine intakes even if per capita salt intakes are reduced to $<5 \text{ g day}^{-1}$.

In some regions, iodization of salt may not be practical for control of iodine deficiency, at least in the short term. This may occur in remote areas where communications are poor or where there are numerous small-scale salt producers. In these areas, other options for correction of iodine deficiency should be considered, such as iodized oil. Iodized oil is prepared from unsaturated fatty acids in seed or vegetable oils, by addition of iodine to the double bonds. It can be given orally or by intramuscular injection. The intramuscular route has a longer duration of action (up to 2 years), but oral administration is more common because it is simpler.

Iodized oil is recommended for populations with moderate-to-severe iodine deficiency that do not have access to iodized salt, and may be targeted toward women of child-bearing age, pregnant women, and children. The recommended dose is 400 mg of iodine per year for women and 200 mg of iodine per year for children 7–24 months of age. Iodine can also be given as potassium iodide or iodate as drops or tablets, and in drinking or irrigation water. Iodine supplements ($\sim 150 \mu\text{g day}^{-1}$) are recommended for pregnant and lactating women residing in areas of mild-to-moderate iodine deficiency.

Iodine Excess and Toxicity

Ingestion of excess amount of iodine leads to acute iodine poisoning, which causes gastrointestinal irritation, abdominal pain, nausea, vomiting, and diarrhea, as well as cardiovascular symptoms, coma, and cyanosis. Most people are remarkably tolerant to high dietary intakes of iodine. The US Food and Nutrition Board of the National Academy of Sciences has set a Tolerable Upper Intake Level (UL) for iodine. The UL is the highest level of daily intake that is likely to pose no risk of adverse health effects in almost all individuals. The UL is $200 \mu\text{g day}^{-1}$ for ages 1–3 years, $300 \mu\text{g day}^{-1}$ for ages 4–8 years, $600 \mu\text{g day}^{-1}$ for ages 9–13 years, $900 \mu\text{g day}^{-1}$ for ages 14–18 years, and $1100 \mu\text{g day}^{-1}$ thereafter. Individuals with autoimmune thyroid disease or chronic iodine deficiency may respond adversely to intakes lower than these.

A rapid increase in iodine intake in populations with chronic iodine deficiency may precipitate iodine-induced hyperthyroidism (IIH). This is more likely to occur if the

iodine is given in excess, for example, if the iodine content of iodized salt is too high, or when iodine-containing medication is given. IIH occurs mainly in older people with nodular goiter. Thyrocytes in nodules often become insensitive to TSH control, and if iodine supply is suddenly increased, these autonomous nodules may overproduce thyroid hormone. Symptoms of IIH include weight loss, tachycardia, and muscle weakness. It is dangerous when superimposed on underlying heart disease, and may be lethal. To reduce risk for IIH, the iodine level in salt should be monitored and reduced if too high.

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Physiology, Dietary Sources, and Requirements

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Glossary

Goiter Abnormally enlarged thyroid gland; often resulting from underproduction or overproduction of thyroid hormones or from iodine deficiency.

Goitrogens Dietary substances that interfere with thyroid metabolism and aggravate the effect of iodine deficiency.

Iodine An essential element in the diet needed by the thyroid gland for the synthesis of thyroid hormones.

Thyroglobulin A protein that is the precursor to iodine-containing hormones and is typically present in the colloid of thyroid gland. Abbreviated as T_g .

Thyroid stimulating hormone Anterior pituitary hormone that stimulates the thyroid gland to produce thyroid hormones. Abbreviated as TSH.

Thyroxin Hormone containing four atoms of iodine produced by the thyroid gland. Abbreviated as T_4 .

Triiodothyronine Bioactive thyroid hormone with one less iodine atom per molecule than thyroxin. Abbreviated as T_3 .

Iodine is an essential component of the hormones produced by the thyroid gland. Inadequate intake of iodine impairs thyroid function and results in a spectrum of disorders, including goiter, impaired cognitive development, and congenital abnormalities, collectively referred to as iodine deficiency disorders (IDDs). Two billion people worldwide are at risk of iodine deficiency, with the highest risks in south Asia and sub-Saharan Africa. In nearly all countries the best strategy to control iodine deficiency is iodization of salt.

Ecology of Iodine

Although iodine is widely present in the environment, it is distributed unevenly. The median concentration of iodine in soils worldwide is $5 \mu\text{g g}^{-1}$, but can range from 1 to $150 \mu\text{g g}^{-1}$. The highest amounts are found in soils rich in organic components and located near the coast. Iodine was present during the primordial development of the Earth, but large amounts were leached from the surface soil by glaciations, snow, or rain and were carried by rivers and floods to the ocean. Thus, most of the world's iodine resides in the ocean. The major mechanism of iodine transfer from the ocean to land is based on volatilization of seawater iodine into the atmosphere. Another major source seems to be the release of volatile methyl iodide by marine organisms. However, the return of iodine to soil is insufficient compared to the original loss, leaving soils and drinking water iodine depleted. Iodine-deficient soils are common in mountainous areas (e.g., the Alps, Andes, Atlas, and Himalayan ranges) and areas of frequent flooding, especially in South and Southeast Asia. Many inland regions in central Asia and Africa and central and eastern Europe are also affected by iodine-deficient soils. Populations in these areas that depend on locally grown food, consequently, become iodine deficient unless additional iodine is provided through imported iodine-rich foods, iodine fortification, or supplementation.

Absorption and Metabolism

Iodine is present in food in different chemical forms. Most ingested iodine is reduced to iodide in the gut and is absorbed almost completely in the duodenum. Iodide is cleared from the circulation mainly by the thyroid and kidney. The thyroid adjusts the amount of iodide uptake to amounts required for adequate thyroid hormone synthesis. In conditions of adequate iodine supply, no more than 10% of the absorbed iodine is taken up by the thyroid. In chronic iodine deficiency, this fraction can exceed 80%. Similarly, during lactation, the mammary gland regulates iodine uptake for secretion in breast milk. Several other tissues can also concentrate iodine, including the salivary glands, choroid plexus, and gastric mucosa, but these are minor pathways of uncertain significance. Once the thyroidal iodine requirement has been met, excess iodine is excreted by the kidney.

The body of a healthy adult contains 15–20 mg of iodine, of which 70–80% is in the thyroid. In chronic iodine deficiency, the iodine content of the thyroid can fall below 20 μg . In iodine-sufficient areas, the adult thyroid traps approximately 60 μg of iodine per day to balance losses and maintain thyroid hormone synthesis. A transmembrane protein, the sodium/iodide symporter (NIS), mediates the first and key step in the process of supplying iodide to the gland. This protein transfers iodide into the cytoplasm of the follicular cells at a concentration gradient 20 to 50 times that of serum. Iodine in the thyroid gland then participates in a complex series of reactions to produce thyroid hormones. Iodide must first be oxidized to a higher oxidation state before it can act as an effective iodinating agent. Only H_2O_2 is sufficiently potent to oxidize iodide. At the apical membrane, thyroid peroxidase (TPO) catalyzes the iodination of tyrosyl residues of thyroglobulin (T_g) producing either monoiodotyrosine (MIT) or diiodotyrosine (DIT), the precursors of thyroid hormones. TPO then catalyzes the coupling of the phenyl groups of the iodotyrosines through a diether bridge to

form the thyroid hormones. Two residues of DIT couple within T_g to form thyroxine (T_4), or one DIT and one MIT to form triiodothyronine (T_3). In the thyroid, mature T_g , containing 0.1–1.0% of its weight as iodine, is stored extracellularly in the luminal colloid of the thyroid follicle. Approximately one-third of T_g 's iodine is in T_4 and T_3 , the remainder being stored in the inactive precursors DIT and MIT. The iodine in the inactive precursors is not released into circulation, but instead is removed from the tyrosine moiety by a specific deiodinase and then recycled within the thyroid gland. This process is particularly important for iodine conservation in situations of iodine deprivation (Figure 1).

Before secretion from the thyroid, T_4 and T_3 must be released from peptide linkage within T_g . T_g retrieved by micropinocytosis passes first through the endosomal system, where proteolysis and hormone release is initiated, then into lysosomes, where the process is completed and T_4 and T_3 are released into the circulation. T_4 is secreted in higher quantities from the thyroid compared with T_3 . Once in the circulation, T_4 and T_3 rapidly attach to several binding proteins synthesized in the liver, including thyroxine-binding globulin, transthyretin, and albumin. The bound hormone then migrates to target tissues where T_4 is deiodinated to T_3 , the metabolically active form.

T_3 is essential for the development and differentiation of all cells of the human body. It acts mostly through nuclear receptors regulating gene expression, although other

mechanisms have also been described. T_3 plays a key role in normal skeletal development, linear growth, and the acquisition and maintenance of bone mass. T_3 also has a critical role in the development and function of the human central nervous system. Moreover, thyroid hormones are the major endocrine regulators of basal metabolic rate. Hypothyroidism results from insufficient production of thyroid hormones or a defect in thyroid hormone receptor activity, whereas hyperthyroidism results from an excessive secretion of thyroid hormones.

Adaptations of Thyroid Metabolism to Iodine Deficiency

When dietary iodine intake is low, the thyroid may still achieve adequate secretion of thyroid hormones by modifications of metabolism. Thyroid stimulating hormone (TSH) is the primary factor that regulates the function of thyroid follicular cells and, ultimately, thyroid hormone secretion. In a classic negative feedback system, thyroid hormone inhibits the synthesis of TSH directly at the pituitary level and indirectly at the hypothalamic level by reducing the secretion of thyrotropin-releasing hormone (TRH). TSH stimulates the trapping of iodine into the thyroid, the breakdown of T_g , and preferential synthesis and release of T_3 into the blood. As a greater fraction of circulating iodine enters the thyroid, renal iodide excretion is reduced. As long as the iodine intake remains above approximately $50 \mu\text{g day}^{-1}$, the absolute uptake of

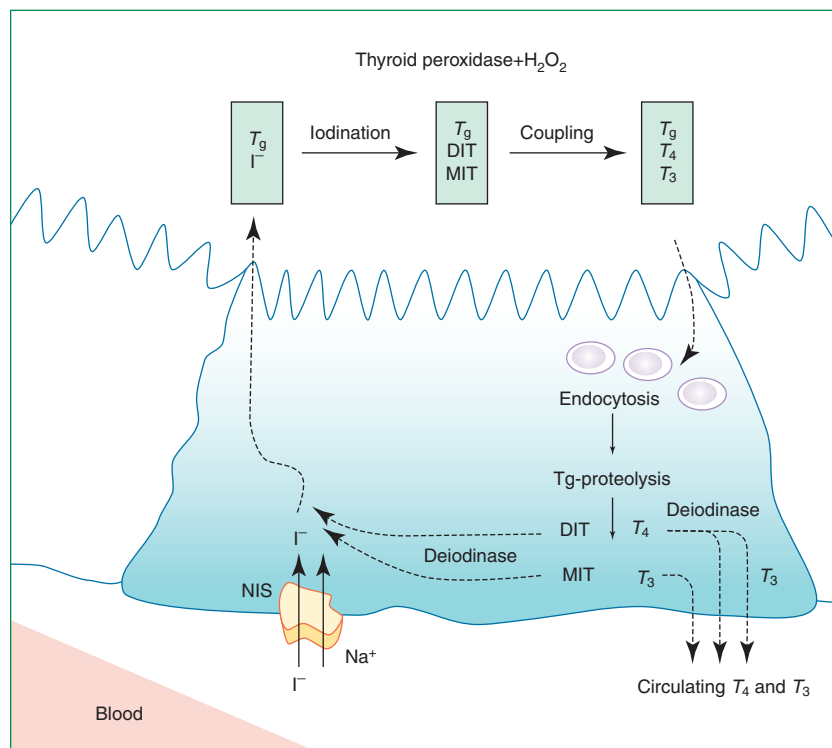


Figure 1 Iodine pathway in the thyroid cell. Iodide (I^-) is transported into the thyrocyte by the sodium/iodide symporter (NIS) at the basal membrane and migrates to the apical membrane. I^- is oxidized by the enzymes thyroid peroxidase (TPO) and hydrogen peroxide (H_2O_2) and attached to tyrosyl residues in thyroglobulin (T_g) to produce the hormone precursors monoiodotyrosine (MIT) and diiodotyrosine (DIT). Residues then couple to form thyroxine (T_4) and triiodothyronine (T_3) within the T_g molecule in the follicular lumen. T_g enters the cell by endocytosis and is digested. T_4 and T_3 are released into the circulation, and iodine on MIT and DIT is recycled within the thyrocyte. Reprinted from Zimmermann MB, Jooste PL, and Pandav CS (2008) Iodine-deficiency disorders. *The Lancet* 372: 1251–1262.

iodide by the thyroid remains normal and organic iodine content of the thyroid is within the normal limits (i.e., 10–20 mg), despite the decrease in serum iodine concentration. Below this threshold, there is an increased risk of iodine depletion in the thyroid, and many individuals develop goiter.

The basic process in the transformation of the normal thyroid to a goiter is the generation of new thyrocytes and follicles (hyperplasia) in addition to increasing cell volume (hypertrophy). The optimal thyroid response to iodine deficiency would be an increase in thyroid blood flow, in iodide trapping capacity, and in iodination rate, and a rather low T_g content in a much reduced colloid space. However, the goitrous thyroid is often large and filled with colloid. The low iodine and high T_g concentration lead to a lesser iodination of T_g , thus reducing the efficiency of thyroid hormone synthesis. In children in areas of moderate-to-severe iodine deficiency, the characteristic pattern of circulating thyroid hormones is a variably elevated TSH, a low serum T_4 , and a normal or high-normal T_3 concentration. The serum T_g concentration is typically elevated. Thyroid failure and cretinism usually develop only in regions of chronic, severe iodine deficiency where individuals show low circulating T_4 and T_3 and dramatically elevated TSH. The effects of iodine deficiency on the development of goiter and thyroid hypofunction are extremely variable among populations and individuals, even in endemic areas. The dietary, environmental, and genetic factors that account for this variability remain largely unknown.

Adaptations of Thyroid Metabolism to Iodine Excess

Intakes up to 600 $\mu\text{g day}^{-1}$ in the European Union and 1100 $\mu\text{g day}^{-1}$ in the United States are defined as safe for adults. Nevertheless, most individuals tolerate higher intakes, whereas a few may have untoward effects at lower intakes. The NIS is key in maintaining normal thyroid hormone concentration as it reduces the transport of iodide into the thyroid cells under conditions of excessive iodine intake (Table 1). Even before NIS reacts, a sudden iodine overload paradoxically blocks the second step of hormone synthesis, the organification of iodide. This so-called Wolff–Chaikoff effect requires a high ($\geq 10^{-3}$ molar) intracellular concentration of iodide and provides a short-term block against further thyroidal iodine uptake. Another response to iodine excess occurs in the first step of thyroid hormone synthesis. When iodine is abundant, DIT predominates over MIT in favor of T_4 biosynthesis, which is less active than T_3 . Thus, a euthyroid state is maintained despite an increased amount of iodine taken up by the gland. Moreover, MIT and DIT are excreted as nonhormonal by-products, thereby ridding the gland of excess iodine.

There are no clinical symptoms specific to these adaptations of excessive intake. Although most individuals suffer no disturbance from iodine excess, some persons develop thyroid dysfunction despite the multiple control systems. Iodine excess may cause hyperthyroidism, hypothyroidism, euthyroid goiter, or thyroid autoimmunity. Factors responsible for this variety of responses are mostly unknown.

Table 1 Overview of different mechanisms involved in maintaining normal thyroid function in iodine deficiency and iodine excess

Mechanism ^a	Role in iodine deficiency ^b	Role in iodine excess ^b
Sodium/iodide symporter (NIS)	+++	+++
Wolff–Chaikoff effect	0	+++ (short term, in acute excess)
Block of hormone secretion from stores	0	++
Redistribution of organic iodine in colloid	++	+
Secretion of nonhormonal iodine	0	++

^aComplex interactions of TSH with each of these mechanisms.

^bContribution ranges from none (0) to important (+++).

Source: Reprinted from Bürgi H (2010) Iodine excess. *Best Practices and Research Clinical Endocrinology and Metabolism* 24: 107–115.

Impact of Other Micronutrients on Thyroid and Iodine Metabolism

Besides iodine, other highly prevalent micronutrient deficiencies, such as deficiencies of iron, selenium, and vitamin A, adversely affect thyroid function. Although there is little information on the prevalence of concomitant iodine deficiency with iron, selenium, or vitamin A deficiency, in view of the high prevalence of these individual micronutrient deficiencies in low-income countries, it is highly likely that a substantial number of individuals may be affected by multiple micronutrient deficiencies.

Numerous studies in animals have shown that iron deficiency anemia impairs thyroid metabolism. Iron deficiency anemia may influence thyroid metabolism by altering the central nervous system control and reducing TPO activity. Iron deficiency anemia could also impair thyroid metabolism through lowered oxygen transport. It is likely that these mechanisms jointly contribute to the impairment of thyroid function in iron deficiency. There is strong evidence for such an interaction between iron and iodine and thyroid metabolism from randomized, controlled intervention trials in humans, which have repeatedly shown that providing iron along with iodine, either as an iron supplement or as dual-fortified salt, result in significantly greater improvements of thyroid metabolism.

Selenium is an integral component of two important enzymes – iodothyronine deiodinase and glutathione peroxidase (GPX) – that are present in many tissues, including the thyroid gland. Briefly, there are three types of deiodinases. Two 5'-deiodinases (5'DI and 5'DII) catalyze the activation of the prohormone T_4 to the active thyroid hormone T_3 . 5'DI is also involved in the degradation of reserve T_3 . The third selenocysteine-containing deiodinase inactivates thyroid hormones, both the prohormone T_4 and its active metabolite such as T_3 and 3,5- T_2 . GPX and thioredoxin reductase are expressed in thyroid tissue and protect the thyroid gland from hydrogen peroxide produced during the synthesis of thyroid hormone, thereby protecting against oxidative damage. In conditions of inadequate supply of both iodide and selenium, complex

rearrangements of thyroid hormone metabolism enable adaptation by increasing retention of selenium in the brain, endocrine tissues, and especially in the thyroid gland. However, to date, most controlled intervention trials in humans have failed to confirm an effect of selenium supplementation on thyroid metabolism.

Vitamin A deficiency has multiple effects on the pituitary–thyroid axis. Vitamin A modulates thyroid hormone metabolism in the thyroid gland and the periphery, and the production of TSH by the pituitary. Similarly to iron, but with slightly less evidence from randomized clinical trials in children, vitamin A supplementation may provide a beneficial impact on thyroid metabolism, either when given alone or in combination with iodized salt.

Requirements and Dietary Sources of Iodine

Recommendations for iodine intake by age and population group, as defined by the U.S. Institute of Medicine (IOM) and the World Health Organization (WHO), are shown in Table 2. The most vulnerable population groups for iodine deficiency are young children and pregnant women due to their increased physiological needs. The tolerable upper intake level (UL) for adults is set at 1100 μg iodine per day in the US and 600 μg iodine per day in the European Union. For most people, iodine intake from foods and supplements is unlikely to exceed the UL.

The native iodine content of most foods and beverages is low. Most foods consumed contain 3–80 μg per serving. Plant-based foods are generally low in iodine and are affected by the soil content, irrigation, and use of fertilizers. Seafood and seaweed are generally rich sources of iodine. However, the iodine content of fish varies greatly depending on the water they inhabit. In some countries, such as Japan and Iceland, where there is a high consumption of seafood, some population groups have been found to consume excessive amounts of iodine.

The most important source of dietary iodine in many countries is iodized salt. The addition of iodine to salt is the most effective way to control iodine deficiency, and WHO, the United Nations Children's Fund (UNICEF), and the International Council for Control of Iodine Deficiency Disorders (ICCID) recommend that salt in regions of iodine deficiency

should be fortified at a concentration of 20–40 mg iodine per kg salt, depending on local salt intake. Since 1990, the percentage of households worldwide using iodized salt has risen from less than 20% to more than 70%, thereby markedly reducing the problem of iodine deficiency. The iodine content of breast milk varies depending on maternal iodine status and dietary intake. Colostrum contains the greatest amount of iodine, with concentrations as high as 200–400 $\mu\text{g l}^{-1}$. Studies in women from iodine-deficient areas found mean iodine content of 9–32 $\mu\text{g l}^{-1}$ in breast milk. In contrast, mean iodine content in breast milk of women living in areas of salt iodization or supplementation ranges between 25 and 155 $\mu\text{g l}^{-1}$.

Dietary and Environmental Factors that Affect Iodine Requirements

Goitrogens are dietary substances that interfere with thyroid metabolism and can aggravate the effect of iodine deficiency. Most goitrogens do not have a major clinical effect unless iodine deficiency is present. Vegetables of the Brassica family (i.e., broccoli, cabbage, cauliflower, kale, turnips, rapeseed) contain glucosinolates, which are potent goitrogenic substances. The metabolites of glucosinolates compete with iodine for thyroidal uptake. More important, however, are the naturally occurring goitrogens cyanoglucosides in several staple foods, such as cassava, maize, bamboo shoots, sweet potatoes, and lima beans. Cyanoglucosides are metabolized to thiocyanates which are anions that compete with iodine in thyroid hormone synthesis. Several studies have shown that cassava plays a role in the etiology of endemic goiter along with iodine deficiency. Flavonoids in millet and soy may impair TPO activity, which has raised concerns about potential adverse effects of soy-based infant formulas on thyroid function of young children. However, evidence from clinical trials on soy consumption remains inconclusive.

Some substances that are commonly found in the environment may also affect thyroid function. The anions perchlorate, thiocyanate, and nitrate are competitive inhibitors of NIS at pharmacological doses. When present in high amounts, these substances can decrease the active transport of iodine into the thyroid and thereby reduce thyroid hormone synthesis. The most vulnerable population groups are the developing fetus and the newborn, as sufficient iodine is essential for their normal thyroid function at this crucial time

Table 2 Recommendations for iodine intake ($\mu\text{g day}^{-1}$) by age and population group

Age or population group	U.S. Institute of Medicine ^a ($\mu\text{g day}^{-1}$)	Age or population group	World Health Organization ^b ($\mu\text{g day}^{-1}$)
Infants 0–12 months	110–130	Children 0–5 years	90
Children 1–8 years	90	Children 6–12 years	120
Children 9–13 years	120		
Adults ≥ 14 years	150	Adults ≥ 12 years	150
Pregnant women	220	Pregnant women	250
Lactating women	290	Lactating women	250

^aAdequate intake for infants ≤ 12 months; Recommended Daily Allowance for children > 1 year.

^bRecommended Nutrient Intake.

Sources: Reproduced from U.S. Institute of Medicine (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington, DC: National Academy Press, with permission from World Health Organization. Reproduced with permission from World Health Organization, International Council for the Control of the Iodine Deficiency Disorders, United Nations Children's Fund (2007) *Assessment of the Iodine Deficiency Disorders and Monitoring their Elimination*, 3rd edn. Geneva: World Health Organization.

of neurodevelopment. A low level of perchlorate exposure appears to be ubiquitous. However, at present, the potential effects of environmental perchlorate exposure on thyroid function remain controversial, and more research is needed. Cigarette smoke contains cyanide that is metabolized to thiocyanate. Smoking has been shown to adversely affect thyroid hormone status and iodine concentration in breast milk. Nitrates occur naturally in soil, groundwater, and plants, and sodium nitrite is used as a preservative in cured meats and other foods. Although several studies have found no association between dietary nitrate consumption and thyroid function, studies in areas of very high nitrate contamination of water have found an increased risk of goiter in school-aged children. Other substances may interact adversely with thyroid hormone synthesis, but additional studies are needed to better characterize the thyroidal effects. Continued monitoring of chemical exposures is important to detect potential thyroidal inhibitors as industrial practices and governmental regulations change over time.

Assessment of Iodine Status

Four indicators are generally recommended for assessment of iodine status: thyroid volume, urinary iodine, serum TSH, and serum T_g concentrations. Although thyroid volume reflects long-term iodine nutrition, urinary iodine concentration is an indicator of recent and T_g concentration of medium-term iodine intake. More details are provided in the following sections.

Thyroid Volume

As described in the section on Adaptations of Thyroid Metabolism to Iodine Deficiency above, increased thyroid volume is a common consequence of long-term iodine deficiency. Thyroid volume can be determined by neck inspection and palpation or by ultrasonography. Goiter surveys are usually done in school-aged children. By palpation, a thyroid is considered goitrous when each lateral lobe has a volume greater than the terminal phalanx of the thumbs of the individual being examined. Because palpation has poor sensitivity and specificity in areas of mild iodine deficiency, measurements of thyroid volume by ultrasound is preferable in these areas. Thyroid ultrasound is non-invasive, quickly done, and feasible even in remote areas using portable equipment. Thyroid volume can be classified as goiter according to international reference criteria.

Although thyroid size decreases in children in response to iodine repletion, thyroid size may not return to normal values for months or years after correction of iodine deficiency. Therefore, because of the lack of sensitivity to acute changes in iodine intake, this method is of limited usefulness in assessing the impact of salt iodization programs.

Urinary Iodine Concentration

Because the absorption of dietary iodine is high and approximately 90% of iodine consumed is excreted in urine, the

urinary iodine concentration serves as a good reflection of iodine nutrition. Urinary iodine concentration can be measured in spot urine specimens from a representative sample of the target group, and expressed as the median, in $\mu\text{g l}^{-1}$. If the aim of the assessment is to plan and evaluate larger-scale, population-based interventions, the results of the assessment do not need to provide certainty with regard to any particular individual's true iodine status and the collection of spot urine specimens is adequate. The collection of urine for 24 h is only required when the iodine status of an individual would need to be assessed. Several analytical methods with varying levels of sophistication are available for determining the iodine concentration in urine. During the sample collection and analyses, great care must be taken to avoid contamination of the urine sample because iodine is ubiquitous in the environment. For program evaluation, urinary iodine concentration is the most useful indicator because it reflects the current dietary iodine intake, and WHO has proposed the cut-off points for classifying population iodine nutrition into different degrees of public health significance (Table 3).

Table 3 Epidemiological criteria for assessing population iodine nutrition based on median urinary iodine concentrations in different population groups

Median urinary iodine ($\mu\text{g l}^{-1}$)	Iodine intake	Iodine status
<i>School-aged children^a</i>		
<20	Insufficient	Severe iodine deficiency
20–49	Insufficient	Moderate iodine deficiency
50–99	Insufficient	Mild iodine deficiency
100–199	Adequate	Adequate iodine nutrition
200–299	Above requirements	Risk of iodine-induced hyperthyroidism in susceptible groups
≥ 300	Excessive	Risk of adverse health consequences
<i>Pregnant women^a</i>		
<150	Insufficient	
150–249	Adequate	
250–499	Above requirements	
≥ 500	Excessive ^b	
<i>Lactating women^{cd}</i>		
<100	Insufficient	
≥ 100	Adequate	
<i>Children <2 years^c</i>		
<100	Insufficient	
≥ 100	Adequate	

^aReproduced with permission from World Health Organization, International Council for the Control of the Iodine Deficiency Disorders, United Nations Children's Fund (2007) *Assessment of the Iodine Deficiency Disorders and Monitoring their Elimination*, 3rd edn. Geneva: World Health Organization.

^bThe term excessive implies that iodine intake is in excess of the amount needed to prevent and control iodine deficiency.

^cReproduced from Zimmermann MB, Jooste PL, and Pandav CS (2008) Iodine-deficiency disorders. *The Lancet* 372: 1251–1262.

^dIn lactating women, the cut-offs for median urinary iodine concentrations are lower than the iodine requirement because of the iodine excretion in breastmilk.

Thyroid Hormone and Thyroglobulin Concentrations

Thyroid hormone concentrations are poor indicators of iodine status due to the strong regulatory adaptations of thyroid metabolism. In iodine-deficient populations, serum T_3 increases or remains unchanged, and serum T_4 usually decreases. However, these values are often within normal range and overlap with iodine-sufficient populations.

Similarly, WHO does not recommend the use of blood TSH concentration in school-age children and adults. Although serum TSH increases slightly in iodine deficiency, the concentrations often stay within the normal range. In contrast, neonatal TSH concentration reflects iodine nutrition more accurately. TSH increases when the supply of thyroid hormone and iodine from the maternal circulation to the fetus has been compromised. WHO suggests that when a sensitive assay is used on samples collected during the first few days of life, a <3% frequency of TSH concentrations $>5 \text{ mIU l}^{-1}$ (milli-international units per liter) indicates iodine sufficiency in a population.

When iodine intake is adequate, small amounts of T_g are secreted into the circulation. In iodine deficiency, thyroid hyperplasia and goiter increase serum T_g levels. T_g has been found to be a good indicator of iodine status reflecting iodine nutrition over months or years. T_g can be assessed in serum or whole blood stored on filter paper. The reference interval in iodine-sufficient school-aged children is $4\text{--}40 \mu\text{g l}^{-1}$.

See also: Energy Metabolism. Iodine: Deficiency Disorders and Prevention Programs. Iron: Physiology, Dietary Sources, and Requirements. Selenium. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements

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Relevant Websites

www.iccidd.org
International Council for the Control of Iodine Deficiency (ICCIDD).

Physiology, Dietary Sources, and Requirements

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Iron Chemistry and Physiology

Body Content, Forms, and Function

Iron, the 26th element of the periodic table, has an atomic weight of 55.85. Two common aqueous oxidation states, ferrous (Fe^{2+}) and ferric (Fe^{3+}), enable iron to participate in oxidation/reduction reactions that are essential to energy metabolism by accepting or donating electrons. However, this property also enables free iron to catalyze oxidative reactions, resulting in reactive and damaging free radicals. Accordingly, body iron must be chemically bound to facilitate appropriate physiological function, transport, and storage, with minimal opportunity for free ionic iron to catalyze harmful oxidative reactions.

Most of the body's iron functions in heme protein complexes that transport oxygen as hemoglobin and myoglobin. Approximately two-thirds of the body iron is in hemoglobin, a 68 000 MW structure containing four subunits of heme, a protoporphyrin ring with iron in the center (**Figure 1**), and four polypeptide chains (two chains each of α - and β -globin). For transport by hemoglobin, oxygen bonds directly to the iron atom, stabilized in an Fe^{2+} oxidation state surrounded by the protoporphyrin ring and histidine residues. Hemoglobin iron easily binds and releases oxygen, circulating in blood erythrocytes. Myoglobin, consisting of a single heme molecule and globin, enables oxygen transfer from erythrocytes to the mitochondria in muscle cells.

Smaller quantities of iron in the heme form function in mitochondrial cytochromes involved in electron transfer, oxygen utilization, and the production of ATP. A small fraction of body iron functions in heme-containing hydrogen peroxidases such as catalase that protect against excessive hydrogen peroxide accumulation by catalyzing its conversion to hydrogen and oxygen.

Iron also functions in nonheme proteins that contain an iron–sulfur complex, a cubical arrangement of four iron and four sulfur atoms. This is the principal form of iron in the mitochondria, functioning in enzymes of energy metabolism such as aconitase, NADH dehydrogenase, and succinate dehydrogenase. In both mitochondria and cytosol, aconitase is sensitive to iron concentrations. When iron is abundant, the aconitase enzyme assumes the full iron–sulfur cubic structure that is associated with carbohydrate metabolism. However,

when intracellular iron concentrations are reduced, the protein loses aconitase activity and functions as an iron-binding protein (IRP). IRPs interact with iron response elements (IREs) located in the mRNA to regulate the synthesis of proteins involved in iron transport, storage, and use, in response to changes in cellular iron concentrations.

Absorption, Excretion, Transport, and Storage

Absorption

Both heme and nonheme (inorganic) iron are absorbed in an inverse proportion to body iron stores (indicated by serum ferritin; **Figure 2**). Heme iron is absorbed more efficiently than the nonheme form. Nonheme iron absorption can vary from 0.1% to >35% and that of heme iron from 20% to 50%, depending on body iron status (stores, erythropoiesis, and hypoxia) and bioavailability from the diet. These ranges indicate greater control of nonheme compared with heme iron

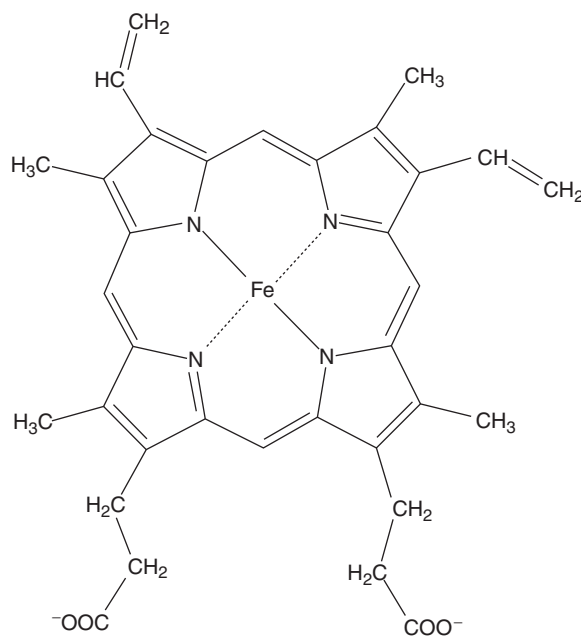


Figure 1 Heme (ferroprotoporphyrin).

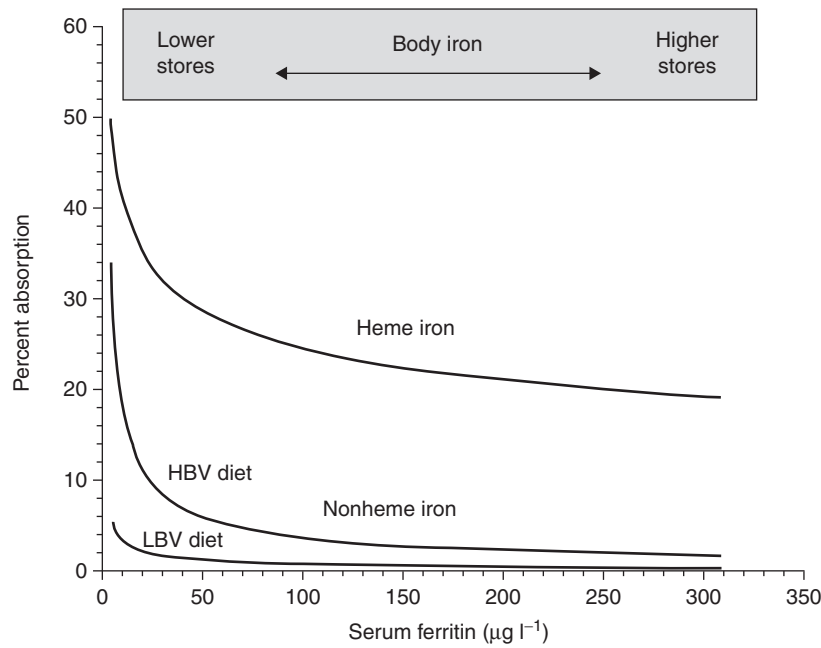


Figure 2 Heme and nonheme iron absorption as influenced by body iron stores and dietary bioavailability. HBV and LBV indicate high and low dietary bioavailability, respectively.

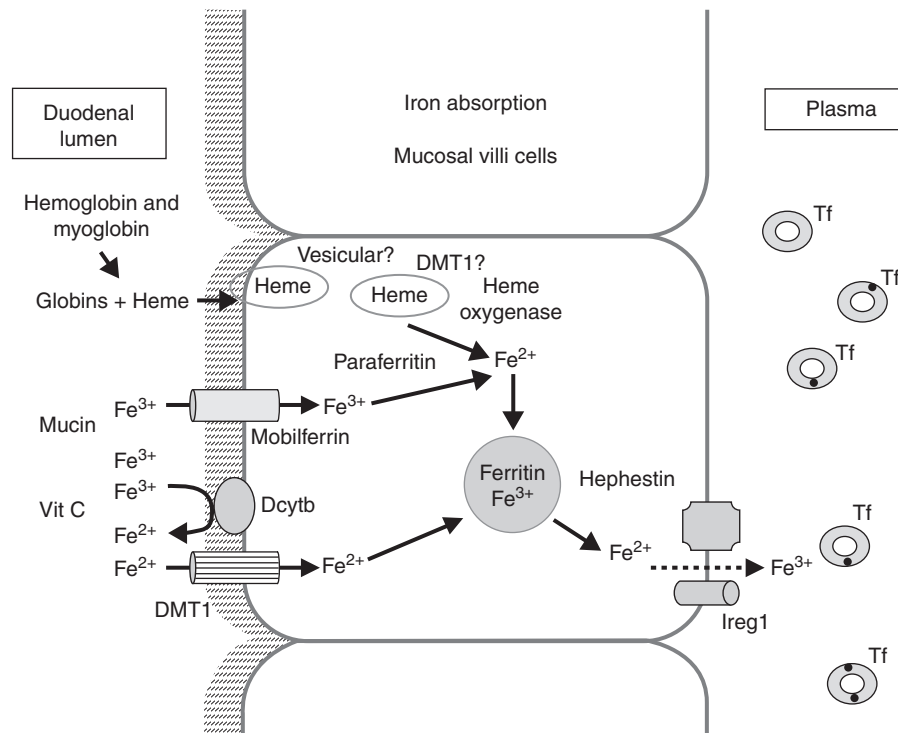


Figure 3 Absorption of iron in the intestinal mucosa.

absorption. When iron stores are high, absorption of nonheme iron can be minimized more completely, and when iron stores are low, nonheme iron is absorbed nearly as efficiently as heme iron. Because there is considerably more nonheme iron in the diet (usually ~85–100%), this form of iron

contributes most to the physiological control of iron absorption in relation to iron needs.

The upper portion of the duodenum, with its low pH luminal conditions, is the primary site for both heme and nonheme iron absorption (**Figure 3**). Nonheme iron

absorption is better understood than heme iron absorption, but a heme-carrying protein that may be responsible for mucosal uptake of heme iron has been identified. The globin proteins of hemoglobin are proteolytically digested in the intestinal lumen, producing peptide remnants that may enhance the absorption of the heme molecule by preventing heme polymerization. The heme molecule is absorbed as an intact porphyrin structure, most likely *via* a heme-carrying protein (HCP1), possibly involving endocytosis. In the mucosal cell, heme iron is split into ferrous iron and bilirubin by heme oxygenase, adding to a common pool of cellular iron for transport into plasma or intracellular storage and exfoliation.

Nonheme iron is best absorbed if presented to the intestinal villi as soluble ions (preferably reduced, ferrous ions) or as low-affinity, low-molecular-weight iron ligands. Gastric acid facilitates these conditions. Ascorbic acid concurrently ingested with iron helps to maintain the iron in a soluble, reduced, low-molecular ligand form in the intestinal lumen.

Proteins involved in mucosal uptake and transfer of nonheme iron and possible regulatory molecules have been identified (Figures 3 and 4). These include duodenal cytochrome *b* (Dcytb), which converts ferric to ferrous iron at the apical mucosal surface. A divalent metal transporter (DMT-1) transfers ferrous iron into the mucosal cell. Mutations in DMT-1 impair iron absorption and produce microcytic anemia in rodents. Ferrous iron has the highest affinity for DMT-1, but it can also transport other divalent ions, such as manganese, lead, cadmium, zinc, and copper. This may contribute to competitive inhibition observed in the absorption of these metals. Iron transported into the enterocyte may be

further transported to the body at the basolateral membrane, completing absorption, or may be held and returned to the intestinal lumen with cellular desquamation. Ferroportin, or Ireg-1, is involved in efflux of iron from the mucosal cell at the basolateral membrane. A mutation in ferroportin results in an uncommon form of hemochromatosis, an iron storage disorder. The mRNA for both DMT-1 and ferroportin contains an IRE, enabling the regulation of mRNA translation by intracellular iron concentrations. Dcytb, DMT-1, and ferroportin are all upregulated in iron deficiency. Intestinal transfer of iron to the circulation also involves hephaestin, an intestinal ferroxidase with a protein sequence similar to that of ceruloplasmin (a copper-containing ferroxidase in serum). A defective hephaestin gene in mice results in anemia and accumulation of iron in intestinal cells.

Iron absorption is responsive to recent iron intake, iron stores, erythropoiesis, hypoxia, pregnancy, and inflammation. A newly identified peptide, hepcidin, is related to several of these stimuli of regulatory control. Hepcidin is an antimicrobial peptide found in human blood and urine that serves as a signal for limiting iron absorption. Some control of absorption also likely involves the HFE protein located in the basolateral membrane of intestinal crypt cells. A specific point mutation in the HFE gene is associated with the most common form of hemochromatosis, a disorder involving excessive iron absorption and accumulation. The HFE protein interacts with β_2 -microglobulin (B2M) and transferrin receptor, apparently influencing iron uptake from serum transferrin, the primary protein involved in serum iron transport (Figure 4). Knowledge of the control of iron absorption is growing rapidly.

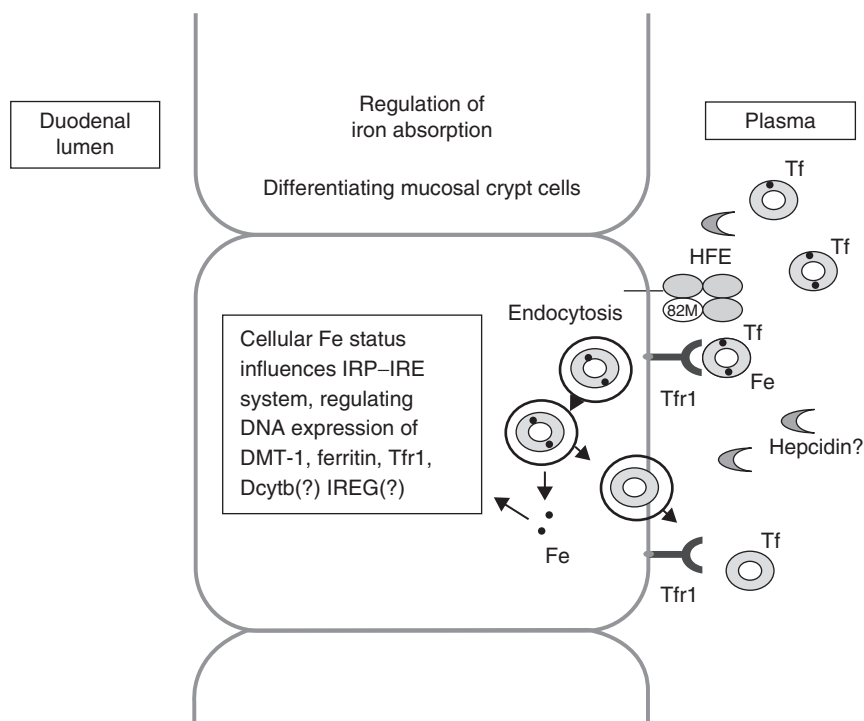


Figure 4 Regulation of iron absorption in the mucosal crypt cells before differentiation and development into actively absorbing intestinal villi cells.

Transport

Transferrin transports essentially all of the 3 or 4 mg of iron in serum, including dietary iron absorbed from the duodenum and iron from macrophages after the degradation of hemoglobin. Each transferrin molecule binds two ferric ions; the transferrin in serum is normally approximately one-third saturated with iron. The amount of iron that can be bound by transferrin is measured as the total iron-binding capacity (TIBC). In iron deficiency, serum iron is reduced, and TIBC is elevated; expressing serum iron as a fraction of the TIBC defines the transferrin saturation, which is reduced in iron deficiency. As iron deficiency develops, these measures of iron transport signal iron deficiency before the functional pool of circulating hemoglobin is reduced (Figure 5).

Membrane transferrin receptors enable the cellular uptake of iron. Transferrin receptors complex with transferrin, the complex is internalized by endocytosis, and the iron is released from transferrin inside the cell on vesicular acidification (Figure 4). Transferrin receptors are abundant in erythrocyte precursors, placenta, and liver, and the number of receptors changes inversely with cellular iron status. Serum transferrin receptors are soluble, truncated forms of the cellular receptors, present in proportion to the cellular receptors, which serve as a clinical indicator of cellular iron status that is useful in distinguishing between iron deficiency and other causes of anemia.

Other proteins involved in iron transport include lactoferrin, which is the major IRP in human milk. Haptoglobin and hemopexin proteins clear hemoglobin and heme, respectively, from circulation as they are released from senescent red blood cells or by disease.

Storage

Iron is primarily stored in the liver, spleen, and bone marrow in the form of ferritin or hemosiderin. Ferritin is a water-soluble protein complex of 24 polypeptide subunits in a spherical cluster with a hollow center that contains up to 25% iron by weight or 4000 atoms of iron per molecule. Hemosiderin is a water-insoluble complex, immunologically similar to ferritin, containing up to 35% iron. Ferritin and hemosiderin each account for approximately half of the storage iron in the liver.

Excretion

The approximately 1 mg of iron lost daily by men and postmenopausal women represents mainly obligatory fecal losses from exfoliated mucosal cells, bile, and extravasated red cells, with minor additional amounts in desquamated skin cells and sweat. Urine contains minimal amounts of iron.

Adolescent girls and premenopausal women lose considerable amounts of iron through menstruation. Menstrual

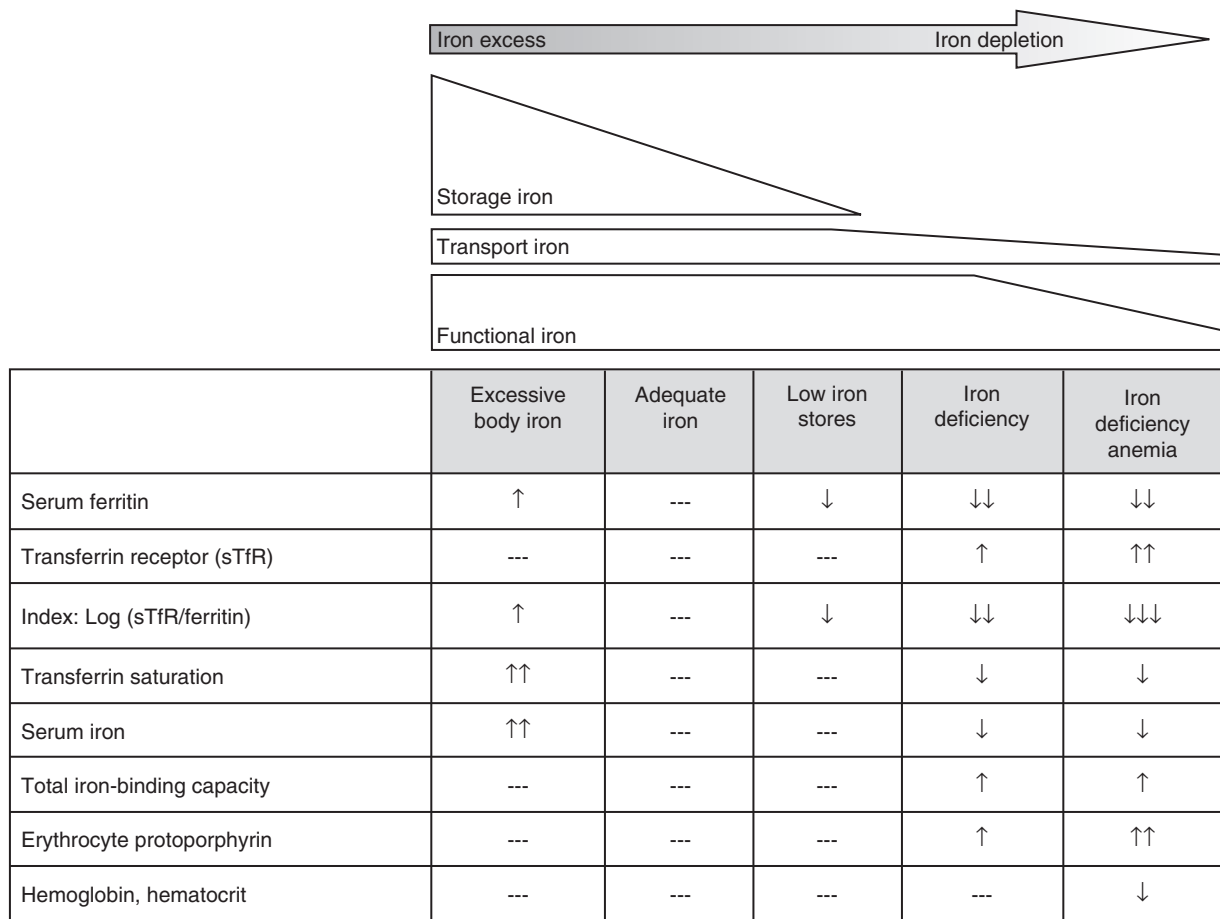


Figure 5 Clinical indicators of body iron status.

losses of individual women vary considerably; half of women lose less than 14 mg of iron per menstrual period, but the distribution is skewed, and 5% lose 50 mg or more. Iron deficiency among women in affluent countries is commonly attributable to these high iron losses rather than to differences in dietary intakes.

Body Iron Balance

The adult human has 2–4 g of total iron, or approximately 50 mg kg⁻¹ in men and 40 mg kg⁻¹ in women. Red blood cells contain approximately two-thirds of body iron and have an average life span of 120 days; consequently, approximately 20 mg of iron daily is efficiently recycled from senescent to newly formed erythrocytes through the reticuloendothelial system.

In contrast to other nutrients, controlled through both absorption and excretion, body iron balance is controlled almost exclusively by absorption. Adults consume approximately 10–20 mg iron daily from food. The average absorption and excretion of iron for adult men or postmenopausal women is approximately 1 mg daily. Menstruation can more than double iron losses in women of child-bearing age, increasing their requirement for absorbed iron. Pregnancy also increases the iron requirements considerably. Total body iron in fetuses and newborns is approximately 75 mg kg⁻¹ translating into an iron accretion rate of 1–1.5 mg kg⁻¹ per day, which, however, does not apply to newborn infants because the normal decline in hemoglobin concentration after birth causes significantly increased iron stores. Therefore, a healthy, term infant is initially independent of external iron and can double its birth weight before iron stores are depleted. Breast milk is low in iron (0.2–0.4 mg l⁻¹), and even though this iron is well utilized, infants breastfed for longer than 4–6 months without receiving iron supplements or iron-fortified complementary foods are at a risk of developing iron deficiency anemia. After that age and through the first years of life, when the growth rate continues to be high, the iron requirements are higher than during any period later in life. Therefore, the introduction of iron-rich complementary food is essential to prevent iron deficiency. Compared to term infants, preterm infants have lower body iron and hemoglobin contents at birth, as well as serum and storage iron. Iron stores may be depleted already during the first months of life, coinciding with the onset of erythropoiesis and catch-up growth. In very low birth weight infants, iron losses due to phlebotomy can amount to 6 mg kg⁻¹ per week. Therefore, in contrast to term infants, in whom iron deficiency typically develops after the first half of infancy, preterm infants are at a risk of iron deficiency already during the first half of infancy. Preterm infants of short gestational age or of lower birth weight are at a particular risk of developing iron deficiency as are preterm infants in low-income countries and those exclusively breastfed without iron supplementation. Maternal iron deficiency does not appear to compromise the iron endowment of their infants, but severe iron deficiency, i.e., iron deficiency anemia, does have an adverse effect on iron status of the newborn. Infants of moderately and severely anemic mothers have lower iron stores and a threefold increased risk of low birth weight, placing them at a higher risk of iron deficiency at an early age. The timing of umbilical cord clamping also affects the iron

endowment of the newborn. Early cord clamping decreases iron transfer to the infant, whereas delayed cord clamping increases the red cell volume in the infants and, in turn, increases the iron endowment.

Clinical Assessment of Iron Status

With adequate iron status, there is sufficient iron to meet all of iron's functional roles and a small reserve of storage iron that can be mobilized when needed (Figure 5). Excessive body iron, stored in the liver and bone marrow, is marked by elevated serum ferritin and also serum iron and transferrin saturation. Ferritin in plasma corresponds well with body iron stores, but its use as an indicator is limited under inflammatory conditions, which increase circulating ferritin concentrations. Iron deficiency occurs when iron stores are depleted and the iron transported for physiological function is reduced. Iron deficiency without anemia is commonly detected by abnormal values for two out of three blood indices, usually serum ferritin, transferrin saturation, and free erythrocyte protoporphyrin. The ratio of serum transferrin receptor to serum ferritin is considered a single, sensitive indicator of iron status across the full range of body iron status, except under conditions of inflammatory stress and during infancy (Figure 5). As iron deficiency becomes more severe, iron deficiency anemia results, with small, pale erythrocytes and reduced blood hemoglobin and hematocrit. The cut-off values used for different age groups when defining anemia and iron deficiency, respectively, may need some further consideration. A study on Swedish adolescents has pointed out how the cut-off values chosen to define anemia (hemoglobin) and iron deficiency (serum ferritin) affect the prevalence of these conditions. This dilemma is even more pronounced in infants and young children for whom appropriate reference values largely are missing and few attempts have been made to establish the appropriate cut-off values. Attempts have recently been made to establish age-appropriate cut-off values for infants with the conclusion that the prevalences of iron deficiency and iron deficiency anemia may have been overestimated.

Iron Nutrition

Iron Deficiency

Iron deficiency is the most common nutrient deficiency, affecting as many as two-thirds of all children and women of child-bearing age worldwide. It has been estimated that iron deficiency severe enough to cause anemia affects 20–25% of infants and as many as 40% of women and 25% of men. Iron deficiency occurs in industrially developed and developing countries. In the United States, 9–11% of toddlers, adolescent girls, and women of child-bearing age have iron deficiency, and 2–4% have iron deficiency anemia. The prevalence of iron deficiency is approximately doubled in US black and Hispanic women. The magnitude of this problem, however, is related not only to the socio-economic status of the population studied, but also how iron deficiency and anemia are defined (see Section on Clinical Assessment of Iron Status). UNICEF estimated that 40–50% of children below 5 years of age in

low-income countries suffer from iron deficiency. Some estimates indicate that even in affluent societies it can be as high as 30% or as low as 5%, or less.

Consequences of Iron Deficiency

Iron deficiency adversely affects pregnancy, impairs early childhood development, and cognitive function, and reduces the ability to do physical work. These serious problems are almost exclusively associated with iron deficiency severe enough to cause anemia; however, small reductions in exercise capacity, detectable in a laboratory setting, are also detectable in women with low iron stores and no anemia.

Physical Work Capacity

Iron deficiency anemia adversely affects physical work capacity, reflecting the element's key role in oxygen and energy utilization. Maximal oxygen consumption during exercise is reduced, in association with decreased muscle myoglobin and other iron-containing enzymes. Iron supplementation has improved productivity among Guatemalan sugar and coffee plantation workers, Indian tea pickers, and Indonesian road construction workers and rubber tappers. Iron supplementation programs are clearly cost-effective in addition to providing a positive impact on human health and well-being.

Cognitive Development

In infants, iron deficiency anemia has been associated with reduced mental and motor test scores and behavioral changes. This impaired mental and motor functioning appears to persist after treatment with iron, emphasizing the need for early detection and treatment and preferably prevention of iron deficiency during early development. However, it should be noted that iron deficiency in children is associated with a large number of psychosocial and economic disadvantages, which could account for some or all of the children's functional deficits. There are relatively few double-blind randomized-controlled trials of iron supplementation with adequate power, but there is considerable evidence showing that children with iron deficiency anemia usually have poor cognitive and motor development and that iron supplementation usually has beneficial effects on motor development in children with iron deficiency anemia under 3 years of age and on cognition in iron-deficient anemic school-aged children.

Reproduction

Iron deficiency anemia has been associated with premature birth and low birth weight. Iron supplementation during pregnancy is not always completely effective in preventing maternal anemia in women with low iron stores early in gestation, leading to suggestions for promoting adequate iron stores in all women of child-bearing age before conception.

Other

Iron deficiency increases the susceptibility to lead poisoning. It may also impair resistance to infection and regulation of body temperature. Iron deficiency has been associated with the eating of nonfood material (pica) or ice (pagophagia). Clinical signs

may include spoon-shaped fingernails and abnormalities of the mucosa of the mouth and gastrointestinal tract.

Recommended Dietary Intakes

The US and Canadian recommended iron intakes are intended to meet the requirements of 97.5% of the healthy population, replacing excreted iron and maintaining essential iron functions with a minimal supply of body iron stores. They also assume a relatively high bioavailability of the dietary iron. The recommended 8 mg daily for adult men and postmenopausal women can easily be met with varied Western-style diets. More careful food choices are needed to obtain the 18 mg recommended to meet requirements for 97.5% of adult menstruating women. This higher recommendation reflects the high menstrual iron losses of some women; the median iron requirement is 8.1 mg for menstruating women.

During pregnancy, dietary iron recommendations are increased to 27 mg daily, based on the iron content of the fetus and placenta (approximately 320 mg) and the expanded blood volume associated with a healthy pregnancy. Meeting this recommendation generally requires iron supplementation. Supplementation with 30–60 mg daily is commonly recommended. Lactation has minimal impact on maternal iron balance and recommendations, largely due to lactational amenorrhea.

The high iron requirements of early growth put infants and toddlers at a risk of iron deficiency. Breast-feeding is recommended for the first year of life. Although iron in breast milk is relatively low (0.35 mg l^{-1} , or 0.27 mg daily), it is well absorbed, possibly mediated by lactoferrin. Breast milk alone is assumed to be adequate for the first 6 months of infancy, with the addition of iron-rich foods in the next 6 months. When infant formula is used, iron fortification of the formula is recommended. Most infant formulas contain 4–12 mg of iron per liter, which is at least 10–30 times higher than the level of iron in breast milk. It may be questioned whether infant formula used during the first 6 months of life should contain a vast excess of iron, which provides no benefit in order to cover perceived increased iron requirements during 6–12 months of age. In areas where the same type of infant formula is used during the first 12 months of age, increasing the level of iron fortification in complementary foods may be an alternative possibility, whereas in areas where different types of infant formulas are used between 0–6 and 6–12 months of age, the follow-on formula may have a higher level of iron fortification. Reflecting the high but decreasing growth rate, the recommended daily intake of iron is 11 mg for infants 7–12 months, 7 mg for children between 1 and 3 years, and 10 mg for children between 4 and 8 years of age. There is no recommended intake for infants 0–6 months old, only an adequate intake (AI), which is 0.27 mg day^{-1} and based on the mean iron intake of exclusively breastfed infants. Iron stores are low in preterm infants as they are built during the last trimester of pregnancy. Iron supplementation and blood transfusion are therefore routinely used to prevent iron deficiency anemia in this population. The proper level and timing of iron supplementation is still controversial, but the ESPGHAN Committee on Nutrition recommends an intake of $2\text{--}3 \text{ mg kg}^{-1}$ per day, corresponding to $1.8\text{--}2.7 \text{ mg per}$

100 kcal, and that prophylactic enteral iron should be started at 2–6 weeks of age (2–4 weeks in extremely low birth weight infants).

Western dietary recommendations have been based on mixed diets with a relatively high bioavailability of iron and may need to be increased twofold or more for low-meat, plant-based diets with greater phytic acid content (see section Bioavailability). Other factors that may increase dietary requirements include achlorhydria, which decreases iron absorption, hookworm, or other parasites that increase gastrointestinal blood loss, or intrauterine contraceptive devices that may increase menstrual losses by 30–50%. In contrast, hormonal contraceptives reduce iron requirements by reducing menstrual losses by approximately 50%.

Dietary Iron

Food Sources

Typical Western diets contain approximately 6 mg iron per 1000 kcal. Men and women consume approximately 16–18 and 12–14 mg daily, respectively. In the United States, 24% of dietary iron is supplied by breads, pasta, and bakery products. An additional 21% comes from (mostly fortified) cereal products. Other abundant dietary sources are red meats (9% from beef), poultry, legumes, and lentils. In countries such as the United States, fortification practices increase the influence of grain and cereal products as sources of iron. In countries without fortification to at least replace the iron lost during milling, the refinement of grain products considerably reduces dietary iron content. The populations of developing countries that eat little meat and do not include legumes or lentils as a dietary staple are at an increased risk of inadequate iron intake.

Bioavailability

In underdeveloped countries, diets may be inadequate in both iron content and bioavailability (the amount that is absorbed and utilized by the body). However, the bioavailability of iron can be more important than the iron content in determining the amount of iron absorbed from food. Diets with similar total iron contents can differ 8–10-fold in the amount of absorbable iron. Dietary iron bioavailability is high from refined Western diets containing meat, poultry, and fish and abundant sources of ascorbic acid with low consumption of phytic acid from whole grains and legumes and limited drinking of coffee and tea with meals. On average, men absorb 1 mg daily from such diets, and women, with their lower iron stores, absorb approximately 2 mg. Individuals may absorb considerably more or less, depending on their body iron stores (Figure 2). It has been shown in adults that iron status is the strongest regulator of iron absorption. However, iron homeostasis in infants may not be fully developed.

It has been shown that iron absorption was similar in iron-supplemented and unsupplemented 6-month-old healthy, exclusively breast-fed infants born at term, whereas at 9 months of age, unsupplemented infants had considerably higher iron absorption. This strongly suggests that homeostatic

regulation of iron absorption is absent in young infants but matures and is present at 9 months of age.

Heme Iron

Approximately 10%, or 1–2 mg, of the iron in a mixed, Western diet is in the well-absorbed heme form. Heme iron accounts for approximately 40% of the iron in meat, poultry, or fish flesh. There is little to no heme iron in dairy products, or foods of plant origin. Heme iron is absorbed as an intact porphyrin structure. Heme iron absorption is enhanced by meat, poultry, or fish but it is not influenced by the other enhancers and inhibitors of nonheme iron absorption. Meat can be a significant source of heme iron for older infants and children and has a positive effect on iron status.

Nonheme Iron

Nonheme iron usually accounts for 85–100% of dietary iron. In contrast to heme iron, the absorption of nonheme iron is substantially influenced by dietary enhancers and inhibitors consumed concurrently. These factors appear to affect the solubility of a single exchangeable pool of nonheme iron absorbed from the intestinal digesta.

Absorption of nonheme iron is enhanced by ascorbic acid, which reduces ferric iron to ferrous iron, resulting in a soluble iron–ascorbic acid complex. Enhanced absorption has been demonstrated with synthetic and several food sources of ascorbic acid. The enhancement increases logarithmically with the dose, approximately doubling absorption with as little as 25 mg of ascorbic acid and increasing absorption by nearly 10-fold with 1000 mg of ascorbic acid.

Nonheme iron absorption is also enhanced by concurrently consuming meat, poultry, or fish. Despite intensive study, the factor responsible for this enhancement by animal flesh has not been identified and may involve the general matrix of low-molecular-weight peptides released during digestion.

Nonheme iron absorption is reduced by phytic acid (inositol hexaphosphate), present in legumes, rice, and grains, that binds iron and makes it insoluble. Both phytate and iron are concentrated in the aleurone layer and germ of grains, and they are reduced with milling, which increases the bioavailability of the remaining iron. An additional unidentified factor in soy beans, independent of the phytic acid, also impairs iron absorption. Polyphenols in grains, fruits, and vegetables, and including the tannins in tea and coffee, also inhibit nonheme iron absorption. Ascorbic acid consumed concurrently can partially reduce the inhibition of nonheme iron absorption by both phytic acid and polyphenols. Calcium in supplemental quantities has been shown to inhibit both heme and nonheme iron absorption from foods in short term studies but shows no effect on long-term iron status.

Supplementation and Fortification

The serious international problem of iron deficiency has been met with limited success by supplementation and fortification efforts. Both approaches suffer from difficulties in delivery and acceptance. Supplements that readily ionize into the ferrous form, such as ferrous sulfate, ferrous fumarate, or ferrous gluconate, are highly bioavailable but may cause gastrointestinal discomfort. Iron injections are poorly tolerated

and can result in serious infections. Because daily supplementation reduces the physiological efficiency of iron absorption, routine weekly iron supplementation with 60 mg iron has been suggested in developing countries for women of child-bearing age, beginning in adolescence. Menstruating women in more affluent countries are advised to undergo an assessment from a health professional before taking iron supplements in excess of 20 mg daily.

Fortification of staple foods with 3–10 mg iron daily, depending on the needs of the population, is a long-term preventative strategy. In the United States, bread and cereal products are routinely fortified with 20 mg iron per pound (460 g) of flour, and additional fortification at the option of food suppliers is common. However, fortification is difficult when food processing is decentralized, as is common in low-income countries. Food fortification carries the additional challenge that the chemical forms of iron most bioavailable also tend to be the most reactive with the food fortified, resulting in adverse changes in flavor, color, and shelf-life. Promising approaches include the fortification of food sauces with iron chemically bound with amino acids or with EDTA (sodium iron ethylenediaminetetraacetic acid), from which it is well absorbed even in the presence of phytic acid. Elemental iron powders, commonly referred to as carbonyl, electrolytic, and reduced forms of iron, are relatively inert in foods and inexpensive, but their bioavailability may be 30–80% less than iron from ferrous sulfate, depending on the dissolution in the gastrointestinal tract. Ferric orthophosphate and ferric pyrophosphate do not adversely affect foods but are poorly bioavailable; however, efforts are under way to enhance their bioavailability by reducing the particle size and encapsulating the particles with various lipids or carbohydrates to prevent agglomeration.

The form of iron given to infants may affect indicators of iron status differently. It has been shown that infants provided iron-fortified cereals between 6 and 9 months of age had significantly higher hemoglobin concentrations than infants given the same amount of iron daily in the form of iron drops. Similarly, it has been found that the addition of meat at 8 months of age had a positive effect on hemoglobin but not on serum ferritin. In contrast, infants given iron drops had significantly higher serum ferritin concentrations than those fed iron-fortified cereals. This suggests that dietary iron and iron from supplements are metabolized differently, with the latter preferentially being deposited in stores, whereas iron in meat and fortified foods is incorporated into erythrocytes. Further studies are needed to elucidate the mechanisms behind these observations.

Excessive Intakes

An extensive biological control system limits the occurrence of free ionic iron that can readily participate in toxic, free radical-producing reactions. Large quantities of ingested iron are acutely toxic, and accidental ingestion of medicinal iron preparations is a cause of poisoning deaths in young children. Iron supplementation is also associated with gastrointestinal irritation. Iron supplements also adversely affect absorption of zinc. Iron absorption is well controlled, but iron overload can result from excessive parenteral iron administration or blood transfusions. Dietary iron overload, possibly exacerbated by

genetic factors, occurs in sub-Saharan tribes that consume a high-iron traditional beer prepared and stored in iron containers. Genetic factors can substantially influence body iron retention, as indicated by hemochromatosis, a relatively frequent iron storage disorder of northern European descendants characterized by excessive iron absorption and leading to life-threatening iron damage of organs in adulthood. The possible association of high iron stores with increased risk of diseases related to oxidative stress, including cardiovascular disease, diabetes, and cancer, is an area of epidemiological investigation.

Iron overload as such has not been recognized in term human infants, and is only implicated in premature infants with a known, or feared, consequence of increased iron-associated oxidative damage. Indications of excessive iron intakes by infants have recently been observed in some studies. An adverse effect of iron supplements on linear growth has been shown in iron-replete infants in both affluent and developing countries. Other studies have shown negative effects of iron supplements on weight gain. However, the nutritional status of the infants in those studies was compromised overall, which is known to decrease linear growth and cause stunting. Thus, when linear growth is compromised, it is possible that the adverse effect of excess iron is manifested differently and instead impairs weight gain. The mechanism(s) behind the adverse effect of excess iron is still not known, but may involve the pro-oxidative effects of excess iron or, possibly, an interaction between iron and nutrients involved in growth, such as zinc.

See also: Adolescents: Requirements for Growth and Optimal Health. Bioavailability. Breast Feeding. Pregnancy: Nutrient Requirements. Supplementation: Developed Countries; Developing Countries; Dietary Supplements

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KETOSIS

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Introduction

The two ketone bodies acetoacetate ($\text{CH}_3\text{COCH}_2\text{COO}^-$) and D-3-hydroxybutyrate ($\text{CH}_3\text{CHOHCH}_2\text{COO}^-$) are the only freely soluble lipids in the circulation.

The name ketone bodies originates from the German *Ketonkörper* (literally, ketones excreted from the body) and refers to their discovery in the urine of diabetic patients in the latter half of the nineteenth century. In reality, the term is a misnomer because 3-hydroxybutyrate is not a ketone. It arose because the reagent originally used reacted positively with ketones in diabetic urine. Acetone (CH_3COCH_3), the product of the spontaneous decarboxylation of acetoacetate, is also a ketone and is present in blood and urine when the plasma concentration of acetoacetate is elevated. It is excreted via the kidneys and lungs and is responsible for the sweet smell on the breath in ketotic states.

The association of ketone bodies with the pathology of diabetes resulted in the view that they were toxic waste products. It is only in the past 30 years that this view has been convincingly reversed. Two factors led to this change, namely the development of an enzymatic method for the determination of acetoacetate and 3-hydroxybutyrate, which in turn allowed the dramatic finding of Cahill and colleagues in 1967 that adult human brain removed appreciable amounts of ketone bodies from the circulation in prolonged starvation.

The aim in this contribution is to review (a) the formation of ketone bodies in physiological and pathological situations and (b) the function of ketone bodies as physiological substrates and signals.

Formation of Ketone Bodies

It is well established that in humans and other mammals the only organ that contributes significant amounts of ketone

bodies to the blood is the liver; this organ, unlike peripheral tissues, is unable to utilize ketone bodies to any appreciable extent. More recently it has been found that during the suckling period (high-fat diet) the intestine also has the capacity (approximately 10% of that of the liver) to produce ketone bodies. Whether ketone bodies are used *in situ* or are transported via the portal blood to supplement the existing hyperketonemia is an open question.

The main blood-borne substrates for the synthesis of ketone bodies (ketogenesis) are the nonesterified fatty acids; others of lesser importance are the branched-chain amino acids, leucine and isoleucine. In addition, acetate (sources: intestinal fermentation, in vinegar, or an oxidation product of ethanol) is a ketogenic substrate.

Long-chain fatty acids contained in dietary lipids do not enter the portal blood directly but are esterified in the intestinal cells, packaged with proteins and phospholipids to form chylomicrons (large lipoproteins), and transported via the lymphatic system to the thoracic duct, where they enter the blood. In contrast, the short- and medium-chain fatty acids (below C_{14}) contained in dairy products or in clinical medium-chain triacylglycerol preparations are directly absorbed as the respective fatty acids and are transported to the liver via the portal blood (Figure 1). The long-chain fatty acids in the plasma are bound to albumin and are released from adipose tissue triacylglycerol stores by the process of lipolysis.

Extrahepatic Regulation

A key factor in the regulation of ketogenesis is the availability of nonesterified long-chain fatty acids to the liver, which in turn is controlled by their release from adipose tissue. The enzyme responsible for the initiation of the hydrolysis of stored triacylglycerols to fatty acids is hormone-sensitive lipase. As its name implies, this enzyme is exquisitely sensitive to hormones: adrenaline (in the plasma) and noradrenaline (released from sympathetic nerve endings) are activators,

[†]Deceased.

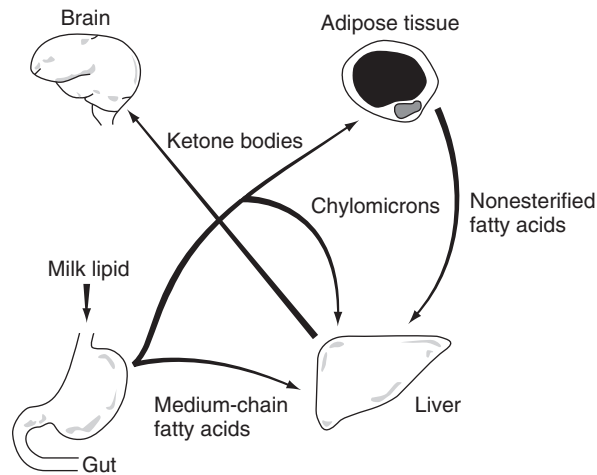


Figure 1 Intertissue fluxes of substrates in the suckling neonate. Thickness of line denotes rate of flux.

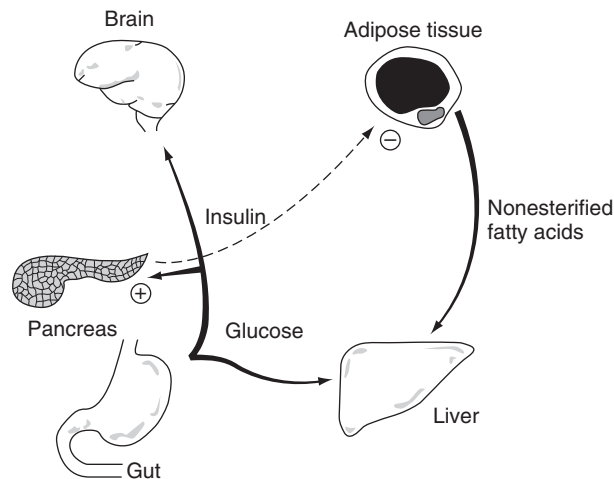


Figure 2 Intertissue fluxes of substrates in the fed state. Thickness of line denotes rate of flux.

whereas insulin inhibits the activity. In small mammals glucagon is also an activator of the enzyme, but this does not seem to be the case in the human.

Insulin has an additional effect on the net release of long-chain fatty acids from adipose tissue in that it stimulates their re-esterification to triacylglycerols. Thus after a high-carbohydrate meal, when insulin secretion and its concentration in the plasma is high, the release of fatty acids from adipose tissue is suppressed and their concentration in the plasma is low (**Figure 2**). In contrast, during stress, when adrenaline and noradrenaline are elevated, the release of fatty acids is increased and their plasma concentration is high.

In experimental animals increased plasma ketone body concentrations (hyperketonemia) can inhibit adipose tissue lipolysis (a) indirectly by increasing the secretion of insulin or (b) by a direct effect on the tissue (**Figure 3**). This can be viewed as a feedback mechanism for controlling the rate of ketogenesis via fatty acid supply to the liver, but whether this is important in the human is not known. In contrast, the supply of short- and medium-chain fatty acids to the liver is

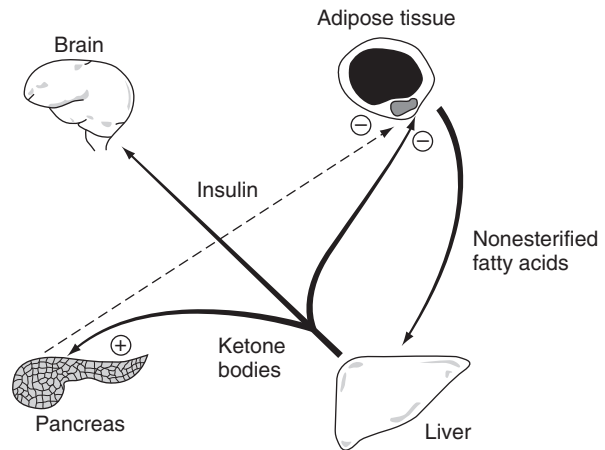


Figure 3 Role of ketone bodies as feedback regulators.

mainly dependent on the dietary intake and on the proportion that escapes further metabolism in the intestinal tract; there is no known involvement of hormones in the process.

Intrahepatic Regulation

There are situations (e.g., stress) where the supply of fatty acids to the liver may be increased, but there is no necessity to increase the availability of ketone bodies to the peripheral tissues. Consequently, there is a requirement that the rate of hepatic ketogenesis should be controlled independently of the supply of fatty acids. However, it must be stressed that without an increase in the supply of fatty acids the rate of ketogenesis cannot increase.

Much of the current interest is concerned with how the intrahepatic metabolism of fatty acids (**Figure 4**) is regulated. Long-chain fatty acids entering the liver have three main fates:

1. They can be re-esterified to phospholipids and triacylglycerols and then be secreted as very-low-density lipoproteins (VLDL).
2. They can be oxidized via the mitochondrial β -oxidation complex to acetyl-CoA. The latter can combine with another molecule of acetyl-CoA in the reaction catalyzed by acetoacetyl-CoA thiolase and then enter the hydroxymethylglutaryl-CoA pathway to form acetoacetate.
3. The acetyl-CoA derived from the fatty acids can be completely oxidized in the tricarboxylate cycle.

The short- and medium-chain fatty acids cannot be re-esterified to any appreciable extent in mammalian liver and therefore they are either metabolized to ketone bodies or completely oxidized. In addition, unlike the long-chain fatty acids, they are transported directly into the mitochondrial matrix without the need to be converted first to the corresponding acyl-CoA derivatives.

Role of Malonyl-CoA

The entry of free long-chain fatty acids into the hepatocyte is via a specific carrier on the plasma membrane. Once inside the cytosol the long-chain fatty acids are bound to binding proteins, converted to the acyl-CoA derivatives, and then can either

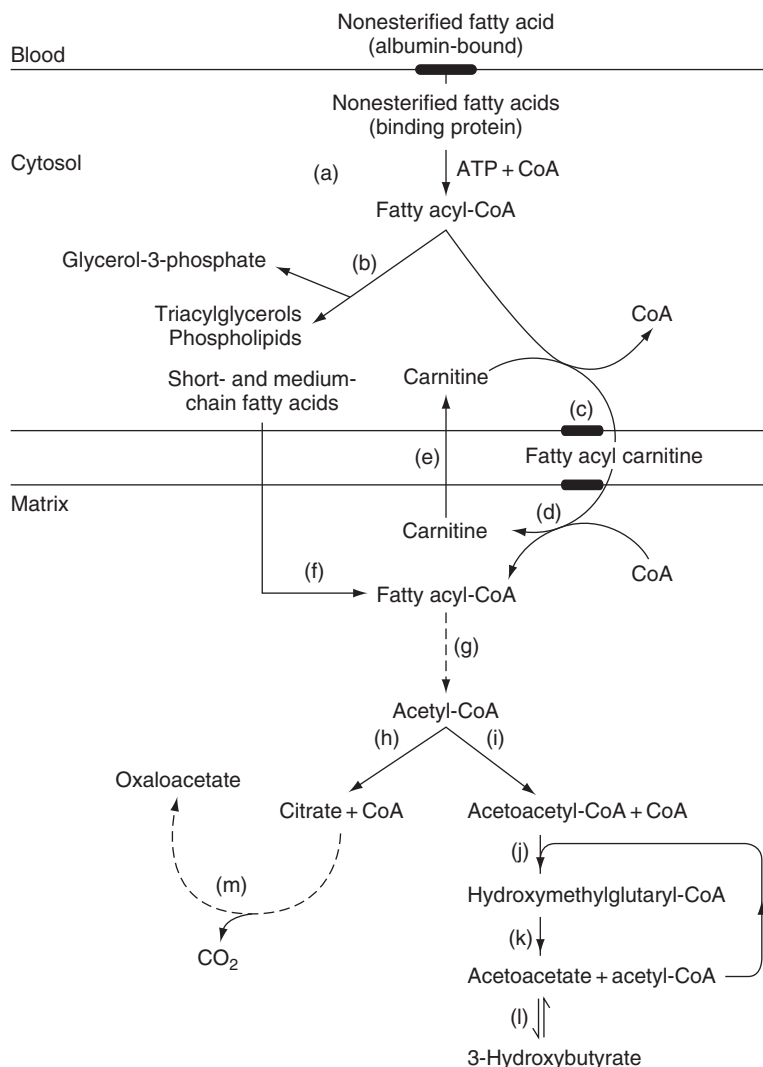


Figure 4 Pathway of fatty acid catabolism in liver. Enzymes involved: (a) long-chain fatty acyl-CoA synthetase; (b) glycerol-3-phosphate acyl-CoA transferase; (c) CAT I; (d) CAT II; (e) carnitine exchange; (f) short- and medium-chain fatty acyl-CoA synthetase; (g) fatty acid oxidation complex; (h) citrate synthase; (i) acetoacetyl-CoA thiolase; (j) hydroxymethylglutaryl-CoA synthase; (k) hydroxymethylglutaryl-CoA lyase; (l) hydroxybutyrate dehydrogenase; and (m) tricarboxylate cycle.

be esterified or enter the mitochondria via a complex transport system, the carnitine-acyl-CoA transferase (CAT) system. This consists of two proteins: CAT I located on the outer mitochondrial membrane and CAT II on the inner mitochondrial membrane (Figure 5). The overall action of the two enzymes results in the transfer of a long-chain fatty acyl-CoA to the mitochondrial matrix and the return of free carnitine to the cytosol via an exchange mechanism. Although carnitine is not consumed in the reaction, the available concentration can be critical. In nutritional carnitine deficiency there is impairment of long-chain fatty acid oxidation and ketogenesis.

The activity of CAT I is the key to the intrahepatic regulation of fatty acid metabolism in most situations. Its activity increases in ketogenic situations. More importantly, CAT I is inhibited by malonyl-CoA and the sensitivity of CAT I to this inhibitor changes in various pathophysiological situations such as fasting or diabetes.

As malonyl-CoA is a key intermediate in the synthesis of fatty acids (lipogenesis) from products (pyruvate and lactate) of glucose metabolism, this interaction provides a regulatory link between lipid and carbohydrate metabolism (Figure 5). Thus on high-carbohydrate diets, when the rate of hepatic lipogenesis, and consequently the cytosolic concentration of malonyl-CoA, is high, the activity of CAT I will be inhibited and fatty acids will be diverted to esterified products and secretion as VLDL rather than oxidation and conversion to ketone bodies. Conversely, on high-fat diets or in starvation, when lipogenesis is inhibited, malonyl-CoA concentration is low and CAT I is active. The sensitivity of CAT I to malonyl-CoA generally correlates with the prevailing concentration of the latter.

The short- and medium-chain fatty acids do not utilize the CAT I and II system to enter the mitochondrial matrix and therefore their oxidation is not greatly influenced by the prevailing 'carbohydrate status' (amount of glycogen, direction of

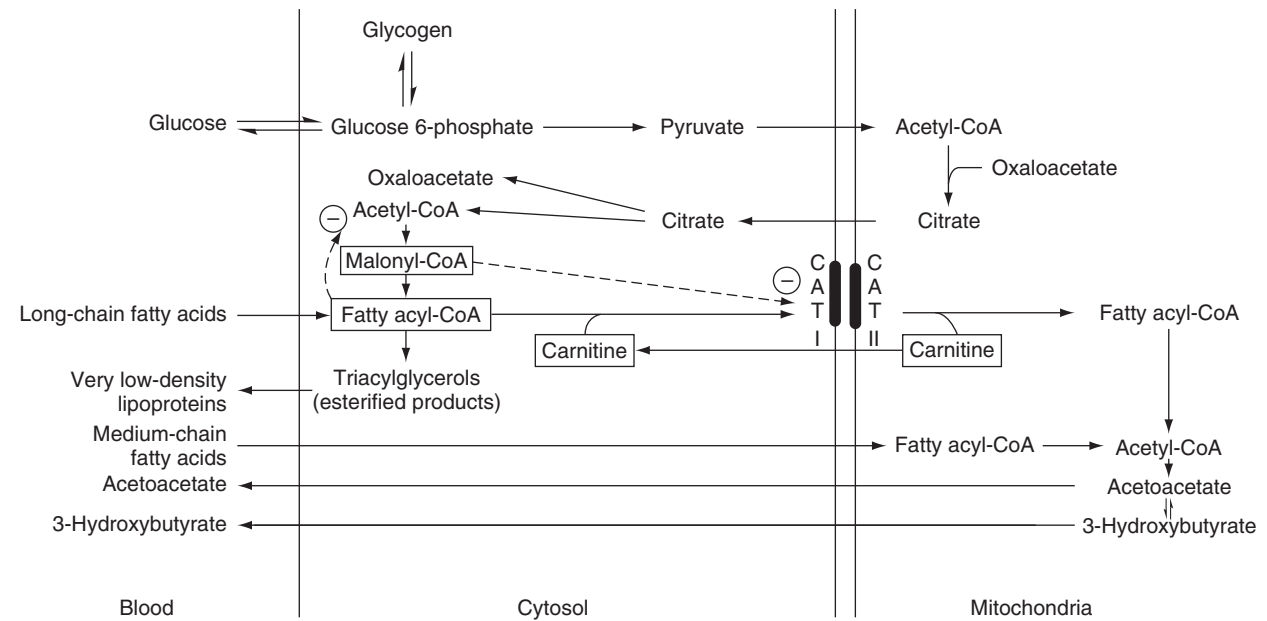


Figure 5 Inter-relationship between hepatic carbohydrate metabolism, lipogenesis, and ketogenesis. Circled minus signs indicate inhibition by the metabolite.

carbohydrate flux, glycolysis, or gluconeogenesis) of the liver (Figure 5).

Insulin can rapidly depress the rate of ketogenesis *in vitro*. This effect is thought to result mainly from its stimulatory action on a key enzyme of lipogenesis, acetyl-CoA carboxylase, which in turn increases the concentration of malonyl-CoA. Glucagon and catecholamines have the opposite effect. Thus hormonal effects can be exerted at both the extrahepatic (lipolysis) and intrahepatic (modulation of lipogenesis) levels.

Intramitochondrial Regulation

Once the fatty acyl-CoA molecule is attached to the mitochondrial β -oxidation complex there appears to be little regulation exerted until release of the acetyl-CoA fragments. As indicated above, the acetyl-CoA can enter the tricarboxylate cycle and be oxidized to CO_2 or can be converted to ketone bodies via the hydroxymethylglutaryl-CoA pathway.

It appears that in most experimental situations the complete oxidation of fatty acids proceeds at a low, but relatively similar, rate and it is the activity of the hydroxymethylglutaryl-CoA pathway that shows larger changes. This has led to the view that the pathway might be regulated by mechanisms other than substrate supply.

Studies on the expression of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) synthase have shown that both the mRNA coding for the protein and the amount of protein increase during the onset of ketogenic states (fasting, diabetes) and that these changes are rapidly reversed (refeeding, insulin treatment). However, the finding that rates of ketogenesis from medium-chain fatty acids (CAT I and II) do not alter greatly with change in physiological state, if the rate of fatty acid supply is held constant, would seem to rule out appreciable regulation within the hydroxymethylglutaryl-CoA pathway. Indeed, current thinking suggests that the activity of CAT I is

the primary intrahepatic site for the regulation of fatty acid oxidation and ketogenesis. If there is another important site, particularly during situations associated with the reversal of ketogenesis, it is likely to be proximal to the step catalyzed by this protein (e.g., the supply of fatty acids to the liver). Thus *in vivo* there is little doubt that the primary step that controls ketogenic flux is the rate of long-chain fatty acid release from adipose tissue.

Function of Ketone Bodies

The major role of ketone bodies is to supply an alternative oxidizable substrate to glucose for the brain in situations where the availability of the latter is impaired (e.g., starvation). In addition, ketone bodies can act as precursors for the acetyl-CoA required in neural lipid synthesis (myelin). Other mammalian tissues, including heart, skeletal muscle, kidney, and lactating mammary gland, can utilize ketone bodies but, in contrast to glucose utilization, no energy can be obtained in the absence of oxygen. In these tissues metabolism of ketone bodies results in the inhibition of glucose utilization and inhibition of the oxidation of pyruvate. The net result is a sparing of carbohydrate for the brain and the strictly glycolytic tissues (erythrocytes, retina).

Pathways of Ketone Body Utilization

Mitochondrial Pathway

The major site of ketone body utilization in peripheral tissues is the mitochondria (**Figure 6**). Although transporters for ketone bodies have been described on the plasma and inner mitochondrial membranes of some tissues, these do not appear to limit the flux. The initiating enzyme for acetoacetate

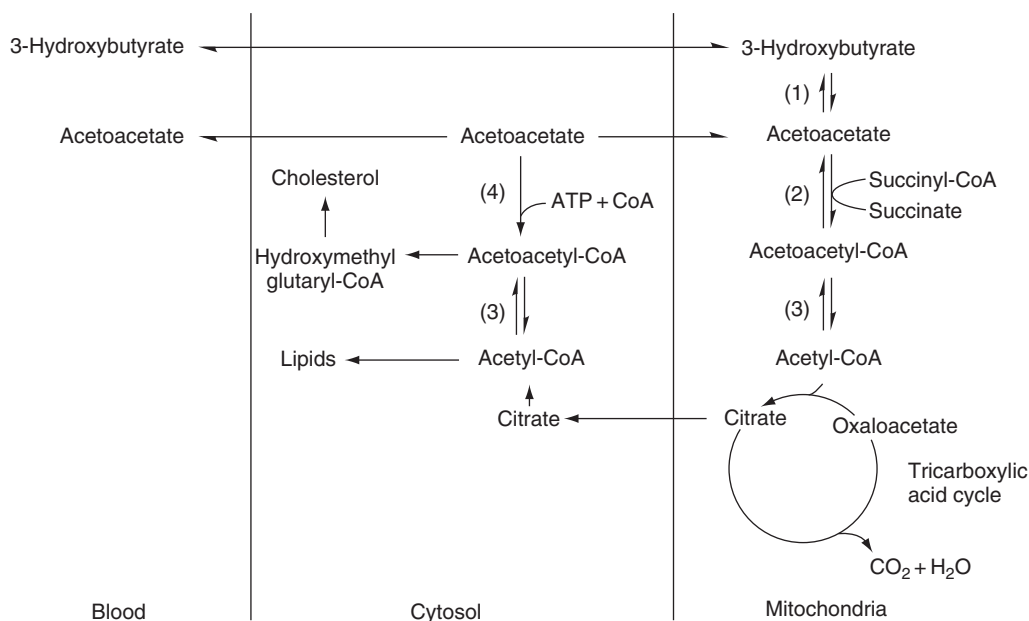
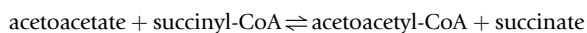


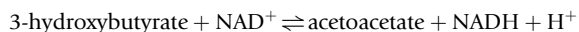
Figure 6 Pathways of ketone body utilization in peripheral tissues: (1) hydroxybutyrate dehydrogenase; (2) 3-oxoacid-CoA transferase; (3) acetoacetyl-CoA thiolase; and (4) acetoacetyl-CoA synthetase.

metabolism is 3-oxoacid-CoA transferase:



The resulting acetoacetyl-CoA is cleaved to two molecules of acetyl-CoA by acetoacetyl-CoA thiolase; they are then oxidized in the tricarboxylate cycle.

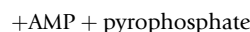
3-Hydroxybutyrate is converted to acetoacetate by 3-hydroxybutyrate dehydrogenase:



The ready reversibility of the three enzymes of the mitochondrial pathway (Figure 6) means that if the overall system is near equilibrium within the cell *in vivo*, the utilization of the ketone bodies will be dependent on their respective concentrations and on the rate of removal of the products. Thus acetoacetate utilization will be promoted when mitochondrial acetyl-CoA is decreased, whereas an increase in the latter will have the opposite effect. Similarly, oxidation of hydroxybutyrate will increase if the concentrations of NADH₂ and acetoacetate fall. Unlike the hepatic hydroxymethylglutaryl-CoA pathway for ketogenesis, which is essentially irreversible, the free reversibility of this pathway in peripheral tissues can be viewed as means of buffering the mitochondrial acetyl-CoA pool and hence energy production. Some of the acetyl-CoA can be transported to the cytosol in the form of citrate to act as a precursor for lipogenesis (Figure 6).

Cytosolic Pathway

The cytosol of tissues where active lipogenesis occurs (adipose tissue, developing brain, lactating mammary gland, and liver) contains an enzyme, acetoacetyl-CoA synthetase, which converts acetoacetate to acetoacetyl-CoA (Figure 6):



Its activity is at least an order of magnitude lower than that of the mitochondrial 3-oxoacid-CoA transferase, whereas its affinity for acetoacetate is appreciably higher. The presence of acetoacetyl-CoA thiolase in the cytosol allows the conversion of acetoacetate to acetyl-CoA and then to lipids without the involvement of the mitochondria.

Brain cytosol also contains 3-hydroxy-3-methylglutaryl-CoA synthase, allowing acetoacetate to act as a direct precursor for sterol synthesis. Evidence from *in vivo* experiments with ¹⁴C-labeled acetoacetate has confirmed the existence of this pathway in developing brain and liver. The cytosolic route for acetoacetate utilization can be seen as a mechanism for directing this substrate to lipid or sterol synthesis rather than to oxidation.

Ketosis

The concentration of ketone bodies in the blood at any time represents a balance between the rate of hepatic ketogenesis and the rate of utilization by peripheral tissues. It is generally assumed that an increase in ketogenesis leads to a rise in blood ketone bodies, which in turn results in their increased utilization. In rare situations, such as congenital absence of key enzymes involved in ketone body utilization (e.g., 3-oxoacid-CoA transferase) or inhibition of these enzymes by pharmacological agents, blood ketone bodies may increase without any concomitant increase in ketogenesis.

The concentration of ketone bodies in the blood is exquisitely sensitive to changes in pathophysiological state. It is therefore useful to define *normoketonemia* in mammals as a concentration of total ketone bodies in blood below 0.2 mmol l⁻¹, *hyperketonemia* as above this level, and *ketoacidosis* (ketosis; by analogy to the definition of lactic acidosis) as above 7 mmol l⁻¹. In adult mammals there are small but

Table 1 Range of blood ketone body concentrations in humans

<i>Situation</i>	<i>Ketone body concentration (mmol l⁻¹)</i>
Fed normal diet	About 0.1
Fed high-fat diet	Up to 3
Fasted: 12–24 h	Up to 0.3
Fasted: 48–72 h	2.0–3.0
Postexercise	Up to 2
Late pregnancy	Up to 1
Late pregnancy: fasted 48 h	4.0–6.0
Neonate: 0–1 day	0.2–0.5
Neonate: 5–10 days	0.7–1.0
Hypoglycemia	1.0–5.0
Untreated diabetes mellitus	Up to 25

Table 2 Comparison of factors influencing ketogenesis in suckling and fasted states

<i>Factor</i>	<i>Suckling</i>	<i>Fasted</i>
Plasma nonesterified fatty acids	Increased	Increased
Plasma insulin	Decreased	Decreased
Plasma glucagon	Increased	Increased
Hepatic carnitine	Increased	Increased
Hepatic lipogenesis	Decreased	Decreased
Hepatic malonyl-CoA	Decreased	Decreased
Hepatic CAT I activity	Increased	Increased
Sensitivity to malonyl-CoA	Decreased	Decreased

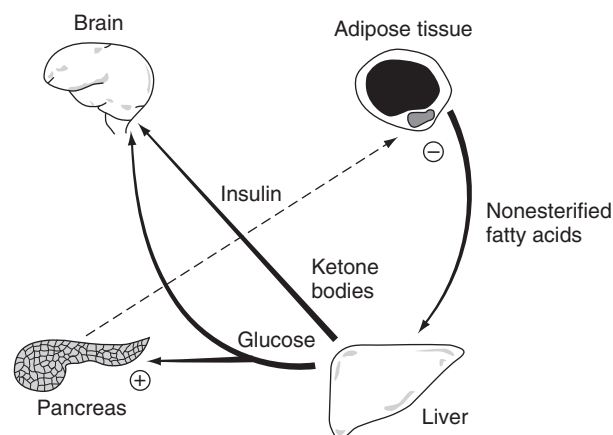


Figure 7 Intertissue fluxes of substrates in the starved state. Thickness of line denotes rate of flux.

characteristic diurnal variations in ketone body concentrations. Larger increases in concentration occur in humans in response to change in pathophysiological state (**Table 1**). The concentrations span a 200-fold range and it is this which underlines the important role of ketone bodies as substrates and signals.

Physiological Ketosis

Physiological hyperketonemia is found in the suckling neonate (high-fat diet of the milk; **Figure 1**), postexercise (depletion of hepatic glycogen reserves), and after prolonged fasting (more than 24 h; **Figure 7**). All these situations have in common a low hepatic carbohydrate status (depletion of glycogen or activation of gluconeogenesis) and therefore from a physiological standpoint one would expect an increased rate of ketogenesis. Comparison of the factors that can influence ketogenesis in suckling and fasting (**Table 2**) shows the expected broad agreement.

More detailed information on the hierarchy of the regulatory factors during onset and reversal of ketogenesis has been obtained for the fasting state by measurements at short time intervals. The first event after withdrawal of food is a lowering of plasma insulin accompanied by an increase in plasma fatty acids (stimulation of lipolysis). However, for an appreciable period (8–10 h) there is no increase in blood

ketone bodies or in the *in vitro* rates of hepatic ketogenesis (measured with saturating fatty acid concentrations). The major increment in ketogenic rate occurs at the nadir of the hepatic malonyl-CoA concentrations and when the sensitivity of CAT I to malonyl-CoA starts to increase rapidly. This long time lag before a change in sensitivity of the protein to malonyl-CoA inhibition is thought to be due to the time required to bring about alterations to the lipid environment of the outer mitochondrial membrane.

Confirmation of this view is that on refeeding, when insulin rapidly increases and plasma fatty acids decrease with a parallel decrease in blood ketone bodies, there is again a time lag before malonyl-CoA concentrations rise and a longer one before sensitivity returns. In physiological and nutritional terms this delay of return to the normal feeding settings of intrahepatic regulation makes excellent sense. It is only when the refeeding consists primarily of large amounts of carbohydrate that the starved liver needs to inhibit the activity of CAT I to prevent the oxidation of newly synthesized fatty acids. If the meal consists mainly of lipid with little carbohydrate the activity of CAT I needs to remain high to allow oxidation of the excess fatty acids. Thus the liver must sense a prolonged increase in plasma insulin before the high activity of CAT I is suppressed.

Pathological Ketosis

The major example of pathological ketosis is of course insulin-dependent or type 1 diabetes. Essentially the changes in this condition are similar to those that occur during fasting, but they are more pronounced. Insulin is absent or very low in the plasma and therefore there is no antagonistic action to restrain the opposing hormones, adrenaline, noradrenaline, and glucagon. Consequently, lipolysis in adipose tissue is greatly stimulated and plasma fatty acids increase to high levels.

The lack of insulin and the large flux of fatty acids to the liver means that lipogenesis is inhibited at the level of acetyl-CoA carboxylase and there is the expected decrease in malonyl-CoA concentration. In addition, the sensitivity of CAT I to inhibition by malonyl-CoA is considerably decreased. The level of expression of hepatic CAT I and II proteins also increases several-fold in diabetes. Thus the liver is in the ideal mode for producing excessive amounts of ketone bodies.

It has been suggested that diversion of oxaloacetate to hepatic glucose synthesis (which is also increased in insulin deficiency) may also play a role in the increased rate of

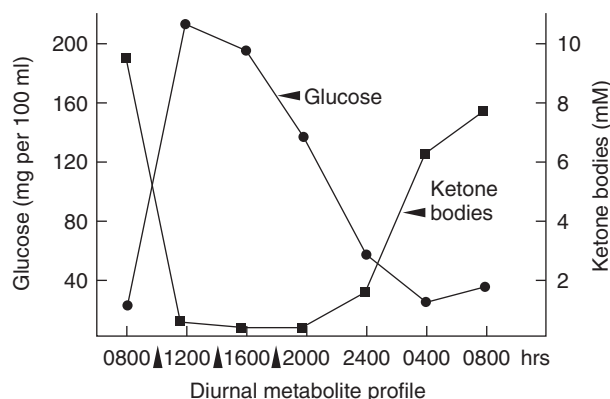


Figure 8 Diurnal blood metabolite profile of a child with glycogen synthetase deficiency.

ketogenesis by diverting acetyl-CoA from the tricarboxylate cycle. However, the present evidence suggests that this makes a minor contribution. Although the excessive output of ketone bodies by the liver undoubtedly makes the major contribution to their high levels in the blood, it is likely that there is also a degree of underutilization by peripheral tissues. The net result is ketoacidosis and excretion of large amounts of energy as ketone bodies in the urine.

A rare, but intriguing, example of pathological ketosis (ketone bodies up to 10 mmol l^{-1}) is the inborn error of hepatic glycogen synthase deficiency (Figure 8). Here glycogen is virtually absent from the liver so that after short-term fasting (5–10 h) the glucose falls to hypoglycemic levels, plasma insulin is decreased, plasma fatty acids increase, and ketogenesis is switched on. On consuming a meal the pattern is reversed until the blood glucose falls again. This case illustrates the importance of hepatic glycogen (and its mobilization) in the smooth transition of substrate supply from the fed to the fasted state. Treatment in this case was to recommend the consumption of more frequent high-carbohydrate snacks. It is of interest that this particular child suffered no ill effects from the daily exposure to high concentrations of ketone bodies, underlining their role as normal substrates for the brain when available.

Metabolic Acidosis

The great disadvantage of ketone bodies is that both acetoacetate and hydroxybutyrate are relatively strong acids. When they increase to high concentration there is the expected decrease in the blood pH, the plasma hydrogen carbonate concentration, and the partial pressure of carbon dioxide in blood and body fluids. The symptoms of acidosis include malaise, weakness, anorexia, and vomiting and these may eventually lead to coma. Treatment of diabetic ketoacidosis is to give insulin as soon as possible, usually as a continuous intravenous infusion. This rapidly decreases the raised plasma fatty acids and more slowly lowers the blood glucose and ketone

bodies. Prolonged starvation, where the blood ketone bodies may reach $8\text{--}10 \text{ mmol l}^{-1}$, does not usually cause a serious disturbance of the acid–base balance. Loss of ketone bodies via the urine occurs but is not excessive. The nonenzymic decarboxylation of acetoacetate to acetone and carbon dioxide can be seen as a primitive mechanism for removing the potential acidotic effects of ketone bodies. The fact that acetone can be converted to glucose by the liver at low rates is an extra bonus.

The other common form of metabolic acidosis is lactic acidosis. This can arise because of infection, tissue hypoxia (anaerobic glycolysis), can be drug induced (ethanol, hypoglycemic biguanides), or can arise because of a congenital defect (pyruvate dehydrogenase or pyruvate carboxylase deficiency). In addition to the acidosis caused by lactic acid or ketone bodies there is a group of organic acidurias (some 25–30 different types) in which an inborn error results in the accumulation of an organic acid in the blood and urine. However, frank acidosis is not always associated with these conditions. The key investigation is chromatographic identification of the organic acid.

See also: Adipose Tissue: Structure, Function and Metabolism. Carbohydrates: Regulation of Metabolism. Cholesterol: Sources, Absorption, Function and Metabolism. Fatty Acids: Metabolism. Lactation: Physiology. Starvation and Fasting: Biochemical Aspects

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LACTATION

Contents
Dietary Requirements
Physiology

Dietary Requirements

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Introduction

Human lactation causes a major increase in the mother's requirement for most nutrients. The lactating woman must meet the challenge of producing milk adequate in volume and nutrient composition to meet the requirements of her infant, while maintaining her own nutritional status. This is mainly achieved by an increased maternal dietary intake of energy and nutrients, otherwise nutrient depletion may occur due to excessive mobilization of maternal stores.

Because of major gaps in our knowledge about the nutrient composition of breast milk, and especially its content of essential fatty acids (EFAs), vitamins, and minerals, the impact of lactation on maternal and infant nutritional status, the recommended nutrient intakes for lactating and breastfeeding infants are somewhat uncertain. They are based mainly on the usual volume of milk secreted by well-nourished women and its nutrient content – although data are sparse concerning the latter. Recommended intakes of most nutrients for lactation are 10–90% higher than for non-pregnant, non-lactating women.

The dietary recommendations for lactating women considered in this article are those of the Food and Agriculture Organization/World Health Organization Reports on fats (2008) and micronutrients (2001), and the dietary reference intakes (DRIs) of the Institute of Medicine (USA and Canada)

for micronutrients (1997, 1998, 2000, 2001), and macronutrients (2002), with an update on calcium and vitamin D in 2011. The rationales for the recommended nutrient intakes, and requirements and dietary recommendations for energy, fats, protein, calcium, zinc, folate, and vitamin A are addressed specifically.

Rationale for Recommended Nutrient Intakes

Recommendations on dietary nutrient intakes for lactating women by different scientific authorities are typically based on the estimated total amount of each nutrient secreted daily into breast milk, taking into account, where known, the efficiency of milk synthesis and the bioavailability of the nutrient from the maternal diet. This estimate for each nutrient is then added to the recommended nutrient intake for nonpregnant, non-lactating women.

The onset of lactation after parturition is brought about by the major hormonal changes that occur in this period. During the first 2–7 days postpartum a thick yellow fluid (colostrum) is secreted. With the progress of lactation, the volume of milk secreted increases and its nutrient composition changes, with an increase, decrease, or no change in concentration, depending on the nutrient. After approximately 21 days, the milk secreted is considered mature milk. The volume of breast milk

secreted daily increases rapidly in the first postpartum days, from ~500 ml on day 5, ~650 ml at 1 month, and ~750 ml at 3 months, thereafter remaining relatively stable until decreasing during weaning. In industrialized countries, the average volume of breast milk produced is 750–800 ml day⁻¹ in the first 4–5 months postpartum and decreases to 600 ml day⁻¹ during 6–12 months after delivery. In this period, the volume of milk produced may be even lower and is quite variable, depending on the weaning practices for the infant. The FAO/WHO and DRI committees considered 750 and 780 ml, respectively, as the average milk volume produced during full lactation and the basis for recommendations. For most nutrients, average concentration in mature milk multiplied by the average milk volume was used to estimate the total amount of nutrient secreted daily into breast milk. A correction factor was then applied when the bioavailability of a nutrient in the maternal diet is less than 100%, and, where known, for the anabolic cost of milk synthesis. The final value was added to the recommended intake for nonpregnant, nonlactating women. The stage of lactation was considered to be a factor for some nutrients and, where applicable, separate values were given according to the period of time postpartum.

The volume of milk secreted during lactation is not influenced by maternal nutritional status, unless maternal undernutrition is severe. The composition of breast milk for most nutrients is adequate to support infant growth and development over a wide range of maternal nutritional status. However, maternal diet and nutritional status do have an influence on the concentration of some micronutrients such as vitamins A, D, thiamin, riboflavin, vitamins B₆ and B₁₂, choline, iodine, and selenium. Also, the fatty acid composition of breast milk can be affected by maternal diet.

When setting recommendations the DRI committees take maternal age into account, so there are separate values for adolescent (≤ 18 years) and adult (19–50 years) lactating women. For some nutrients, adolescent lactating women may have greater requirements than adult women because they are still growing and they need to cover their own nutrient demands. Recommended intakes of vitamins A and C, calcium, phosphorus, magnesium, iron, and zinc are higher for adolescent than for adult women during lactation.

In general, there is considerable uncertainty about dietary nutrient recommendations for lactation due to high intra- and interindividual variability in breast milk volume output, specific nutrient concentrations in milk, and the temporal changes in milk volume and nutrient concentrations during a day and across the period of lactation. The composition of breast milk is affected by several factors depending on the nutrient, such as stage of lactation, changes during a feeding, diurnal rhythm, maternal diet, gestational age of the infant at birth, and parity. Moreover, the total amount of nutrients secreted into breast milk depends on the extent and duration of breast feeding. In addition, physiological adaptations to the high nutrient demands for lactation may include increased nutrient absorption and conservation, and use of maternal nutrient stores. These adaptations are quite specific for each nutrient and not easily quantified, which contributes to the degree of uncertainty. Maternal age and maternal nutritional status during pregnancy and lactation may also influence the homeostatic adaptations during lactation including the

efficiency of nutrient absorption and the degree of mobilization of maternal nutrient stores.

Requirements and Dietary Recommendations

Macronutrients

Energy

The dietary energy intake recommended for healthy adults of normal weight (body mass index between 18.5 and 25 kg m⁻²) is that required to maintain energy balance, considering gender, age, weight, height, and level of physical activity. The energy requirements of lactating women include the additional energy that is necessary for milk production, which differ by the stage and extent of breastfeeding.

The energy density of human milk is mainly determined by its fat content, which represents 50–60% of the total energy in mature milk and is the most variable energy-yielding component. Protein and lactose contribute to approximately 5% and 38% of energy, respectively. The mean energy density of representative 24-h pooled mature milk samples from well-nourished women ranges from 0.64 to 0.74 kcal g⁻¹ (2.7–3.1 kJ g⁻¹).

The estimated energy requirements (EER) for lactating women set by the DRI committee are based mainly on studies done in the 1990s, using the doubly labeled water method. The main findings in women who were fully breastfeeding their infants up to 6 months of age were: total energy expenditure of 2109–2580 kcal day⁻¹ (8860–10840 kJ day⁻¹) or 35.8–41.0 kcal kg day⁻¹ (150–172 kJ kg⁻¹ day⁻¹), milk energy output of 483–538 kcal day⁻¹ (2030–2260 kJ day⁻¹), and energy mobilization from tissue stores of 72–287 kcal day⁻¹ (300–1200 kJ day⁻¹). It was concluded that the energy requirements of lactating, well-nourished women are met primarily from the diet and partially by mobilization of tissue stores, without evidence for adaptations in basal metabolism and physical activities. The EER for lactating adult women during the first 6 months of lactation is calculated as the sum of the EER obtained from the equation for adult nonlactating women (using current age, weight, and physical activity level), the energy secreted in milk energy (500 kcal day⁻¹ or 2100 kJ day⁻¹), and subtracting the energy derived from tissue mobilization during the maternal weight loss that normally occurs during lactation (170 kcal day⁻¹ or 714 kJ day⁻¹). The committee assumed a milk production rate of 0.78 l day⁻¹ from birth through 6 months of age, a milk energy density of 0.67 kcal g⁻¹ (2.8 kJ day⁻¹), and an average maternal weight loss of 0.8 kg month⁻¹. For the second 6 months of lactation, the incremental EER is calculated assuming a milk energy output of 400 kcal day⁻¹ or 1680 kJ day⁻¹ (a milk production rate of 0.6 l day⁻¹) and no maternal weight loss. The EER for lactating adolescents (14–18 years) is calculated in the same manner as for adult lactating women, but the increment for lactation is added to the appropriate equation for estimating the EER of nonlactating adolescents.

The acceptable macronutrient intake distribution ranges, expressed as percentage of total dietary energy, are the same as for the general adult population: 10–35% protein, 20–35% fat, and 45–65% carbohydrates. Natural simple sugars, such as

those present in fruit, and complex carbohydrates (polysaccharides), such as in cereals (rice, wheat), cereal products (flour, pasta) and starchy roots, should be the preferred sources of carbohydrates in the diet. Added sugars, usually sucrose, should not be higher than 25% of dietary energy. Many of the energy-yielding carbohydrate food sources are also sources of dietary fiber that are beneficial for reducing the risk of coronary heart disease, ameliorating constipation, and other health outcomes. A total fiber intake of 29 g day⁻¹ is recommended for lactating women. Whole grain cereals, nuts, legumes, and fruit are good fiber and energy sources, and are also nutrient-rich foods. Restriction of energy intake during lactation to values below 1800 kcal (7500 kJ) per day may lead to low intakes of other nutrients including vitamins and minerals.

Fat

Total fat content of human milk is affected by several factors, including stage of lactation, stage of feeding, and parity, but maternal intake of energy, fat, or fatty acids and maternal nutritional status have little influence, except when there is long-term or severe maternal undernutrition. Milk fat content is highly variable, averaging 35–40 g l⁻¹ in mature milk from well-nourished women delivering at term gestation. The content of individual fatty acids in milk is also highly variable, especially for the long-chain polyunsaturated fatty acids (LCPUFA, especially docosahexenoic acid, DHA), and more dependent on maternal diet than total fat. The concentration of DHA can vary from 0.2 to 1% of total fatty acids and is proportional to the usual intake of the mother. Fatty acid intake and relative contribution of carbohydrate and fat to the mother's total energy intake, as well as maternal body stores and endogenous synthesis, influence the fatty acid composition of human milk. In well-nourished mothers, the polyunsaturated EFA linoleic acid (18: 2n-6) and α -linolenic acid (18: 3n-3) represent approximately 11 and 1% (wt/wt), respectively, of the total fatty acids in milk. LCPUFA of the n-6 and n-3 series account for 1.2 and 0.6% of the fatty acids, respectively.

The adequate transfer of polyunsaturated fatty acids from maternal circulation to milk and the maternal synthesis of LCPUFA, especially arachidonic acid (20: 4n-6), dihomo- γ -linolenic acid (20: 3n-6), eicosapentenoic acid (EPA, 20: 5n-3), and DHA (22: 6n-3), from their respective EFA precursors, are important for infant growth, neurodevelopment, and visual function. These polyunsaturated fatty acids are structural components of all cell membrane phospholipids. Arachidonic acid and DHA are the two quantitatively most important LCPUFA in the brain and retina, and the LCPUFA with 20 carbon atoms are precursors for the synthesis of eicosanoids, a group of signaling molecules. The major part of the polyunsaturated fatty acids in human milk (70–85% in women consuming an omnivorous diet) is derived from maternal body stores which reflect dietary intake over the long term, and not from direct dietary transfer.

The metabolic fate of individual fatty acids depends on dietary energy intake and energy balance. Therefore, the intake and requirements for fat, EFA, and LCPUFA are usually expressed as a percentage of the total energy in the diet (en %), rather than total intake (g). The fat intake recommended for lactating women is in the range of 20–35 en %, which is the

same range as recommended for the adult population. Concerning the fatty acid intake, FAO/WHO recommends an additional maternal intake of 1–2 en % as EFA (3–4 g day⁻¹) during the first 3 months of lactation, and up to 4 en % (about 5 g day⁻¹) thereafter due to depletion of maternal fat stores. Based on the median linoleic and α -linolenic acid intakes of lactating women in the US, the DRI committee recommends an intake of 5–10 en % (average 13 g day⁻¹) of n-6 (as linoleic acid) and of 0.6–1.2 en % (average 1.3 g day⁻¹) of n-3 (as α -linolenic acid) polyunsaturated fatty acids throughout lactation, with a 10% contribution from LCPUFA in the n-6 and n-3 series to these ranges. The ratio of n-6: n-3 unsaturated fatty acids in the diet is important because these fatty acids are desaturated and elongated, and incorporated into membranes, using the same series of enzymes. Increased intake of linoleic acid reduces the conversion of α -linolenic acid to EPA and DHA, whereas the conversion of linoleic acid to arachidonic acid is inhibited by EPA and DHA, as well as by arachidonic acid, α -linolenic acid, and linoleic acid itself. The n-6:n-3 ratio recommended for adults by both DRI and FAO/WHO committees is 5:1 to 10:1. Vegetable oils are the main dietary source of n-6 fatty acids, and also of n-3 fatty acids although in lower amounts. Fish such as herring, mackerel, and salmon are good sources of n-3 fatty acids.

The intake of trans fatty acids (trans isomers of oleic and linoleic acid) present in hydrogenated food fats and oils, deep-fried foods, and meats are of special concern in lactating women when intake is excessively high, or when EFA intake is low during pregnancy and lactation. An inverse correlation of arachidonic acid and DHA with trans fatty acids in plasma lipids has been reported in infants, suggesting impaired LCPUFA synthesis and metabolism.

Protein

The average protein content in colostrum is 15–20 g l⁻¹ decreasing to approximately 8–10 g l⁻¹ in mature human milk during the first 6 months of lactation. The protein concentration in human milk is not affected by diet, body composition, or maternal undernutrition.

The recommended dietary allowance (RDA) of protein for adolescent and adult lactating women set by the DRI committee is 1.1 g kg⁻¹ of body weight per day. This corresponds to an increment of 25 g day⁻¹ above the RDA for nonlactating women, and is the same as for pregnant women. Recent data have shown that protein intakes of 1 g/kg/day are able to maintain good milk production, and promote conservation of maternal skeletal muscle apparently by down-regulating protein metabolism. The recommended range of percentage of energy from dietary protein is the same as for the general adult population (10–35%).

The factorial approach was used to estimate the protein RDA for lactation, assuming that the maintenance protein requirement of the lactating woman is not different from that of nonlactating women, and that the additional protein and/or amino acid requirements are proportional to milk production. The additional protein requirement for lactation is defined as the output of total protein and nonprotein nitrogen (the latter converted to protein by multiplying by 6.25) in milk. Nonprotein nitrogen represents 20–25% of total milk nitrogen, mainly as urea. It is taken into account because it is assumed

that the nitrogen needed to cover the total nitrogen loss in milk should be derived from dietary protein. The total protein output, approximately 10 g day^{-1} , is divided by the incremental efficiency of nitrogen utilization (0.47), which is assumed to be the same in adult and adolescent lactating women. The additional estimated average requirement due to milk production is therefore 21.2 g day^{-1} . After correction by the coefficient of variation and rounding off, the RDA for lactation is $+25 \text{ g day}^{-1}$, which corresponds to $+0.46 \text{ g protein kg day}^{-1}$ (based on a reference woman of 57 kg) above the RDA for nonlactating women.

Recommendations for individual indispensable amino acids for lactation by the DRI committee assume that the incremental needs correspond to the amino acids secreted in milk, because there are no specific data on the amino acid requirements of lactating women. Therefore the RDA for amino acids in lactation is calculated by adding the average amounts of amino acids in human milk in the first 6 months of lactation (expressed as $\text{mg kg}^{-1} \text{ day}^{-1}$) to the respective RDA for nonlactating women. Overall, recommendations for indispensable amino acids for the lactating women are 36% (histidine) to 80% (tryptophan) higher than those for nonlactating women. High-quality protein from sources such as eggs, milk, meat, and fish provides the requirements for all indispensable amino acids. Individuals who restrict their diets to plant proteins (cereals, legumes, nuts, starchy roots, vegetables, and fruits) may be at risk of inadequate intakes of certain indispensable amino acids. However, adequate complementary mixtures of plant proteins, with increased digestibility through processing and preparation, can provide high-quality protein.

Minerals and Vitamins

Daily requirements for several micronutrients (riboflavin, vitamins B₁₂, C, A, and E, copper, iodine, manganese, selenium, and zinc) are higher during lactation than during pregnancy, indicating that lactation is a very demanding process. The only micronutrients needed in lower amounts during lactation are iron, due to the small amount of iron secreted into breast milk and to the usual amenorrhea of nursing women, and folate. However, iron requirements may be high postpartum for women who need to replace major blood losses during delivery.

The recommended intakes for micronutrients during lactation established by FAO/WHO and DRI committees are summarized in [Table 1](#). The percentages of change from the recommendations for nonpregnant nonlactating women are also shown. To meet these intakes, lactating women should be guided to consume daily a large variety of foods rich in micronutrients, because food diversification contributes to improve the intake of limiting nutrients. Micronutrients most commonly at risk of inadequate intakes by lactating women are calcium, zinc, folate, and vitamin A.

Calcium

It is estimated that lactating women secrete an average of 200 mg of calcium per day into mature breast milk although

this amount is variable among women, usually ranging from 150 to 300 mg day^{-1} . The maternal diet does not affect the milk calcium concentration except when maternal calcium intake is very low ($<300 \text{ mg day}^{-1}$). The primary source of calcium for milk production is the increased mobilization of calcium from maternal bone due to the increased bone resorption that occurs during lactation, favored by the low estrogen concentration. This results in a net loss of maternal bone mass during lactation that is regained after weaning upon return of ovarian function. The decreased urinary calcium excretion during lactation also contributes to the calcium economy for milk secretion. The efficiency of intestinal calcium absorption is not increased during lactation and does not contribute to the extra calcium needed for milk production.

Several studies have shown that the adaptive changes in calcium homeostasis during lactation are independent of maternal calcium intake. It was demonstrated that the loss of bone mass during lactation was not affected by calcium supplementation (1000 mg day^{-1}) of nursing women with habitual dietary calcium intakes of 300, 800, or 1200 mg day^{-1} . Because the loss of maternal bone calcium that occurs during lactation is not prevented by increased dietary calcium, and the calcium lost appears to be regained after weaning, the recommended intake of calcium of lactating women is the same as that for nonpregnant nonlactating women of the same age, being 1000 and 1300 mg day^{-1} for adult and adolescent women, respectively. Even if not increased during lactation, the recommended calcium intake may be difficult to obtain by women with a low habitual intake of dairy products. Therefore lactating women should be guided to consume dairy products such as milk, yogurt, cheese, and other calcium-rich foods such as fish with edible bones, broccoli, and kale.

Lactating adolescents are a group of special concern regarding calcium intake due to the already high calcium requirements of non-pregnant nonlactating adolescents. These young women are still increasing their own bone density as well as needing the increased calcium requirement to support lactation. Studies are needed to investigate if these women are able to regain bone after weaning to the same level as when they were nonpregnant and nonlactating, and if they would benefit from increased calcium intake.

Zinc

Zinc concentrations in human milk are highest in colostrum, decrease rapidly during the first 3 months postpartum, and continue to fall but more gradually at later stages of lactation. Typical milk zinc concentrations are 4 mg l^{-1} at 2 weeks, 3 mg l^{-1} at 4 weeks, 2 mg l^{-1} at 8 weeks and 1.2 mg l^{-1} at 24 weeks. These concentrations are not influenced by either maternal dietary intake or zinc supplementation, at least in well-nourished women. Less is known about the effect of low maternal zinc intakes on milk zinc concentrations, but some reports indicate that concentrations in developing countries may be slightly lower than those in developed countries at comparable times postpartum.

Average daily losses of zinc in breast milk range from 2.2 mg during the first month postpartum to 1 mg at 6 months. The average estimate of daily output of zinc in milk during the first 3 months of lactation is 1.6 mg, which would

Table 1 Daily recommended mineral and vitamin intakes for adult lactating women

Nutrient	FAO/WHO ^a		IOM ^b	
	Recommended value	% change ^c	Recommended value	% change ^c
Vitamin A (μg RAE)	—	—	1300	↑ 86
Vitamin A (μg RE)	850	↑ 70	—	—
Vitamin D (μg)	5	No change	15	No change
Vitamin E (mg α-TE)	7.5	No change	19	↑ 27
Vitamin K (μg)	55	No change	90	No change
Thiamin (mg)	1.5	↑ 36	1.4	↑ 27
Riboflavin (mg)	1.6	↑ 45	1.6	↑ 45
Niacin (mg NE)	17	↑ 21	17	↑ 21
Vitamin B ₆ (mg)	2.0	↑ 54	2.0	↑ 54
Pantothenate (mg)	7.0	↑ 40	7.0	↑ 40
Biotin (μg)	35	↑ 17	35	↑ 17
Folate (μg DFE)	500	↑ 25	500	↑ 25
Vitamin B ₁₂ (μg)	2.8	↑ 17	2.8	↑ 17
Vitamin C (mg)	70	↑ 55	120	↑ 60
Calcium (mg)	1000	No change	1000	No change
Iodine (μg)	200	↑ 82	290	↑ 93
Iron (mg)	15 ^d	↓ 49	9	↓ 50
Zinc (mg)	9.5 ^e	↑ 94	12	↑ 50
	8.8 ^f	↑ 80		
Magnesium (mg)	270	↑ 23	310	No change
Selenium (μg)	35	↑ 35	70	↑ 27
Chromium (μg)	—	—	45	↑ 80
Copper (μg)	—	—	1300	↑ 44
Fluoride (mg)	—	—	3	No change
Manganese (mg)	—	—	2.6	↑ 44
Molybdenum (μg)	—	—	50	↑ 11

^aFAO/WHO (2001) Human vitamin and mineral requirements. *Report of a Joint FAO/WHO Expert Consultation*. Rome: Food and Agriculture Organization.

^bInstitute of Medicine (IOM) (2001) *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Also (2011) *Dietary Reference Intakes for Calcium and Vitamin D*. Washington, DC: National Academies Press.

^cChanges from recommendations for nonpregnant nonlactating women: ↑, increase; ↓, decrease.

^dAssuming 10% bioavailability.

^e0–3 months postpartum, assuming moderate bioavailability.

^f4–6 months postpartum, assuming moderate bioavailability.

RAE, retinol activity equivalent; α-TE, alpha-tocopherol equivalent; NE, niacin equivalent; DFE, dietary folate equivalent.

theoretically double the minimum endogenous zinc losses in lactating women compared to those of nonlactating nonpregnant women. However, maternal homeostatic mechanisms such as enhanced zinc absorption and reduced urinary zinc excretion compensate for the secretion of zinc into human milk, independent of maternal zinc intake. Intestinal conservation of endogenous fecal zinc appears to contribute to zinc homeostasis during lactation at low zinc intakes (<8 mg day⁻¹). Involution of the uterus, decreased maternal blood volume, and increased resorption of trabecular bone in the postpartum period also contribute to mobilizable zinc pools to compensate for the increased needs. These sources appear to provide up to 0.5 mg day⁻¹ of zinc during the first 3 months of lactation. Taking all these adaptive mechanisms into account, the average estimated for the increased requirement for absorbed zinc during the first 6 months of lactation is 1.35 mg.day⁻¹. Therefore, dietary zinc requirements during lactation are substantially increased compared to those of nonpregnant nonlactating women, both for adults and adolescents.

Bioavailability is an important factor in setting dietary zinc recommendations because the efficiency of dietary zinc

utilization may vary up to fivefold depending on the overall composition of the diet, and particularly on the negative effect of dietary phytate. The efficiency of absorption is inversely related to the level of dietary zinc, except when phytate intake is high. Dietary zinc recommendations during lactation are set at 12 mg day⁻¹ for adult lactating women consuming a mixed diet, but requirements will be higher for those whose diet is high in phytate because it is based mainly on unrefined cereals and legumes. Red meat, milk, poultry, eggs, and seafood provide highly available zinc, and their consumption should be encouraged in lactating women.

Vitamin A

Vitamin A is present in human milk as retinyl esters (95%) and free retinol. Vitamin A activity is also provided as carotenoid precursors, mainly as β-carotene, which accounts for up to 30% of total carotenoids in breast milk. The concentration of vitamin A in human milk is high in early lactation (600–2000 μg l⁻¹) and declines thereafter to 200–1100 μg l⁻¹. It is responsive to maternal intake, particularly in nursing women with poor vitamin A status. Such women are at risk of providing insufficient amounts of vitamin A to their infant.

Dietary recommendations for vitamin A during lactation are based on replacing the amount of the vitamin secreted into breast milk during the first 6 months of lactation, while preserving maternal vitamin A stores. Because the bioconversion of carotenoids in human milk is still uncertain, the contribution of maternal carotenoids in breast milk to meeting the vitamin A lactation is not considered.

Based on the average milk vitamin A milk concentration of $485 \mu\text{g l}^{-1}$, an additional intake of $400 \mu\text{g}$ retinol activity equivalents (RAE) per day is recommended for lactating women, which represents an increase of over 70% compared to the recommendations for non-pregnant nonlactating adolescent and adult women. A RAE is defined as $1 \mu\text{g}$ all-trans-retinol, $12 \mu\text{g}$ beta-carotene, or $24 \mu\text{g}$ α -carotene or β -cryptoxanthin. The amount of carotenoids equivalent to 1 RAE is double the equivalent to 1 RE (retinol equivalent).

The vitamin A intake recommended for lactating women can be obtained as the preformed vitamin from foods of animal origin (primarily milk products, eggs, and liver) and as carotenoid precursors in green leafy vegetables and ripe, colored fruits. However, meeting the recommended intake by consumption of plant sources alone, as is the case in many developing countries, may be difficult unless the diet contains some carotenoid-rich foods such as sweet potatoes.

Folate

The concentration of folate in breast milk increases during the lactation period, with lower values for colostrum ($10\text{--}40 \mu\text{g l}^{-1}$) than for mature milk ($79\text{--}133 \mu\text{g l}^{-1}$). These concentrations are several-fold higher than in maternal plasma, independent of maternal folate status, indicating that the mammary gland actively transports and regulates the secretion of this vitamin into milk. Folate concentration in breast milk is maintained with the concomitant depletion of maternal folate when maternal dietary intake is low. Maternal supplementation during lactation has little effect on milk folate but it benefits maternal folate status. Dietary folate requirements during lactation are based on the average milk folate concentration of $85 \mu\text{g l}^{-1}$ and assume that the mother absorbs 50% from a mixed diet. The average extra amount of dietary folate needed to cover lactation is thus estimated as $133 \mu\text{g day}^{-1}$, an increase of approximately 40% above the nonpregnant nonlactating average folate requirements. Dietary folate recommendations during lactation are set at $500 \mu\text{g}$ dietary folate equivalents (DFEs) daily. A DFE is defined as $1 \mu\text{g}$ of food folate, or $0.6 \mu\text{g}$ of folic acid from fortified food or as a supplement taken with meals, or $0.5 \mu\text{g}$ of folic acid as a supplement taken on an empty stomach. Thus, in order to meet lactation requirements, less of this vitamin is needed when given as pure folic acid than as natural food folate.

Although folate is found in a variety of foods, such as fresh green vegetables, oranges, legumes and nuts, several servings per day of these foods are needed to meet recommended intake. Moreover, considerable losses of folate can occur during food harvesting, storage, and cooking. Fortification of wheat flour with folic acid has become mandatory or encouraged in many countries in order to reduce the risk of neural tube defects in women at risk of this condition.

Other B vitamins

Maternal deficiency of thiamin, riboflavin, vitamin B₆, vitamin B₁₂, and choline can result in low concentrations in breast milk and subsequent infant deficiency. It has been estimated that intakes of infants exclusively breastfed by a deficient mother may be in the range of 16% (vitamin B₁₂) to 80% (vitamin B₆) of their recommended Adequate Intakes. Further information is needed about the status of these vitamins in lactating women and their infants because intakes of most B vitamins are inadequate where animal source food consumption is low – a common situation in poor populations. Increasing maternal intake through supplementation increases the milk content of these vitamins and improves the status of the mother and the infant.

See also: Adolescents: Requirements for Growth and Optimal Health. Breast Feeding. Calcium. Dietary Guidelines, International Perspectives. Energy Requirements. Fatty Acids: Metabolism. Folic Acid. Lactation: Physiology. Protein: Requirements and Role in Diet. Zinc: Physiology, Dietary Sources, and Requirements

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Glossary

Arborizing ductal network The system of ducts composed of epithelial cells that connect lobuloalveolar units to the nipple.

Junctional complex Tight junctions branching networks of transmembrane protein strands that seal adjacent epithelial cells, preventing passage of ions and other substances from blood to milk.

Lobuloalveolar unit The portion of the mammary gland specialized for producing and secreting milk. The lobuloalveolar unit is composed of a single layer of secretory epithelial cells that synthesize and secrete milk. Secretory epithelial cells are surrounded by a network of

myoepithelial cells that contract in response to oxytocin stimulation to stimulate milk ejection.

Paracellular transport The passage of substances between epithelial cells. Paracellular transport is regulated by tight junctions and is closed during lactation.

Secretory activation The hormone regulated process of maturation of gene expression, secretory mechanisms and transport pathways that occurs after parturition, and that is required for copious milk secretion. Secretory activation is also called lactogenesis II.

Transcytosis The endocytic uptake of substances at the basal membrane and their transport to the apical membrane for secretion.

Lactation is a uniquely mammalian physiological process in which the caloric and nutrient reserves of the mother are transformed into a complex fluid capable of supporting the nutritional demands of newborns for sustained periods. Milk, the product of lactation, is a mixture of solutes whose composition reflects the activities of distinct secretion and transport processes of the mammary gland and mirrors the differing nutritional requirements of mammalian neonates. In humans, this fluid is capable of providing the full-term infant with all the nutrients required for the first 4–6 months of life as well as offering significant protection against infectious disease. Although artificial formulas are widely utilized for human infant nutrition in developed countries, many components of human milk, including critical growth factors, long-chain polyunsaturated fatty acids (PUFA), antiinfectious oligosaccharides and glycoconjugates, and the protein lactoferrin, are not duplicated in formula. Although it is likely that such substances are beneficial even to healthy infants in well-protected environments, they are particularly important for infants living under conditions of inadequate sanitation, as well as for preterm infants and infants with feeding problems. Despite the obvious importance of milk to neonatal nutrition and the selective advantage of lactation in mammalian evolution, the physiological mechanisms underlying milk secretion and utilization are not well understood and the molecular mechanisms involved in the production of individual milk components are still poorly characterized. In this article, the functional anatomy of the mammary gland is described, followed by a brief description of human milk composition and a review of the transport mechanisms involved in the secretion of individual milk components. The authors then summarize the functional differentiation of the mammary gland and the initiation of lactation – a process that involves a series of carefully programmed functional changes that transform a prepared, but nonsecretory, gland into a fully functioning organ during the first week postpartum in humans.

Functional Anatomy of Lactation

The lactating mammary gland consists of an arborizing ductal network that extends from the nipple and terminates in grape-like lobular clusters of alveoli forming the lobuloalveolar unit, which is the site of milk secretion. A stylized diagram of these structures is shown in **Figure 1**. Alveoli are composed of a single layer of polarized secretory epithelial cells that possess specialized features indicative of highly developed biosynthetic and secretory capacities, including numerous mitochondria, an extensive rough endoplasmic reticulum network, and a well-developed Golgi apparatus. Secretory components including lipid droplets and casein-containing secretory vesicles are found juxtaposed to the apical membrane of these cells. The epithelial cells are connected to each other through a junctional complex composed of adherens and tight junctional elements that function to inhibit the transfer of extracellular substances between the vascular system and milk compartments during lactation (**Figure 2**). The basal portion of alveolar epithelial cells is surrounded by a meshwork of myoepithelial cell processes that contract to bring about milk ejection and by a connective tissue stroma that supports and separates the lobules. The stromal component also contains lymphatics and becomes extensively vascularized during lactation to sustain the biosynthetic demands of alveolar epithelial cells. In nonpregnant, nonlactating animals, the stroma contains a large adipose component.

The nipple, which is the termination point of the mammary ductal network, is innervated by the fourth intercostal nerve. Afferent sensory stimuli from suckling are transmitted to the spinal cord and the brain, resulting in the release of prolactin and oxytocin from the pituitary. Prolactin, secreted from the anterior pituitary, acts directly on alveolar epithelial cells to foster the synthesis and secretion of milk components. Oxytocin, secreted from the posterior pituitary, stimulates contraction of the myoepithelial cells that surround the alveoli

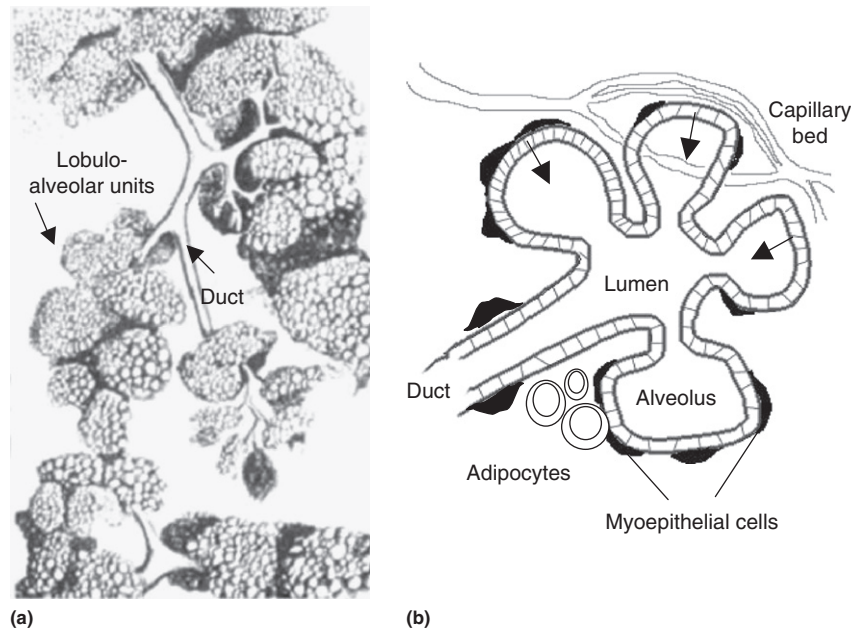


Figure 1 Camera lucida drawing of a section of the breast of a woman who died 2 days after last suckling her infant (a). The drawing clearly shows collecting ducts and the grape-like lobuloalveolar units, which are engorged with milk. (b) Cross-sectional diagram showing the relationships of the lobuloalveolar unit composed of milk-secreting alveoli and ducts with the other cellular compartments of the mammary gland. Arrows indicate milk secretion by the alveolar epithelial cells into the lumen. Camera lucida drawing is reproduced from Dabelow A (1941) *Der Entfaltungsmechanismus der Mamma. II. Die postnatale Entwicklung der menschlichen Milchdrüse und ihre Korrelationen. Morphology Journal* 85: 361–416, with permission from Wiley.

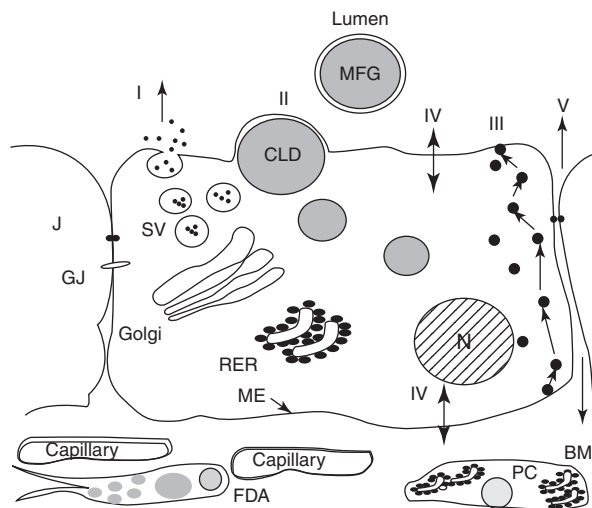


Figure 2 Diagram of a mammary epithelial cell showing pathways for milk secretion described in the text. Abbreviations: SV, secretory vesicle; RER, rough endoplasmic reticulum; BM, basement membrane; N, nucleus; PC, plasma cell; FDA, fat-depleted adipocyte; JC, junctional complex containing the tight and adherens junctions; GJ, gap junction; ME, myoepithelial cell; CLD, cytoplasmic lipid droplet; MFG, milk fat globule. Redrawn from Neville MC, Allen JC, and Watters C (1983) *The mechanisms of milk secretion*. In: Neville MC and Neifert MR (eds.) *Lactation: Physiology, Nutrition and Breast-Feeding*, p. 50. New York, NY: Plenum Press.

and ducts. This process, called the 'letdown reflex,' forces the milk from the alveoli through ductules into ducts draining several clusters of alveoli. In humans, the small ducts converge into 15–25 main ducts that drain sectors of the gland and

open directly on the nipple. The secretory product is stored in the alveolar space until myoepithelial cell contractions force it through the ducts toward the nipple, where it is available to the suckling infant.

Milk Composition

The major macronutrients in milk are lactose (a disaccharide unique to milk); lipids; proteins, including casein, α -lactalbumin, lactoferrin, secretory immunoglobulin A (sIgA), and many others present at much lower concentrations; and minerals such as sodium, chloride, calcium, and magnesium. Other nutritionally important substances in milk are enzymes, vitamins, trace elements, and growth factors. The lipid content of milk varies considerably between species. In human and cow's milk, the fat accounts for approximately 4% of milk volume, whereas in whales and seals it can account for as much as 60% of milk volume. Milk fat is primarily composed of triglycerides, a major source of neonatal calories, and it also contains cholesterol and phospholipids, essential for early neonatal development. Casein micelles form a separate phase that can be pelleted by high-speed centrifugation or acidification. These micelles have a high calcium and phosphate content. The aqueous fraction of milk, often called whey, is a true solution that contains all the milk sugar as well as the major milk proteins lactoferrin, α -lactalbumin, and sIgA and nonprotein nitrogen compounds (mostly urea); the monovalent ions sodium, potassium, and chloride; citrate; calcium; free phosphate; and most of the water-soluble minor components of milk. The casein fraction from cow's milk, usually obtained by rennin precipitation, is used in cheese making,

Table 1 Comparison of the macronutrient contents of human and bovine milk

Component	Human milk	Bovine milk
Carbohydrates (g dL⁻¹)^a		
Lactose	7.3	4.0
Oligosaccharides	1.2	0.1
Proteins (g dL⁻¹)^a		
Caseins	0.2	2.6
α -Lactalbumin	0.2	0.2
Lactoferrin	0.2	Trace
Secretory IgA	0.2	Trace
β -Lactoglobulin	0	0.5
Nonprotein nitrogen (NPN) (g L⁻¹)		
Total NPN	0.42 ^b	0.29 ^c
Urea	0.16 ^b	0.14 ^c
Milk lipids (%)^a		
Triglycerides	4.0	4.0
Phospholipids	0.04	0.04
Minerals and other ionic constituents (mM)^a		
Sodium	5.0	15
Potassium	15.0	43
Chloride	15.0	24
Calcium	7.5	30
Magnesium	1.4	5
Phosphate	1.8	11
Bicarbonate	6.0	5

^aReproduced from Neville MC (1998) Physiology of lactation. *Clinical Perinatology* 26: 251.

^bReproduced from Atkinson SA and Lonnerdal B (1995) In: Jensen RG (ed.) *Handbook of Milk Composition*. San Diego: Academic Press.

^cReproduced from Alston-Mills B (1995) In: Jensen RG (ed.) *Handbook of Milk Composition*. San Diego: Academic Press.

whereas the whey has a multiplicity of uses, most notably as the base for infant formula. Urea and other nonprotein nitrogen components of milk are a source of nitrogen for amino acid and protein synthesis. Isotope utilization studies indicate that on average 10–20% of urea nitrogen is converted into protein by breast-fed infants. Significantly higher utilization rates, however, have been measured in children recovering from infection, suggesting that alterations in urea nitrogen utilization may be a homeostatic response. Human and bovine milk differ primarily in their concentrations of lactose, mono- and divalent ions, and casein levels and the existence of antiinfectious agents in human milk (Table 1). These differences are related to the specific needs of these species. Human milk, for example, possesses higher concentrations of lactose and lower divalent ion concentrations than cow's milk. The high lactose concentration provides a large amount of 'free water,' via osmotic regulation, that serves as a reserve for temperature regulation via sweating in human infants. Human milk also contains a number of agents that protect against gastrointestinal and respiratory infections, including oligosaccharides that interact specifically with pathogen receptors, lactoferrin, and sIgA. Bovine milk, however, contains high concentrations of casein, which provides protein and associated calcium and phosphate needed to support the rapid growth of young calves.

Synthesis and Secretion of Milk Components

Solutes enter milk through five general pathways (Figure 2). Endogenously generated substances, including the major milk proteins, oligosaccharides, and nutrients such as lactose, citrate, phosphate, and calcium, are secreted through an exocytotic pathway (pathway I). Lipids and lipid-associated proteins are secreted by a process that is unique to mammary epithelial cells (pathway II). The transcytosis pathway (pathway III) transports a wide range of macromolecular substances derived from serum or stromal cells, including serum proteins such as immunoglobulins, albumin, and transferrin; endocrine hormones such as insulin, prolactin, and insulin-like growth factor-1; and stromal-derived agents such as immunoglobulin A (IgA) cytokines, and lipoprotein lipase. In addition, various membrane transport pathways (pathway IV) exist for the transfer of ions and small molecules, such as glucose, amino acids, and water across basal and apical plasma membranes. Finally, there is a paracellular pathway (pathway V) that provides a direct route for entry of serum and interstitial substances into milk. This pathway, however, closes during the first few days of lactation in humans. Transport through these pathways is affected by the functional state of the mammary gland and regulated by direct and indirect actions of hormones and growth factors. The general cellular and physiological properties of these pathways are summarized next.

Exocytotic Pathway (I)

Like exocytotic secretion mechanisms found in other cells, proteins, oligosaccharides, and nutrients such as lactose and citrate are packaged into secretory vesicles within the Golgi that are then transported to the apical region of the cell, where they fuse with the apical plasma membrane, discharging their contents into the extracellular space. A unique feature of this pathway in the mammary gland is the presence of high concentrations of lactose, phosphate, citrate, and calcium within the vesicles. Lactose is synthesized in the Golgi from UDP-galactose and glucose, which have entered from the cytoplasm using specific transporters, by the enzyme β -galactosidase, with α -lactalbumin acting as a cofactor. The high concentration of lactose present in the Golgi during lactation osmotically stimulates the influx of water that contributes to the fluidity of milk. Casein micelle formation begins in the terminal Golgi with condensation, and simultaneous phosphorylation, of casein molecules. The addition of calcium, possibly in the secretory vesicle, leads to maturation of casein micelles into particles sufficiently dense to be seen in the electron microscope. This complex thus delivers an efficient package of protein, calcium, and phosphate that provides the nutrients necessary for bone growth, among other things. Calcium enters the cytoplasm from the plasma by a poorly defined transport process. Cytoplasmic calcium is then transported into secretory vesicles by an ATP-dependent Ca^{2+} pump localized on Golgi and secretory membranes. The phosphate in secretory vesicles is derived from the hydrolysis of UDP-galactose during the synthesis of lactose. Citrate is generated endogenously within the cytoplasm of alveolar epithelial cells and transported into the Golgi lumen by an undefined process.

Lipid Secretion Pathway (II)

Estimates of the quantity of milk lipid secretion during lactation in humans and rodents indicate that in many species, the lactating mammary gland may be one of the most lipogenic organs in the body. In a fully lactating woman secreting 800 ml day⁻¹ of milk containing 4% fat, the mammary gland synthesizes approximately 32 g of triglyceride daily or approximately 6 kg, 10% of the weight of the woman, in a typical 6-month lactation. The fatty acids for triglyceride synthesis are synthesized from glucose or derived from the plasma lipids by the action of lipoprotein lipase. Once available in the mammary alveolar cells, fatty acids are either bound to a fatty acid-binding protein or activated by combination with coenzyme A (CoA) and then bound to an acyl-CoA-binding protein. Activated fatty acids are joined with glycerol-3-phosphate by transacylases located in the endoplasmic reticulum to form triglycerides, which enter the cytoplasm as protein-coated structures called cytoplasmic lipid droplets.

These structures are translocated to the apical membrane, where they are enveloped by a novel budding process that leads to their release as membrane-bound lipid droplets known as milk fat globules. The fatty acid composition of milk triglycerides reflects differences in maternal diet. Medium-chain (C8–14) fatty acids are synthesized only in the mammary gland using glucose (or acetate in ruminants) as a substrate, whereas long-chain fatty acids are derived from the plasma. Nigerian women who have high-carbohydrate, low-fat diets have significantly more medium-chain fatty acids in their milk than Western women who consume a high-fat diet (Table 2).

Transcytosis Pathway (III)

Transport of proteins and other macromolecules by transcytotic pathways involves endocytic uptake of substances at the basal membrane, formation and maturation of endosomes, and sorting to lysosomes for degradation or to the apical recycling compartment for exocytosis at the apical membrane. The best-studied molecule in this regard is IgA. IgA is synthesized by plasma cells in the interstitial spaces of the mammary gland or elsewhere in the body and binds to receptors on the basal surface of the mammary alveolar cell; the entire IgA–receptor complex is endocytosed and transferred to the apical membrane, where the extracellular portion of the receptor is cleaved and secreted together with the IgA. It is thought that many other proteins, hormones, and growth factors that find their way into milk from the plasma are secreted by a similar mechanism.

Transmembrane Pathway (IV)

Transport processes for sodium, potassium, and chloride exist on the basal and apical plasma membranes of alveolar epithelial cells. Uptake mechanisms for calcium, phosphate, and iodide, however, are thought to be limited to the basal membrane. The mammary epithelial cells possess a GLUT1 glucose transporter and a sodium-dependent glucose transporter. The GLUT1 transporter is thought to mediate glucose transport at the basal and Golgi membranes, but it does not contribute to glucose transport at the apical membrane. Both sodium-dependent and sodium-independent amino acid

Table 2 Major fatty acids of human and bovine milk (wt%)

Fatty acid	Human milk		Bovine milk
	Western diet	Nigerian diet	
Saturated fatty acids			
Medium and intermediate chain (formed in the mammary gland)			
8:0, octanoic acid	0.46		1.3
10:0, decanoic acid	1.03	0.54	2.7
12:0, lauric acid	4.40	8.34	3.0
14:0, myristic acid	6.27	9.57	10.6
Long chain			
16:0, palmitic acid	22.0	23.35	28.2
18:0, stearic acid	8.06	10.15	12.6
Monounsaturated fatty acids			
16:1 n-7 (cis), palmitoleic acid	3.29	0.91	1.6
18:1 n-9 (cis), oleic acid	31.3	18.52	21.4
18:1 n-9 (trans), oleic acid	2.67	0.86	1.7
Polyunsaturated fatty acids (PUFA) (essential fatty acids)			
18:2 n-6, linoleic acid	10.76	11.06	2.9
18:3 n-3, linolenic acid	0.81	1.41	0.3
Long-chain PUFA (n-6)			
18:3 n-6, γ -linolenic acid	0.16	0.12	2.9
20:2 n-6,	0.34	0.26	0.03
20:3 n-6, dihomo- γ -linolenic acid	0.26	0.49	0.1
20:4 n-6, arachadonic acid	0.36	0.82	0.2
Long-chain PUFA (n-3)			
20:5 n-3, eicosapentenoic acid	0.04	0.48	0.08
22:5 n-3	0.17	0.39	
22:6 n-3, docashexenoic acid	0.22	0.93	0.09

transport mechanisms analogous to those found in other organs are located in the basolateral component of the mammary epithelium. It is unclear whether apical membranes have similar transport mechanisms for amino acids, and it is unknown how amino acids enter milk.

Paracellular Transport Pathway (V)

Pathway V (Figure 2) involves the passage of substances between epithelial cells rather than through them, and for this reason it is designated the paracellular pathway. During full lactation the passage of even low-molecular-weight substances between alveolar cells is impeded by the gasket-like tight junction structures that join the epithelial cells tightly, one to another. During pregnancy, with mastitis and after involution, the tight junctions become leaky and allow components of the interstitial space, such as sodium and potassium, to pass unimpeded into the milk, which is sometimes useful in diagnosing breast-feeding problems.

Regulation of Milk Synthesis, Secretion, and Ejection

Milk volume production is a primary indicator of lactational function; the most precise methods for measuring the volume of milk produced involve weighing infants before and after each feed for 24 h or longer or using an isotope dilution technique with stable isotopes. Clinically, the amount of milk that can be expressed with a breast pump or the change in infant weight after a single feed can be used as a rough index. The volume of milk secreted by women exclusively breast-feeding a single infant at 6 months postpartum is remarkably constant at approximately 800 ml day^{-1} in populations throughout the world. Mothers of twins, and occasionally even triplets, are able to produce volumes of milk sufficient for complete nutrition of their multiple infants, and studies of wet nurses indicate that at least some women are capable of producing up to 3.5 l of milk per day. However, if infants are supplemented with foods other than breast milk, milk secretion is proportionately reduced. This point is illustrated in Figure 3, which shows that milk volumes gradually decline during weaning and increase as the feeding frequency increases. These observations illustrate the important principle that the volume of milk secretion in lactating women is regulated by infant demand. If milk cannot be removed from the breast, local mechanisms cause an inhibition of milk secretion and downregulation of milk synthetic machinery. With partial removal of milk on a consistent basis, these local factors adjust milk secretion to a new steady-state level. If milk removal ceases for extended periods, involution sets in and the gland loses its competency to secrete milk.

Hormonal Control of Milk Synthesis and Secretion

In most species, the presence of high levels of plasma prolactin appears to be essential for lactation. In rats, the ergot alkaloid bromocriptine (an inhibitor of prolactin release from the pituitary) inhibits lactation, and in women it inhibits the onset of lactation when given in appropriate doses. How prolactin

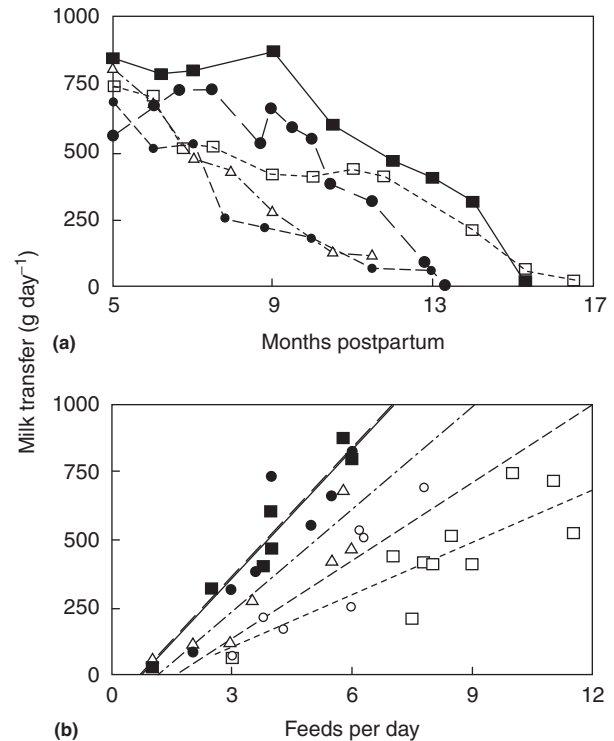


Figure 3 Changes in milk volume during weaning and in response to increased feeding frequency. (a) Milk volume transfer as a function of time postpartum. (b) Relation between feeding frequency (feeds per day) and the milk volume. Data from five breast-feeding dyads; each symbol represents an individual dyad. Reproduced from Neville MC, Allen JC, Archer PC, *et al.* (1991) Studies in human lactation: milk volume and nutrient composition during weaning and lactogenesis. *The American Journal of Clinical Nutrition* 54: 81–92.

influences lactation is not known in any detail. However, it appears to promote both the survival of mammary epithelial cells and the synthesis of macromolecular milk components. In addition, prolactin is an osmoregulator in some species of fish, birds, and amphibians and may function to maintain solute transport in the mammary gland. Maintaining high calcium levels in milk is also dependent on parathyroid hormone-related protein-dependent mobilization of calcium from maternal stores.

Local Control of Synthesis and Secretion

The neurotransmitter, serotonin, functions as an intrinsic homeostatic regulator of lactation, reducing milk production when production rates exceed removal rates, such as during weaning. Serotonin has been shown to be synthesized by mammary epithelial cells in mice and cattle and is found in milk. Elevated levels of serotonin have been demonstrated to directly reduce milk production and inhibit the expression of genes encoding milk protein components. In humans, elevated serotonin levels associated with the use of serotonin reuptake inhibitor-based antidepressants have been shown to delay secretory activation. Understanding this regulatory system may be very important in helping women to increase or

maintain their milk supply, particularly in the postpartum period.

Regulation of Milk Ejection

When the infant is suckled, afferent impulses from sensory stimulation of nerve terminals in the areolus travel to the central nervous system, where they promote the release of oxytocin from the posterior pituitary. This neuroendocrine reflex can be conditioned, and in women, oxytocin release is often associated with stimuli such as the sight or the sound, or even the thought, of the infant. The oxytocin is carried through the bloodstream to the mammary gland, where it interacts with specific receptors on myoepithelial cells, initiating their contraction and expelling milk from the alveoli into the ducts and subareolar sinuses. The passage of milk through the ducts is facilitated by longitudinally arranged myoepithelial cell processes whose contraction shortens and widens the ducts, allowing free flow of milk to the nipple. Milk is removed from the nipple not so much by suction as by the stripping motion of the tongue against the hard palate. This motion carries milk through the teat into the baby's mouth. The letdown response is decreased by psychological stress or pain, which interferes with oxytocin release. Oxytocin also appears to be involved in regulating maternal behavior in laboratory animals and may play a similar role in humans.

Initiation of Lactation

Pregnancy transforms the mammary gland from a simple ductal tree into a highly efficient exocrine organ with expansive lobuloalveolar structures. This transformation is hormonally regulated and involves changes in the cellular composition of the mammary gland and alterations in the structural, cellular, and biochemical properties of alveolar cells that are critical to the development of efficient solute transport and secretory functions. Alveolar epithelial cells begin to differentiate into secretory cells at midpregnancy in most species. The differentiation process occurs heterogeneously and has been divided into initiation and activation phases based on differences in the composition of mammary secretions, gene expression, and the structural and functional properties of alveolar cells. Alveolar cells become capable of limited secretion of some milk components during the initiation phase, which in humans is detected by measurement of increased concentrations of lactose and α -lactalbumin in the plasma. Copious milk secretion, however, is induced during the secretory activation phase (sometimes called lactogenesis II) that occurs in response to the decrease in serum progesterone levels. In rodents and ruminants, this decrease is closely associated with parturition; in humans, it occurs after parturition.

Changes in Milk Composition during Secretory Activation

Secretory activation is reflected in dramatic modifications of the solute composition of milk and increased secretory volume, which in turn reflect the maturation of secretory mechanisms and transport pathways during this period. In women,

there are three temporally distinct changes in milk composition at the onset of lactation. The earliest is a decrease in sodium and chloride concentrations and an increase in the lactose concentration of milk (Figure 4). These modifications occur immediately after delivery and are largely complete by 72 h postpartum. They precede increases in milk volume by at least 24 h and can be explained by closure of the tight junctions that block the paracellular pathway. Blocking this pathway prevents lactose, generated by the epithelial cells, from passing from the lumen of the alveolus to the plasma, and it prevents sodium and chloride from directly entering the lumen from the interstitial space. These changes result in the reduction of sodium and chloride and an elevation of lactose concentrations in the mammary secretion. The increased lactose concentration is reflective of decreased water entering the lumina as monovalent ion secretion decreases rather than increasing the lactose secretion rate. Secondly, the rates of secretion of sIgA and lactoferrin into milk of women are elevated soon after delivery. The concentrations of these two important protective proteins remain high, comprising as much as 10% of milk, for the first 48 h after birth. The concentration of each protein diminishes rapidly after day 2, both from dilution as milk volume secretion increases and from actual reduction in their rates of secretion, particularly of immunoglobulins.

Although both these proteins are found at high concentrations in colostrum, they are likely to be secreted by different mechanisms; lactoferrin, an endogenous protein of alveolar cells, is secreted by the exocytotic pathway (pathway I), whereas sIgA, a plasma-derived protein, is secreted by receptor-mediated transcytosis (pathway III). In addition, the peak secretion rate of lactoferrin occurs at the same time as that of

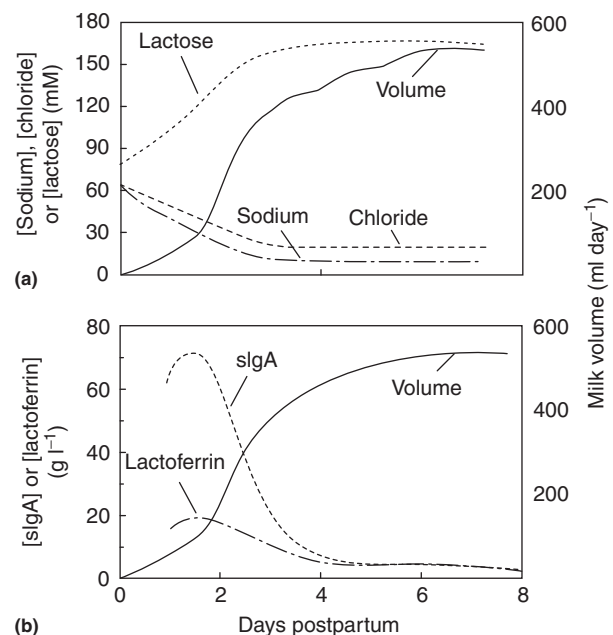


Figure 4 Changes in milk composition and volume in women during secretory activation and early lactation. Reproduced from McManaman JL and Neville MC (2003) Mammary physiology and milk secretion. *Advanced Drug Delivery Reviews* 55: 630–641.

lactose and the major milk proteins, whereas sIgA secretion peaks 1 day earlier, indicating the possibility that the exocytotic and transcytosis pathways are regulated differently during early lactation. The third phase occurs approximately 36 h postpartum and is associated with massive and concerted increases in milk volume and the rates of synthesis and secretion of almost all the components of mature milk, including, but not limited to, lactose, protein (mainly casein), lipid, calcium, sodium, magnesium, potassium, citrate, glucose, and free phosphate. Considering that the secretion of these substances involves the actions of several distinct transport pathways and biosynthetic processes, such tightly synchronized increases imply the presence of a common activation switch for coordinating their activities.

Hormonal Control of Secretory Activation

The decrease in progesterone around parturition is generally agreed to be required for the onset of milk secretion. In humans, it is known that removal of the placenta, the source of progesterone, is necessary for the initiation of milk secretion. In swine, timing of the increase in milk lactose correlates closely with timing of the decrease in plasma progesterone at parturition. Exogenous progesterone prevents lactose and lipid synthesis in mammary glands of pregnant rats and sheep after the removal of their ovaries, the source of progesterone in these species. Progesterone also suppresses β -casein expression in the rat mammary gland during pregnancy and the decrease in progesterone levels is linked to increased β -casein synthesis at parturition. Receptors for progesterone are not detected in lactating mammary tissues, which explains why progesterone does not inhibit established lactation. It is likely that the decline in progesterone is insufficient to activate secretion and that the actions of other hormones, including prolactin and glucocorticoids, are necessary to complete this process. In all *in vitro* mammary systems, insulin and corticoids, in addition to prolactin, are necessary to maintain the synthesis of milk components. Furthermore, cortisol replacement is required for maintenance of milk production in adrenalectomized animals. An early notion that a surge of glucocorticoids is the initiator of lactation is likely incorrect because the increase in cortisol seen in unanesthetized women associated with the stress of labor is complete by the time milk volume begins to increase to any extent. Because secretory activation proceeds at parturition in severely diabetic rats, a role for insulin in lactogenesis as opposed to metabolic adjustments during

lactation seems improbable. In summary, the most reasonable interpretation of the data from both animal and human studies is that the hormonal trigger for lactogenesis is a decrease in progesterone in the presence of maintained prolactin. Because postpartum prolactin levels are similar in both breast-feeding and non-breast-feeding women, the basic process appears to be initiated whether or not breast-feeding occurs. The caveat, of course, is that the mammary epithelium must be sufficiently prepared by the hormones of pregnancy to respond with milk synthesis.

Delays in Secretory Activation

A delay in the onset of milk secretion is a problem for the initiation of breast-feeding in a significant number of parturient women. A number of pathological conditions may delay secretory activation in women, including cesarean section, diabetes, obesity, and stress during parturition. The role of cesarean section is controversial, but if there is one, it is likely to have only a modest effect. However, poorly controlled diabetes, stress from delivery, or obesity are associated with significant decreases in early milk production. Because each of these conditions is related to higher blood glucose, hyperglycemia may be an underlying factor in the delay in lactation. However, once it is established, women with diabetes do not have a problem in maintaining lactation. Thus, compensatory factors may override initiation defects to ensure infant nutrition in these disorders.

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LACTOSE INTOLERANCE

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Overview

Lactose maldigestion and intolerance results from an inability to digest varying amounts of the milk sugar, lactose. This is a result of an inadequate amount of the genetically regulated milk sugar enzyme commonly referred to as lactase and more precisely identified as lactase-phlorizin hydrolase. The most common reason for lactose maldigestion is a decline of lactase activity with increasing age. Lactose maldigestion may also occur secondary to intestinal tract infection and diarrhea. A rare form of alactasia, an absence of the milk sugar enzyme, can occur at birth. The symptoms associated with lactose maldigestion are a result of the incomplete hydrolysis, or splitting, of the disaccharide lactose into its absorbable monosaccharide components, glucose and galactose. The most common form of lactose maldigestion observed in the majority of the world's adult population, is due to genetically determined low lactase levels. Lactose maldigestion may result in abdominal bloating or pain, flatulence, loose stools, and diarrhea, singly or in combination. The symptoms associated with lactose maldigestion result in lactose intolerance.

Low lactase levels due to genetic nonpersistence is reported in approximately 70% of the world's adult population. The prevalence is lowest in individuals of northern European descent (15%) and highest in many Asian populations with reports approaching 100%. The prevalence of lactase nonpersistence in individuals of African descent is approximately 70% to 80%. Similar levels are reported for Latinos and those of Eastern European and South American ancestry. Not all individuals with a reduced level of the enzyme lactase experience symptoms with the ingestion of dietary lactose. The presence or absence of symptoms varies with the amount and type of food consumed, intestinal transit time and level of residual intestinal lactase. Individuals with low lactase levels may tolerate a moderate intake of lactose.

Uniform agreement regarding the application and definition of terms identifying lactose nonpersistence has been lacking and has led to confusion and controversy. The 2010 National Institutes of Health Consensus conference on lactose intolerance has underscored the use of an agreed terminology. Hypolactasia is defined as a relative diminution, or very low levels of lactase enzyme activity. Lactose malabsorption, identifies a lactose test result following a lactose challenge resulting in an abnormal rise in breath hydrogen resulting from undigested lactose reaching the colon. The undigested lactose may result in one or more of the following symptoms: bloating, abdominal cramps, flatulence, and diarrhea. Lactose intolerance, is the term used to identify individuals with any

of the above clinical symptoms resulting from unhydrolyzed lactose. It is a reliable indicator of unhydrolyzed lactose when properly used and interpreted. Milk intolerance, self reported, may be due to lactose maldigestion but may result entirely or partly for other reasons.

The most reliable method for diagnosing lactase deficiency is determining lactase activity in the small bowel. The test is invasive and expensive. Lactose maldigestion can generally be identified by a breath hydrogen test, the most commonly used test to measure the level of undigested lactose reaching the colon. Bacterial fermentation of the undigested lactose is responsible for the volume of breath hydrogen production. A lactose tolerance test measuring blood sugar rise is also used. Genetic testing is available. Lactose elimination trials represent a noninvasive, no cost alternative, albeit often difficult to carry out and interpret. Small bowel bacterial overgrowth can confound results. Individuals experiencing discomfort with lactose ingestion can elect to consume commercially hydrolyzed milk that is readily available, milk substitutes or alternative food sources equally rich in calcium.

Historical and Geographic Perspective

The first herd animals, sheep, are reported to have been domesticated approximately 10 000 BC. Herd animals were primarily used for meat and perhaps certain other purposes. The historical record suggests that herd animals during this period were not milked. Evidence that humans milked domesticated animals dates to approximately 4000 to 3000 BC, in northern Africa and southwest Asia. Following that time, dairying spread across Eurasia and into sub-Saharan Africa. Dairying was not, however, adopted by all groups in Asia and Africa who had suitable herd animals. Even as late as AD 1500, the beginning of the great European overseas expansion, there were sizable areas occupied by nonmilking groups. In Africa the zone of nonmilking centered on the Congo Basin but extended beyond to cover approximately one-third of the continent. In Asia the zone of nonmilking covered the bulk of the eastern and southeastern portions of the continent, including Thailand, Vietnam, China, and Korea as well as the islands to the east. Dairying remained unknown in the Pacific region and in the Americas in pre-European times. Animal milk was not part of their diet. At that time the nonmilking people of Asia, Africa, and the Americas consumed mother's milk as infants, but normally ingested no milk after weaning.

It was striking that adults of all groups whose origins lay in the traditional zone of nonmilking were predominantly

maldigesters, usually from 75% to 100% of the individuals tested. Also striking was the fact that the people with low prevalences of lactose maldigestion (northwest Europeans and certain East African pastoral groups) came from a long tradition of consuming milk, much of it in lactose-rich forms. This suggests the geographic or culture–historical hypothesis. The hypothesis is based on the assumption that in the hunting and gathering stage, human groups everywhere were like most other land mammals in their patterns of lactase activity. That is, in the normal individual lactase activity would drop at weaning to low levels, which prevailed throughout life. With the beginning of dairying, however, significant changes occurred in the diets of many human groups. As a result, there may have been a selective advantage for those aberrant individuals who experienced high levels of intestinal lactase throughout life. That advantage would have occurred only in certain situations: Where milk was an especially critical part of the diet, where the group was under dietary stress, and where people did not process all their milk into low lactose products such as aged cheese. Under those conditions, most likely to occur among pastoral groups, such aberrant individuals would drink more milk, would benefit more nutritionally as a result, and would enjoy increased prospects of survival, wellbeing, and of bearing progeny and supporting them. In classic evolutionary terms, the condition of high intestinal lactase activity throughout life, or lactase persistence, would come to be typical of such a group.

Lactase Nonpersistence

In its pure form, lactose can not be transported across the mucosa of the small intestine. To be absorbed, it must be hydrolyzed by lactase to yield glucose and galactose. These two simple sugars are rapidly and completely absorbed in the normal small intestine. The rate of lactase synthesis is high from birth until the age of 3–5 years. However, between ages 5 and 14 years, many people undergo a genetically programmed reduction in lactase synthesis resulting in only 5–10% of the lactase levels in infancy. This reduction, known as lactase nonpersistence or primary lactase deficiency, is not related to the continued intake of milk or lactose. As noted, less than one-third of the world's adult population is genetically predisposed to maintain a high degree of lactase activity or lactase persistence throughout adulthood.

Lactase persistence in the human population is inherited as a dominant genetic trait. It has been observed that low lactase level is “ancient and globally distributed” predating the appearance of a persistent lactase variant that was naturally selected in dairying regions. Hollox *et al.* report, “the continued adult production of lactase results from the persistent expression of the protein lactase-phlorizin hydrolase which is encoded by the lactase gene (LCT) on chromosome 2”. Swallow notes, “the distribution of different lactase phenotypes in human populations is highly variable and is controlled by a polymorphic element cis-acting to the lactase gene. A putative causal nucleotide change has been identified and occurs on the background of a very extended haplotype that is frequent in northern Europeans, where lactase persistence is frequent”.

Lactase persistence is a likely result of the advent of dairying and the result of natural selection. Samples of ancient human mitochondrial deoxyribonucleic acid (DNA) sequences from ancient skeletons in the early Neolithic Europeans support the hypothesis. Investigators did not observe the allele most often identified with lactase persistence in Europeans suggesting lactase persistence was uncommon in early European farmers thereby reinforcing the cultural–historical hypothesis.

Lactose Digestion and Gastrointestinal Function

Lactose is hydrolyzed at the intestinal jejunal brush border by the enzyme lactase into its absorbable monosaccharides glucose and galactose. Lactase activity is robust during infancy and as is the case in humans along with most mammals declines after weaning. Accordingly, the general pattern of lactase nonpersistence is a continuous decline in genetically programmed populations. A shifting pattern of lactose digestion and gastrointestinal function are the result of lactase nonpersistence. The pattern can be described and monitored during three distinct clinical phases.

First, there is a decreasing ability to digest the large lactose load consumed during the screening test. It is important to recognize that this is not an all or nothing phenomenon but rather a slowly progressive decline in available lactase activity, and that this decline, as earlier noted, can be influenced by transit time, the vehicle in which the lactose is consumed, and the intake of additional foods along with lactose.

Next, with the continued decline of lactase activity, a point is reached when available lactase activity is no longer sufficient to hydrolyze more modest levels of lactose. Therefore, the consumption of a glass of milk or another product containing the equivalent level of lactose will result in incomplete hydrolysis of the lactose consumed. The individuals so tested frequently do not recognize signs or symptoms associated with the incomplete digestion of lactose.

Finally, with the continued decline of lactase activity with increasing age, individuals become symptomatic as a result of the undigested lactose. The decline in available lactase activity reaches a recognizable clinical threshold with increasing age.

Initially, many reports had treated the population studied as a single unit and had paid incomplete attention to age-specific considerations. Distinctions between secondary lactose malabsorption due to short-term intestinal injury, and primary lactose malabsorption that has a genetic basis, were not always made. This introduced additional confounding variables. Differences in an individual's capacity to hydrolyze and tolerate a lactose challenge dose compared to his or her ability to utilize lesser amounts of lactose found in usually consumed amounts of milk created additional areas of confusion.

When attention is paid to the many factors associated with lactose digestion from infancy to old age, it is possible to place many of the seeming contradictions into perspective. What may have appeared to be incongruities in reported data appear to merge into a relatively predictable pattern of lactose digestion.

Lactose maldigestion and intolerance are influenced by age, infection, size of the lactose bolus, gastric emptying time, intestinal transit time, individual sensitivities, eating habits,

genetics, environment, food ideologies, and cultural patterns. Further, symptoms of lactose malabsorption may also be the result of bacterial fermentation of undigested carbohydrate in the colon. The type and extent of the colonic bacterial profile and the absorption of hydrogen and the volatile fatty acids will influence individual reports of symptoms associated with lactose intolerance. Clearly, lactose malabsorption is not a homogeneous event. Neither is it an all or nothing phenomenon having its origins in a single etiology. Clinical expressions of lactose malabsorption, lactose intolerance, and milk rejection find their origins in one or more of the causes outlined above (Figure 1).

Prevalence

Children

A review of reported data on diverse populations support the conclusion that in later childhood and adolescence an important transition in lactose digestion occurs. Below 3 years of age there is lactase persistence. Between 3 and 11 years of age the beginning of a genetically controlled lactase non-persistence is recognized. Older children and young adults are increasingly unable to digest even modest amounts of lactose. This results in increased symptom production, recognition of discomfort, and avoidance of lactose-containing products that provoke symptoms (Table 1).

A progressive decrease in lactase is noted from approximately 1–5 years of age through adolescence. Reported rates in United States African-American children ranged from 27% lactose maldigestion following lactose testing using a lactose load equivalent to two 8-ounce glasses of milk at 1–2 years to 74% in 11–12-year-old children. The progressive decrease in

the ability to hydrolyze a lactose challenge was observed in children of both high and low socioeconomic status. Studies in white children 1–12 years of age identified only 17% of children maldigesting a lactose challenge. Signs and symptom production associated with a reduction in lactose digestion in a child population is difficult to measure due to the nature of the symptoms being reported and the signs observed and the subjective nature of the reports. This is reinforced by a report of 21 African-American girls of 11–15 years of age indicating 82% had evidence of lactose maldigestion with reports of gastrointestinal symptoms being negligible and breath hydrogen excretion, while remaining high, varied between two time periods. Consistent with the above data, milk consumption studies, both observed and reported, suggest a progressive decline in milk intake with increasing age in the African-American population of children and parallel reports

Table 1 Genetically determined lactase levels in healthy individuals by age and lactase persistence

Age	Lactase level	
	Low lactase nonpersistent individual	High lactase persistent individual
Fetal period	Low	Low
Birth	High	High
Weaning	Decline	High
3–12-year-old child	Reduced	High
Adolescent	Low	High
Adult	Lower	Average
Elderly	Lowest	Decline

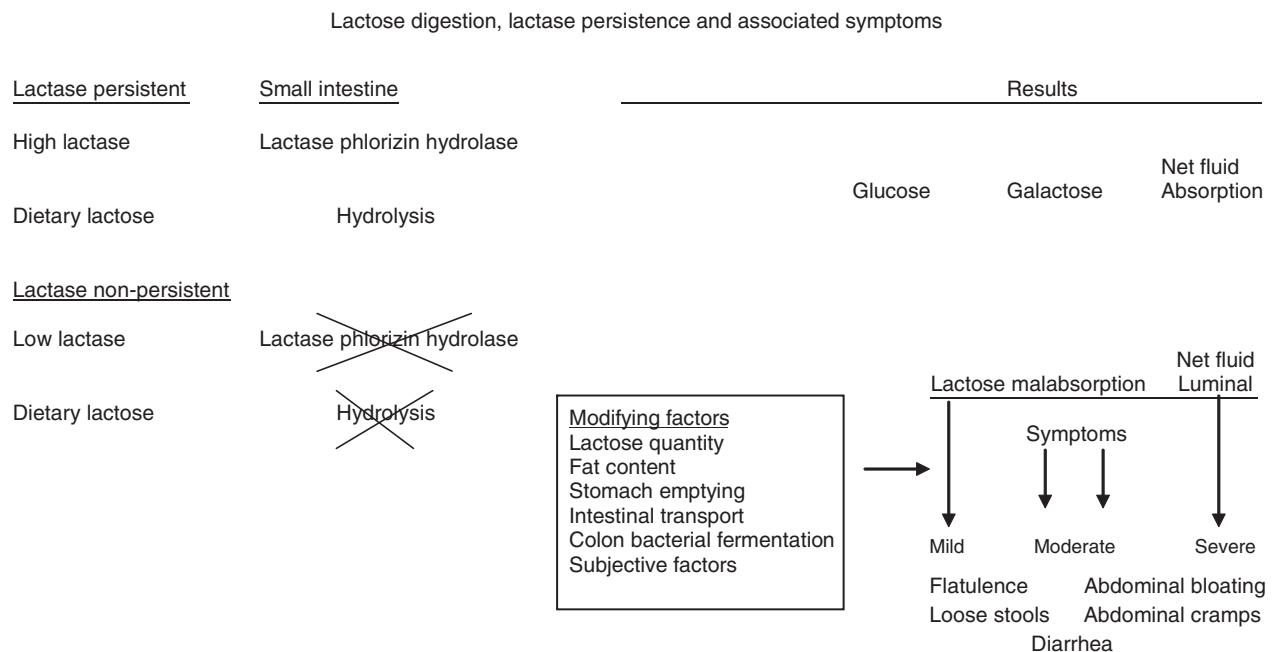


Figure 1 Lactose digestion, lactase persistence, and associated symptoms.

in children from other populations with a high prevalence of lactose maldigestion (Table 2).

Phenotypic lactase nonpersistence needs to be distinguished from secondary lactose maldigestion and intolerance as a result of a variety of other conditions. Secondary lactose maldigestion can be observed with diarrheal disease and infection, celiac disease, allergic enteropathy, Crohns' disease, chemotherapy, radiation, and small bowel resection.

Adults

The progressive increase in prevalence of lactose maldigestion increases with age reaching reported adult levels of approximately 70% of the world's adult population. The exceptions are populations of northern and central Europeans and some Middle Eastern populations as well as groups of primarily European descent in Australia, New Zealand, and North America. Thus, minority populations in North America and Europe, as well as adult populations in most developing countries are lactose maldigesters (Table 3).

Reported milk drinking patterns of individuals classified as maldigesters vary considerably in adults. Data range from 50% reporting symptoms with one 8-ounce glass of milk, to 75% reporting symptoms with two 8-ounce glasses of milk and 30% reporting not drinking any milk. Nevertheless, caution must be exercised in interpreting reported symptoms and making the diagnosis of lactose intolerance. There can be considerable crossover between individuals who self-identify

as intolerant to lactose and are not diagnosed as lactose maldigesters *versus* those in whom the diagnosis was carefully established. More attention to identifying and categorizing symptoms more precisely may help. A recent Finnish study notes flatulence as the most severe symptom in maldigesters whereas abdominal bloating is most frequently reported by individuals self-identifying as lactose maldigesters. Moreover, microbiota may play a role in the presence and intensity of lactose-related symptoms. Data suggest that increased levels of colonic bacteria, as well as their diversity, may play a role, as a result of increased fermentative capacity in reducing the symptoms associated with lactose intolerance.

Pregnant Women

The role of lactose digestion in pregnant women is of special interest. Despite the nutritional value of milk during pregnancy, the lactase levels in some individuals in a number of racial and ethnic groups may be insufficient to hydrolyze commonly consumed amounts of lactose resulting in lactose maldigestion and possibly milk intolerance. The Institute of Medicine report notes, that "lactose intolerance among pregnant African-American women may result in their subsequent avoidance of milk". Other populations may also experience lactose maldigestion and intolerance to milk during pregnancy.

Lactose maldigestion, in pregnant women in our studies as measured by breath hydrogen response to 240 ml of low fat

Table 2 Patterns of lactose digestion by lactase status

<i>Lactase status</i>	<i>Test results</i>	<i>Symptoms</i>	<i>Lactose intolerance</i>	<i>Milk consumption</i>
Adequate	Normal (—)	0	0	Average (+)
Marginal lactase	+	0/+	0/+	+
Deficiency	—	—	—	—
Moderate lactase	+	0/+	0/+	+
Deficiency	—	—	—	—
Severe lactase	+++	++	++	—
Deficiency	—	—	—	—

Sidney, Phillips, Paige & Bayless.

Table 3 Prevalence of lactose maldigestion in selected populations

<i>Population</i>	<i>Country</i>	<i>% Lactose maldigestion</i>	<i>Population</i>	<i>Country</i>	<i>% Lactose maldigestion</i>
African-American 18–54 years	USA	75	General 21–65 years	Finland	15
Asian 23–39 years	USA	100	General 20.3 years	Germany	70
Native American 18–54 years	USA	81	General 16–54 years	Chile	80
African-American 13–19 years	USA	69	Non Caucasian	Peru	94
Mexican 18–94 years	USA	53	General 38–49 years	Brazil	80
Vietnamese 22–63 years	USA	100	Arab adult	Israel	81
Sicilian 25 years average	Italy	71	General male 14–34 years	Egypt	73
Northern 28.7 years average	Italy	52	General 15–78 years	Greece	45
Central 36 years average	Italy	18	Bantu 13–43 years	Uganda	100
Romai	Hungary	56	Yoruba 13–70 years	Nigeria	83
Austrian 22 years average	Austria	20	General adult	India	61
General 20.3 years average	Finland	17	General 17–83 years	Korea	75
Aboriginal	Australia	84	General 15–64 years	Japan	100

Table 4 Lactose maldigestion^a in pregnant and nonpregnant African-American women

<i>African-American women</i>	<i>% Lactose maldigestion</i>
Early pregnancy (13–16 weeks)	66
Late pregnancy (30–35 weeks)	69
Postpartum (8 weeks)	75
Nonpregnant	80

^aBreath hydrogen rise > 20ppm following the consumption of 240 ml of low fat (1%) milk containing 12 g of lactose following an overnight fast.

(1%) milk, reinforces the Institute of Medicine's concern with lactose digestion among pregnant African-American women. We report the prevalence of lactose maldigestion in early (13–16 weeks), late (30–35 weeks) and 8 weeks postpartum as 66%, 69%, and 75% respectively. The prevalence in non-pregnant control women was 80% (Table 4).

Accordingly, healthcare providers instructing African-American women on the optimal dietary pattern during pregnancy need to be mindful of a high rate of lactose maldigestion. Implications for fetal growth and development remain to be answered by further study. Furthermore, health providers need to be aware that the presence or absence of symptoms may be unevenly reported by pregnant African-American women; and symptoms do not represent a reliable guide to lactose digestion. Less than 25% of pregnant lactose maldigesting women reported any symptoms with 240 ml of low fat (1%) milk. Symptoms may be further reduced when milk is consumed with other foods. Unanswered is the level of digestion and absorption of a range of nutrients in the consumed milk. Health care providers should discuss with the pregnant woman, her ability to tolerate milk, and where and when appropriate, should educate her as to other food options. In this regard, Kingfisher and Millard report that "Euroamerican staff tended to give advice that was biologically appropriate for them but not for many of their patients, a process reflecting biocentrism".

Secondary Lactase Deficiency

Secondary lactase deficiency is distinct from genetically determined loss of lactase with age. Secondary lactase deficiency is frequently associated with diseases of the small intestine. Enteric viruses such as rotavirus and Norwalk agent can induce lactase deficiency by penetration of the enterocyte in the small intestine. Rotaviruses are a principal cause of diarrhea and lactose intolerance in infancy. Denudation of the brush border of the jejunal mucosa associated with diarrhea can lead to the loss of the other two disaccharides, maltase and sucrose. Continued diarrhea may also lead to severe complications such as monosaccharide intolerance. Giardiasis and *Ascaris lumbricoides* have also been implicated as contributing to lactose maldigestion. Severe protein malnutrition is frequently associated with lactose maldigestion. Other disease conditions that give rise to secondary lactose maldigestion are celiac disease, gluten-induced enteropathy, and tropical and non-tropical sprue. The mucosal brush border of the small intestine is severely damaged in each case.

Lactose Digestion and Diet

Calcium

Dietary calcium is an important element in skeletal development. Dairy products can account for up to three-quarters of dietary calcium in some populations. Milk is a rich source of calcium. Nevertheless, many minorities in the United States and population groups throughout the world drink decreasing amounts of milk after early childhood and little milk as adults. Given the high prevalence of lactose intolerance, alternatives to cow's milk should be identified for those who cannot tolerate lactose and desire a milk alternative. Lactose-intolerant individuals ultimately attribute their discomfort to lactose-containing foods and voluntarily reduce or eliminate their milk intake. Data from National Studies in the United States indicate African-American and Hispanic women have lower intakes of calcium compared with non Hispanic women. An Institute of Medicine Report concludes that the disparity in calcium intake "may be explained in part by the much higher prevalence of lactose intolerance among African-Americans and Hispanics, sometimes resulting in their subsequent avoidance of milk". In general, populations at risk for lactose intolerance report a lower calcium intake as a result of the decline in the intake of milk and milk products. One solution to this problem is to educate lactose-intolerant groups as to alternative calcium-containing foods, reinforce appropriate cultural patterns and dietary practices that include alternatives to milk and identify other culturally acceptable calcium-containing foods. Meeting the calcium requirement with an alternative diet is a challenge but nevertheless is required for many in the community. Although milk may serve as a primary source of calcium, appreciable quantities of calcium can be found in nondairy foods (Table 5).

Clearly it is more difficult to meet the published calcium recommendation with a diet low in whole cow's milk. A review of the tables of food composition reveals a variety of foods that contain acceptable levels of calcium per 100-g portion or other standard portions. Other lactose-modified dairy products including hard cheeses, yogurts, and lactose-modified milk are good calcium sources.

In addition, lactose digestive aids are available and are increasingly used. The digestive aids commercially available include lactase tablets, lactase preparations, lactose-free milk, and prehydrolyzed milk. Live culture yogurt is another alternative to milk. Lactose in yogurt is better digested than lactose in milk. Tolerance to yogurt is thought to be due to the microbial beta-galactosidase activity that digests the lactose.

Osteoporosis

The role of lactose maldigestion, calcium intake and osteoporosis has been studied. Osteoporosis and osteoporotic fractures are major public health problems. The role of lactose maldigestion and osteoporosis remains unsettled. For example, minority populations consuming small amounts of milk should be at greater risk for osteoporosis. Nevertheless, African-American and Hispanic populations in the United States appear to have a lower risk of developing osteoporosis. Caucasian and Asian women were found to have the highest

Table 5 Calcium content in milligrams per 100 g portion or as noted^a

Canned sardines (3 oz.)	372	Brewer's yeast (2 tbs)	66
Buckwheat pancakes	249	Lobster	65
Kale (raw)	225	Green beans	63
Mustard greens	220	Flounder	61
Muffins ^b	206	Bran flakes	61
Waffles ^b	192	Canned apricots (1 cup)	57
Figs (dry)	186	Gingerbread (1 piece)	57
Canned salmon (3 oz. with bones)	167	Plain rolls	55
Collard greens	162	Toaster pastry (1 piece) ^b	54
Oat breakfast cereal ^b	160	Prunes (dry)	54
Wheat pancakes	158	Orange	54
Almonds	152	Whole egg	54
Tofu (8 oz.)	143	Peanuts	54
Egg yolk	147	Artichokes	51
Corn bread ^b	139	Cod	50
Kale (frozen)	134	Brussels sprouts	50
Filberts	120	Clams (3 oz.)	47
Beet greens	118	Lima beans	47
Oysters (½ cup)	113	Puffed wheat ^b	46
Whole cow's milk (100 g)	113	Whole wheat bread (2 slices)	46
Swiss chard	105	Sweet potato	46
Rhubarb (cooked ½ cup)	105	Fruit cocktail (1 cup)	46
Canned shrimp (3 oz.)	98	Raisins (1/2 cup)	45
Okra	92	Apricots	44
Soy beans (1 cup)	90	Farina (1 cup)	44
Sunflower seeds	88	Fig bars (4 cookies)	44
Broccoli	88	Pecans	43
Sauerkraut (1 cup)	85	White bread (2 slices)	42
Potato salad (1 cup)	80	Pecans	43
Peanut butter	74	White bread (2 slices)	42
Spinach	73	Tangerine	40
Dates (dry)	72	Raspberries (raw)	40
		Apple sauce	21

^aOski and Paige modified from Krause and Mahan & Burton.^bEnriched, fortified, or restored to legal standard when one exists.

risk for osteoporosis, with fracture rates of 140.7/1 00 000 and 85.4/1 00 000, respectively. Hispanic and African-American females had lower age-adjusted rates, at 49.7/1 00 000 and 57.3/1 00 000, respectively. A study of gene-identified lactose intolerance in a Dutch Caucasian elderly population is associated with lower dietary Calcium intake and serum Calcium levels but not associated with bone mineral density or fractures. The paradox reinforces the complexity of the disease and the importance of biologic, genetic and as yet undetermined factors in the etiology of osteoporosis.

Nutrition policy

Apart from the nutritional implications outlined above, there are policy considerations that require attention. Clearly milk has important economic, nutritional, and emotional significance in Western culture, a culture strongly committed to the concept that milk is an ideal food. Yet, lactose digestion

should be an important consideration in developing a suitable policy regarding the use of milk and dairy products by the lactose malabsorber and by ethnic or racial groups, among whom high rates of malabsorption prevail. Accordingly, a balance must be struck between dietary guidance and the interests of a diverse population with a large number of lactose maldigesters. For many the continued use of a limited amount of milk may be appropriate and comfortable. For others dietary modification and lactose reduction or elimination may be warranted. The substitution of low-lactose products or alternative foods may be successful. Traditional diets among lactose-maldigesting populations, using little or no milk or dairy products should be respected.

Summary

In summary, the principles of genetics and evolution help to explain the emergence of continued lactase activity beyond weaning. Darwin referred to food as a major factor in selective pressures. Lactose digestion illustrates how a certain food, by indirectly favoring the survival of those able to digest that substance, can influence the evolutionary process.

Clinical and nutritional consequences of lactose digestion in adults must be examined in relation to digestion, intolerance, milk rejection, symptoms, and their recognition. Estimates of how frequently milk intolerance is a clinically significant problem in adults vary. The protocol of individual scientific studies can influence interpretation. A balance of factors tend to prevent or minimize symptoms when the stomach, small intestine, and colon can compensate for an increased solute load, but abdominal discomfort or diarrhea occur when these small intestinal and colonic physiologic mechanisms are loaded beyond their capacity. The role of the colonic flora in metabolizing unabsorbed sugar and the importance of colonic salvage of unabsorbed carbohydrate is an important variable in the symptom complex. Secondary lactase deficiency due to infectious gastroenteritis and malnutrition represents a distinct clinical syndrome and must be distinguished from lactose intolerance.

Dietary recommendations must take account of lactose maldigestion. Milk and dairy product consumption will vary among lactose-maldigesting and milk-intolerant individuals. Lactose-reduced or lactose-free products are available to lactose-maldigesting and -intolerant individuals who wish to drink milk and milk-based products. Nevertheless, dietary recommendations must be modified and respectful of those who do not drink milk. Accordingly, appropriate alternatives to milk and other lactose-containing foods must be identified and guidance provided in developing nutritionally equivalent diets.

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LEGUMES

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Glossary

Food legume Any vegetative or reproductive structures from legume plants that are utilized for human food.

Legume A plant belonging to the Fabaceae (Leguminosae) family. The name legume comes from the Latin word *legumen*, meaning seeds that are harvested from pods.

Introduction

Legumes have been an important component of the human diet for several millennia and are used throughout the world today. They are a diverse group of plants that belong to the Fabaceae family (sometimes also referred to as the Leguminosae) and are estimated to include approximately 20 000 species in 700 genera. However, only a handful of these species have been developed as crops that are in common culture. Some of the more extensively grown legumes are listed in [Table 1](#).

Legumes are consumed primarily as seed foods, but pods, leaves, and roots or tubers of various species are also eaten. The pod is an enveloping structure that protects the seeds as they develop and mature, and it is a characteristic feature of this group of plants. In fact, the name legume comes from the Latin word *legumen*, which means seeds that are harvested from pods. Other names used for legume seeds are pulse, which is derived from the Latin word *puls*, meaning pottage, or the phrase grain legume, used in reference to leguminous seeds. The more general phrase, food legume, is used to represent any vegetative or reproductive structures from legume plants that are utilized for human food.

An important nutritional aspect of legume foods is their high concentration of protein, which in most legume seeds is at least twice that of cereal seeds. Legumes can produce more protein because the plants are generally well nourished with nitrogen, even in soils with limited inorganic nitrogen. Legume roots have the ability to form symbiotic associations with particular microbial species, in a structure called the root nodule. This symbiosis allows the plant to readily acquire atmospheric nitrogen and use it for the synthesis of amino acids. These protein precursors are transported to the developing seeds and are deposited there for later use. Legume seeds also contain a broad mix of energy reserves (starch or oil), minerals, and various phytochemicals – all of which are stored in seeds to provide nourishment to the young developing seedling.

As omnivores, humans have been able to take advantage of the nutrient and phytochemical reserves in legume seeds for

dietary requirements and health benefits. This is especially important in the developing world, where malnutrition is always a concern, and legumes can provide an inexpensive source of dietary protein (relative to animal food products), among other nutrients. The protein in legume seeds, although somewhat lacking in sulfur amino acids and tryptophan, is still an important complement to energy-rich carbohydrate staples, such as rice, wheat, maize, and various root and tuber crops. However, when eating legumes, we also must deal with the various antinutrients and toxic compounds found in seeds. These seed components include various enzyme inhibitors, tannins, phenolics, alkaloids, and neurotoxins. Some of these can cause debilitating consequences in humans, although cooking and other processing techniques can be used to reduce or alleviate their negative effects.

Legume Types

Legumes are grown throughout the world, with some adapted to warmer tropical and subtropical climates and others preferring temperate to cooler climates. The 20 species listed in [Table 1](#) are some of the more commonly cultivated legumes and include those whose annual production reaches levels that allow for worldwide marketing. In developing countries, many locally adapted legume species are cultivated on a small scale or are harvested from wild sources. These less cultivated legumes are usually harvested as mature seeds, but immature pods, leaves, roots, or tubers can also be collected.

Most of the common legume species are grown agro-nomically and harvested as mature seeds. These can be cooked and consumed in their entirety, or they are cracked and used as split seeds with the hulls (seed coats) removed. Seeds of some species are milled to produce a flour product, or they can be processed to yield protein isolate (e.g., soybean and lupine), extracted oils (e.g., soybean and peanut), or starch (e.g., pea).

For those legumes also cultivated as vegetable crops, immature seeds or immature pods can be harvested. These are canned, frozen, or sold as fresh products. Immature pods are

Table 1 Commonly cultivated legume species

Scientific name	Common names
<i>Arachis hypogaea</i> L.	Peanut, groundnut
<i>Cajanus cajan</i> (L.) Millsp.	Pigeon pea, red gram, Congo pea
<i>Cicer arietinum</i> L.	Chickpea, garbanzo, Bengal gram
<i>Glycine max</i> (L.) Merr.	Soybean, soya, edamame
<i>Lablab purpureus</i> (L.) Sweet	Hyacinth bean, Indian bean, Egyptian bean
<i>Lathyrus sativus</i> L.	Grass pea, chickling pea
<i>Lens culinaris</i> Medik.	Lentil
<i>Lupinus albus</i> L.	White lupine
<i>Macrotyloma uniflorum</i> (Lam.) Verdc.	Horse gram, Madras gram
<i>Phaseolus lunatus</i> L.	Lima bean, butter bean
<i>Phaseolus vulgaris</i> L.	Common bean, black bean, kidney bean, pinto bean, snap bean, string bean, French bean
<i>Pisum sativum</i> L.	Pea, garden pea, English pea
<i>Psophocarpus tetragonolobus</i> (L.) DC.	Winged bean, Goa bean, four-angled bean
<i>Vicia faba</i> L.	Broad bean, fava bean
<i>Vigna aconitifolia</i> (Jacq.) Marechal	Moth bean, mat bean
<i>Vigna mungo</i> (L.) Hopper	Urd bean, black gram
<i>Vigna radiata</i> (L.) Wilczek	Mung bean, green gram, golden gram
<i>Vigna subterranea</i> (L.) Verdc.	Bambara groundnut
<i>Vigna umbellata</i> (Thumb.) Ohwi and Ohashi	Rice bean, Mambi bean
<i>Vigna unguiculata</i> (L.) Walp. ssp. <i>unguiculata</i>	Cowpea, black-eyed pea, southern pea

Source: Reproduced from Rubatzky VE and Yamaguchi M (1997) *World Vegetables: Principles, Production, and Nutritive Values*. New York: Chapman & Hall, with permission from Taylor and Francis.

nutritionally similar to leafy vegetables in that they contain various carotenoids and other phytochemicals; however, they also contain immature seeds that can provide a modest amount of protein. For some species, young tender leaves or whole shoots are also collected and used as vegetable greens that are eaten fresh or cooked. More detailed information is given on some of the common legume types in the following sections.

Bambara Groundnut

Bambara groundnut (*Vigna subterranea* (L.) Verdc.) is indigenous to west central Africa. Most of its current production is in Africa, but the plant is also cultivated in India, Southeast Asia, Australia, and Central and South America. The plant has an interesting growth habit in that after pollination, the developing pod and seeds are pushed into the ground, where they grow until full maturity. Plants are typically uprooted at harvest to collect the seeds and pods; because of this subterranean growth, they have acquired the common name groundnut. Mature seeds are boiled and consumed as a cooked seed, prepared as porridge, or milled into a flour to form cakes. Immature seeds are also harvested and cooked as a fresh vegetable.

Broad Bean

Broad bean (*Vicia faba* L.), also known as fava bean, is grown from tropical to temperate regions, with production occurring in North and South America, Europe, Africa, and China. This legume is grown for its enlarged, succulent, immature seeds that are removed from its thick, fleshy pod. Mature dry seed is also harvested. Although broad beans are widely consumed,

they do contain storage proteins (vicine and convicine) whose metabolites can lead to acute hemolytic anemia in individuals with a deficiency in glucose-6-dehydrogenase (found predominantly in people of Mediterranean or African descent). Additionally, broad beans contain high levels of L-DOPA, a phenolic compound that can be converted to dopamine. Because of their L-DOPA content, broad beans should be avoided by individuals using monoamine oxidase inhibitors (MAOI-type drugs). The use of these drugs, in combination with high intakes of dopamine (or dopamine precursors), can lead to dangerous increases in blood pressure.

Chickpea

Chickpea (*Cicer arietinum* L.) is grown worldwide and is best adapted to cool, dry climates. Thus, it is a winter crop in some regions of the world. Two seed types are recognized: the large-seeded kabuli type, characterized by its beige-colored seed coat and ram's head shape, and the desi type, with its smaller size and dark-colored irregularly shaped seeds. Kabuli varieties are preferred for consumption as whole seeds, whereas desi types are typically processed into flour. Immature green pods and young tender leaves are also cooked and eaten as vegetables, especially in India.

Common Bean

Common bean (*Phaseolus vulgaris* L.) is grown in temperate zones as well as in temperate regions within the subtropics. As a dry seed, it is an important crop in Africa and in Central and South America. Many bean types are cultivated that exhibit vast differences in seed coat coloration and pod characteristics. Mature seeds are harvested as dry beans (e.g., black bean, pinto bean, and kidney bean); immature pods are used as a

vegetable (e.g., snap bean and French bean). Pod types have been bred to have few fibers in the pod wall.

Cowpea

Cowpea (*Vigna unguiculata* (L.) Walp. ssp. *unguiculata*) is grown throughout the tropics and subtropics. It is an important crop in Africa, its probable center of origin, but is also grown in Brazil, India, Southeast Asia, and the United States. There are three major subspecies of *V. unguiculata*; in addition to ssp. *unguiculata*, there is an appreciable production of ssp. *cylindrica* (common names: catjang cowpea and Bombay cowpea) and ssp. *sesquipedalis* (common names: yardlong bean and asparagus bean), especially in Asia. All types are harvested as vegetables (shoots, leaves, and immature pods) or as dry, mature seeds.

Grass Pea

Grass pea (*Lathyrus sativus* L.) is a hardy, cool-weather adapted legume that is cultivated in India, Africa, the Middle East, and South America. It is harvested primarily as a dry, mature seed, although young leaves and immature pods are edible. Grass pea is quite tolerant of limited moisture and does well in nutrient-poor soils; thus, in times of drought it is one of the few legumes that produces a harvest, and it is widely consumed by low-income populations during times of famine. Unfortunately, excessive or prolonged consumption of grass pea can lead to lathyrism, a debilitating muscle paralysis that is caused by a neurotoxin in the seeds.

Hyacinth Bean

Hyacinth bean (*Lablab purpureus* (L.) Sweet) is grown in India and in many tropical regions of the world. Mature seeds are consumed as a cooked food or a sprouted seed. The immature pods and seeds are also harvested as vegetable foods. Although this plant is cultivated as an annual, it will persist as a perennial, and when cultivation is extended it will form large, starchy roots that can be eaten. Some varieties (mostly dark-seeded types) contain high levels of a cyanogenic glycoside in their seeds. When cyanogenic glycosides are hydrolyzed by plant enzymes during cooking, or possibly by intestinal enzymes after ingestion, cyanide can be released and lead to cyanide poisoning.

Lentil

Lentil (*Lens culinaris* Medik.) is another of the world's important pulse crops, especially for populations in developing countries. The plant is adapted to cool climates; Canada, India, and Turkey account for nearly 70% of its production. Lentils are harvested primarily as a dry, mature seed, but immature pods are also used as a vegetable in India.

Mung Bean

Mung bean (*Vigna radiata* (L.) Wilczek) is grown in tropical climates and is an important legume in India, China, and other

Asian countries. Dry seeds are harvested and consumed as split, whole, boiled, or roasted forms. Immature pods are eaten, and there has been interest in developing the tuberous root as a food because of its high protein content (nearly 15%).

Pea

Pea (*Pisum sativum* L.) is grown primarily in cooler regions of the world. Different varieties have been developed to produce mature, dry seeds; succulent, well-developed immature seeds; or succulent, immature edible pods. Dry seed varieties are sometimes referred to as field peas. The names garden pea and English pea are used for the varieties harvested as immature seeds, whereas the edible pod types are commonly known as snow pea or sugar snap pea. In some Asian cuisines, the shoots of pea plants are also used as vegetable greens.

Peanut

Peanut (*Arachis hypogaea* L.) is grown throughout the tropics, much of the subtropics, and even in some temperate zones. As with Bambara groundnut, its pods have a subterranean growth habit, and thus it also has acquired the common name, groundnut. Peanut is one of the few commonly grown legumes whose seeds contain high levels of oil. Most legume seeds have less than 5% oil, but for some peanut cultivars seed oil content is as high as 40–50%. Roasted seed and extracted oil is used and marketed worldwide; in some regions, young shoots and leaves of the plant are used as greens, and immature pods are consumed as a cooked vegetable. Although a nutritious legume, peanut has recently gained much attention and scientific interest due to the low, but nonetheless significant, incidence of individuals who are allergic to peanut proteins. For those extremely hypersensitive to this food, violent and life-threatening reactions can occur in response to exposure to as little as 0.1 mg of peanut. In fact, peanut is believed to be the most common cause of death due to foods.

Pigeon Pea

Pigeon pea (*Cajanus cajan* (L.) Millsp.) is broadly adapted to many climatic regions and soil types, and thus its production occurs over a huge area of crop land. It is an important food legume in India, other Asian countries, Africa, and South America. Mature grains are usually consumed as split, dehulled seeds. Immature seeds and pods are also consumed in large quantities.

Soybean

Soybean (*Glycine max* (L.) Merr.) is undoubtedly the most important food legume today, being a major source of protein and extracted oil. Soybean is believed to have originated in eastern Asia as a subtropical plant, but plant breeders have helped develop varieties adapted to several climatic zones. The crop is grown in many countries, but more than 70% of the world's production comes from the United States, Brazil, and China. Most soybeans are harvested as dry seed; a typical variety contains 20% seed oil and 35% protein (although some varieties can be as high as 45% protein). Both soy oil

and soy protein isolate are found as ingredients in many processed foods. In eastern Asia, the immature seed is also harvested extensively and used as a vegetable.

Winged Bean

Winged bean (*Psophocarpus tetragonolobus* (L.) DC.) is adapted to tropical conditions and is grown in Southeast Asia, Papua New Guinea, various Pacific Islands, and Africa. The tender pods are the most widely consumed part of the plant, especially throughout Asia, but the leaves, stems, flowers, seeds, and tuberous roots are all nutritionally valuable and are used as food. Winged bean is another of the legumes with elevated seed oil content; varieties typically average 15% oil, with protein levels of 30–37%. The tuberous roots are a good source of energy in the form of starch, and they contain 8–10% protein.

Grain Legume Nutritional Value

As noted previously, many parts of legume plants are consumed by humans. However, the seeds are the predominant food type across all species, and their nutritional value is discussed in the following sections.

Protein

Protein content in legume seeds is governed both by genotype and by environment. Seed protein levels can vary across varieties of a given species and even among seeds on an individual plant. In general, however, food legumes contain 20–30% protein by proximate analysis (Table 2). The exceptions to this are soybean and winged bean, which contain up to 37% and 45% protein, respectively.

Legume proteins are primarily of two types: storage proteins, which account for approximately 70% of total seed nitrogen, and enzymatic, regulatory, and structural proteins,

which are present for normal cellular activities, including the synthesis of storage proteins. Legume storage proteins are soluble in dilute salt solutions but insoluble in water and therefore fall into the classical globulin group of protein fractions. Legume protein types are further characterized by their sedimentation coefficients, which in most species approach 11S and 7S; these are commonly referred to as the legumins and vicilins, respectively. Most legumes contain both types of storage protein, but the proportion of the two types varies from species to species.

In terms of protein quality, as defined by an optimal proportion of amino acids required by humans, legume proteins are deficient in the sulfur-containing amino acids and tryptophan but are rich in lysine. Cereals, however, are relatively deficient in lysine; thus, the combination of legumes with cereals often can improve the overall protein quality of the mixed foods. The nutritive value (or biological value) of legume proteins has been investigated quite extensively and has been shown to be rather low in some legumes, with the amount of utilizable protein ranging from 32% to 78%. In other words, not all of the protein available in a given legume (see Table 2) is converted into new protein when consumed by humans. The reasons for this are the general deficiency of essential amino acids (sulfur-containing and tryptophan) and the presence of many inhibitors of protease activity that are found in legume seeds. These enzyme inhibitors are primarily proteinaceous in character, and many have an effect on the digestive enzymes trypsin or chymotrypsin. The inhibition of these enzymes leads to a reduction in protein digestibility and thus the gut's ability to absorb amino acids. Fortunately, because many of these inhibitors are proteinaceous, cooking, heating, fermenting, and, in some cases, germination can inactivate and significantly lower their inhibitory effect. However, not all of the inhibitors found in legume seeds are proteins (e.g., other inhibitors include tannins and polyphenols).

Lipids

Grain legumes generally contain higher concentrations of lipids than cereals. In legumes, lipids are stored in oil bodies in the cotyledons (the bulk of the seed), whereas most oils in cereals are limited to the outer bran layer. Most common legumes contain 1–7% lipid, based on proximate analysis. Exceptions to this range are soybean, peanut, and winged bean, which average 20%, 45%, and 15%, respectively. Legumes are good lipid sources for humans because they contain high amounts of essential fatty acids. Although composition varies across species, most legumes contain some quantity of oleic, linoleic, and linolenic acids. Phospholipids and glycolipids are also found in legume seeds.

Carbohydrates

Legume seeds contain starch, mono- and oligosaccharides, and other polysaccharides. Total carbohydrates range from 25% to 65% across the commonly grown legume species. Starch is the predominant carbohydrate in most cases, with exceptions in the oilseeds soybean and peanut. Legumes generally contain low amounts of monosaccharides (usually

Table 2 Protein contents of food legume seeds

Legume	Protein range (% dry weight)
Broad bean	22.9–38.5
Chickpea	14.9–29.6
Common bean	21.1–39.4
Cowpea	20.9–34.6
Grass pea	22.7–29.6
Horse gram	18.5–28.5
Lentil	20.4–30.5
Moth bean	21.0–31.3
Mung bean	20.8–33.1
Pea	21.2–32.9
Peanut	23.5–33.5
Pigeon pea	18.8–28.5
Rice bean	18.4–27.0
Soybean	33.2–45.2
Urd bean	21.2–31.3
Winged bean	29.8–37.4

Source: Reproduced from Salunkhe DK, Kadam SS, and Chavan JK (1985) *Postharvest Biotechnology of Food Legumes*. Boca Raton, FL: CRC Press, with permission from Taylor and Francis.

1% or less) and only slightly higher amounts of disaccharides, such as sucrose (1–3%). However, some soybean varieties have been reported to contain as much as 7% sucrose.

Various oligosaccharides have been characterized in legume seeds, including raffinose, stachyose, and verbascose, which are galactosides of sucrose. Because humans do not express the enzyme α -galactosidase, these compounds remain undigested in the small bowel and pass through to the large bowel, where they can be fermented by anaerobic microbes. This leads to flatulence, or gas production, which is experienced following the consumption of some legumes. The concentration of raffinose-type oligosaccharides varies among legume species and, not surprisingly, the capacity to induce flatulence also varies.

Fiber

Legume seeds are a source of dietary fiber, containing both crude fiber and neutral detergent fiber. Most legumes contain 3–5 g of fiber per 100 g of dry seed, with most of the fiber found in the seed coat fraction. Exceptions are grass pea and hyacinth bean, which contain 8 and 10 g of fiber, per 100 g of dry seed, respectively. Compositionally, legume seeds contain varying quantities of lignin, cellulose, hemicelluloses, pectins, gums, and mucilage.

Minerals

Legume seeds contain a broad mix of minerals, many of which are essential both for plants and for animals. In fact, almost all essential minerals for humans can be found stored in the seeds. In comparison to cereals, legumes tend to have higher concentrations of calcium and potassium, as well as the micronutrients iron, zinc, and copper. Most of the calcium is sequestered as calcium oxalate crystals, however, and this form of calcium has extremely low bioavailability. Also, the majority of phosphorus in legume seeds is stored as phytic acid, which can complex calcium, iron, and zinc and thereby diminish their bioavailability. Other compounds found in legume seeds, including tannins, phenols, organic acids, protein, and fiber, can also interact with minerals and lower their bioavailability. As a result humans absorb only approximately 2% of the iron from beans, although absorption from soybeans may be higher. Fortunately, certain processing procedures, such as fermenting or sprouting seeds, can reduce the levels of some of these mineral chelators. Owing to these various problems, there is a significant effort under way in the plant science community to increase the absolute mineral levels in various legume seeds, i.e., through biofortification, as well as to lower the levels of several major inhibitory compounds.

Vitamins

Most food legumes are good sources of thiamin, riboflavin, and niacin but are poor sources of ascorbic acid. This vitamin is present at only low levels in newly harvested dry seeds, and it disappears after long storage. In some species, varieties exist that produce green- or orange-colored cotyledons, and β -carotene, a pro-vitamin A carotenoid, can be found in some

cases. The amounts of this vitamin precursor, however, are generally quite low. Tocopherols (vitamin E) are also found in some legume seeds, and folate, which is present in all legumes, can be quite high in certain species (e.g., lentil). Because folate is important in the prevention of neural tube defects, legume consumption is recommended for women of childbearing age, especially in regions of the world where folate fortification is limited.

Health-Promoting Phytonutrients

There is much interest in the role of various phytonutrients to promote good health and to reduce the risk of various cancers. As with many plant foods, legume seeds contain a number of these types of compounds. Prominent in this group are the isoflavonoids, such as genistein and daidzein, which are found at high levels in soybeans. Epidemiological studies have suggested a positive association between the consumption of soy isoflavones and reduced risk of breast and prostate cancer in humans. These and other related isoflavones are found in seeds of most of the commonly grown legumes. In addition, various saponins, catechins, epicatechins, and anthocyanidins have been measured in various legume seeds, and these compounds have also been suggested to have health-promoting qualities. Plant biochemists and human nutritionists are actively working to manipulate the levels of these and other compounds in legumes. Components of soybeans and other legumes also lowered serum cholesterol and triglycerides in clinical trials, actions attributed to the soluble fiber and phytonutrients such as phytosterols. The 2010 Dietary Guidelines for Americans encourage an increased intake of peas and beans and state that because of their high nutrient content, legumes can be considered either a vegetable or a protein food.

See also: Bioavailability. Cereal Grains. Dietary Fiber: Physiological Effects and Health Outcomes. Phytochemicals: Health Effects. Protein: Quality and Sources. Vegetarian Diets. Whole Grains

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LIPOPROTEINS

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Classification of Lipoproteins

Classification of Serum Lipoproteins According to Their Electrophoretic Mobilities

With the development of techniques to separate proteins according to their electrophoretic behavior, it could be demonstrated that most of the lipid present in serum was associated with proteins migrating with α_1 - and β -globulin mobilities. This resulted in the first classification of lipoproteins as α_1 - and β -lipoproteins. The ratio of lipid to protein on the α_1 -lipoproteins was approximately 1:1, whereas the β -lipoproteins had a greater relative content of lipids. Application of more advanced electrophoretic techniques resulted in further discrimination among the lipoprotein classes and for many years lipoproteins were classified as β -, pre- β -, and α -lipoproteins. Careful observation of the electrophoretic lipoprotein profiles in normals and subjects with familial lipoprotein disorders gave rise to the first classification of lipoprotein disorders by Fredrickson and colleagues. The equivalence between electrophoretic and ultracentrifugal separation is presented in Table 1.

Several electrophoretic supports have been used to separate plasma lipoproteins. These include paper, cellulose acetate, agarose, and polyacrylamide. Agarose gel electrophoresis remains the most commonly used for easy and rapid assessment of lipoprotein patterns in the clinical laboratory. This technique is especially useful for identifying the presence of a broad β band in the diagnosis of type III hyperlipidemia. Gradient agarose-polyacrylamide gel electrophoresis under non-denaturing conditions has been an essential tool to analyze low-density lipoprotein (LDL) and high-density lipoprotein (HDL) subclasses, providing a greater resolution than ultracentrifugation. LDL subfractions have been resolved by non-denaturing polyacrylamide gradient gel electrophoresis (2–16%) in up to seven LDL subclasses with densities ranging from 1.020 to 1.063 g ml⁻¹ and diameters ranging from 22.0 to 28.5 nm. Usually a major subpopulation and several (one–four) minor LDL subpopulations are found in most subjects examined. A predominance of smaller, more dense LDL, versus larger, more buoyant LDL particles in plasma has been associated with increased coronary heart disease (CHD) risk. There is evidence supporting the genetic origin of the distribution of LDL subfractions; however, age, gender, and environmental factors strongly influence the penetrance. HDL subfractions have been resolved using a similar technique, with a polyacrylamide gradient ranging from 4% to 30%, into

five subclasses (HDL_{3c}, HDL_{3b}, HDL_{3a}, HDL_{2a}, and HDL_{2b}). More recently 11–14 subclasses have been described, including β -migrating particles, using an improved electrophoresis technique. The clinical importance of these subfractions is still under investigation.

Classification of Serum Lipoproteins According to Their Ultracentrifugal Characteristics

The presence of lipids within the lipoprotein particles confers these macromolecular complexes with a lower density compared with other serum proteins. With the arrival of the analytical ultracentrifugation in the 1940s, this characteristic allowed its initial separation as a discrete peak using this technique. During the following years, it was demonstrated that this fraction was made up of a wide spectrum of particle sizes and densities (d) ranging from 0.92 to 1.21 g ml⁻¹.

Lipoproteins were classically separated into four major classes designated as chylomicrons (exogenous triacylglycerol-rich particles of $d < 0.94$ g ml⁻¹), very low-density lipoproteins (VLDL, endogenous triacylglycerol-rich particles of $d = 0.94$ –1.006 g ml⁻¹), LDL (cholesteryl ester-rich particles of $d = 1.006$ –1.063 g ml⁻¹), and HDL (particles containing approximately 50% protein of $d = 1.063$ –1.21 g ml⁻¹). With subsequent improvements to the ultracentrifugation techniques, further heterogeneity was detected within each of those major lipoprotein classes; this resulted in the need for further subdivision into several density subclasses such as HDL_{2a} ($d = 1.10$ –1.125 g ml⁻¹), HDL_{2b} ($d = 1.063$ –1.10 g ml⁻¹), and HDL₃ ($d = 1.125$ –1.21 g ml⁻¹).

There is no doubt that the separation of lipoproteins by ultracentrifugation has been essential for the advances in this field; however, this technique is very labor intensive and the isolated lipoproteins are usually modified due to the high g force and salt concentrations used in this process. The development of new vertical and near vertical rotors has shortened considerably the runs and thus diminished some of these negative effects.

Classification of Serum Lipoproteins According to Their Apolipoprotein Composition

Recent interest in the study of lipoprotein subfractions has resulted in an increased use of methods of separation based on affinity chromatography, especially those using immunoaffinity. Using columns containing antibodies against

Table 1 Classification of plasma lipoproteins

<i>Lipoprotein</i>	<i>Diameter (nm)</i>	<i>Density (g ml⁻¹)</i>	<i>Electrophoretic mobility</i>	<i>Major lipids</i>	<i>Major apolipoproteins</i>
Chylomicrons	80–500	<0.95	Origin	Dietary triacylglycerols, cholesteryl esters	A-I, A-II, A-IV, B-48, C-I, C-II, C-III, E
Remnants	>30	<1.006	Origin	Dietary cholesteryl esters	B-48, E
VLDL	30–80	<1.006	pre- β	Endogenous triacylglycerols	B-100, C-I, C-II, C-III, E
IDL	25–35	1.006–1.019	pre- β and β	Cholesteryl esters, triacylglycerols	B-100, E
LDL	18–28	1.019–1.063	β	Cholesteryl esters	B-100
HDL ₂	9–12	1.063–1.125	α	Cholesteryl esters, phospholipids	A-I, A-II
HDL ₃	5–9	1.125–1.210	α	Cholesteryl esters, phospholipids	A-I, A-II

Table 2 Classification and properties of apolipoproteins

<i>Apolipoprotein</i>	<i>Amino acids</i>	<i>Tissue expression</i>	<i>Chromosomal localization</i>	<i>Functions</i>
apo A-I	243	Liver Intestine	11	Major structural component of HDL Ligand for HDL binding Activator of LCAT
apo A-II	77	Liver	1	Reverse cholesterol transport Structural component of HDL
apo A-IV	377	Intestine Liver	11	Activator of hepatic lipase Regulator of LPL activity Activator of LCAT
apo B-48	2152	Intestine	2	Intestinal lipid absorption Structural component of TRL
apo B-100	4536	Liver	2	Secretion of chylomicrons Structural
apo C-I	57	Liver Intestine	19	Activator of LCAT Inhibitor of the LRP
apo C-II	79	Liver Intestine	19	Activator of LPL
apo C-III	79	Liver Intestine	11	Inhibits LPL
apo D	169	Most tissues	3	Radical scavenger Reverse cholesterol transport
apo E	299	Liver Macrophage	19	Binding of heme-related compounds Ligand for the LDL receptor Ligand for the LRP
apo(a)	Variable	Liver	6	Reverse cholesterol transport ?

specific apolipoproteins (**Table 2**), a large number of HDL subpopulations have been resolved. Similarly, this technique allows the separation of several triacylglycerol-rich lipoproteins subfractions.

Lipoproteins containing apo A-I can be separated into two major species: those containing both apo A-I and apo A-II, known as LpAI:AI, and those containing apo A-I but not apo A-II (LpAI). Small numbers of particles containing apo A-II, but not apo A-I, have been detected in normal subjects; however, these particles could become predominant in the presence of rare genetic disorders associated with HDL deficiency. Another HDL species containing apo A-I and apo E is important in reverse cholesterol transport by transporting

cholesterol from the cell membranes to the liver for elimination from the body.

Lipoproteins containing apo B consist of four lipoprotein families. Lipoproteins containing apo B only (Lp(B)) are cholesteryl ester-rich and are found primarily within the LDL density range, but they have also been detected within the VLDL range. Particles containing both apo B and apo C (LpB:C), apo B and apo E (LpB:E), and all three apolipoprotein groups (LpB:E:C) are triacylglycerol-rich and are found within the VLDL and intermediate-density lipoprotein (IDL) density range. The apo C and apo E contents decrease as density increases.

More recently, the affinity for lectins of Lp(a), a lipoprotein containing apo B-100 as well as an antigenically unique

apolipoprotein (apo(a)), has been used to develop a new technique to measure the levels of this lipoprotein in plasma.

Synthesis and Catabolism of Lipoproteins

Metabolism of Lipoproteins Carrying Exogenous Lipids

Dietary fats absorbed in the intestine are packaged into large, triacylglycerol-rich chylomicrons for delivery through the bloodstream to sites of lipid metabolism or storage. These lipoproteins interact with lipoprotein lipase (LPL) and undergo lipolysis, forming chylomicron remnants. The major sites of LPL activity are adipose tissue, skeletal muscle, the mammary gland, and the myocardium. In these sites, the fatty acids from the triacylglycerols are used for storage, oxidation, or secretion back to the circulation. The triacylglycerol-depleted particles resulting from the lipolysis, known as chylomicron remnants, pick up apo E and cholesteryl ester from HDL and are rapidly taken up by the liver via a process mediated by the apo E receptor. This is a fast process and chylomicron particles are not usually present in the blood after a prolonged fasting period. The occurrence of chylomicronemia can be easily detected by the presence of a creamy supernatant floating on top of the plasma or serum kept for several hours at 4 °C.

Transport of Endogenous Lipids

The liver cell secretes triacylglycerol-rich VLDL, which can be converted first to IDL and then to LDL through lipolysis by a mechanism similar to that described for chylomicrons. The excess surface components are usually transferred to HDL, and the triacylglycerol-depleted VLDL becomes an IDL. Some of these particles may be taken up by the liver via an apo E receptor, whereas others are further depleted of triacylglycerols, becoming cholesteryl ester-enriched particles known as LDL, which contain apo B as their only apolipoprotein. Consumption of fat-rich meals or glucose enhances VLDL production.

Some primary causes of elevated VLDL or IDL levels are familial endogenous hypertriglyceridemia (type IV according to Fredrickson's classification) and familial dysbetalipoproteinemia (type III hyperlipidemia). Genetic mutations at the apo E gene locus are responsible for the type III phenotype. Some secondary causes for elevated VLDL levels are obesity, diabetes mellitus, and alcohol consumption, as well as the use of high doses of certain drugs (e.g., thiazide diuretics and estrogens). The presence of elevated levels of IDL has been associated with an increased atherosclerotic risk.

LDL particles are major carriers of cholesteryl ester in the blood. An LDL receptor that recognizes apo B-100 and apo E, but not apo B-48, allows the liver and other tissues to catabolize LDL. High-fat and high-cholesterol diets can decrease the activity of the LDL receptor, leading to increased levels of circulating LDL. These particles supply cholesterol to cells in the periphery for synthesis of cell membranes and steroid hormones. Modified or oxidized LDL can also be taken up by the scavenger receptor on macrophages in various tissues, including the arterial wall. This process is a potential initiator of foam cell formation and atherosclerosis.

Several LDL subclasses have been identified using gradient gel electrophoresis. Large, less dense LDL particles are commonly found in premenopausal women and men at low risk for CHD, whereas the small, more dense particles have been associated with a significant increased risk for myocardial infarction. The distribution of these particles appears to have a significant genetic component modulated by age and environmental factors.

Reverse Cholesterol Transport

HDL is synthesized by both the liver and the intestine. Its precursor form is discoidal in shape and matures in circulation as it picks up unesterified cholesterol from cell membranes and other lipids (phospholipid and triacylglycerol) and proteins (A-I, E, and C apolipoproteins) from triacylglycerol-rich lipoproteins (chylomicron and VLDL) as these particles undergo lipolysis. The cholesterol is esterified by the action of the lecithin-cholesterol acyltransferase (LCAT) and the small HDL₃ particle becomes a larger HDL₂ particle. The esterified cholesterol is either delivered to the liver or transferred by the action of cholesteryl ester transfer protein (CETP) to other lipoproteins (such as chylomicron, VLDL remnants, or LDL) in exchange for triacylglycerols. This cholesterol may then be taken up by the liver via receptors specific for these lipoproteins, or it can be delivered again to the peripheral tissues. The triacylglycerol received by HDL₂ is hydrolyzed by hepatic lipase and the particle is converted back to HDL₃, completing the HDL cycle in plasma. In the liver, cholesterol can be excreted directly into bile, converted to bile acids, or reutilized in lipoprotein production.

Several genetic disorders have been identified associated with low levels or total deficiency of HDL.

Effects of Dietary Fats and Cholesterol on Lipoprotein Metabolism

The cholesterolemic effects of dietary fatty acids have been extensively studied. The saturated fatty acids C_{12:0}, C_{14:0}, and C_{16:0} have a hypercholesterolemic effect, whereas C_{18:0} has been shown to have a neutral effect. Monounsaturated and polyunsaturated fatty acids in their most common *cis* configuration are hypocholesterolemic in comparison with saturated fatty acids. The effects of *trans* fatty acids on lipid levels are under active investigation. Our current knowledge shows that their effect is intermediate between those of saturated and unsaturated fats. The effect of dietary cholesterol on lipoprotein levels is highly controversial. This may be due in part to the dramatic interindividual variation in response to this dietary component. Specific effects of dietary fats and cholesterol on each lipoprotein fraction are the focus of other articles and they are only briefly summarized below and in Table 3.

Effects of Diet on Chylomicron Metabolism

Diets very high in saturated fat have been associated with increased postprandial chylomicrons and chylomicron remnants compared with diets rich in n-6 polyunsaturated fats;

Table 3 Effects induced on the major lipoprotein fractions by different dietary components following isoenergetic replacement of saturated fatty acids

	MUFA	PUFA n-6	PUFA n-3	trans FA	Simple carbohydrate	Carbohydrate plus fiber
VLDL-C	≈	≈/↓	↓	↑	↑	≈
LDL-C	↓	↓	≈/↓	↑	↓	↓
HDL-C	≈/↑	≈/↓	↓	↓	↓	≈/↓

≈ Equivalent effect; ↓ concentration reduced; ↑ concentration increased.

Table 4 Classification of hyperlipidemias according to Fredrickson

Type	Plasma cholesterol	Plasma triacylglycerol	Lipoprotein fraction(s) affected	Atherosclerosis risk	Genetic disorder
I	Normal to elevated	Very elevated	Chylomicrons	No	Familial LPL deficiency Apo C-II deficiency
IIa	Elevated	Normal	LDL	High	Familial hypercholesterolemia Familial combined hyperlipidemia Polygenic hypercholesterolemia
IIb	Elevated	Elevated	LDL and VLDL	High	Familial hypercholesterolemia Familial combined hyperlipidemia
III	Elevated	Very elevated	IDL	High	Familial dysbetalipoproteinemia
IV	Normal or elevated	Elevated	VLDL	Moderate	Familial hypertriglyceridemia Familial combined hyperlipidemia
V	Normal or elevated	Very elevated	VLDL and chylomicrons	Moderate	Familial hypertriglyceridemia

however, human experiments carried out using moderate to high fat intake have not shown significant effects of different types of dietary fat or dietary cholesterol on postprandial lipoproteins.

The effects of dietary carbohydrates on postprandial lipoproteins have also been studied. Most protocols have used diets very high in simple carbohydrates. In general, high carbohydrate intake has been associated with increased levels of fasting triacylglycerols and increased postprandial levels of chylomicrons and chylomicron remnants.

Effects of Diet on VLDL Metabolism

It is well-known that diets high in simple carbohydrate increase hepatic secretion of VLDL. This carbohydrate induction of hypertriglyceridemia is the source of the current controversy regarding the optimal diet for subjects at high risk for cardiovascular disease. Some authors have demonstrated that the increased hepatic triacylglycerol secretion induced by high-carbohydrate diets was not accompanied by parallel increases in apo B-100 secretion. In other words, the consumption of low-fat, high-carbohydrate diets did not affect the number of particles but resulted in larger, more triacylglycerol-enriched VLDL particles.

Intake of saturated fat results in an increased secretion of the number of VLDL particles by the liver, whereas the opposite effect is observed with polyunsaturated fat. Of special note are the dramatic effects on VLDL production found following high intakes of n-3 fatty acids. These diets are associated with marked decreases in triacylglycerol secretion by mechanisms not fully understood. It has been speculated that n-3 fatty acids may stimulate intracellular degradation of apo B in hepatocytes. Dietary cholesterol,

within the physiological range, appears to play a minor role in hepatic VLDL production.

Effects of Diet on LDL Metabolism

The effects of dietary fat and cholesterol on LDL metabolism have been extensively studied. However, the effects of dietary cholesterol are still highly controversial. Whereas some studies have demonstrated increased LDL production and decreased catabolism associated with high cholesterol intakes, others have failed to find such associations.

Replacement of saturated by polyunsaturated fats has been associated with decreased LDL apo B production in some studies, whereas in other studies, increased ratios of polyunsaturated to saturated fats resulted in increased LDL apo B catabolism. Unlike the effects described for VLDL metabolism, intake of n-3 fatty acids appears to play a minor role on LDL metabolism.

Effects of Diet on HDL Metabolism

Diets high in simple carbohydrates reduce HDL cholesterol levels. This effect appears to be mediated by increases in the catabolism of apo A-I; however, one study has also demonstrated an additional decrease in apo A-I production.

Disorders of Lipoprotein Metabolism

For historical reasons the classification of disorders of lipoprotein metabolism will be presented according to the classical Fredrickson's classification (Table 4).

Type I or Familial Chylomicronemia

This disorder is characterized by greatly elevated levels of exogenous triacylglycerols and it is the result of impaired lipolysis of chylomicrons due to a deficiency of LPL or its activator, the apo C-II. Several genetic mutations at the structural genes for both LPL and apo C-II have been reported. These are autosomal recessive traits. In the heterozygous state, subjects have normal to slightly elevated plasma triglycerides, whereas homozygotes have triacylglycerol levels that may exceed 1000 mg dl^{-1} in the fasting state. The diagnosis of the homozygous state takes place during the first years of life from the presence of recurrent abdominal pain and pancreatitis. Eruptive xanthomas and lipemia retinalis may also occur.

The recommended treatment includes a diet low in simple carbohydrates and with a fat content below 20% of total energy. The use of medium-chain triglycerides (MCT) has also been reported to be efficacious. Body weight should be maintained within the normal limits and alcohol consumption should be avoided.

Other secondary causes leading to the presence of chylomicrons in the fasting state include uncontrolled diabetes mellitus, alcoholism, estrogen use, and hypothyroidism.

Fasting chylomicronemia has not been clearly associated with increased risk for atherosclerosis; however, there is considerable evidence supporting the atherogenic properties of chylomicron remnants.

Type II or Familial Hypercholesterolemia

Familial hypercholesterolemia (FH) is an autosomal dominant disorder characterized by elevation of plasma LDL cholesterol levels. Mutations at the LDL receptor gene locus on chromosome 19 are responsible for this disorder. Multiple different mutations have been described at this locus resulting in the FH phenotype. In the heterozygous state, subjects develop tendinous xanthomas, corneal arcus, and CHD. Elevations of LDL can result from well-characterized genetic disorders such as FH or familial defective apo B-100.

The ranges of LDL cholesterol levels in plasma of FH subjects are $200\text{--}400 \text{ mg dl}^{-1}$ in heterozygotes and above 450 mg dl^{-1} in homozygotes. The frequency of defects at the LDL receptor locus is approximately 1 in 500 for the heterozygous state and 1 in a million in the homozygous state.

Inhibitors of 3-hydroxy-3-methylglutaryl (HMG) coenzyme A are useful in the treatment of hypercholesterolemia. Most pharmacological therapies are ineffective in the homozygous state. FH homozygotes may be treated with LDL apheresis, liver transplantation, and portacaval shunt. More recently, encouraging results have been obtained using *ex vivo* gene therapy.

The genetic defect(s) associated with a common form of hypercholesterolemia present in most subjects with cholesterol levels between 250 and 300 mg dl^{-1} has (have) not been elucidated. This disorder may be due to a combination of minor gene defects (i.e., presence of apo E-4 allele) that in combination with the environment (i.e., diet, lack of exercise) predispose individuals to moderately elevated LDL cholesterol levels. This disorder has been also named polygenic hypercholesterolemia.

Familial Defective apo B-100

Familial defective apo B-100 is an autosomal dominant genetic disorder that presents with a phenotype similar to FH. The frequency of this disorder may be similar to that of FH; however, it varies considerably depending on the ethnicity of the population studied. The specific mutation responsible for this disorder is a point mutation at amino acid 3500 of the mature apo B. The diagnosis of this disorder requires molecular biology techniques.

Type III or Familial Dysbetalipoproteinemia

In this disorder both plasma triacylglycerol and cholesterol are increased. Several mutations within the apo E gene locus have been found to be responsible for this disease; however, in most patients the complete expression of the clinical genotype needs additional interactions such as age, obesity, and diabetes. In addition to the accumulation in plasma of VLDL remnants and chylomicrons, other characteristics of this disorder are tuberoeruptive xanthomas and in some cases also planar xanthomas. Therapies include diet and hypolipidemic agents such as fibrates, statin, or nicotinic acid. In most cases, diagnosis can be carried out first by agarose gel electrophoresis, followed by molecular biology techniques to detect the presence of the apo E-2 allele.

Familial Type IV and Type V Hypertriglyceridemias

These two disorders may have overlapping phenotypes. In type IV or familial endogenous hypertriglyceridemia, triacylglycerol levels are increased and HDL is usually decreased. This disorder appears to be autosomal dominant and relatively frequent in populations consuming high-fat diets. The precise molecular defect has not been defined; however, the increase in triacylglycerol is associated with overproduction of triacylglycerol by the liver and often with consequent reduced clearance. Diet should be the first step in therapy, followed if necessary by pharmacotherapy using fibrates or nicotinic acid. Premature CHD has been seen in some but not all cases presenting with this phenotype.

Type V hyperlipidemia is a much more rare disorder. Usually the first signs of this abnormality are abdominal pain or pancreatitis. VLDL levels are high and chylomicrons are present in the fasting state. This abnormality has not been linked to any specific molecular defect. Besides the primary genetic defect, other secondary causes of type V hyperlipidemia are poorly controlled diabetes mellitus, nephrotic syndrome, hypothyroidism, glycogen storage disease, and pregnancy. Recent data indicate increased susceptibility to atherosclerosis.

Familial Dyslipidemia

Familial dyslipidemia may be a variant of the familial hypertriglyceridemias described previously. It is characterized by hypertriglyceridemia in combination with low HDL cholesterol. Patients are generally overweight, with male pattern obesity, insulin resistance, diabetes, and hypertension. These subjects have both increased hepatic triacylglycerol secretion and increased HDL apo A-I catabolism.

Familial Combined Hyperlipidemia

Familial combined hyperlipidemia (FCH) was initially described as the combination of hypercholesterolemia and hypertriglyceridemia within the same kindred, and with kindred members having one of these abnormalities or both. Moreover, most subjects with FCH have HDL cholesterol levels below the 10th percentile. Affected subjects have elevation in VLDL, LDL, or both. This disorder has a frequency of approximately 10% in survivors of premature myocardial infarction (less than 60 years of age) and approximately 14% in kindred with CHD.

It has been reported that affected subjects have overproduction of apo B-100. The precise molecular defect has not been elucidated, although there are already several candidate gene loci, including the LPL. The expression of this disorder may be triggered by other factors, such as overweight, hypertension, diabetes, and gout. The treatment should include diet and exercise and, if necessary, niacin, HMG CoA reductase inhibitors, or fibrates, depending on the major lipid present in excess.

Familial Hyperapobetalipoproteinemia

Familial hyperapobetalipoproteinemia is characterized by apo B values above the 90th percentile in the absence of other lipid abnormalities; it has been suggested to be a variant of FCH. This disorder is relatively common (5%) in kindreds with premature CHD. The molecular defect is not known, but metabolic studies suggest overproduction of apo B-100.

Familial Hypoalphalipoproteinemia

Severe HDL deficiency, characterized by HDL cholesterol levels $<10 \text{ mg dl}^{-1}$ is rare and may be due to Tangier disease, apo A-I deficiencies, LCAT deficiency, or fish-eye disease. The apo A-I deficiency states are due to rare deletions, rearrangements, or point mutations within the apo A-I/C-III/A-IV gene complex. Familial hypoalphalipoproteinemia is relatively common and is characterized by HDL cholesterol levels below the 10th percentile of normal. These subjects have been reported to have either decreased HDL production or increased HDL apo A-I catabolism. This phenotype is present in approximately 4% of kindred with premature CHD.

The genetic defect or defects are not known; however, it has been suggested that FCH, familial hyperapobetalipoproteinemia, familial dysbetalipoproteinemia, and familial hypoalphalipoproteinemia may be variants of a single disorder. This disorder is characterized by a genetic predisposition in subjects consuming high-fat, high-cholesterol diets to an increased secretion of apo B-containing lipoproteins and an increased catabolism of apo A-I-containing lipoproteins. The expression of the phenotype is usually enhanced by the presence of male pattern obesity.

Familial Lipoprotein (a) Excess

Lipoprotein (a) (Lp(a)) is an LDL particle with one molecule of apolipoprotein (a) attached to it. Elevated levels of Lp(a) ($>35\text{--}40 \text{ mg dl}^{-1}$ or 90th percentile) have been associated

with premature CHD. This increased risk appears to result from two different mechanisms: cholesterol deposition in the arterial wall and inhibition of fibrinolysis.

Lp(a) concentrations are highly variable among individuals; however, they tend to remain constant during a person's lifetime. Between 80% and 90% of the variability appears to be of genetic origin, owing, for the most part, to variations at the structural apo(a) gene locus. Lp(a) concentrations are inversely associated with a size polymorphism of apo(a). This polymorphism is due to differences in the number of a multiple repeat of a protein domain highly homologous to the kringle 4 domain of plasminogen. Diets and medications used to lower LDL cholesterol levels do not appear to have a significant effect on Lp(a) concentrations; however, niacin has been reported to decrease Lp(a) levels. There have been reports suggesting that diets high in *trans* fatty acids have some raising effects on Lp(a) levels, whereas estrogen replacement lowers Lp(a) in postmenopausal women.

General Guidelines for the Treatment of Lipoprotein Abnormalities for CHD Prevention

There is a clear benefit from lowering LDL cholesterol with diet or drug therapy in patients with hyperlipidemia or CHD or both. Dietary therapy includes using diets that are restricted in total fat ($<30\%$ of calories), saturated fat ($<7\%$ of calories), and cholesterol ($<200 \text{ mg day}^{-1}$). Pharmacological therapies include anion exchange resins, niacin, and HMG CoA reductase inhibitors. The latter agents have been demonstrated to also lower CHD mortality. It should be noted that dramatic inter-individual variations have been demonstrated in response to diet and drug therapies. Consequently the efficacy of hypolipidemic therapies will vary from individual to individual. More information is needed about the benefits of HDL cholesterol raising in patients with low HDL cholesterol levels as well as the benefits of lowering triacylglycerol plasma concentrations, and more specifically the triacylglycerol carried in lipoprotein remnants. This is also true regarding the benefits of Lp(a) lowering using niacin in patients with elevated Lp(a) levels.

See also: Body Composition. Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Coronary Heart Disease: Lipid Theory; Prevention. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids; Metabolism. Fertility. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. *Trans*-Fatty Acids: Health Effects, Recommendations, and Regulations

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LIVER DISORDERS

Nutritional Management

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Glossary

Cholestasis A condition where bile cannot flow from the liver to the duodenum. It can occur because of an obstruction of the intrahepatic or extrahepatic biliary system, genetic defects, or be acquired as a side effect of several medications.

Dysgeusia The distortion of the sense of taste.

Gluconeogenesis A metabolic pathway that results in the formation of glucose from noncarbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids.

Glycogenesis The pathway of glycogen synthesis, in which glucose molecules are combined with chains of glycogen for energy storage.

Glycolysis The metabolic process that converts glucose into pyruvate.

HELLP A life-threatening obstetric complication including hemolysis, elevated liver enzymes, and low platelets syndrome.

Hepatoportoenterostomy A surgical procedure performed on young infants with extrahepatic biliary atresia to allow bile drainage from intrahepatic bile ducts to the small intestine.

Steatohepatitis A form of liver disease, characterized by fat accumulation and inflammation in the liver.

Introduction

This review will cover the role of the liver in normal nutrition, including the important functions of bile salt production, macronutrient metabolism, and fat-soluble vitamin absorption, metabolism and storage. Next the pathogenesis of malnutrition in liver disease will be discussed, starting with the mechanisms of malnutrition in both acute and chronic liver failure. Specific nutritional issues in liver failure will be addressed, including metabolic disturbances of carbohydrates, proteins, and fats. Nutritional disturbances in the major types of specific liver diseases will be reviewed: hepatocellular, metabolic liver disease, and biliary tract disorders. Nutritional assessment and management of patients with acute, chronic liver disease, and end stage liver disease will be discussed.

Nutritional Aspects and Liver Physiology in Normal Liver and Liver Diseases

Bile Salts

A normal functioning liver will secrete 600–1200 ml of bile to the gall bladder on a daily basis. Bile is made up of bile salts, lecithin, conjugated bilirubin, phospholipids, cholesterol, electrolytes, and water. Bile salts, which are the predominant component of bile are synthesized from cholesterol

in the hepatocyte. The primary function of bile salts lies in its interaction with lipid digestion. Bile salts bind with large fat particles, which alone are insoluble in water, and act on them as an emulsifier, breaking them down into smaller particles called micelles (**Figure 1**). Micelles, the product of the fat particle and bile salt structure, aid in the transport of fat to the mucosal membrane for absorption. Fat-soluble vitamins and cholesterol are also incorporated into mixed micelles for proper absorption.

Micellar solubilization is only required for long chain fatty acids. Short and medium chain fatty acids (12 carbons or less) do not require micelle formation for absorption; instead they enter the portal circulation directly, bound to albumin, and are transferred to the liver for oxidation. Approximately 94% of the micelle forming bile acids are reabsorbed in the ileum, and shuttled via the hepatic portal vein bound to albumin back to the liver for re-use. Only 6% of the bile acids are lost in excretion.

Macronutrient Metabolism

Carbohydrates

The liver is responsible for maintaining normal blood glucose concentrations under various metabolic conditions. Among the several metabolic processes that allow this fine regulation

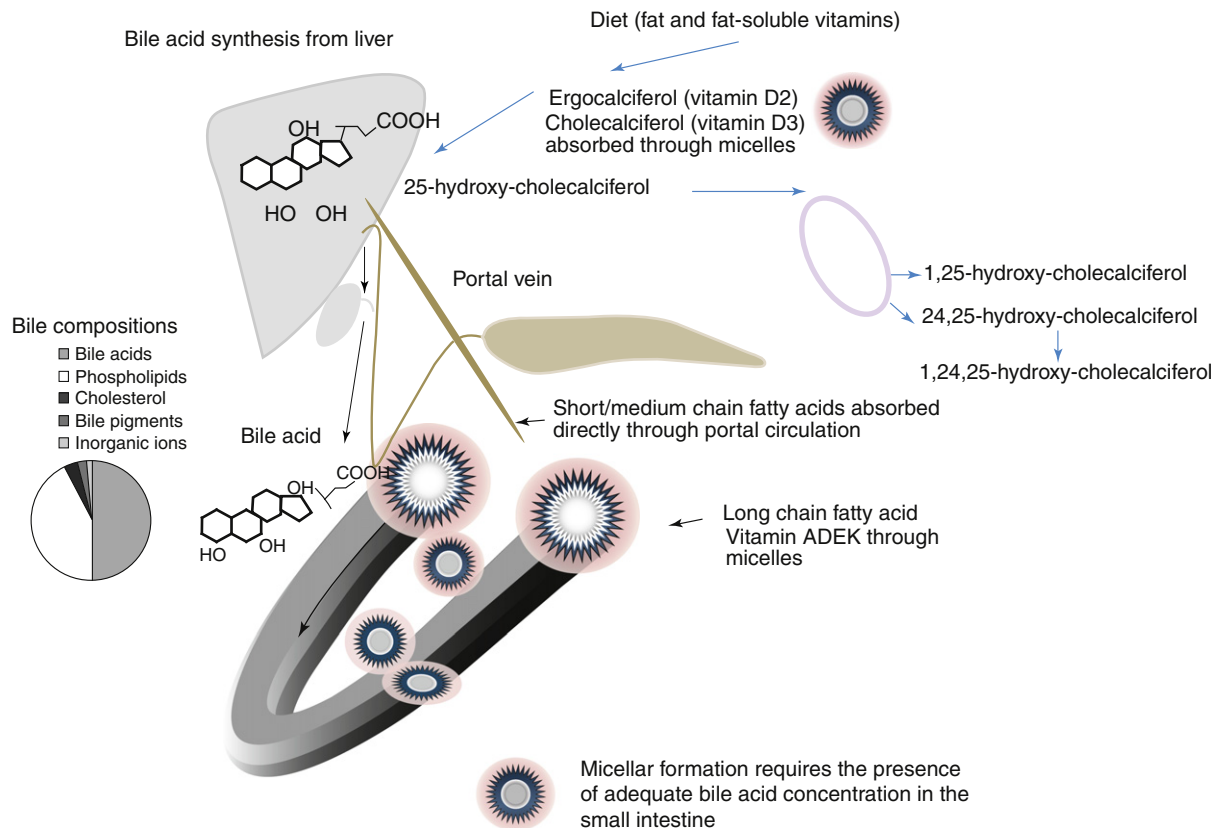


Figure 1 Compositions of bile, bile acid excretion, micellar formation in the small intestine, absorption pathways of short, medium, and long chain fatty acids and fat-soluble vitamin, as well as vitamin D metabolism.

are glycogenesis, gluconeogenesis, and glycolysis. The end product of carbohydrate digestion is 80% glucose, with the remaining 20% being fructose and galactose; the latter two being quickly converted into glucose in the liver. Once transported into the hepatocyte, the glucose molecule is phosphorylated (via glucokinase) and cannot leave the cell unless dephosphorylated with glucose phosphatase. Glucose is either used for immediate energy release, or stored as glycogen. Not surprisingly, in a patient with liver disease glucose intolerance and insulin resistance are common. Cirrhotic patients are prone to developing diabetes. Energy from carbohydrates plays an important role in protein sparing mechanisms, preventing the use of protein as energy.

Proteins

Protein metabolism occurs in liver, specifically, the deamination of amino acids, urea formation for removal of ammonia, plasma protein synthesis, and in the interconversions between amino acids. Ingested protein is the sole source of the ten essential amino acids, and the primary source of nitrogen necessary for the synthesis of other amino acids. Protein is digested and broken down to amino acids which are absorbed into the circulation and taken to cells throughout the body, primarily the liver and quickly become combined by peptide linkages. The plasma level of amino acids is tightly controlled and maintained near a constant level. Once the cellular limit

of protein storage is met, excess amino acids are degraded and used for energy or stored as fat or glycogen. The liver is the primary site of all amino acid catabolism with the exception of branch-chained amino acid catabolism which occurs in the muscle cells. The urea cycle, in which the toxic compound ammonia is converted to urea, occurs solely in the liver. The synthesis of the plasma proteins albumin, fibrinogen, and globulin also occur in the liver.

Plasma proteins such as albumin and coagulation factors constitute approximately 50% of the proteins synthesized in the liver. In liver disease, decreased synthesis of these proteins has important clinical consequences including ascites from hypoalbuminemia and coagulopathy from decreased synthesis of coagulation factors. In end stage liver disease, hypoglycemia can result from decreased hepatic gluconeogenesis from amino acids. Decreased activity of the urea cycle enzymes result in hyperammonemia and hepatic encephalopathy, the ultimate expression of which can be cerebral edema.

Lipids

The liver is responsible for the metabolism of lipids via four key processes: fatty acid oxidation for energy; lipoprotein syntheses; the synthesis of cholesterol and phospholipids; and the conversion of carbohydrate to fat for storage. Digested fat is a key source of energy in which after splitting into fatty acids and glycerol, the fatty acid components further split

via beta-oxidation into acetyl-CoA. Two molecules of acetyl-CoA become paired together to form acetoacetic acid and are transported to other cells to provide energy in the citric acid cycle.

In cholestatic liver disease there is malabsorption of dietary lipid, and consequent malnutrition. There are experimental data in primates showing that chronic ethanol consumption results in a decrease in liver phospholipids and of phosphatidylcholine (PC). Consequently the total phospholipid content in the mitochondrial membranes is decreased; mitochondria are altered both structurally and functionally. There is diminished mitochondrial oxidation because of decreased cytochrome oxidase activity, which can be restored by administration of PC. The extent to which chronic liver disease of etiologies other than chronic ethanol consumption results in similar perturbations is unknown.

Fat-soluble Vitamins

The liver plays a key role in the absorption of the fat-soluble vitamins, A, D, E, and K as they are only successfully absorbed in association with fat and sufficient quantities of bile salts (Figure 1). The liver is also the primary storage site for several vitamins including vitamin A, E, K, and B₁₂. Vitamin A is stored in the largest quantity with amounts sufficient to prevent deficiency for 5–10 months. Vitamin D is stored in amounts sufficient for 2–4 months. Vitamin B₁₂ is stored in amounts sufficient for at least one year. The liver is responsible for the first hydroxylation of vitamin D to the circulation form, 25-OH vitamin D.

Deficiencies of fat-soluble vitamins are common in liver disease associated with steatorrhea due to the concomitant malabsorption of fat. Vitamin A deficiency can result in anorexia, growth failure, decreased resistance to infections, and night blindness. Vitamin D deficiency results in osteopenia or osteoporosis, as well as rickets. The prevalence of fractures is increased in women being treated for alcohol abuse and also following sobriety; deficiencies of vitamin D as well as calcium, phosphorus, and fluoride may all play a role. The deficiency of vitamin E results in neuroaxonal dystrophy, clinically manifesting as peripheral neuropathy, and cerebellar disturbances. Vitamin K deficiency results in hemorrhage because of reduced synthesis in clotting factors.

Trace Elements

Zinc deficiency in cirrhotics may contribute to hypoalbuminemia and dermatitis as well as anorexia from dysgeusia. Deficiency of selenium can lead to decreased synthesis of important antioxidant selenoproteins such as glutathione peroxidase. Little is known about the effect of acute or chronic liver disease on other trace elements.

Liver in Specific Hepatobiliary Disorders and Nutritional Management

Hepatocellular Diseases

Alcoholic liver Disease

The term alcoholic liver disease refers to a spectrum of types of hepatic injury associated with continuous alcohol

consumption, ranging from alcoholic fatty liver, alcoholic steatohepatitis, fibrosis, and cirrhosis. Nutritional disturbances in alcoholics are an important cause of morbidity and mortality; all classes of nutrients are affected. Anorexia leads to decreased food intake and subsequent protein-calorie malnutrition. Maldigestion and malabsorption can occur secondary to chronic alcohol injury to small intestinal mucosa. Alcohol consumption is often associated with chronic pancreatic insufficiency which results in steatorrhea and decreased absorption of dietary protein, fat, and fat-soluble vitamins. Chronic alcohol consumption also results in impaired hepatic amino acid uptake and protein synthesis.

In alcoholics, utilization of lipids and carbohydrates is markedly compromised due to an excess of reductive equivalents and impaired oxidation of triglycerides. Alcoholics are often resistant to insulin and exhibit impaired uptake of glucose into muscle cells. Insulin-dependent diabetes is common. Heavy alcohol consumption is frequently associated with deficiencies of a wide variety of micronutrients including the fat- and water-soluble vitamins, particularly folate, pyridoxal-5'-phosphate, thiamine, and vitamin A.

Table 1 summarizes the five published controlled trials of the effect of oral or enteral nutritional supplements on patients with alcoholic hepatitis. In most, nitrogen balance and protein synthesis improved, although no effect on mortality was shown, perhaps because of the small number of patients studied or the duration of follow-up. In the largest study, at one year of follow-up, the experimental group had a significantly better survival: 2/24 or 8% died as compared to 10/27 or 37% of the controls. In general, the effects of parenteral nutrition in alcoholic liver disease are similar to the studies of enteral nutritional supplements.

Many studies have examined the effect of oral or enteral nutritional supplementation in patients with alcoholic cirrhosis. Results are summarized in Table 2. Many studies are small and of short duration, so it is not surprising that results are inconclusive. Most studies demonstrated an improvement in nitrogen balance and protein synthesis; only one showed increased survival in the treated group. Taken together these studies suggest that there are benefits to nutritional supplementation in this population.

A variety of international associations have made nutritional recommendations for patients with various types of alcoholic liver disease. The primary recommendation is of course abstinence, which may be all that is needed in patients with fatty liver. Patients with alcoholic hepatitis should take 40 kcal kg⁻¹, 1.3–1.5 g protein kg⁻¹, 4–5 g kg⁻¹ of carbohydrates, and 1–2 g kg⁻¹ of lipids per day. Those with cirrhosis without malnutrition should take 35 kcal kg⁻¹, 1.3–1.5 g protein/kg and carbohydrates and lipids as recommended for patients with alcoholic hepatitis. Those with cirrhosis and malnutrition should take higher amounts of protein (1.5–2.0 g kg⁻¹) and lipids (2.0–2 g kg⁻¹) and lower amounts of carbohydrates (3–4 g kg⁻¹). Fluid should be restricted to 2–2.5 l day⁻¹ and B-vitamins, folate, thiamine, vitamins C, and K should be routinely supplemented. In addition patients with cholestasis should take 50% of their dietary lipids as medium chain triglycerides and should be supplemented with the fat-soluble vitamins: A, D, E, and K. The major strategy in the management of alcoholic cirrhotics with ascites and

Table 1 Studies on therapy of alcoholic hepatitis with oral or enteral nutritional supplements

Author	Design	Patients (n)	Duration (days)	Experimental treatment (EXP)	Control Treatment (CTR)	Mortality	Secondary endpoints
Galambos <i>et al.</i> , 1979	Open label	16	16–42	Oral (standard hospital diet) or intravenous supplement (51.6–77.4 g protein)	None	Not assessed	Nitrogen balance + albumin improved in EXP, CTR not assessed improvement of albumin. Transferrin. RBP
Mendenhall <i>et al.</i> , 1985	Historical controls	57	30	Standard hospital diet (2500 kcal day ⁻¹) + 2200 kcal day ⁻¹ BCAA	Standard hospital diet	NS	Positive nitrogen balance in EXP, delayed hypersensitivity improved
Calvey <i>et al.</i> , 1985	Randomized, controlled	64	21	Standard diet (~2000 kcal day ⁻¹) + 65 g standard AA or BCAA	Standard diet, 80 g protein day ⁻¹	NS	Positive nitrogen balance in EXP, delayed hypersensitivity improved
Soberon <i>et al.</i> , 1987	Crossover	14	6	Nasoduodenal tube, 35 kcal kg ⁻¹ day ⁻¹ , fat/carbohydrate/protein 45/40/15%	3 days standard hospital diet (35 kcal kg ⁻¹ day ⁻¹)	0/6 contrs. 3/8 treatm	Nitrogen balance improved fivefold at 2 weeks
Cabre <i>et al.</i> , 2000	Randomized, controlled	71	28	Nasogastric tube, 2000 kcal day ⁻¹ , 72 g protein day ⁻¹ , 31% BCAA	Standard diet (1 g protein per kg) + 40 mg day ⁻¹ prednisolone	11/35 TEN 9/36 PRED NS FU: 2/24 TEN 10/27 (<i>P</i> = 0.04)	No dropouts in PRED, 8 dropouts in TEN; equal improvements of albumin, Child score, Maddrey score; equal rate of infections

Abbreviations are as follows: AA, amino acids; BCAA, branched-chain amino acids; FU, follow-up; NS, not significant; PRED, prednisolone group; TEN, total enteral nutrition group.

Table 2 Studies on treating alcoholic cirrhosis with oral and enteral nutritional therapy

Author	Design	Patients (n)	Duration (days)	Experimental treatment (EXP)	Control treatment (CTR)	Mortality	Secondary endpoints
Smith <i>et al.</i> , 1982	Open label	10	10–60	Three different formulae: oral 76–143 g protein, 2000–3716 kcal day ⁻¹	None	None	Positive nitrogen balance, improved albumin, transferrin, creatinine/height, midarm muscle fat areas
Keohane <i>et al.</i> , 1983	Open label	10	3–23	Oral BCAA formula 80 g protein day ⁻¹ through nasogastric tube	None	1 death (HRF)	Positive nitrogen balance, improved albumin
McGhee <i>et al.</i> , 1983	Randomized, double-blind, crossover	4	11	20 g casein + 30 g BCAA formula	50 g casein day ⁻¹	None	EXP equal to CTR Positive nitrogen balance
Christie <i>et al.</i> , 1985	Randomized, double-blind, crossover	8	12	BCAA (50%) formula	Standard diet (18% BCAA)	1 death (infection)	EXP equal to CTR Positive nitrogen balance
Okita <i>et al.</i> , 1985	Open label	10	4	40 g protein + 40 g BCAA formula day ⁻¹	2100 kcal day ⁻¹ 80 g protein day ⁻¹	None	EXP equal to CTR Positive nitrogen balance
Bunout <i>et al.</i> , 1989	Randomized, controlled	36	28	50 kcal kg ⁻¹ , 1.5 g protein day ⁻¹	Standard diet	EXP 2/17 CTR 5/19 (NS)	No differences
Cabre <i>et al.</i> , 1990	Randomized, controlled	35 (23 alc.)	23–35	211 kcal day ⁻¹ including 71 g BCAA formula	Standard diet	Improved (<i>P</i> = 0.02)	Child score improved, albumin improved
Marchesini <i>et al.</i> , 1990	Randomized	64	90	Standard diet + BCAA supplement (0.24 g kg ⁻¹)	Standard diet + casein supplement	None	Nitrogen balance improved in both. BCAA better than Standard diet
Kerans <i>et al.</i> , 1992	Randomized	31	28	Casein supplement (1.5 g protein day ⁻¹ , 40 kcal day ⁻¹ kg ⁻¹ day ⁻¹)	Standard diet	NS	Both groups improved nitrogen balance and albumin
Hirsch <i>et al.</i> , 1993	Randomized, controlled	51	12 (months)	Standard diet + casein supplement (1000 kcal day ⁻¹ , 34 g protein day ⁻¹)	Standard diet	EXP 3/26 CTR 6/25 (NS)	Fewer hospitalizations, improved albumin and visceral protein

(Continued)

Table 2 Continued

Author	Design	Patients (n)	Duration (days)	Experimental treatment (EXP)	Control treatment (CTR)	Mortality	Secondary endpoints
Nielsen <i>et al.</i> , 1995	Open label	15	38	Increasing amounts of protein via standard diet (1.0–1.8 g kg ⁻¹ day ⁻¹)	None	None	Increased protein retention through gradual or protein intake
Campillo <i>et al.</i> , 1995	Open label	26	30	Standard diet	None	None	Anthropometric ratios improved
Hirsch <i>et al.</i> , 1992	Open label	31	6 (months)	Standard diet + casein supplement. (1000 kcal day ⁻¹ , 34 g protein day ⁻¹)	None	6 deaths/31	Increased albumin, improved cellular immunity

Abbreviations used are as follows: BCAA, branched-chain amino acid; CTR, control group; EXP, experimental group; HRF, hepatorenal failure; NS, not significant.

edema is to restrict fluids to $1\text{--}1.5\text{ l day}^{-1}$ and to restrict sodium as well.

Nonalcoholic Fatty Liver Disease (NAFLD) and Nonalcoholic Steatohepatitis (NASH)

NAFLD and NASH have become very important causes of liver disease in both children and adults, particularly because obesity is now being diagnosed in epidemic proportions in both age groups and both liver disorders are most commonly associated with obesity. Children with NAFLD may present before their fifth birthday, the disorder is more common in males, hepatic fibrosis is common, and may even evolve into cirrhosis during childhood. Treatment consists of weight reduction and aerobic exercise. Vitamin E may be beneficial.

In adults NASH and NAFLD have been recognized for at least a quarter century as chronic liver diseases associated with obesity (with or without noninsulin-dependent diabetes mellitus and with or without hyperlipidemia.) NAFLD may account for as much as 80% of cases of elevated liver enzymes in the US. Most adults with the disorders are 110–130% above ideal body weight. The prognosis of NAFLD is good if weight reduction is achieved. NASH is usually slowly progressive but can lead to cirrhosis and the need for liver transplantation in the minority of individuals affected.

In many patients, NAFLD is a component of the insulin-resistance syndrome known as metabolic syndrome, which is characterized by central obesity, hypertension, hypertriglyceridemia, low levels of high-density lipoprotein-cholesterol, and hyperglycemia. In patients with this syndrome, it is hypothesized that there is greater insulin resistance in muscles and adipose tissue than liver. Those with the BMI class $>30\text{ kgm}^{-2}$ have an increased prevalence of each of the five components of the metabolic syndrome. Patients with NASH exhibited a higher intake of saturated fatty acids, total fat, and cholesterol, and a lower intake of polyunsaturated fat, fiber, and the antioxidant vitamins C and E. These findings provide a strong rationale for specific dietary modifications in NASH patients. The concept of low glycemic index diet could be introduced to these patients and theoretically correct metabolic syndrome. A daily deficit of 500–1000 calories and approximately 150 min per week of aerobic exercise is recommended. Approximately 10% weight reduction from a baseline of approximately 1–2 pounds a week is generally the successful principle of weight management. Primary care physicians should screen this condition in high-risk populations and implement the above therapy along with behavior modification programs. Other therapies including weight-loss medications; protein-sparing modified fasting; and weight-loss surgeries in those who have morbid obesity require a referral to specialists.

Viral Hepatitis

Patients with acute viral hepatitis are not usually at risk for nutritional deficiencies except they experience anorexia and cholestasis resulting in a brief period of malabsorption. Patients chronically infected with hepatitis B and C viruses (HBV, HCV) may develop cirrhosis over 10–20 years with an increased complication of hepatocellular carcinoma. Patients

with chronic viral infection with significant alcohol intake, insulin resistance, obesity, cholestasis, cirrhosis, and cancer require additional nutritional assessment as mentioned in other sections of this article. Hepatic steatosis, obesity, and alcohol consumption are risk factors for antiviral treatment failure to achieve virologic response. Antiviral treatment for HBV and HCV with interferons could cause anorexia and further nutritional deficiency. HIV patients who have chronic HBV and HCV co-infection are expected to acquire more nutrient deficits.

Autoimmune Hepatitis

Autoimmune hepatitis most frequently presents itself in both children and adults of both genders with a female preponderance. The nutritional assessment and therapy are not different from those of other hepatitis. The disease can be accompanied by intestinal diseases such as inflammatory bowel disease or celiac disease, and the nutritional management should take both organ systems into account. Although mild liver function abnormalities are common in celiac disease, there are recent reports of celiac disease in patients with severe liver disease, all of whom demonstrated an improvement in their liver disease with introduction of a gluten-free diet.

Hepatobiliary Disorders

Neonatal and Infantile Cholestatic Disorders

The major differential diagnoses of conjugated hyperbilirubinemia in the first 30 days of life is extrahepatic biliary atresia (EHBA), infectious neonatal hepatitis from viruses, bacteria, and parasites as well as the neonatal hepatitis syndrome, for which a large number of specific genetic disorders have now been identified. These include alpha-1-antitrypsin deficiency, progressive familial intrahepatic cholestasis (PFIC I–III), inborn errors of bile salt synthesis, cystic fibrosis-liver disease, Alagille Syndrome (ALGS), hypothyroidism, panhypopituitarism, and other neonatal cholestatic syndromes. Cholestasis and malabsorptive complications usually resolve when the specific treatment is applied in a timely manner; however children with PFIC and ALGS tend to suffer from cholestasis and malnutrition without specific therapy such as biliary diversion (in some children with PFIC I and ALGS) and liver transplantation. Despite hepatopuertoenterostomy which, if performed before 60 days of age may at least delay disease progression, these infants often develop malabsorption of fat and fat-soluble vitamins secondary to decreased bile flow and fat emulsification (Figure 2). Aggressive formula feeding in early infancy period may be needed via nasogastric tube feeding with a high medium chain triglyceride (MCT) containing infant formula. In other biliary obstructive conditions such as choledochal cyst, bile duct stricture, choledocholithiasis, and tumors or masses (intrinsic and extrinsic) in hepatobiliary regions, a surgical intervention may completely reverse cholestasis and nutrient malabsorption. The nutritional consequences are much the same for all – steatorrhea and malabsorption of the fat-soluble vitamins. Nutritional Management is also much the same for all: use of a hydrolysate or elemental formula rich in MCT and supplementation

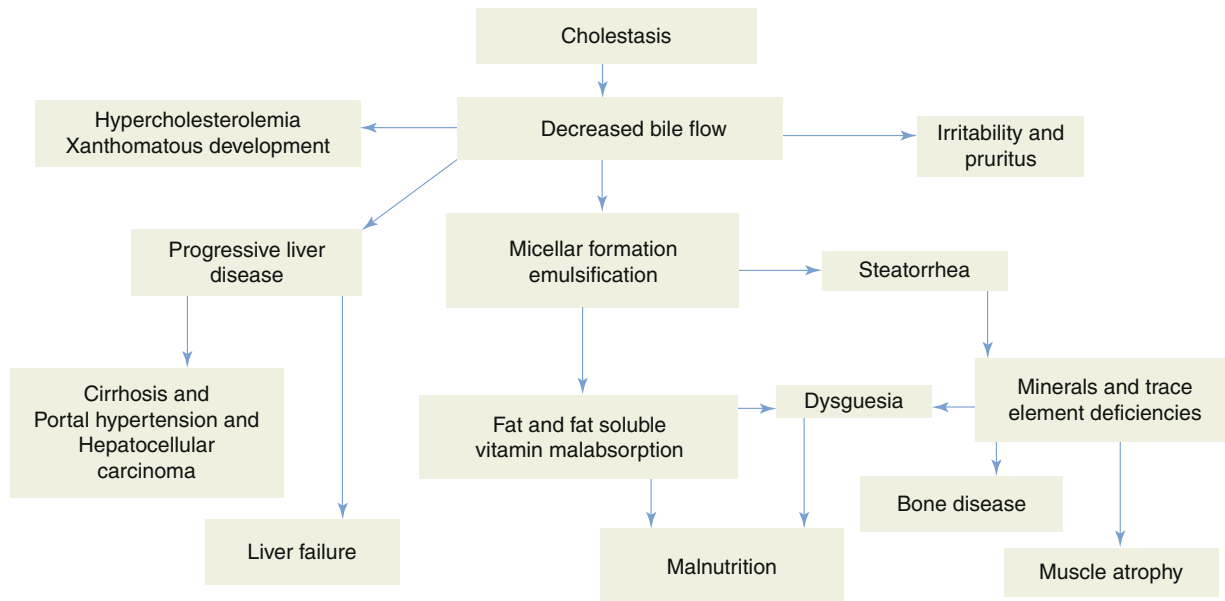


Figure 2 Altered physiology of liver and bile flow (cholestasis) leads to nutrient malabsorption, malnutrition, and progressive liver disease.

with vitamins A, D, E, and K, and possibly with additional micronutrients such as zinc, iron, calcium. Water-miscible vitamin E is poorly absorbed; administration of vitamin E solubilized in polyethylene glycol succinate is a more effective way to administer vitamin E to cholestatic infants. Simultaneous administration of other fat-soluble vitamins with this form of vitamin E is expected to improve them as well. Ursodeoxycholic acid (UDCA) is the major bile acid from the black bear and has been used in traditional Asian medicine for centuries for the treatment of hepatobiliary diseases. Synthetic UDCA has been used effectively in children and adults with cholestasis to improve bile flow and nutrient malabsorption.

Parenteral Nutrition (PN)-associated Liver Disease

Premature infants and children with short bowel syndrome are particularly prone to develop this disorder and in the pediatric age group, PN-associated liver disease is usually cholestatic. The cholestasis can be solely intrahepatic or can be associated with cholelithiasis. PN-associated liver disease can be seen at any age and with any disease etiology resulting in long-term dependence on parenteral nutrition; in older children and adults steatosis is more common as an initial presentation rather than cholestasis. Potential pathogenetic mechanisms include the gastrointestinal dysfunction associated with the lack of enteral nutrients as well as components of the parenteral nutritional solutions as potential hepatotoxins including amino acids, glucose, lipids (particularly peroxidizable lipids), and photo-exposed multi-vitamins. The most effective management is aggressive administration of enteral nutrients and decrease or discontinuation of parenteral nutrition as early as possible. Providing intravenous fat as fish oil, as well as limiting the overall amount of intravenous lipid may also help in minimizing liver damage seen in association with PN.

Cholestatic Diseases in Adults

Primary sclerosing cholangitis most commonly presents itself in association with ulcerative colitis, less commonly with Crohn's disease, or as an isolated entity. The nutritional management of the disorder is essentially like that of other cholestatic disorders; in patients with Crohn's disease of the small bowel, aggressive administration of an elemental diet rich in medium chain triglycerides may be beneficial. Primary biliary cirrhosis (PBC), a disease generally presenting itself in young female adults PBC results in steatorrhea and malabsorption of the fat-soluble vitamins (Figure 2). Osteoporosis and osteopenia are common. Other cholestatic diseases include common bile duct obstruction secondary to stone, stricture, parasitic infestation, and pancreatobiliary tumors or cancers. It is accepted however that endoscopic interventions should be used as needed in the case of significant biliary obstruction. For prevention of severe osteoporosis supplementation with vitamin D and calcium are needed. Vitamin K and alendronate may be beneficial in increasing bone mineral density. Serum levels of the fat-soluble vitamins should be monitored in high-risk patients and vitamins replaced as appropriate.

Metabolic Liver Disorders

Metabolic diseases or inborn error diseases of metabolism mostly are an autosomal recessive disorder which has an enzyme deficiency (Table 3). In general, a restriction of the responsible substances which cause abnormal metabolic pathways and noxious intermediates will diminish organ damages such as galactosemia, hereditary fructose intolerance, tyrosinemia type 1, and urea cycle defects. Liver function improves by galactose and lactose elimination in individuals with galactosemia. Despite restriction, long-term

Table 3 Summary of metabolic liver diseases, management, clinical and laboratory monitoring

<i>Metabolic disorders</i>	<i>Metabolic defects</i>	<i>Management</i>	<i>Laboratory and clinical monitoring</i>
Galactosemia	Galactose-1-phosphate, toxic metabolite accumulates in liver and other organs	<ul style="list-style-type: none"> ● Elimination of galactose and lactose (mainly dairy products and breast milk). ● Appropriate commercial infant formulas (soy formula, lactose-free formulas) for infants. ● For complete elimination grains, fruits, and vegetables contain galactose such as American persimmon, papaya, tomato, watermelon, etc. ● Calcium and vitamin D supplementation possible. ● Breast milk and sucrose-free formulas for infants. ● Restriction of fructose, sucrose, and sorbitol. ● Only certain vegetables are permitted. ● Commercial products, medication, and toothpaste may contain small amounts of sucrose. 	<ul style="list-style-type: none"> ● Serum glucose for hypoglycemia as a complication in acute phase. ● Urine reducing substance, serum galactose for compliant issues. ● IQ test. ● Infertility from ovarian failure. ● Bone mineral density. ● Eye exam for cataracts.
Hereditary fructose intolerance	Accumulation of fructose-1-phosphate in the liver caused by aldolase B deficiency. This substance is a competitive inhibitor of phosphorylase which regulates the conversion of glycogen to glucose	<ul style="list-style-type: none"> ● Restriction of fructose, sucrose, and sorbitol. ● Only certain vegetables are permitted. ● Commercial products, medication, and toothpaste may contain small amounts of sucrose. 	<ul style="list-style-type: none"> ● Monitor growth, liver span, and intellectual development. ● No biochemical method for monitoring fructose restriction.
Glycogen storage disease (GSD) (I, III, IV, VI, and IX)	Correct hypoglycemia (less in GSD IV), lactic acidosis, hyperuricemia (in type I), and hyperlipidemia	<ul style="list-style-type: none"> ● Continuous nocturnal nasogastric/gastrostomy tube feeding with formula in infants. ● Uncooked corn starch ($1-2 \text{ g kg}^{-1}$ every 4 h) in older children and adults. ● High protein diet ($3 \text{ mg kg}^{-1} \text{ day}^{-1}$) in GSD type III only. 	<ul style="list-style-type: none"> ● Monitor growth, liver span, and intellectual development. ● To maintain normal serum glucose, lipid profile, and especially lactic acid and uric acid in GSD I. ● Benign and malignant hepatic adenoma in GSD I and III.
Hepatorenal tyrosinemia I	Defects of tyrosine metabolism	<ul style="list-style-type: none"> ● NTBC in association with dietary treatment. ● Restriction of phenylalanine and tyrosine, and methionine (only if prolonged hypermethioninemia is observed). ● Low phenylalanine and tyrosine containing formula. ● Optimal protein diet. 	<ul style="list-style-type: none"> ● To monitor plasma amino acid profile. ● To normalize plasma tyrosine and ensure normal growth.
Urea cycle disorders	Deficiencies of urea cycle enzymes	<ul style="list-style-type: none"> ● Restriction of upstream essential nutrients to prevent intoxication and supplementation of downstream nutrients. ● Arginine substitution in severe ornithine transcarbamylase (OTC) deficiency and carbamyl-phosphate-synthetase (CPS)-I deficiency. ● Avoid catabolism. ● Ammonia removal with sodium benzoate. ● Vitamin supplementation (folate and trace elements). ● Carnitine supplementation if low. ● Sufficient fluid intake. ● Avoid hidden nitrogen, e.g., liquorice. ● Liver transplantation in severe OTC and CPS deficiency. ● Gene therapy (future). 	<ul style="list-style-type: none"> ● Serum ammonia. ● Urine orotate (defect beyond CPS). ● Plasma amino acid. ● Serum electrolytes (hypokalemia from sodium benzoate).

(Continued)

Table 3 Continued

Metabolic disorders	Metabolic defects	Management	Laboratory and clinical monitoring
Hemochromatosis	HFE mutation	<ul style="list-style-type: none"> ● Routine phlebotomy if serum ferritin $> 200 \mu\text{g l}^{-1}$ in female, $> 300 \mu\text{g l}^{-1}$ in male (limited data in children). ● Low-iron diet. ● Avoid vitamin C and medications or vitamin supplements containing iron. 	<ul style="list-style-type: none"> ● Hemoglobin, serum iron, ferritin, and transferrin saturation. ● To maintain ferritin level $< 50 \mu\text{g l}^{-1}$. ● To document hepatic iron depletion by liver biopsy or Magnetic Resonance Imaging (MRI).
Wilson's disease	Copper accumulation secondary to mutations in ATP7B, a copper-binding ATPase	<ul style="list-style-type: none"> ● Copper-restricted diet (copper enriched diet includes chocolate, nuts, legumes, mushrooms, shellfish, and liver). ● Copper chelating agents (trientine, D-penicillamine, and zinc). ● Vitamin E as adjuvant therapy. ● Vitamin B₆ (pyridoxine) 25–50 mg day⁻¹ for individuals using D-penicillamine as a chelator. ● Liver transplantation in severe or fulminant liver disease. 	<ul style="list-style-type: none"> ● Total serum copper, ceruloplasmin, urinary copper excretion. ● Serial eye exam in individuals with Kayser–Fleisher rings before therapy.
Hepatic porphyrias (acute intermittent porphyria)	HMB-synthase deficiency	<ul style="list-style-type: none"> ● Carbohydrate loading with intravenous glucose. ● Avoid certain drugs (enzyme inducers), stress, fasting, menstruation, and alcohol. ● Hematin. 	<ul style="list-style-type: none"> ● To monitor peripheral neuropathy. ● Long-term risk for hypertension, renal insufficiency, and hepatocellular carcinoma.

complications such as mental disability, speech defects, ovarian failure, and neurologic syndromes as a result of endogenous production of galactose or minimal exposure to nondairy galactose-containing dietary sources. Some patients with galactosemia could tolerate galactose later in life, and diet liberalization should be strictly discussed with their physicians and dietitians. Asymptomatic heterozygous mothers should be recommended to avoid dietary galactose during subsequent pregnancies. Infants with tyrosinemia type 1 not only require instant restriction of phenylalanine and tyrosine but also titration of protein intake and avoidance of tissue catabolism. Urea cycle disorders present varying degrees of hyperammonemia. In the neonatal period, these disorders present dramatically, with somnolence, poor feeding, vomiting, lethargy, seizures, and even hyperammonemic coma. In older children and adults, the presentation may be more subtle and begin with chronic vomiting, developmental delay, seizures, psychiatric illness, postpartum decompensation, and hyperammonemia associated with valproate therapy, protein overconsumption, or increased catabolism. In hepatic porphyrias, patients usually present neurologic, cutaneous, and gastrointestinal symptoms (especially acute intermittent porphyria) and mild elevation of transaminases.

Pregnancy and Liver Disease

Liver diseases that predominantly affect females, such as PBC and autoimmune hepatitis, decrease the chances of conception and demand that pregnant women with these disorders should be managed in high-risk obstetric facilities. During pregnancy liver size and histology do not alter. The development of esophageal varices possibly arises in pregnant women with chronic liver disease as a result of an enlarging uterus creating an increase in venous return from the inferior vena cava to the azygous system. Owing to estrogen effects, the significant increase in the serum concentration of triglycerides, low-density and very-low-density lipoproteins, and cholesterol may be twice the normal limit for nonpregnant women of the same age. A slight reduction in serum albumin contributes to the approximate 20% decline in total serum protein concentration. Liver diseases which can evolve as a consequence of pregnancy include intrahepatic cholestasis of pregnancy, acute fatty liver of pregnancy, and hemolysis, elevated liver enzymes, and low platelets syndrome (HELLP).

Acute Liver Failure

The nutritional status of someone with acute liver failure versus chronic liver failure can differ greatly. The primary goal of the nutritional management in acute liver failure is supportive. An increase in nausea, vomiting, and anorexia may be associated with acute liver disease which may result in a decreased oral intake. If normal nutritional status before the insult is assumed, the patient will have a much higher nutritional reserve than that of a patient in chronic liver failure. Energy needs can be maintained by providing the Dietary Reference Intake (DRI) for infants and children; and approximately 30 kcal kg⁻¹ for adults. Fluid restriction to

70–90% of maintenance may be required if encephalopathy or cerebral edema is present. Hypoglycemia results from impaired gluconeogenesis and depleted glycogen stores. If fluids are provided intravenously, the glucose infusion rate (GIR) may need to be increased with close monitoring of blood sugar levels.

The provision of adequate protein becomes crucial in fulminant hepatic failure and encephalopathy. Adequate protein must be provided to minimize catabolism which could exacerbate any hyperammonemia present. Excessive protein intake should be avoided as it could increase ammonia levels. Protein recommendations for adults and teenagers are 0.5–1.0 g kg⁻¹ day⁻¹; for infants and children 1.2–1.5 g kg⁻¹ day⁻¹. Additional protein restrictions or an increase in the intake of branched-chain amino acids intake may be beneficial. In health, the ratio of branched-chain amino acids/aromatic amino acids (leucine + isoleucine + valine/phenylalanine + tyrosine) = ~3:1 and in liver failure the ratio may decline to ~1, often in association with some degree of hepatic encephalopathy. There is some data to say that normalization of this ratio by administration of branched-chain amino acid formulae can improve hepatic encephalopathy.

Chronic Liver Disease

Chronic liver disease is often accompanied by nutritional deficiencies. The goals of nutritional management are to provide adequate energy and protein to prevent energy deficits and protein catabolism and to promote hepatic cell growth. Recommendations for nutritional management of children with chronic liver disease are presented in Figure 3. Energy needs for adults with chronic liver disease are 30–35 kcal kg⁻¹ day⁻¹. Energy requirements are increased to compensate for the weight loss that often occurs in cirrhosis. Protein should be provided as 0.8–1 g kg⁻¹ for adults and the DRI for protein should be provided for infants and children; unnecessary protein restriction should be avoided as it may only worsen total body protein losses. Energy from fat is best delivered as MCTs due to the frequency of long chain fat malabsorption. Several infant, pediatric, and adult formulas are available with a large percentage of fat in the form of MCTs.

Supplementation with fat-soluble vitamins (A, D, E, and K) in water-miscible solutions is necessary due to the potential for deficiencies associated with fat malabsorption. Serum levels should be monitored regularly to ensure appropriate levels and prevent toxicity. Supplementation with zinc, selenium, iron, calcium should be given as needed. Copper and manganese should not be supplemented as they are excreted via the bile, and may build up to toxic levels. Sodium and fluid restrictions may be necessary in cirrhosis characterized with ascites and edema. This can impose difficulty as this restriction decreases the palatability of the diet, further decreasing oral intake.

End Stage Liver Disease in Pre- and Post-Liver Transplantation

Malnutrition is commonly seen in both alcoholic and non-alcoholic liver disease. The prevalence of malnutrition in

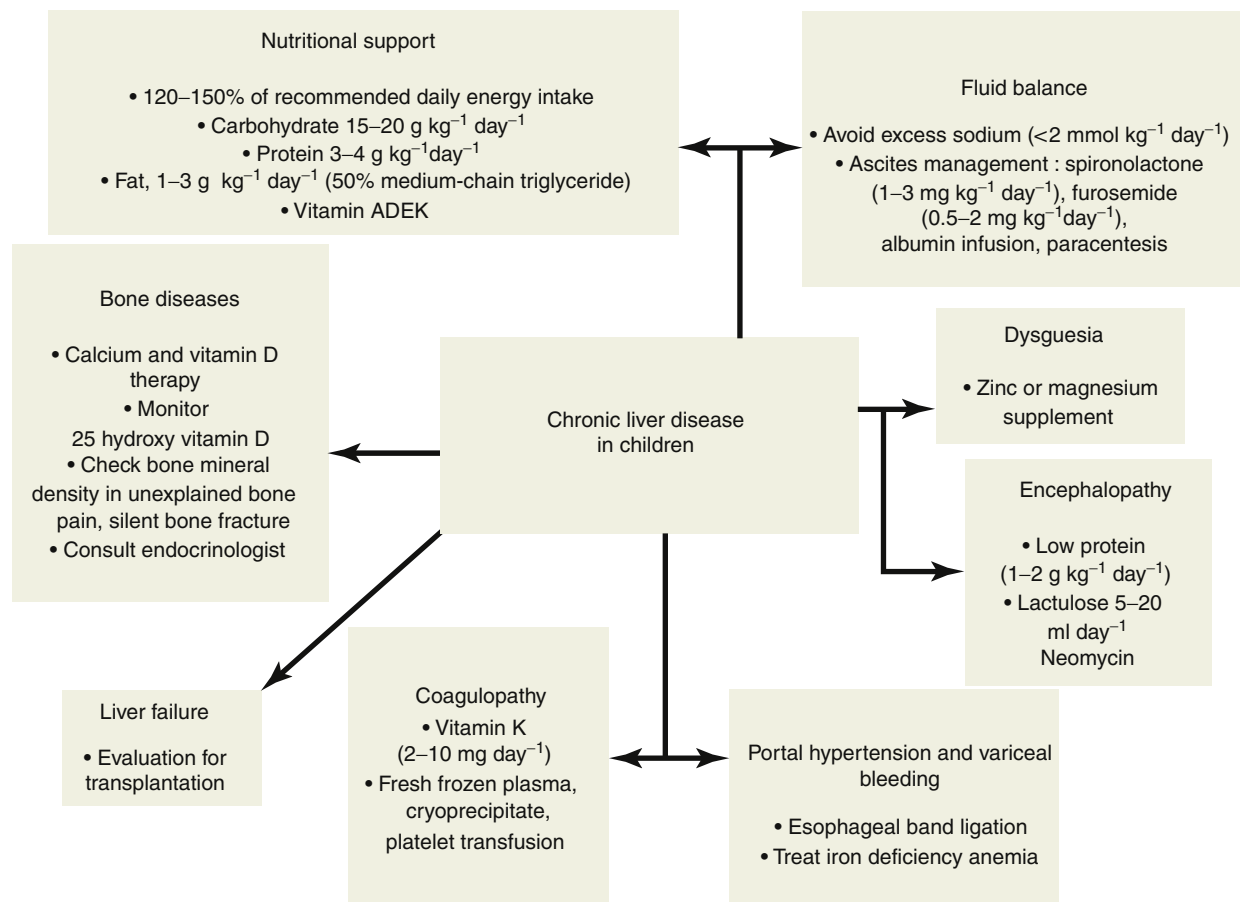


Figure 3 Management in children with chronic liver disease.

cirrhosis is as high as 65–90% and malnutrition is predictive of survival in patients with liver cirrhosis. The severity of malnutrition correlated with that of the liver disease and the development of serious complications such as hepatic encephalopathy, ascites, and hepatorenal syndrome. A variety of mechanisms are considered to contribute to malnutrition in cirrhosis (Table 4). Maintaining optimal nutritional status is important in the patient with end stage liver disease both pre- and post-transplant. However, nutritional assessment in end stage liver disease is particularly problematic. In the pre transplant setting, fluid retention, ascites, and hepatosplenomegaly makes body weight an unreliable nutritional index. True decreases in body weight, due to loss of fat stores and lean body mass may not be fully appreciated in solely following weight trends. In the pediatric population, linear growth is often a better indicator of nutritional status. Chronic malnutrition is often present as is reflected in a decrease in linear growth velocity.

Although anthropometric measurements, twenty-four hour creatinine, bioelectric impedance analysis, and indirect calorimetry have all been used, they are all affected by ascites and peripheral edema. *In vivo* neutron activation analysis and isotope dilution techniques are more accurate ways of assessing body composition but are time-consuming and costly. For practical purposes the indirect assessments of 24 h urinary creatinine excretion to assess body muscle mass and mid-arm

muscle area can be used for patients without high volumes of extracellular fluid; in those with ascites the creatinine-height index is a better way of assessing body muscle mass.

Visceral proteins, including albumin, transferrin, pre-albumin, and retinol binding protein are typically used in monitoring nutritional status due to the decrease seen in inadequate dietary protein intake, but should be used with caution in liver disease as the synthesis of these proteins also decreased in end stage liver disease. Serum levels of fat-soluble vitamins should be monitored closely as well.

Improving nutritional status before a transplant is imperative because malnutrition affects morbidity and mortality post transplant. Although the degree of malnutrition may not be able to be reversed, aggressive nutritional support should be implemented to prevent further worsening of the nutritional state and possibly reduce pre- and post-transplant infection and complications. Reduced protein intakes, lactulose use, use of vegetable protein diets, zinc supplementation, and branched chain amino acid (BCAA)-enriched enteral supplements have been reported to reduce subclinical hepatic encephalopathy confirmed by psychometric test. Although treatment with bisphosphonates is recommended in those with osteoporosis, oral alendronate (not risedronate) may cause esophageal ulcer and could precipitate variceal bleeding.

Posttransplant nutritional support should not be overlooked, as the nutritional deficit is not cured merely by the

Table 4 Factors and mechanisms that results in malnutrition in end stage liver diseases and treatment of the causes of malnutrition

<i>Factors that results in malnutrition in end stage liver diseases</i>	<i>Causes</i>	<i>Management</i>
Decreased dietary intake	<ul style="list-style-type: none"> ● Starvation. ● Nil per os (NPO) status with frequent admissions to hospital. 	<ul style="list-style-type: none"> ● Aggressive nutritional therapy. ● Avoid NPO status unnecessary.
Poor dietary intake from nausea and early satiety	<ul style="list-style-type: none"> ● Gastroesophageal reflux disease. ● Gastroparesis. ● Tense ascites. ● Small bowel dysmotility. ● Small bowel bacterial overgrowth (SBBO). 	<ul style="list-style-type: none"> ● Appetite stimulant. ● Prokinetic use. ● Appropriate diuretic dosages. ● SBBO treatment and prophylaxis with antibiotic (s).
A distortion or decrease in taste sensation (dysgeusia)	<ul style="list-style-type: none"> ● Sodium restriction to control ascites. ● Trace element deficiencies (zinc, magnesium). 	<ul style="list-style-type: none"> ● Supplement of trace elements.
Malabsorption	<ul style="list-style-type: none"> ● Atrophy of intestinal villi from starvation, prolonged NPO, gastroenteritis, SBBO, neomycin use. ● Reduced bile acid pool from cholestyramine for pruritus. 	<ul style="list-style-type: none"> ● Treat causes.
Increased intestinal protein losses	<ul style="list-style-type: none"> ● Protein losing enteropathy from portal hypertension or hidden intestinal diseases (celiac disease, intestinal lymphangiectasia). 	<ul style="list-style-type: none"> ● Treat causes.
Hypermetabolism	<ul style="list-style-type: none"> ● Increased sympathetic nervous system activity. ● Reduced glycogen storage hyperinsulinism sepsis. 	<ul style="list-style-type: none"> ● Measuring the nitrogen balance. ● Adequate glucose or caloric intake to prevent muscle breakdown. ● Frequent meals. ● Treat infection.

transplant. Additionally, the surgery itself poses increased nutritional demand for post surgery healing and support. Nutritional repletion may occur at a more rapid rate than pre-transplant as the patient now has a functional liver in which metabolism and digestion of macro and micronutrients will be improved. Long-chained fat and fat-soluble vitamins therefore should be normally absorbed. Owing to a counter effect of corticosteroid use after liver transplant, growth hormone deficiency should be suspected in persistent growth failure in children despite aggressive nutritional therapy. Recombinant growth hormone therapy successfully treated some of these children without deleterious effect to graft function.

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LOW BIRTH WEIGHT AND PRETERM INFANTS

Contents

Causes, Prevalence, and Prevention

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Causes, Prevalence, and Prevention

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Introduction

It is widely accepted that weight at birth is a key indicator of fetal and neonatal health, both for individuals and populations. The strong association between low birth weight and perinatal mortality and morbidity is well recognized, and there is growing evidence about the different determinants and health consequences of conditions resulting in low birth weight. Knowledge of these epidemiological associations progressively increased over the course of the last 100 years. In the USA, the practice of weighing babies at birth was introduced at the end of the nineteenth century when low-birth-weight babies were categorized as *premature* and usually left unattended with minimal or no intervention attempted to prevent their deaths. This practice and the incorporation of information on birth weight and gestational age into the birth certificate in the mid-twentieth century resulted in irrefutable evidence that *prematurity* was the most significant cause of infant deaths at national level.

Awareness of the importance of low birth weight as a predictor of infant mortality led to the findings suggesting that low birth weight could be due to a restriction of the normal process of fetal growth, to delivery before the full term of gestation, or through a combination of both factors. Based on this evidence, the World Health Organization (WHO) made a distinction between the condition of low birth weight (birth weight less than 2500 g) and prematurity (delivery at less than 37 completed weeks, i.e., 259 days). A further development was the concept of small for gestational age (SGA) that better describes babies affected by intrauterine growth restriction (IUGR). SGA is defined as a birth weight below the 10th percentile for a given gestational age based on a sex-specific reference population. Although these distinctions and definitions are commonly applied in developed countries, their use is more problematic in developing countries where information on gestational age is often nonexistent or unreliable. This data limitation represents a major obstacle to the development of effective prevention and treatment efforts because IUGR and preterm delivery have different determinants and

prognoses, as well as different epidemiological distributions which vary by country and socioeconomic status.

Before discussing the causes, prevalence, and prevention of low birth weight it is important to understand how its two components (gestational age and fetal growth) can be correctly identified and quantified for epidemiological and clinical purposes, and the major methodological limitations in capturing this information.

Assessment of Gestational Age and Fetal Growth. Methods and Limitations

Preterm birth is defined as delivery before 37 completed weeks (259 days). To accurately differentiate between preterm and term delivery it is crucial to have a reliable estimate of gestational age. Sonographic determination is presently the most accurate method to estimate gestational age. When ultrasonography is not available, gestational age can be determined by patient's recall of the time of last menstrual period, physical examination of the size of the uterus, and examination of the neonate. These alternate methods can be used alone or in combination, but are often inaccurate.

Early pregnancy sonographic estimation of gestational age is also crucial for estimation of fetal growth in utero, which is assessed by evaluating the size of several fetal anatomical parameters and comparing those measurements with the normal ranges at specific gestational ages obtained from reference populations with growth that can be considered unaffected by pathological conditions. Alternatively, fetal growth can be assessed by the anthropometrical evaluation of the neonate. Several classification systems have been proposed for newborn birth weight. The simplest categorizes newborns <2500 g as having a low birth weight, but this classification does not enable differentiation between infants born SGA and infants who are small because they are born preterm. A second classification system based on reference charts of birth weight at different gestational ages groups infants into the categories of SGA, adequate for gestational age (AGA), and large for

gestational age (LGA). Because these categories are based on percentile distributions of a reference population, a proportion of normal, constitutionally small, newborns in the lower tail of the normal fetal growth distribution will be miscategorized as growth restricted. The interpretation of the birth weight data using this system is also complicated by inaccuracies in the estimation of gestational age at delivery and by the pathological processes that could affect the size of infants born early in gestation.

Causes

Low birth weight results from either IUGR or preterm delivery, and, in some cases, from a combination of the two. These two conditions are likely caused by various and possibly independent etiopathological factors.

The definitive etiology of preterm delivery has not yet been determined, making it difficult to identify women at risk and to develop and implement effective preventive strategies. Available evidence shows that a complex range of factors such as pregnancy complications, health care practices, and socioeconomic conditions are implicated in preterm births. Preeclampsia, fetal distress, fetal growth restriction, abruptio placenta, fetal death, placenta previa, and multiple gestations, for example, are all associated with preterm delivery, either spontaneous or induced. Developments in obstetric and neonatal care and the consequent increase in obstetric interventions including infertility treatments have been linked with the increase in the rates of preterm delivery observed in recent years. Psychological stress and other socioeconomic factors such as poor nutrition, cigarette smoking, alcohol and drug abuse, young maternal age, poverty, and short stature have also been found to be possible causes. Genetic factors are likely to be involved in the etiopathogenesis of preterm delivery given that the condition tends to recur in families and that prevalence varies across races. The possible role of infection in triggering preterm delivery has been suggested by several studies, which show associations between preterm delivery and amniotic fluid and chorioamniotic infection, bacterial vaginosis, genitourinary chlamydial infection, and periodontal disease. Despite the biological plausibility of these associations, their causal relationship has not been definitely proved by unequivocal scientific evidence.

Conditions associated with IUGR include but are not limited to fetal infections, congenital malformations, chromosomal abnormalities, chemical teratogenes, vascular disease such as preeclampsia, chronic renal disease, chronic hypoxia, placental and cord abnormalities, and multiple fetuses. Present knowledge of IUGR is limited by the challenges of differentiating between constitutional and environmental determinants of fetal growth. This limitation complicates the investigation of the role of maternal size and genetic factors in IUGR. Small women tend to have smaller babies. There is evidence that intergenerational effects on birth weight are transmitted through the maternal line, suggesting a genetic effect. However, poor maternal nutrition and social deprivation have also been proven to be related to small maternal size and impaired fetal growth. Similarly, the relationship between fetal size and race may be mediated by a

combination of genetic and environmental factors. Carefully designed studies are needed to better determine the contribution of genetic and environmental determinants to the process of fetal growth.

Health Consequences

Low birth weight, either due to preterm delivery or IUGR is associated with poor neonatal health outcomes including higher rates of mortality. Neonatal mortality levels are indirectly associated with gestational age at delivery and birth weight.

Preterm birth is one of the major causes of neonatal mortality and morbidity. Of the estimated 8.795 million deaths in children younger than 5 years worldwide in 2008, 41% (3.575 million) occurred in neonates, and the most important single cause was preterm birth complications (12%, 1.033 million, UR 0.717 million–1.216 million). Mortality rates due to preterm birth are correlated with the overall level of neonatal mortality in a specific country. In low resource countries with high neonatal mortality rates (>45 neonatal deaths per 1000 live births), preterm birth is responsible for approximately 20% of all neonatal deaths with the other 80% attributable mainly to infections and birth asphyxia. In more developed countries, where neonatal mortality rates are below 15 deaths per 1000 live births, preterm birth is the cause of up to 40% of neonatal deaths since deaths due to infection and birth asphyxia are largely prevented. Although the proportion of neonatal deaths attributable to preterm birth is higher in developed countries, the majority of preterm birth related deaths occur in low resource settings because of lack of access to preventive and therapeutic interventions. This discrepancy between rich and poor countries is also reflected in the mortality differentials in late preterm births (between 32 and 37 weeks). Although late preterm infants in developed countries have a survival rate similar to full-term infants, the chance of survival of such infants in low resource settings is minimal.

The negative effects of preterm delivery and IUGR often persist throughout infancy and childhood, impacting the individual child, the entire family, the health care system, and society in general. Studies have also shown a relationship between low birth weight and an increased risk of cardiovascular disease, high blood pressure, obstructive lung disease, diabetes, high cholesterol concentrations and renal damage, indicating that the effects of low birth weight may extend into adulthood.

Epidemiology

Aggregate data on low birth weight rates show marked differences in underlying causes between geographical areas. Most low birth weight infants born in developed countries are due to preterm delivery, whereas a substantial proportion of low birth weight in developing countries is related to IUGR.

The first global and regional estimates of preterm birth rates were recently published. We now know that approximately 13 million newborns are born prematurely every year. This figure represents 9.6% of all births and is a conservative measure as it is based on estimates of the risk in normal

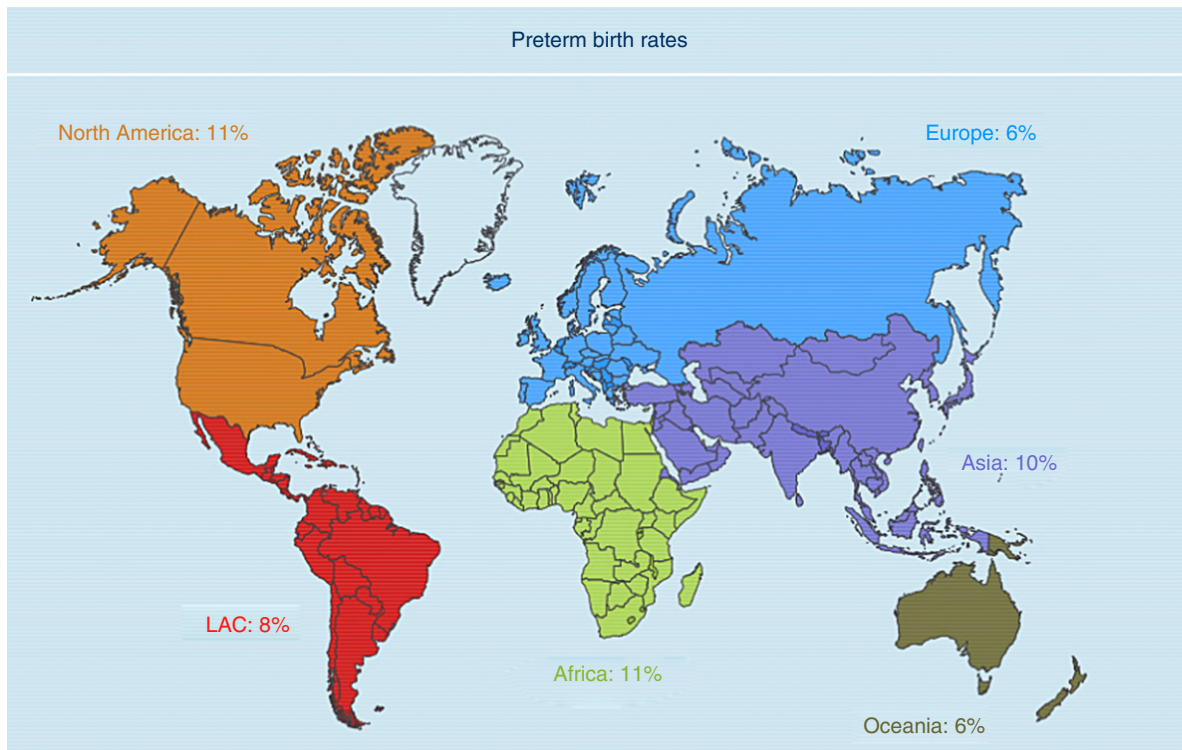


Figure 1 Preterm birthrates.

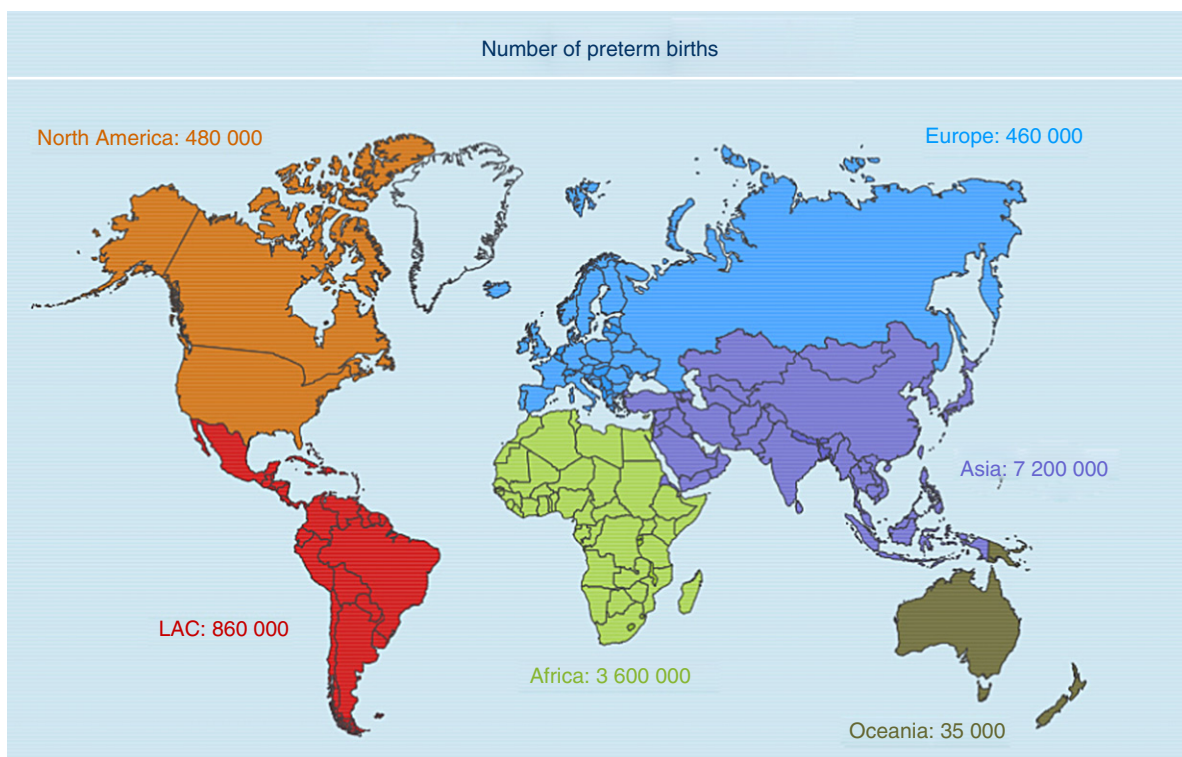


Figure 2 Number of preterm births.

pregnancies. **Figure 1** shows the estimated rates in the six major regions of the world. The highest rates are in North America and Africa. The greatest absolute numbers of preterm births (**Figure 2**) occur in Africa and Asia, the two world regions with the highest number of births and fertility rates.

These global and regional estimates mask important disparities between and within countries. In the US, for example, African and American women are more than twice as likely to deliver preterm than Caucasian women, a difference that accounts for a major proportion of the variation in infant mortality between the two racial groups.

Preterm birth rates are increasing in many developed countries. Although the causes of this trend are not fully elucidated, changes in clinical practices such as increased use of assisted reproductive techniques, and the rising rates of cesarean sections and induced deliveries have been associated with increases in preterm deliveries.

Prevention

Results from clinical trials provide the most powerful scientific evidence to guide policy and program development and implementation. Interventions aimed at preventing low birth weight targeted at preterm delivery and IUGR have not proven to be effective by randomized clinical trials. The multicausal nature of these conditions is likely responsible for single interventions not showing an effect of enough magnitude to be detected by medium sized clinical trials. Thus appropriate combinations of interventions should be a priority for evaluation in the context of large, methodologically sound trials. Available evidence shows that some interventions may be effective and their combined implementation may have a significant public health impact. Interventions likely to be beneficial to prevent IUGR are smoking cessation, antimalarial chemoprophylaxis in primigravide women, and balanced protein energy supplementation. Treatment of urinary tract infection, placement of circumferential stitches on a structurally weak uterine cervix (cerclage), and treatment of bacterial vaginosis in high-risk women have been shown to be effective in preventing preterm birth. These interventions are applicable only to a small number of high risk women and their overall effect on the general population is likely to be limited.

In the next paragraphs, nutritional interventions to prevent preterm delivery and IUGR will be reviewed with the aim of identifying potentially effective interventions and suggesting possible mechanisms that may explain the link between maternal nutritional status and low birth weight. The focus will be on the review of randomized clinical trials which provide the most unbiased epidemiological evidence on the effectiveness of interventions. Clinical trials testing the same or similar interventions can be pooled together to estimate an overall effect by means of systematic reviews of published and unpublished studies and meta-analysis.

Nutritional Interventions to Prevent Preterm Delivery

Of the nutritional interventions during pregnancy that have been tested by clinical trials to prevent preterm delivery, only

calcium and fish oil supplementation appear promising. Nutritional counseling and magnesium supplementation are likely to be effective, but methodological limitations in the analysis of the clinical trial results means definitive conclusions cannot yet be made. Most of the other interventions hypothesized to potentially prevent preterm delivery such as protein and energy supplementation, protein and energy restriction, salt restriction, iron or folate supplementation, zinc supplementation and vitamin A supplementation have not been proven effective.

Nutritional Interventions to Prevent IUGR

Of the interventions tested through randomized clinical trials to prevent IUGR, balanced energy protein supplementation has been shown to reduce the risk of SGA by approximately 30%. On the basis of these results it has been proposed that universal balanced energy supplementation should be provided to women in areas with high prevalence of maternal undernutrition to prevent impaired fetal growth. There is some evidence that magnesium and calcium supplementation may be effective. For calcium supplementation, the evidence is still not clear whether the observed effect on reducing the risk of low birth weight is due to a direct effect on fetal growth or mediated by a prolongation of gestational age at delivery. Other interventions such as nutritional counseling, energy protein restriction, salt restriction, iron or folate supplementation, fish oil supplementation, zinc supplementation, Vitamins E, C, and D supplementation have not shown any preventive effect. High protein supplementation in women of low socioeconomic status in the USA has been associated with an increase in the rate of SGA infants, suggesting that nutritional supplementation may, in some cases, have potentially harmful effects. This finding warrants further investigation.

Conclusions

Low birth weight, due to preterm delivery or IUGR, represents a major public health problem for developing and developed countries. Access to adequate obstetric and neonatal care has been shown to reduce the mortality and morbidity associated with these two conditions. Public health efforts should aim at improving the quality and availability of such services, particularly in developing countries where the absolute numbers of low birth weight babies is highest and access to needed care is lowest. Among nutritional interventions to prevent low birth weight, only balanced energy protein supplementation has been shown to be effective in reducing the risk of SGA and should be provided to all women living in areas with high prevalence of maternal undernutrition.

Research efforts should focus on elucidating the etiological factors responsible for preterm delivery and IUGR. Despite the considerable burden of disease related to these conditions, very little progress has been achieved in identifying their causes. This information is essential for developing and implementing effective preventive and therapeutic interventions with universal application and that could benefit the most vulnerable populations of women and newborns.

Nutritional Management

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Glossary

Low birth weight infant Infant born weighing less than 2500 g; very low birth weight infants weigh less than 1500 g at birth; extremely low birth weight infants weigh less than 1000 g at birth.

Necrotizing enterocolitis (NEC) Death of intestinal tissue.

Parenteral nutrition Intravenous infusion of nutrients and fluid.

Premature infant Infant born before completing 37 weeks gestation.

Trophic feedings Subnutritional quantities (10–20 ml kg⁻¹ day⁻¹) of enteral feeds given for a predetermined time (3–7 days) before beginning the advancement to full enteral feeds.

Introduction

Advances in perinatal medicine have allowed the survival of premature infants born as young as 21 weeks gestation and as small as 244 g. There is no other time in the lifecycle when nutrition is more critical. These infants enter life with their maternal nutrient supply abruptly disconnected, and have numerous nutritional risk factors. Nutrient stores are accumulated during the third trimester of pregnancy; therefore, preterm infants have minimal energy, protein, fat, vitamin, and mineral reserves. The infant may also be a product of a pregnancy complicated by diminished uterine blood flow, thus further compromising the infant's nutrient stores at birth. In fact, infants with birth weights less than 1000 g have energy reserves of less than 200 kcal kg⁻¹ (836 kJ kg⁻¹). The metabolic rate of the preterm infant is elevated due to the predominance of metabolically active tissue and minimal fat stores. Protein, fat, and glucose needs are very high to provide adequate energy for metabolism, fat deposition, and growth. The preterm infant has excessive evaporative losses and increased urinary losses, which greatly increase fluid needs. The gastrointestinal tract of the preterm infant is very immature with minimal production of enzymes and growth factors, poor gastric emptying, and dis-coordinated peristalsis. To further complicate the provision of nutrients, preterm infants have episodes of metabolic instability, including hypo- and hyperglycemia, poor lipid clearance, and electrolyte disturbances. The preterm infant also has high rates of stressful events, including respiratory distress, hypoxemia, hypercarbia, and sepsis. Lastly, premature infants lack the ability to voluntarily consume and process nutrients; therefore, all of the infant's needs must be provided through parenteral and enteral nutrition support. Moreover, early and aggressive nutrition support and subsequent growth in the early neonatal period have been associated with impacting future developmental and health outcomes.

Growth

Similar to full term infants, preterm and low birth weight infants lose 8–15% of their body weight in the first 4–6 days

of life due to diuresis, and subsequently take approximately 2–3 weeks to regain birth weight. The smallest infants lose the greatest percentage of weight, sometimes up to 20% of their birth weight, and subsequently can take the longest time to return to birth weight. The goal of nutrition support is to provide sufficient nutrients to achieve fetal growth rate. **Table 1** shows the goal growth velocity based on weight. Unfortunately, this goal is rarely achieved as regards growth as well as body composition. Most preterm and low birth weight infants show significant delays in growth due to the inability to provide adequate nutrients, especially in the first few weeks following birth, when the infant is most medically unstable. Over the past several years, improvements in neonatal management and a more aggressive approach to nutrition have accelerated growth, but it still lags behind the fetal growth rate. Growth velocity in the infant is the greatest between 25 and 30 weeks gestation, greater than at 40 weeks. If the infant is undernourished after birth, during this key period of accelerated growth, an increased supply of nutrients may be necessary to achieve catch-up growth to prevent the poor outcomes associated with postnatal growth failure; however, achieving adequate catch-up growth may never be possible. Protein and energy are the key nutrients for growth, but they must be provided in appropriate proportions for the optimal utilization of both. Vitamins, minerals, and electrolytes must also be supplied in adequate amounts and proportions to contribute

Table 1 Expected growth velocity

Weight (g)	Goal weight gain (g kg ⁻¹ day ⁻¹)
500–700	21
700–900	20
900–1200	19
1200–1500	18
1500–1800	15
1800–2200	16

Source: Adapted with permission from Table 1 in Ziegler EE, Thureen PJ, and Carlson SJ (2002) Aggressive nutrition of the very low birthweight infant. *Clinical Perinatology* 29(2): 225–244.

to growth. Preterm infants usually initially require parenteral nutrition (PN) to supply nutrition support, with the gradual transition to a combination of parenteral and enteral nutrition, and finally to full enteral nutrition.

Energy Needs

Energy needs of the preterm infant involve several energy-requiring functions, and are summarized in **Table 2**. Resting metabolic rate accounts for the greatest percentage of energy needs. Resting metabolic rate is equivalent to basal metabolic rate plus some of the energy used for growth; estimates have ranged from 45 to 60 kcal kg⁻¹ day⁻¹ (188–251 kJ kg⁻¹ day⁻¹). The energy cost of activity ranges between 2% and 12% of the total energy expenditure. The smaller, more premature infants are at the lower end of the range whereas the larger, less preterm infant has increased activity and therefore a higher expenditure. Although preterm infants are cared for in a thermoneutral environment within an incubator, there is, nevertheless, energy lost to thermoregulation during nursing care and medical procedures. There may also be energy lost to thermoregulation in a stable growing infant during bathing, feeding, and when weaned to a bassinet. The energy cost of growth includes that needed for tissue synthesis as well as the energy stored in tissues. The estimates for growth needs vary widely depending on the composition of weight gain in the infant. For the enterally fed infant, the thermic effect of food and fecal losses also contribute to total energy need. The estimated calorie and protein intakes required to achieve fetal weight gain are listed in **Table 3**.

Table 2 Energy needs of the growing preterm infant

Energy factor	kcal kg ⁻¹ day ⁻¹	kJ kg ⁻¹ day ⁻¹
Resting metabolic rate	45–60	188–250
Activity	10–15	42–63
Thermoregulation	10	42
Thermic effect of food	8	33
Fecal losses	12	50
Growth	25	105
Total	110–130	460–545

PN

Because the gastrointestinal tract of the preterm infant is immature, substantive enteral nutrition adequate to meet nutritional needs and promote growth is usually not possible immediately following birth, especially in those infants whose birth weights are less than 1500 g; therefore, the preterm infant is dependent on PN, an intravenous infusion of a nutrient and fluid solution, to prevent catabolism, maintain lean body mass, support metabolism, and achieve growth until adequate enteral feeds can be established. PN should definitely be considered in infants whose birth weights are less than 1500 g or gestational age less than 30 weeks. It may also be needed for the infant whose birth weight is between 1500 and 2000 g or gestational age 30–32 weeks, especially if the initiation or progression of enteral feeding is likely to be prolonged.

Historically, PN was delayed for several days after birth due to metabolic instability of the infant and concern for tolerance of the components in the solution. More recently, the early use of PN has been recommended as soon as possible, ideally within 24 h, after birth. This practice minimizes the interruption of nutrient delivery and the catabolism that occurs when only dextrose solutions are infused.

PN can be administered by two different routes. There are both risks and benefits associated with each route. In the early days of PN, it was always infused via an indwelling, surgically placed catheter into a central vein. Because some of the complications with this method were related to the catheter, the use of peripheral veins for infusion became popular and is still employed today. The dextrose concentration of peripheral PN is limited to 10–12.5%, and the osmolality is limited to less than 1000 osmoles per liter; thus, the nutrient intake by this route is somewhat limited without excessive fluid intake. Peripheral PN complications include intravenous line infiltrates, with some infants experiencing serious deep sloughing, sometimes requiring skin grafts. Peripheral lines also require vigilance on the part of nursing to prevent infiltrates, and some infants will have multiple intravenous attempts daily because the line needs to be replaced. The advent of the percutaneously inserted central catheter and its liberal use in the last few years has improved and stabilized the delivery of PN to the preterm infant. Central PN is recommended when it is anticipated that it will be used for greater than 5–7 days, usually in infants weighing less than 1000–1250 g. If the infant tolerates glucose and clears lipids well, it is possible to

Table 3 Estimated calorie and protein intakes to achieve fetal weight gain

Body weight (g)	Energy (kcal kg ⁻¹ day ⁻¹) (parenteral–enteral)	Protein (g kg ⁻¹ day ⁻¹) (parenteral–enteral)	Protein/energy (g 100 kcal ⁻¹)
	(Parenteral–enteral)		
500–700	89–105	3.5–4	3.9–3.8
700–900	92–108	3.5–4	4.1–3.7
900–1200	101–199	3.5–4	3.5–3.4
1200–1500	108–127	3.4–3.9	3.1–3.1
1500–1800	109–128	3.2–3.6	2.9–2.8

Source: Adapted with permission from **Table 1** in Ziegler EE, Thureen PJ, and Carlson SJ (2002) Aggressive nutrition of the very low birthweight infant. *Clinical Perinatology* 29(2): 225–244.

Table 4 Risks and benefits of parenteral nutrition routes

<i>Peripheral</i>	<i>Central</i>
Adequate for short-term use	Recommended with PN required for greater than 5–7 days
Dextrose limited to 10–12.5%	Requires placement of central line/PICC
Can provide 80–85 kcal kg ⁻¹ day ⁻¹ if adequate fluid available	Able to meet estimated needs if adequate fluid available
Possible complications	Possible complications
Intravenous line can infiltrate and cause deep skin sloughing	Sepsis
Requires nursing vigilance to care for intravenous line	Line complications: pleural effusions, pneumothorax
Can require multiple intravenous attempts	

meet estimated nutritional needs using this route. Complications such as pneumothorax, pleural effusions, and increased risk of sepsis are associated with central lines. **Table 4** summarizes a comparison of peripheral PN and central PN.

Components of PN

PN solutions contain dextrose, amino acids, lipids, electrolytes, vitamins, and minerals.

Glucose

Glucose, provided as a dextrose solution, is the predominant energy source in PN. It is the main energy substrate for the fetus as well as the neonate after birth. Preterm infants often require more glucose than the term infant due to the higher brain to body weight ratio and the need for additional energy for central nervous system energy requirements. Measurements of glucose utilization in the preterm infant range from 6 to 10 mg kg⁻¹ min⁻¹ (0.033–0.055 mmol kg⁻¹ min⁻¹). Glycogen stores are very limited in the preterm infant; therefore, they require a large and continuous source of glucose to prevent hypoglycemia. This should be initiated at a rate of 6 mg kg⁻¹ min⁻¹ (0.033 mmol kg⁻¹ min⁻¹) and can be advanced 1–2 mg kg⁻¹ min⁻¹ (0.005–0.011 mmol kg⁻¹ min⁻¹) each day to an optimum of 12–14 mg kg⁻¹ min⁻¹ (0.066–0.78 mmol kg⁻¹ min⁻¹) as long as the infant does not become hyperglycemic. Above this rate, glucose is not used for energy, but rather fat deposition, an inefficient process that can result in increased energy expenditure and carbon dioxide production.

Difficulties with glucose metabolism are a common problem in preterm infants due to decreased energy stores, increased gluconeogenesis due to stress, decreased insulin secretion, and insulin resistance. When hyperglycemia occurs, the glucose infusion rate should be decreased; however, the rate should not be decreased below 4–6 mg kg⁻¹ min⁻¹ (0.022–0.33 mmol kg⁻¹ min⁻¹) as this is the minimum supply rate necessary to provide adequate energy to the brain. Usually, the infusion of amino acids improves glucose tolerance by decreasing glucose production, stimulating insulin secretion, and enhancing insulin action. The use of continuous insulin infusions to treat hyperglycemia is controversial. If used, the insulin infusion should be initiated at a rate of 0.05 units kg⁻¹ h⁻¹ and titrated to achieve and maintain a plasma glucose concentration between 80 and 120 mg dl⁻¹ (4.44–6.66 mmol l⁻¹).

Protein

The early administration of protein in the form of crystalline amino acids to the preterm infant is one of the changes that

have occurred over the last decade. Early studies of amino acid administration in preterm infants in the 1960s and 1970s raised the concern for protein toxicity because these infusions were associated with acidosis, azotemia, and hyperammonemia, thus causing a delay in the routine administration of protein. However, the above conditions were probably the result of the preparations being casein or fibrin hydrolysates and of suboptimal quality. Since the 1980s, crystalline amino acid solutions have been used. In the late 1980s, amino acid solutions specifically for use in infants were designed to produce a plasma amino acid level comparable to that of a postprandial breast-fed infant. TrophAmine is currently the recommended amino acid solution for use with preterm and low birth weight infants because it contains taurine, a semiessential amino acid in premature infants, and has a higher ratio of essential to nonessential amino acids, thus resulting in improved nitrogen balance and protein synthesis. Moreover, TrophAmine has been shown to reduce the incidence and degree of PN-associated cholestasis, and has a lower pH, thus enhancing calcium and phosphorus solubility.

The early administration of amino acids is crucial because studies have shown that the preterm infant suffers protein losses of between 0.6 and 1.2 g kg⁻¹ day⁻¹, or 15% of total body protein, when they should be accumulating 2% daily. A number of studies have demonstrated that the infusion of amino acids along with glucose decreases protein catabolism. As little as 1–1.5 g kg⁻¹ day⁻¹ of amino acids has been shown to prevent negative nitrogen balance. Studies have also shown that the infusion of 3 g kg⁻¹ day⁻¹ within the first 2 days of life resulted in increased protein synthesis, suppressed protein breakdown, and produced plasma aminograms similar to those of the breast-fed infant.

The provision of adequate energy is needed for protein metabolism and deposition. Most infants can achieve positive nitrogen balance at 2 g kg⁻¹ day⁻¹ of protein intake when given 50–60 kcal kg⁻¹ day⁻¹ (209–251 kJ kg⁻¹ day⁻¹) of energy. Additionally, approximately 22 kcal (92 kJ) per gram of protein (15–20% of kcal) results in reasonable amino acid utilization.

Therefore, protein should be started on the first day of life, ideally as soon as the infant is admitted to the NICU and intravenous access is obtained, at 3 g kg⁻¹ day⁻¹ and advanced to 3.5–4 g kg⁻¹ day⁻¹ to achieve *in utero* accretion rates.

Cysteine

The amino acid cysteine is a conditionally essential nutrient in preterm infants because they have low cystathionase activity.

Cystathionase, an enzyme, is necessary to convert methionine to cysteine. This amino acid is unstable in liquid solutions, so commercially available crystalline amino acid solutions do not contain cysteine. Plasma levels of cysteine are low in infants receiving cysteine-free PN. Cysteine hydrochloride is soluble and is stable in aqueous solutions for a short period of time, so 40 mg g⁻¹ protein is often added to PN solutions when prepared. The addition of cysteine may result in acidosis, necessitating an increase in acetate. An additional advantage is that the addition of cysteine decreases the pH of the PN solution, which allows for the addition of more calcium and phosphorus.

Lipids

Lipids are the most concentrated source of calories in the PN solution. They are available as lipid emulsions of soy bean and safflower oil. In all, 20% emulsions are recommended for use in preterm and low birth weight infants because they contain less phospholipid than the 10% emulsion, and phospholipid interferes with the rate of lipid hydrolysis, leading to elevated serum triglycerides. Lipids are critical for central nervous system development. Additionally, when infused with the PN solution, they may also prevent phlebitis. Lipids are infused to prevent essential fatty acid deficiency and as an energy source. Maximum lipid clearance occurs when lipids are infused over 24 h. Starting recommendations vary, but it is generally accepted to start with 1 g kg⁻¹ day⁻¹ within the first 24 h of life and advance to an optimum of 3 g kg⁻¹ day⁻¹; however, studies have demonstrated that it is safe to initiate lipids at 3 g kg⁻¹ day⁻¹. Preterm infants have optimal protein retention when approximately 30–40% of calories are provided as lipids. Plasma triglycerides can be used to monitor lipid clearance. It is generally accepted that levels below 150–200 mg dL⁻¹ indicate adequate clearance. Lipoprotein lipase and hepatic lipase are the major enzymes for clearance of intravenous lipid. These activities are inducible by low-dose heparin, which is usually present in central PN solutions. Administration of heparin should be considered in those infants receiving peripheral PN showing poor lipid clearance. In infants with hypertriglyceridemia, the provision of 0.5–1 g kg⁻¹ day⁻¹ of lipid is adequate to prevent essential fatty acid deficiency and is a dose likely to be tolerated by most infants.

Carnitine

Carnitine is necessary for the transport of long-chain free fatty acids into the inner mitochondrial membrane, and for the oxidation of the fatty acids in the mitochondria. Carnitine is considered a conditionally essential nutrient, because the preterm infant has decreased carnitine synthesis capability and has low plasma and tissue concentrations. Moreover, carnitine is found only in human milk and formula; therefore, deficiencies can develop in 6–10 days after birth in less than 34 weeks' gestation infants without enteral feedings. Studies are conflicting as to whether there is benefit to adding it to PN. Its use should be considered in all less than 34 weeks' gestation infants, those receiving long-term PN without enteral feedings, and those with hypertriglyceridemia at 2.5–20 mg kg⁻¹ day⁻¹.

Electrolytes

Electrolytes are added to the PN solution only on day of life one to two after the infant starts diuresing and losing electrolytes in the urine. Hyper- and hyponatremia within the first 48 h of life usually reflect fluid status and not excess or sub-optimal provision of electrolytes. The electrolyte content of PN solutions is usually similar to that found in normal intravenous solutions: usually 3–4 mmol kg⁻¹ day⁻¹ of sodium and 2–3 mmol kg⁻¹ day⁻¹ of potassium. Very immature infants and those on diuretics may require additional amounts to maintain normal plasma concentrations. Chloride and acetate need to be dosed based on electrolyte levels. The very young preterm infant may need a higher proportion of acetate due to urinary bicarbonate losses. Later, when chronic diuretics are used, a greater proportion of chloride may be needed.

Calcium, Phosphorus, and Magnesium

Calcium and phosphorus are relatively insoluble in solution together, making it difficult to provide adequate levels of these minerals to meet the needs of the preterm infant. When PN solutions are advanced to 10% dextrose and 2 g protein per 100 ml, usually 60–80 mg (1.5–2 mmol) calcium and 40–60 mg (1.3–1.9 mmol) phosphorus can be added to the solution. Because the accretion rate of calcium in the fetus is normally 100 mg kg⁻¹ day⁻¹ (2.5 mmol kg⁻¹ day⁻¹), infants on prolonged PN may develop osteopenia and fractures. The usual dose of magnesium is 0.3–0.5 mEq kg⁻¹ day⁻¹ (0.3–0.5 mmol kg⁻¹ day⁻¹).

Trace Minerals

Zinc and copper deficiencies occurred in some preterm infants before these trace elements were routinely added to PN solutions. There is very little research that defines the parenteral requirements of trace minerals in preterm infants. The current recommendations for trace minerals are summarized in [Table 5](#).

Vitamins

Like trace minerals, the recommendations for intake of vitamins are not based on randomized trials, but are based on the best information available. Infants receiving these parenteral intakes in [Table 6](#) do not develop deficiencies or evidence of excessive intake.

The suggested initiation and advancement of PN in the preterm infant is summarized in [Table 7](#).

Enteral Nutrition

The provision of adequate enteral nutrition is the goal of those caring for the preterm infant. However, a fear of the development of necrotizing enterocolitis (NEC), a serious intestinal disease of preterm infants associated with enteral feedings, has influenced feeding practices. NEC is a major cause of morbidity and mortality in preterm infants. The incidence of this disease is estimated to be between 8% and 10% of preterm infants. The cause of NEC is multifactorial, including enteral feeds, hypoxia, ischemia, patent ductus arteriosus, and infection. Approximately 90% of infants who develop NEC have been enterally fed and several studies have shown that the

Table 5 Suggested parenteral intakes of trace minerals

Trace mineral	$\mu\text{g kg}^{-1} \text{ day}^{-1}$
Zinc	400
Iron	200
Copper	20
Selenium	1.5–2
Manganese	1
Iodide	1
Molybdenum	0.25
Chromium	0.2

Table 6 Suggested parenteral intake of vitamins

Vitamin	Amount (kg day^{-1})
Vitamin A (μg)	280–500
Vitamin E (mg)	2.8
Vitamin K (μg)	100
Vitamin D (IU)	400
Ascorbic acid (mg)	25
Thiamin (μg)	350
Riboflavin (μg)	150
Pyridoxine (μg)	180
Niacin (mg)	6.8
Pantothenate (mg)	2
Biotin (μg)	6
Folate (μg)	56
Vitamin B ₁₂ (μg)	0.3

Note: Total dose should not exceed the amounts provided by 5 ml of reconstituted MVI Pediatric (Armour Pharmaceutical Co., Chicago, IL, USA): 700 μg vitamin A, 7 μg vitamin E, 200 μg vitamin K, 10 μg vitamin D, 80 mg ascorbic acid, 1.2 mg thiamin, 1.4 mg riboflavin, 1.0 mg pyridoxine, 17 mg niacin, 5 mg pantothenic acid, 20 μg biotin, 140 μg folic acid, and 1 μg vitamin B₁₂.

Table 7 Suggested initiation and advancement of parenteral nutrition for the preterm infant

Component	Initial	Advancement per day	Goal
Dextrose ($\text{mg kg}^{-1} \text{ min}^{-1}$)	6–8	1–2	12–14
Protein ($\text{g kg}^{-1} \text{ day}^{-1}$)	2–3	1	3.5–4
Lipids ($\text{g kg}^{-1} \text{ day}^{-1}$)	1	1	3

rapid advancement of enteral feedings is associated with NEC. With the advent of PN, the tendency was to delay enteral feeding for prolonged periods of time in order to prevent this disease and to use PN as the sole source of nutrition. However, it is known that delayed enteral feeding has a negative effect on gastrointestinal structure and function. Lack of enteral nutrition induces gastrointestinal atrophy, depresses gut hormone secretion, and delays the maturation of gastrointestinal motility. There are now numerous studies that demonstrate the benefits of early enteral feeding, including the promotion of endocrine adaptation, the accelerated maturation of gut motility patterns, the provision of luminal nutrients, and possible benefits to the immune system. In fact, early enteral

nutrition may enhance feeding tolerance and may actually decrease the incidence of NEC.

Trophic Feedings

Even though it is recognized that early enteral feeding is beneficial, there is still hesitation to begin feedings in the first few days following birth. One of the strategies that has been extensively studied since the late 1980s is trophic feeding, also referred to as minimal enteral nutrition or gut priming. This method involves giving the infant small volumes of feedings, approximately $10\text{--}20 \text{ ml kg}^{-1} \text{ day}^{-1}$, for a period of 3–7 days before beginning to advance to full enteral feedings. The benefits found are greater energy intake, earlier attainment of full enteral feedings, improved growth, less PN-related complications, reduced risk of infection, and earlier hospital discharge. Furthermore, infants who received trophic feedings had no increased incidence of NEC. Many clinicians have adapted variations of this practice, some with a shortened period of trophic feeds, others reserving this practice for the smallest, most preterm and liable infants while employing advancement of feeds in larger, more stable infants. Once minimal enteral nutrition has been established and the infant is stable enough to advance feedings, it is generally considered a safe practice to increase feedings by $20 \text{ ml kg}^{-1} \text{ day}^{-1}$ while using PN for the balance of intake until an adequate enteral intake has been established and tolerated. Although fast feeding advancement has been associated with NEC, one study has shown no increase in the incidence of NEC amongst preterm infants whose feeds were advanced by $35 \text{ ml kg}^{-1} \text{ day}^{-1}$.

Feeding Route

Because preterm infants lack the ability to coordinate sucking, swallowing, and breathing until 32–34 weeks gestation, tube feedings must be used. Jejunal feeding was a popular method for feeding infants during the 1970s to early 1980s. It was felt that this method would minimize the risk of reflux and aspiration. This method is now generally reserved for infants in whom reflux and aspiration is complicating chronic lung disease or those who have poor gastric emptying. Now, most infants are fed using an orogastric or nasogastric tube; the former is usually selected for the tiniest babies as the feeding tube may occlude one naris and impair nasal breathing.

Feeding Selection

Human milk expressed by the infant's mother is the preferred type of feeding for most preterm infants. It is nutritionally superior to artificial formula in many respects, including improved gastric emptying, more stool frequency, and improved fat absorption when breast milk is used. There are also many trophic factors found in human milk that enhance the development of the gastrointestinal tract. Human milk contributes to host defense and reduces the risk of NEC. Preterm infants who have been fed expressed human milk also show a neurodevelopmental advantage, but it is difficult to isolate this from the social variables also associated with mothers willing to express their milk. The use of expressed human milk also enhances

mother–infant bonding, as this is one task that only the baby’s mother is able to perform. However, there are also nutritional concerns related to the use of breast milk in infants born at less than 33 weeks’ gestation or less than 2000-g infants. Protein supplementation is necessary for optimal growth and maintenance of optimal protein status. Supplementation of calcium and phosphorus is also needed for adequate bone mineralization. There are multinutrient fortifiers available that can be added to human milk to improve nutrient intake. The use of these fortifiers has been associated with improved intake of protein and subsequent nitrogen retention, improved intake of minerals, subsequent bone mineralization, and improved growth.

If human milk is not available, the feeding of choice becomes either donated human milk or preterm infant formulas. The use of donated human milk has been associated with decreased incidence of NEC. Preterm infant formulas have greater protein content and are cow’s milk whey predominantly. The carbohydrate is a mixture of lactose and glucose polymers, and the fat a mixture of both long-chain and medium-chain triglycerides for improved nutrient absorption. The concentration of minerals, electrolytes, and vitamins is increased to meet the estimated nutrient needs of the preterm infant when fed in an amount to provide $120 \text{ kcal kg}^{-1} \text{ day}^{-1}$. Studies have shown that infants fed preterm infant formulas have improved growth over those fed term formula or even fortified mother’s own and donor human milk.

Feeding Delivery

The decision regarding how to feed must also be made: continuous versus bolus feeding. The preferred method is controversial. Some clinicians feel that continuous feedings are better tolerated whereas others feel that bolus feedings are more physiologic. In studies, bolus feedings have been associated with improved gastric emptying, and more mature intestinal motility patterns. It is difficult to compare feeding tolerance between continuous and bolus feeds due to differences in the criteria used. Comparison feeding studies have found fewer gastric residuals in those infants given bolus feedings than those fed continuously. A more recent study has found that feeds given as a slow bolus, over 2 h, resulted in a normal duodenal motility pattern, suggesting that some infants may benefit from slow intermittent feedings. Regardless of the method chosen, if an infant does not tolerate one method it may be beneficial to try a different one. **Table 8** summarizes the indications for bolus versus continuous enteral feeding.

Monitoring Feeding Tolerance

Feeding tolerance among preterm infants must be closely monitored because NEC is associated with enteral feedings. The presence of gastric residuals is one factor that is frequently used, but because preterm infants have poor gastric emptying, amounts less than 50% of a previous feed should not be considered significant. Other indicators that should be used in conjunction with gastric residuals include the increase in abdominal girth, the absence of active bowel sounds, the presence of blood in the stool, a change in the number or quality

Table 8 Indications for bolus versus continuous feeding delivery

<i>Bolus</i>	<i>Continuous</i>
Appropriate for most infants	Very small, preterm infants
Usually every 3 h feeds until greater than 1800 g	Hypoglycemia
May need to be infused over 1 h with signs/symptoms of gastroesophageal reflux (GER)	Malabsorption
	Gastroesophageal reflux (GER)
	Feeding intolerance

Table 9 Periodic monitoring of nutritional status

<i>Indicator</i>	<i>Frequency</i>
Weight	Daily
Length	Weekly
Head circumference	Weekly
Electrolytes (PN)	Daily until stable, then 2 times weekly
Albumin	Weekly
Bili/transaminases (PN)	Weekly
Calcium, phosphorus, magnesium, alkaline phosphatase	Weekly
Hemoglobin/hematocrit	Weekly

of stools, and the presence of emesis. A careful exam is warranted if these symptoms are present.

Monitoring Nutritional Status

The nutritional status and growth of the preterm infant should be monitored throughout the hospitalization. The daily fluid and caloric intake should be monitored daily, body weight should be recorded daily, length and head circumference should be measured weekly, and all three measurements plotted on standardized growth charts. If growth is inadequate, the volume or caloric density of feeds and or the protein content should be increased. Biochemical measurements should also be assessed periodically. **Table 9** summarizes the recommended schedule for monitoring anthropometrics and biochemical measurements.

Preparation for Discharge

Approximately 1 week before discharge, preterm infants should be converted to the feeding regimen that will be used at home. Infants who have been fed expressed breast milk should demonstrate the ability to directly breast-feed or to feed supplemented breast milk or formula from the bottle as needed to gain adequate weight. The infants who weigh less than 2500 g at discharge, especially those infants born at less than 30 weeks’ gestation, may require the supplementation of some breast milk feedings with postdischarge formula powder or the feeding of a concentrated postdischarge formula for some of the daily feedings.

For those infants who were fed preterm formula, conversion to a nutrient-enriched postdischarge formula is recommended. These formulas contain additional protein, vitamins, and minerals compared to term formulas. Studies have shown that infants fed these formulas until 9 months corrected age have improved gains in weight, length, and head circumference.

If growth is inadequate with either feeding regimen, then alteration in caloric density may be needed. Arrangements should be made for the nutritional status of these infants to be monitored after discharge.

Conclusions

Preterm infants have specialized nutritional needs. Aggressive PN or enteral nutrition must be initiated as soon as possible after birth and be carefully and continuously assessed to ensure that the best possible nutritional support is provided to promote optimal growth and subsequent developmental outcomes without causing additional morbidity and mortality.

See also: Breast Feeding. Energy Requirements. Growth and Development: Physiological Aspects. Growth Monitoring. Low Birth Weight and Preterm Infants: Causes, Prevalence, and Prevention. Nutritional Requirements of Infants. Nutritional Support: Infants and Children, Parenteral. Pediatric Feeding Disorders: Feeding Children Who Can't or Won't Eat

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LUNG DISEASES

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Glossary

Fat-free mass index Index of muscle mass in relation to height.

Forced expiratory volume Volume exhaled with force usually measured after 1 s.

Resting energy expenditure (REE) Amount of energy needed to maintain normal body functions at rest,

excluding the thermal effect of food and physical activity.

Spirometry A pulmonary function test measuring the amount (volume) and speed (flow) of air that can be inhaled and exhaled.

Introduction

Adequate nutrition is vital for the development and continued health of our lungs. Evidence suggests that prenatal and early-life nutrition affect lung function in later life. Malnutrition is observed in several chronic lung conditions, such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF), where there is both an increased energy expenditure and a reduced intake leading to reduced lung function, increased morbidity, and decreased quality of life. Malnutrition with associated multiple micronutrient deficiencies increases the risk of acute respiratory infections. Conversely, obesity may compromise lung function in conditions such as asthma. This article gives a taste of the complex interaction between nutrition and lung disease and presents some of the evidence base for nutritional support.

Chronic Obstructive Pulmonary Disease

Chronic obstructive pulmonary disease is defined as a preventable and treatable disease characterized by chronic airflow obstruction that is not fully reversible. The impairment of lung function is usually progressive and associated with an abnormal inflammatory response of the lung to noxious particles or gases. The recent inclusion of 'preventable and treatable' in the definition represents a shift toward a more positive attitude.

COPD includes 'chronic bronchitis' defined by chronic bronchial secretions sufficient to cause expectoration occurring on most days for a minimum of 3 months for 2 consecutive years, and 'emphysema,' the pathological process of permanent destructive enlargement of the airspace distal to the terminal bronchioles without obvious fibrosis. Although pure forms of these two conditions exist, there is considerable overlap in the majority of patients, hence grouping them under COPD.

Epidemiology

The Global Burden of Disease Study has projected that COPD, ranking sixth as the cause of death in 1990, will become the

third leading cause of death worldwide by 2020. Studies around the world estimate that the prevalence of COPD ranges from 7% to 19% and the disease is increasing in women. In developed countries, exacerbations of COPD present the greatest burden on the health-care systems, accounting for 10% of all hospital medical admissions in the United Kingdom and direct costs of COPD are estimated to be 38.6 billion of the European and \$18 billion of the United States health care budgets.

Etiology

The single most important risk factor for COPD is cigarette smoking with a direct correlation between number of cigarettes smoked and the likelihood of developing the disease. However, nonsmokers also develop COPD (never-smokers comprise approximately 23.3% of those classified as stage II+) and the individual susceptibility to smoking is very wide with only 15% of smokers likely to develop clinically significant COPD. Other risk factors include occupational exposure to dust and chemicals, air pollution, particularly in women in developing countries, and severe hereditary α_1 -antitrypsin deficiency (Figure 1). Severe childhood respiratory infections have been associated with reduced lung function and increased respiratory symptoms in adulthood, which may, however, in turn be related to factors like low birth weight.

Clinical Features

Clinical features include repeated attacks of productive cough, progressive dyspnea, exertional breathlessness, recurrent infections, wheeze, and occasional chest tightness. Clinical respiratory examination may be normal in mild-to-moderate cases. In severe cases, clinical signs reflect pulmonary hyperinflation, hypoxemia, and the development of pulmonary hypertension and right heart failure (*cor pulmonale*) and polycythemia. Clinical classification according to spirometry results is widely used (Table 1).

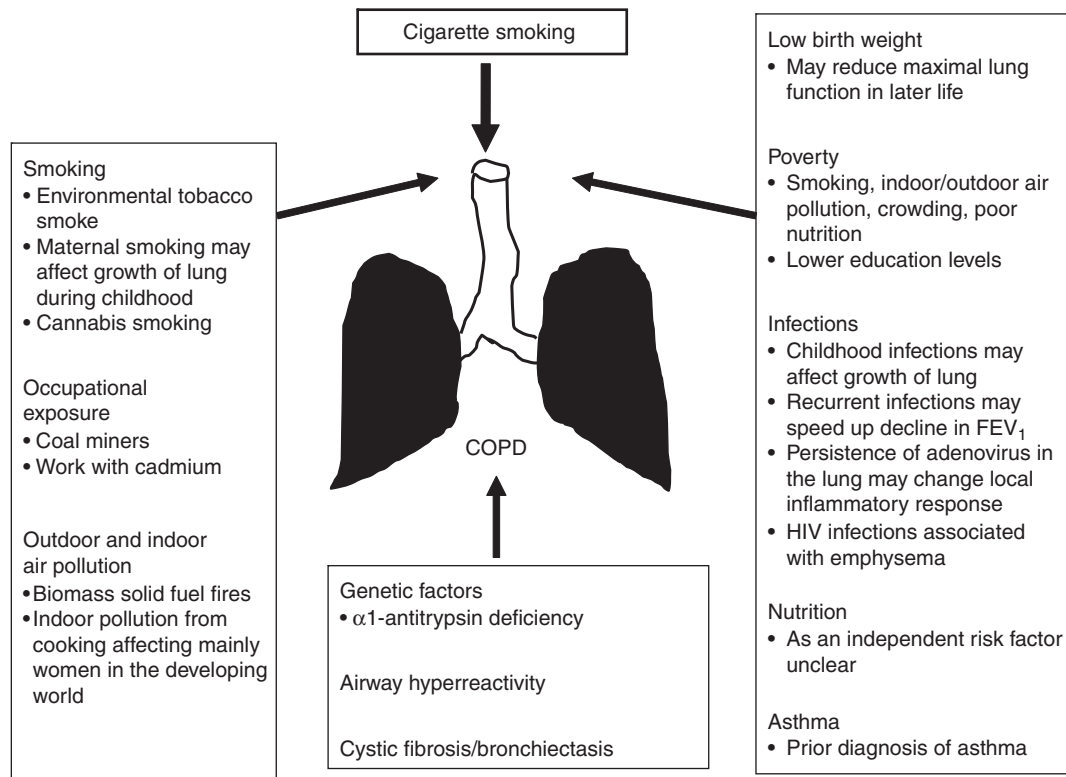


Figure 1 Risk factors for COPD. FEV₁, forced expiratory volume in 1 s.

Table 1 Spirometric classification of COPD

Stage	Description	Definition
I	Mild	FEV ₁ \geq 80% predicted
II	Moderate	50% \leq FEV ₁ < 80% predicted
III	Severe	30% \leq FEV ₁ < 50% predicted
IV	Very severe	FEV ₁ < 30% predicted or FEV ₁ < 50% predicted plus chronic respiratory failure

Differential Diagnoses

Asthma can usually be distinguished from COPD based on reversible rather than irreversible airflow limitation. In the developing world, pulmonary tuberculosis and COPD are common and making the correct diagnosis is essential for management.

Pathology

Pathological features include chronic air wall inflammation with airway smooth muscle hyperplasia and bronchial wall thickening, hypertrophy of mucus secreting glands, and a decrease in ciliated cells causing a less efficient transport of mucus in the airways. Small airways become obstructed; alveolar attachments and pulmonary elastic recoil are lost causing restriction of airflow. Emphysema is usually centriacinar with distension and damage affecting the respiratory

bronchioles, alveolar ducts, and centrally located alveoli. More rarely, panacinar emphysema or paraseptal emphysema develops in the distal airway structures causing later blebs on the lung surface, giant bullae, or both.

Nutrition and COPD

Management of COPD needs a multicomponent approach with particular attention to existing comorbidities. Indices of airflow obstruction only poorly predict prognosis although used in classification. Body mass index (BMI), airflow obstruction, dyspnea, and exercise capacity (BODE) index has been proposed to account for the multiple components of COPD. Malnutrition represents an important clinical problem in a subpopulation with COPD and poor indices of nutrition such as low BMI and low fat-free mass index (FFMI) independently confer a poorer prognosis in patients with COPD in terms of mortality, risk of hospitalization, length of hospitalization, and health-related quality of life.

Between 24% and 71% of all COPD patients show some evidence of malnutrition. However, the percentage is higher in those with more severe disease and those in need of hospital admission. BMI is an indicator of poor prognosis, but even patients with a normal BMI may be undernourished. FFMI is a better marker of lean body mass compared to BMI, as it is associated with other prognostic indices such as exercise capacity, dyspnea, and percentage of predicted FEV₁ (FEV₁=forced expiratory volume in 1 s). In up to 25% of COPD patients with normal weight, depletion of FFM can be noted. In malnourished patients, there may be loss of

respiratory muscle and diaphragm mass as well as altered regulation of the oxidative phenotype and mitochondrial dysfunction affecting respiratory muscle strength and endurance. However, this does not imply a causal relationship; dyspnea may in turn prevent patients from exercising, contributing to muscle atrophy.

Reasons for Malnutrition in COPD

The mechanisms of weight loss in COPD are not fully understood. It may be the result of an imbalance between an elevated resting energy expenditure (REE), an elevated total daily energy requirement, and inadequate dietary intake (Figure 2). This imbalance may be further affected by altered protein metabolism suggested by the selective wasting of FFM. Decreased protein intake, particularly during the first days of acute exacerbation, decreased protein synthesis, and increased protein balance turnover have been reported. An increased systemic inflammatory state with elevated proinflammatory markers such as TNF- α may also adversely affect protein metabolism.

Obesity in COPD

Some COPD patients are obese, resulting in an increased energy cost for physical activity, and extreme obesity decreases lung function. A recent study showed higher survival rates among obese COPD patients (BMI > 25) who lost weight. Another study showed that in those patients with a normal BMI or above, a high-carbohydrate diet may be of benefit to promote strength and function.

Nutritional Support

A 2008 Cochrane meta-analysis of 14 studies on simple nutritional supplementation in stable COPD patients did not identify improvements in anthropometric measures or functional exercise capacity in treated subjects versus control subjects. However, most studies did not use lean body mass as a primary outcome or health-related quality of life, which may be of more importance in COPD. In those studies showing any improvement in functional exercise, the improvements were not maintained when supplementation was discontinued. Nutritional supplementation as part of a multi-component intervention was not reviewed.

More recently, it has been shown that a combination of nutritional supplementation with low-intensity exercise training increased weight and energy intake as well as exercise capacity and decreased REE and inflammatory cytokines in moderate to severe clinically stable malnourished COPD patients. However, further larger studies are required to examine the potential role of the combination of nutritional supplementation and exercise in the management of malnourished patients with COPD.

Initial examination of the combination of nutritional support and substances like creatine, L-carnitine, and growth hormone releasing factor has shown an increase in lean body mass but has not shown a consistent improvement in endurance or health-related quality of life. Ghrelin stimulates growth hormone secretion, food intake, and weight gain without increasing adiposity unlike other appetite stimulants. It decreases muscle wasting via inhibition of production

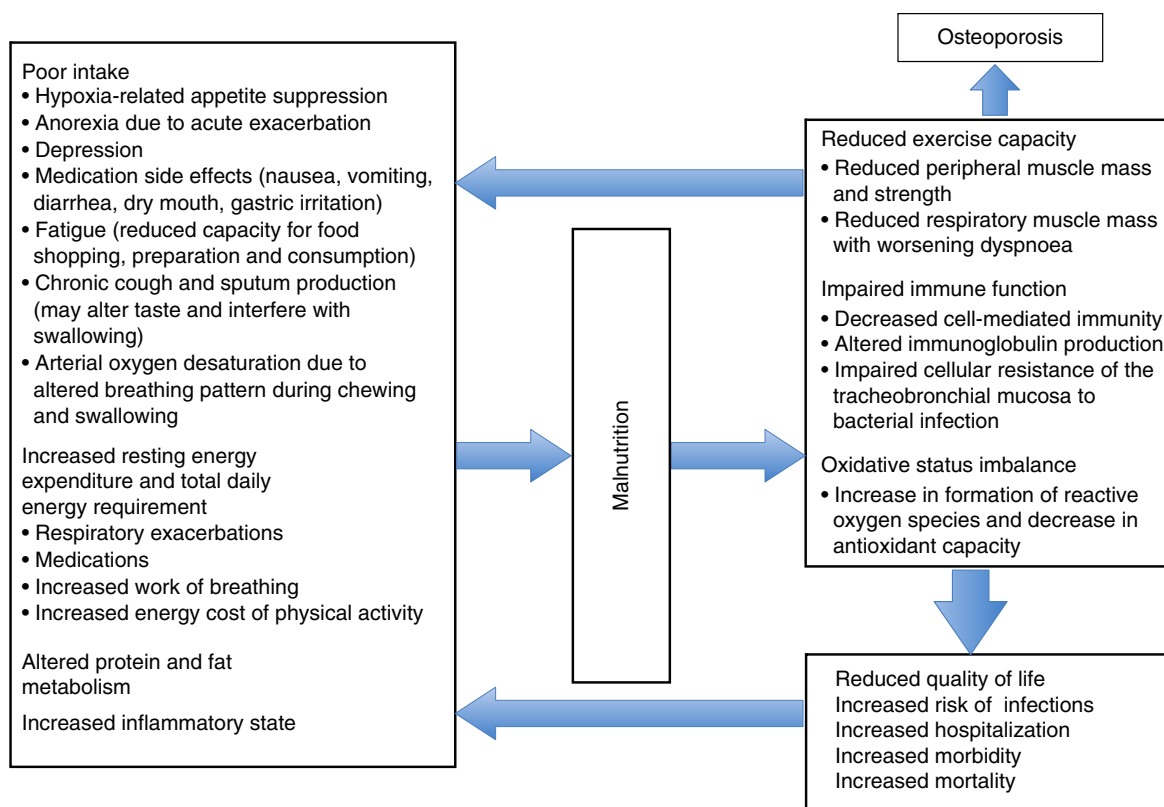


Figure 2 Malnutrition and COPD.

of anorectic proinflammatory cytokines. Appetite stimulants may, however, exacerbated by steroid medication, cause hyperglycemia.

A higher antioxidant food intake may improve lung function in COPD patients and may also have protective effects on the development of COPD. Studies have also shown a high prevalence of vitamin D deficiency among COPD patients, adversely affecting lung function. Supplementation trials of antioxidants and vitamin D are required before recommendations for routine supplementation can be made.

Type of Nutritional Support

Nutritional Advice, Exercise, and Supplementation

Despite the findings of the 2008 Cochrane review, the general logical advice is that nutritional support should be considered in all patients with COPD and it should be included in multidisciplinary pulmonary rehabilitation programs, which have shown some benefits. Individual dietary advice based on a dietary history and an analysis of nutritional status should be arranged for those who are underweight (BMI < 18.5) or obese (BMI > 30), and those whose weight is changing over time (weight loss greater than 10% in the past 6 months or more than 5% in the last month) (Figure 3). Furthermore, if the BMI is low, high-energy, high-protein nutritional

supplements should be given in frequent, small amounts (to avoid postprandial dyspnea and satiety and to improve compliance) to increase the total calorific intake. Exercise should be encouraged to augment the effects of nutritional supplementation.

Tube Feeding

Enteral tube feeding should not be used in COPD patients unless oral intake is unsafe or oral methods of maintaining nutritional status have failed. One should be aware of the risks (Table 4). Although a high-carbohydrate diet produces more carbon dioxide (VCO₂) and therefore requires increased ventilation to expel the excess CO₂, there is no additional advantage in stable COPD patients of formulating low-carbohydrate, high-fat enteral feeds compared to standard high-protein, high-energy supplements.

However, in patients with acute respiratory problems requiring artificial ventilation, the composition of feeds has a profound effect on gas exchanges. Feeding formulas during ventilation should have low-carbohydrate, high-fat content to reduce VCO₂ and ventilation requirements. Overfeeding negates any beneficial response to high-fat feeds because the conversion of energy into fat involves disproportionately large

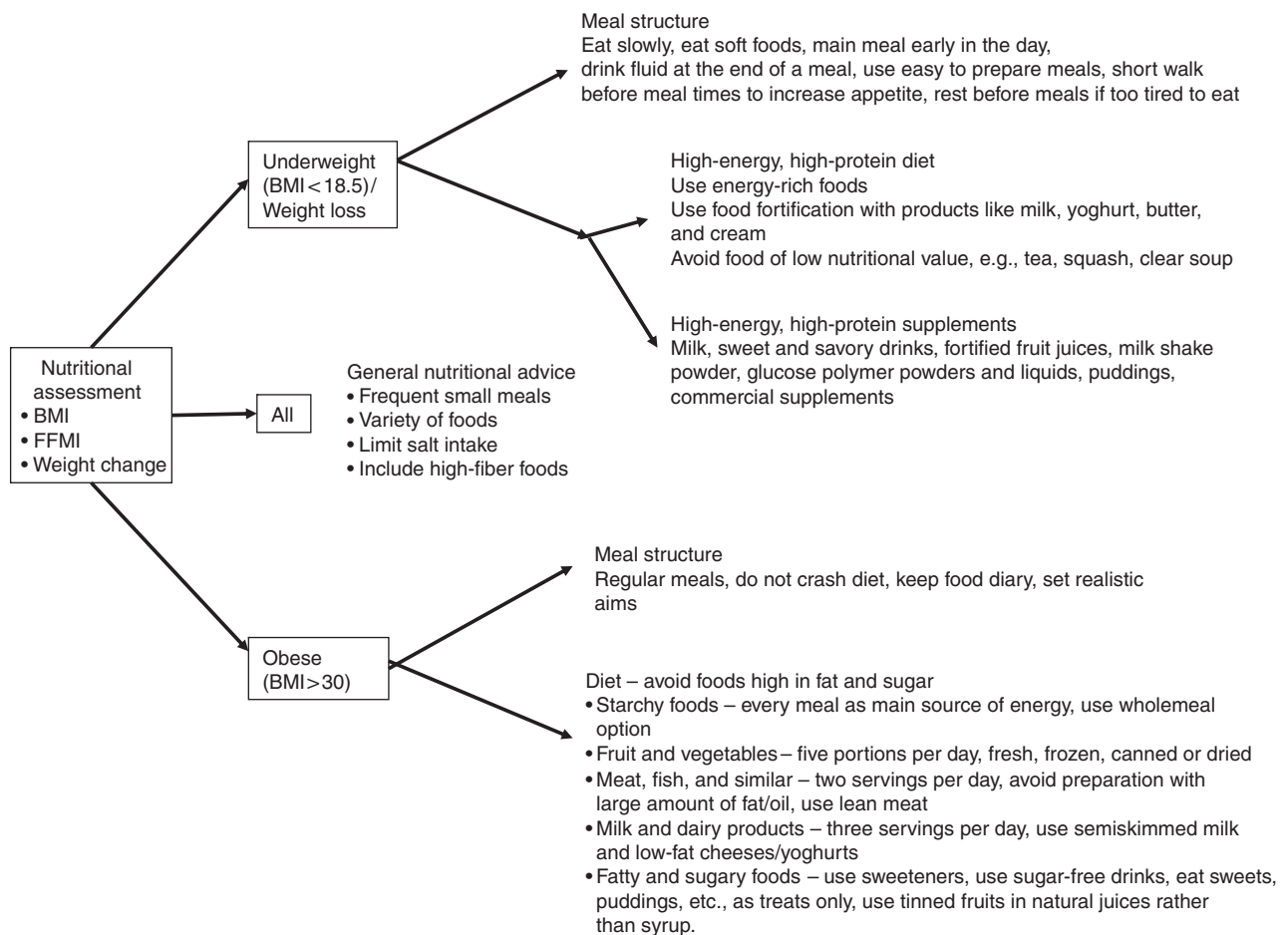


Figure 3 Nutritional advice in COPD.

production of CO₂. Bolus feeds during ventilation are as effective as continuous feeds.

Cystic Fibrosis

Definition

Cystic fibrosis is an autosomal recessive genetic disorder resulting from a mutation of a gene located on chromosome 7q31.3, which codes for a cyclic-AMP-activated chloride channel known as the cystic fibrosis transmembrane conductance regulator (CFTR) expressed in several epithelia. Over 1200 different mutations and 200 polymorphisms have been identified. Mutation ΔF508 accounts for 77% of CF chromosomes in the white population, whereas 3210 + 1G > A accounts for 46% of mutations in the black population.

Epidemiology

CF is the most common fatal genetic condition in Caucasians with a carrier rate of 1 in 25 and an incidence of 1 in 2500 live births. It is less common in the non-white races (1 in 20 000 and 1 in 1 million live births in the black and Oriental populations, respectively), but little is known about its prevalence in developing countries as CF often remains underdiagnosed. Early diagnosis with neonatal screening, aggressive treatment of lung infections, and nutritional support has led to a dramatic improvement in life expectancy. Now, there are more adults than children with CF in many developed countries.

Pathogenesis

The CFTR mutations lead to dysfunction of the exocrine glands in multiple systems including the skin, gut epithelium, pancreas, liver, and reproductive tract; in the respiratory epithelium, it results in increased resorption of sodium and water. Relative dehydration of the airway lining may predispose to chronic bacterial infection and ciliary dysfunction, leading to bronchiectasis and reduction of lung function.

Clinical Features

Most children, if not already identified by neonatal screening, present with malabsorption and failure to thrive as well as recurrent chest infections. Lung disease is the primary cause of morbidity and mortality in CF patients. At birth, the lungs appear normal macroscopically, although studies have shown the presence of an active inflammatory process. Progressive cycles of infection and inflammation lead to bronchiectasis in childhood, and progressive lung damage eventually leads to respiratory failure and death. Initially, the lungs are infected with *Staphylococcus aureus* and nontypable *Haemophilus influenzae*, but later approximately 80% are infected with *Pseudomonas aeruginosa*. Approximately 90% of CF patients have pancreatic insufficiency requiring pancreatic enzyme replacement therapy (PERT). Other features are summarized in Table 2.

Table 2 Complications of cystic fibrosis

Respiratory	
● Bronchiectasis	● Respiratory failure
● Pneumothorax	● <i>Cor pulmonale</i>
● Wheeze	● Lobar collapse due to secretions
● Hemoptysis	● Allergic bronchopulmonary aspergillosis
● Nasal polyps	
Gastrointestinal	
● Meconium ileus	● Malabsorption and steatorrhea
● Rectal prolapse	● Distal intestinal obstruction syndrome
● Intussusception	● Gastroesophageal reflux
● Abdominal distension	● Biliary cirrhosis
● Colonic strictures	● Hepatomegaly
● Cholelithiasis	● Portal hypertension
● Obstructive jaundice	● Cholecystitis
● Pancreatitis	
Others	
● Diabetes	● Salt depletion
● Male infertility	● Growth failure/weight loss/failure to thrive
● Amyloidosis	● Psychosocial problems
● Arthropathy	● Osteopenia/osteoporosis
● Delayed puberty	● Stress incontinence (repeated cough)
● Cutaneous vasculitis	

Malnutrition and CF

Malnutrition in CF patients is still one of the main clinical manifestations despite increased knowledge and improved management. Of adults with CF, 60.8% have a BMI below the recommended levels and 15.7% of patients under 20 are below the fifth centile weight-for-age and gender.

A clear link has been shown between good nutrition and prognosis of CF. BMI percentiles in children and BMI values in adults are also directly and strongly correlated with pulmonary function in terms of FEV₁ (Figure 4).

Furthermore, based on multiple longitudinal and cross-sectional studies, there is an assertion that growth, particularly linear growth, is connected to the evolution of lung health in children who have CF. However, the causal relationship between malnutrition and pulmonary dysfunction remains unclear in CF. There is a suggestion that nutritional status early in life is a determinant for the progression of the lung disease. Studies in infants looking at later lung disease are obviously difficult in terms of ethics but retrospective studies have shown that appropriate nutritional status early in life has a positive effect on lung function later on, suggesting a causal relationship. Malnutrition may affect mechanical lung properties including alveolar remodeling in addition to factors including the activation of cytotoxic T lymphocytes and natural killer cells.

A variety of complex organic and psychosocial factors contribute to malnutrition in CF, which in turn worsens pulmonary dysfunction (Figure 5). Similar to COPD, there is an imbalance of decreased nutrient intake and increased energy expenditure in CF.

Decreased Intake

Pancreatic insufficiency and other complications may contribute to moderate and severe malabsorption affecting protein and fat-soluble vitamins despite adequate use of enzyme

supplements (Figure 5). Reduced appetite due to chronic respiratory infections and other complications, as well as psychosocial factors such as eating disorders in teenagers, further lead to inadequate energy intake in CF patients.

Increased Energy Expenditure

The REE in severe obstructive lung disease such as CF and COPD is increased partly because of the increased work of breathing. REE is 10–20% greater in CF patients than in healthy controls and this increase appears to be closely

associated with declining pulmonary function and subclinical infection. Bronchial sepsis leads to local release of leukotrienes, free oxygen radical, and cytokines, including TNF- α . Interestingly, antibiotics reduce energy requirements of moderately ill patients with chronic *P. aeruginosa*. Furthermore, the presence of even mild lung disease is associated with elevations in REE, indicating that REE could be a sensitive marker of clinical status before lung disease becomes clinically overt. The relationship between protein metabolism and pulmonary function in CF remains unclear.

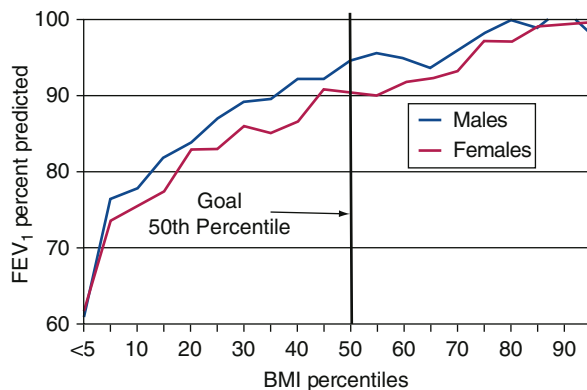


Figure 4 FEV₁ percent predicted vs. BMI percentile in children 6–20 years of age. Reproduced with permission from Cystic Fibrosis Foundation Patient Registry (2009) *2008 Annual Data Report*. Bethesda, Maryland: Cystic Fibrosis Foundation Patient Registry.

Nutritional Support

Nutritional support should be based on complete individual nutritional assessment rather than a generalized approach. Current guidelines advise using weight-for-length in less than 2-year-olds, BMI percentiles for 2- to 18-year-olds, and BMI in the adult population (Table 3). The age of greatest risk of malnutrition appears to be during the first 2 years of life and during early adolescent years. Treatment for nutritional failure may include nutritional advice and behavioral interventions, oral supplements, enteral feeds, and parenteral nutrition (Table 3).

High-Energy/High-Protein Diet

The encouragement of a high-energy, high-protein diet aiming at 110–200% of recommended daily allowance will produce growth in the majority of children and adults with CF (Figure 6). Malnourished children achieve higher energy

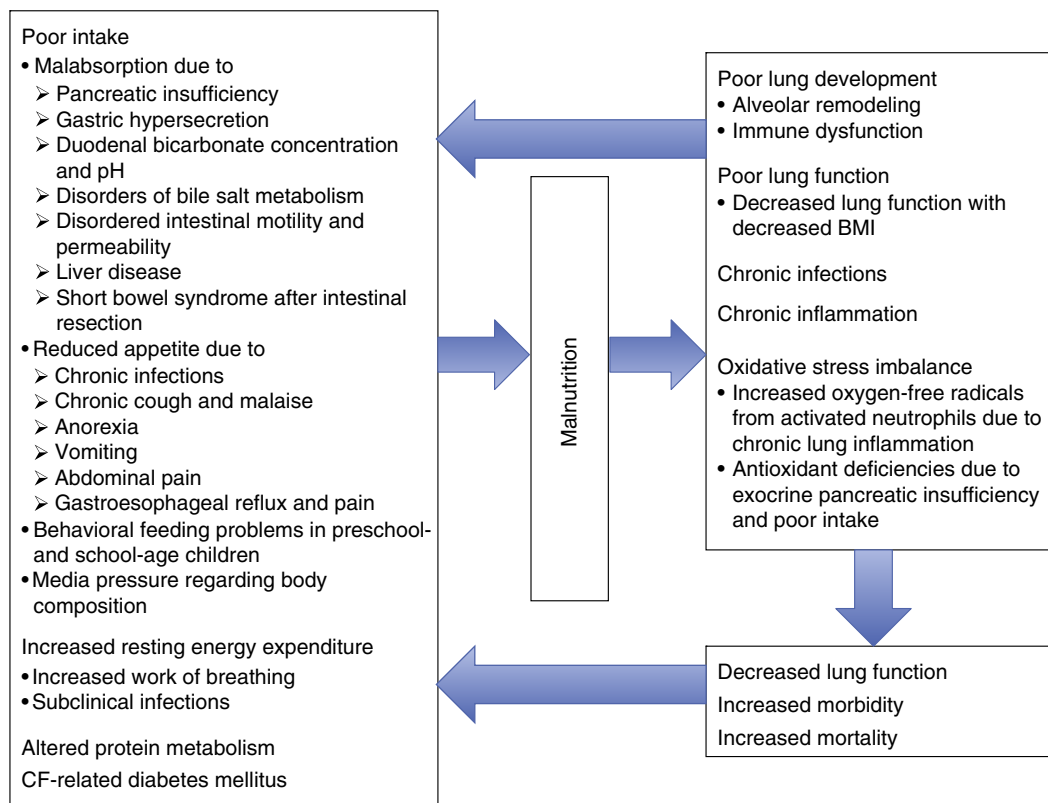


Figure 5 Cystic fibrosis and malnutrition.

Table 3 Indications for nutritional interventions in cystic fibrosis

Age group	0–2 years	2–18 years	> 18 years
Nutritional assessment indicator	Weight-for-length percentiles	BMI percentiles	BMI
<i>Care categories</i>			
Normal/routine nutritional care	Normal weight-for-length percentiles and within 2 centile bands of each other	BMI 25–95th percentile	BMI 20–27
At risk/noninvasive nutritional intervention	Weight and height percentiles decreasing with time or no weight gain	BMI 10–25th percentile OR Weight loss over 1–3 months OR Plateau in weight gain over 2–4 months	BMI 18.5–20 OR Weight < 45 kg regardless of BMI OR 5% weight loss over 2 months
Malnutrition/aggressive nutritional support	Weight 2 or more centile bands below length OR Failure of noninvasive interventions to improve nutritional status	BMI < 10th percentile OR Weight falling 2 or more percentile positions OR Plateau in weight gain for 6 months OR Failure of noninvasive interventions to improve nutritional status	BMI < 18.5 OR Weight < 40 kg regardless of BMI OR 5% weight loss over 2 months despite noninvasive nutritional interventions
Overweight/nutritional advice	N/A	BMI > 95th percentile	BMI > 27
<i>Nutritional interventions</i>			
Advice for routine energy intake as indicated for age and sex	Demand breast-feeding or whey-based artificial formulae. Intake of > 200 ml kg ⁻¹ is not unusual. Early weaning not advised	110–200% standards for healthy population	110–200% standards for healthy population
Combined behavioral and nutritional intervention indicated for weight gain	Recommended for children aged 1–2 years	Recommended for age 2–12 years; insufficient evidence for age 13–18 years	Insufficient evidence for effectiveness for adults
Nutritional supplementation (oral and enteral) intervention if indicated for weight gain	Unrestricted demand feeds. Nutritional supplementation if poor growth or surgery for meconium ileus	Recommended	Recommended
Caloric supplementation (per day)	1–2 years 200 kcal	3–5 years 400 kcal 6–11 years 600 kcal > 12 years 800 kcal	800 kcal
Pancreatic enzyme preparations (based on pancreatic status assessment)	Recommended Upper limit: 10 000 IU lipase kg ⁻¹ body weight day ⁻¹	Recommended Upper limit: 10 000 IU lipase kg ⁻¹ body weight day ⁻¹	Recommended Upper limit: 10 000 IU lipase kg ⁻¹ body weight day ⁻¹
Vitamin Supplementation (fat-soluble vitamins A, D, and E)	Recommended in pancreatic insufficient patients	Recommended in pancreatic insufficient patients	Recommended in pancreatic insufficient patients
Vitamin K	Recommended in liver disease and with prolonged prothrombin time	Recommended in liver disease and with prolonged prothrombin time	Recommended in liver disease and with prolonged prothrombin time
Water-soluble vitamins, iron, and calcium supplements	Recommended if deficient or evidence of low intake	Recommended if deficient or evidence of low intake	Recommended if deficient or evidence of low intake
Sodium supplements (only in hot conditions and evidence of sodium deficiency)	Recommended < 1 year 500 mg day ⁻¹	Recommended 1–7 years 1 g day ⁻¹ , > 7 years 2–4 g day ⁻¹	Recommended 2–6 g day ⁻¹

intake when more frequent meals are offered. Attention should be given to psychological, social, behavioral, and developmental aspects of feeding. There is evidence that particularly for children 1–12 years of age, behavioral intervention and nutrition counseling should be implemented when a risk of poor growth is present (Table 3).

Dietary Supplements

For children with growth deficits, adults with inappropriate weight status, and during acute chest infections, oral and enteral supplements should be used to improve weight. Dietary calorie supplements should complement normal food intake and not replace food, following recommended

age-dependent quantities (Table 3). A Cochrane review concluded that oral calorie supplements, on top of standard dietary advice and monitoring, do not achieve any additional benefit in the nutritional management of moderately malnourished children with CF in addition to dietary advice and monitoring.

Enteral Feeding

When oral high-calorie diets and supplements are ineffective, enteral tube feeding may be considered, which is better tolerated by gastrostomy than by nasogastric tube in the long term (Table 4). Reported use of enteral tube feeding suggests

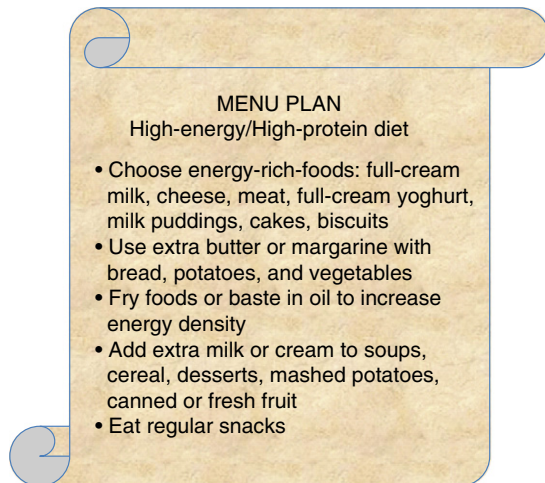


Figure 6 Menu plan for a high-calorie/high-protein diet.

that it results in nutritional and respiratory improvements. However, a Cochrane review concluded that its efficacy has not been fully evaluated and data are limited and randomized trials are needed to investigate efficacy as well as when to start enteral tube feeds. Furthermore, these interventions are not free from complications and should be balanced against the possible gains (Table 4). Enteral feeding may lead to gastroesophageal reflux, formula intolerance, and hyperglycemia. Patients receiving supplemental feeds who demonstrate repeated blood sugar levels higher than 11.1 mmol l^{-1} during the feed may benefit from insulin given before the feeds. Enteral feeds are usually given for 8–10 h overnight, with at least 40–50% of the estimated energy requirement given via the feed. Most patients tolerate an energy-dense polymeric feed providing at least 1.5 kcal ml^{-1} with additional pancreatic enzymes.

Parenteral Nutrition

Parenteral nutrition should be reserved for those with a nonfunctioning gastrointestinal tract (e.g., due to prolonged bowel obstruction or gastrointestinal surgery) and the critically ill as it is costly and associated with several risks, including catheter sepsis and metabolic complications such as hyperglycemia.

Vitamin, Mineral, and Pancreatic Enzyme Supplementation

Malabsorption and pancreatic insufficiency are treated with oral PERT and vitamins (for details, see Table 3). Low fat-soluble vitamin concentrations are associated with poorer clinical status and reduced lung function. In hot conditions and in proven deficiency, sodium supplements are

Table 4 Advantages and disadvantages of different enteral feeding routes

Method	Advantages	Disadvantages
Nasogastric	<ul style="list-style-type: none"> • Short-term feeding 	<ul style="list-style-type: none"> • Tube reinsertion may be <ul style="list-style-type: none"> ○ distressing to patient/caregiver/nurse ○ easily removed • Risk of aspiration
Nasojejunal	<ul style="list-style-type: none"> • Less risk of aspiration • Short-term feeding 	<ul style="list-style-type: none"> • Psychosocial implications • Difficulty of insertion • Radiographic check of position • Easily removed • Risk of perforation • Abdominal pain and diarrhea unless continuous infusion of feed • Discomfort in nasopharynx • Reflux of bile is facilitated
Gastrostomy	<ul style="list-style-type: none"> • Cosmetically more acceptable • Long-term feeding 	<ul style="list-style-type: none"> • Increase reflux if present • Local skin irritation • Stoma infection • Granulation tissue • Leakage • Gastric distension • Stoma closes within a few hours if accidentally removed
Jejunostomy	<ul style="list-style-type: none"> • Reduced risk of aspiration • Long-term feeding 	<ul style="list-style-type: none"> • Surgical/radiology procedure • Risk of perforation • Must be constant infusion of feed • Bacterial overgrowth • Dumping syndrome can occur

recommended. Anorexia and poor growth may result from chronic salt depletion, and significant hyponatremia may be accompanied by vomiting. Pancreatic enzymes should be given at the smallest dose possible to control steatorrhea and achieve normal patterns of growth and weight gain. Total fat excretion should be quantified in order to assess efficacy of PERT.

Appetite Stimulants, Growth Hormones, and Omega-3

Small randomized controlled trials of the appetite stimulant megestrol acetate noted significant improvement in weight and pulmonary function during the treatment period but gains seem to be short-lived. A Cochrane review is under way to examine the use of appetite stimulants in the management of CF. Important side effects include glucose intolerance and adrenal suppression. Cyproheptadine, another appetite stimulant, may be more promising in terms of side effect profile and long-term effect on weight gain.

Small trials with growth hormone given for 1 year showed improvements in respiratory status and exercise capacity and gains in lean body mass, but larger trials are required to make recommendations. Similarly, although small studies suggested an improvement in lung function and clinical status, there is not enough evidence on the safety and effectiveness of fish oil supplements in the routine care of CF.

Asthma

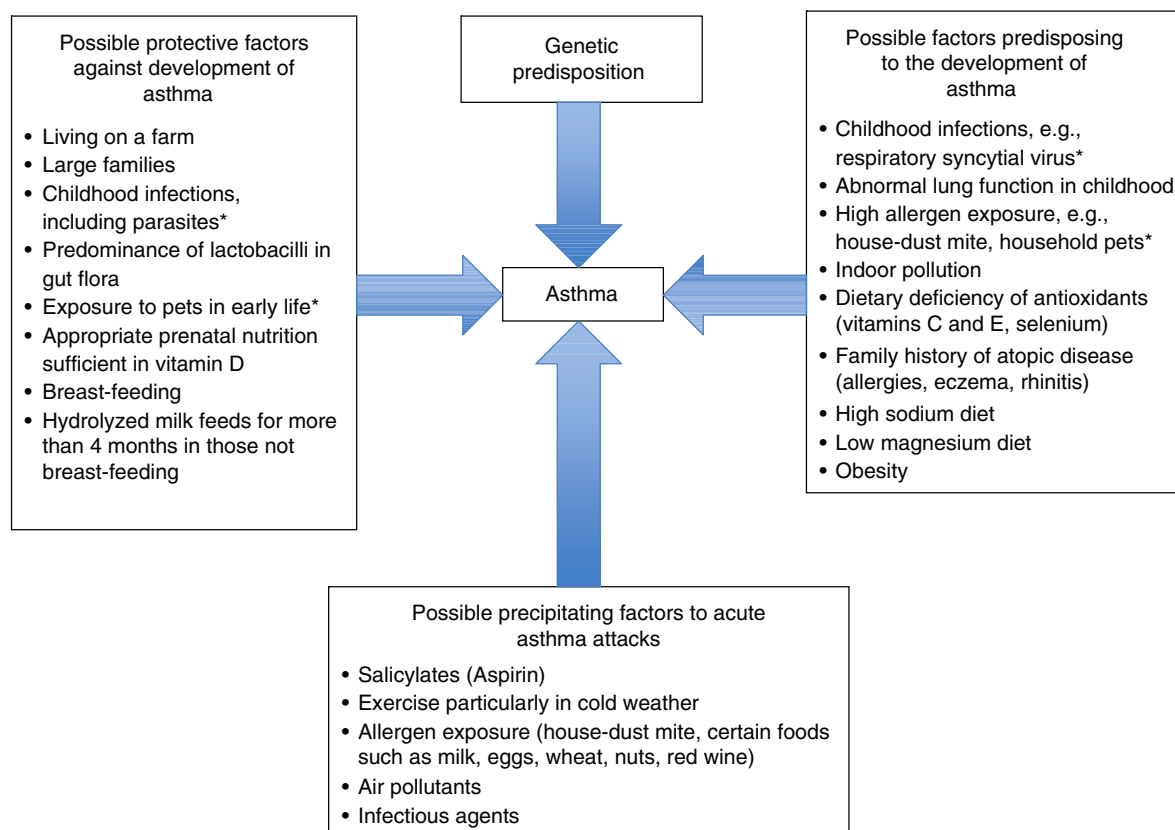
Definition

Asthma (ancient Greek for 'panting') has no universally agreed definition. It is a heterogeneous disease described in terms of clinical, physiological, and pathological characteristics. Chronic airway inflammation and increased airway hyper-responsiveness to various stimuli lead to paroxysmal airway narrowing that may be reversible with or without treatment.

Epidemiology

Since the 1980s, there has been a worldwide increase in the prevalence of asthma in both children and adults with associated increases in morbidity and mortality. It currently affects 300 million people worldwide, and an additional 100 million persons will be diagnosed by 2025, with huge socioeconomic implications in terms of days lost from school or work, health care visits, etc. For most of the developing world, little or no standardized data are available.

A series of environmental factors and genetic predisposition have been implicated in the development of, or protection from, asthma (**Figure 7**). Of people with asthma, 50% develop the condition before age 10, and the majority before age 30. The earlier the onset of wheeze, the better the



*There is controversy and conflicting evidence regarding prenatal and early-life exposures.

Figure 7 Factors implicated in the development of, or protection from, asthma and trigger factors for acute asthma attacks.

prognosis for disease progression. Boys are more likely to 'grow out' of their asthma.

Clinical Features

Central to all definitions of asthma is the presence of more than one of the following symptoms: recurrent episodes of wheeze, breathlessness, chest tightness, and cough. Precipitants include exercise in cold weather and viral upper respiratory infections (Figure 7). There is huge variability in symptom presentation between patients, ranging from those with periodic wheezing attacks with asymptomatic periods between exacerbations and patients with persistent, but fluctuating symptoms of breathlessness and wheeze. There is usually a diurnal pattern, with symptoms and lung function being worse early in the morning.

Pathogenesis

Asthma represents a spectrum of pathophysiologic processes involving different degrees of airway inflammation and remodeling. At the mild end of the spectrum, there is allergic asthma mainly driven by Th2-mediated inflammatory

responses and associated with other allergic comorbidities such as rhinitis and atopic dermatitis. In moderate disease, altered epithelial–mesenchymal communication is observed, leading to the generation of growth factors and cytokines with subsequent sustained inflammation. When the epithelial–mesenchymal component becomes dominant in more severe disease, tissue damage and remodeling can be observed as well as increased mucus secretion with obstruction of peripheral airways.

Nutrition and Asthma

The relationship between nutrition and asthma has had increased interest over the past 10 years. Some studies have looked at nutritional changes influencing the establishment of asthma, whereas others have concentrated on possible secondary prophylactic measures once the diagnosis of asthma has been made (Table 5).

Possible Preventative Effects of Nutrition

The evidence for possible preventative effects of nutritional changes is based mainly on observational studies. Most studies are multifaceted and it is often difficult to differentiate the

Table 5 Nutritional effects on the development of asthma (primary preventative effects) and symptom control (secondary prophylactic effects)

Nutritional intervention	Evidence	Comments
<i>Primary preventative effects</i>		
Maternal food allergen avoidance during pregnancy and lactation	● Not related to subsequent development of asthma	● May adversely affect maternal and fetal nutrition ● High-dose exposure may reduce subsequent sensitization
Early introduction of allergenic foods during weaning	● Not related to subsequent development of asthma	● Small study showed a nonsignificant increase in preschool wheezing with late introduction of egg
Fish oil supplementation during pregnancy	● Marginal effects on the reduction in wheeze and cough at 1 year	● Reduction in cytokine response to allergens in cord blood
Fish oil supplementation in early infancy	● Reduces wheeze at 18 months but no effect on asthma by 5 years of age	● No adverse effects observed
Breast-feeding	● Potential protective effect in relation to early asthma	● No adverse effects observed
Hydrolyzed formula	● Suggested reduction of risk of asthma or wheeze in the first year of life	● In line with WHO recommendation
Low intake of antioxidants	● Possible association with higher prevalence of asthma	● Formula recommended when breast-feeding is not possible and the infant is at high risk
Higher intake of fresh fruit and vegetable	● Improved pulmonary function and lower prevalence of asthma	● Disputed by recent meta-analysis
<i>Secondary prophylactic effects</i>		
Supplementation with antioxidants (vitamins C, E, selenium)	● Limited evidence of clinical benefits	● In line with healthy diet promotion for the prevention of cancer and cardiovascular disease
Supplementation with or diet high in fish oil	● No significant improvement in asthma control	● Selenium supplementation showed improvements in terms of 'clinical evaluation' but not in objective parameters of lung function and airway hyperresponsiveness
Probiotics	● No effect on clinical parameters	● No known adverse effects
Low sodium intake	● Minimal clinical improvements	● Decrease in eosinophilia
Low magnesium intake	● Associated with higher prevalence of asthma	● High sodium intake associated with increased bronchial hyperresponsiveness
Weight reduction in obese asthma patients	● Improved asthma control	● Magnesium used as muscle relaxant in acute asthma attacks
Food additive avoidance	● No effect on asthma control	● Limited evidence of a strict calorie-controlled diet
		● Restrictive diet seldom used in asthma

Note: The evidence is largely based on observational studies that are often multifaceted making it difficult to differentiate the effects of one exposure or intervention from another.

effects of one exposure or intervention from another. Allergic food avoidance during pregnancy, lactation, and weaning has no effect on the subsequent development of asthma. Supplementation with, or diet high in, fish oil during pregnancy and infancy has only limited effect on asthma development. Low vitamin D status in pregnant mothers is associated with a higher occurrence of wheeze in their children but further studies are required. Similarly, a higher prevalence of asthma has been reported with lower intake of antioxidants, although a recent meta-analysis does not support the hypothesis of linking vitamin C and E intake to a risk of asthma.

Breast-feeding is recommended over other types of feeding, and in infants at high risk of developing asthma (first-degree relative with atopy) where breast-feeding is not possible, hydrolyzed formula for a minimum of 4 months together with other preventative measures may reduce the risk of developing asthma or wheeze in the first year of life. Inconclusive evidence exists that growth rate during infancy may be related to the risk of development of asthma.

Secondary Prophylactic Effects of Nutrition in Asthma

Intervention studies could not confirm that supplementation with antioxidants produce clinical benefits in asthma. Better pulmonary function and less asthma have been observed, though, when there is higher intake of fresh fruit and vegetables and it seems to be reasonable to advise a healthy diet. Supplementation with, or a diet high in, fish oil as well as probiotics in asthmatic patients did not show any significant clinical improvement but equally showed no adverse effects with the latter showing a decrease in eosinophilia.

High sodium intake is associated with increased bronchial hyperresponsiveness; however, reducing salt intake results in only minimal clinical improvements. Low magnesium intakes may be associated with higher prevalence of asthma. Magnesium is used in the treatment of acute attacks for smooth muscle relaxation. Studies of long-term oral supplementation trials are limited and further larger randomized trials are required.

Obese asthma patients are advised to reduce weight based on evidence of an association between increased BMI and increased incidence and symptoms of asthma. Not enough evidence exists to recommend a strict calorie-controlled diet as a concomitant intervention with drug-based therapy.

According to a recent Cochrane review, there is no evidence that tartrazine, a commonly used food additive, makes asthma worse or avoiding it improves asthma control. Rarely, intolerance to certain foods may act as a trigger for asthma attacks and they should be avoided like other trigger allergens (Figure 7). A simple exclusion diet may be the most useful diagnostic diet, but strict food diets are seldom used in asthma.

Other Lung Diseases

Obstructive Sleep Apnea Syndrome

Obstructive sleep apnea syndrome (OSAS) is strongly associated with obesity and visceral fat mass and linked to serum

leptin, insulin, and IL-6 and TNF- α levels. Early weight reduction with a very low-calorie diet and supervised lifestyle counseling is effective in improving symptoms in obese OSAS patients.

Bronchiectasis

Bronchiectasis unassociated with other conditions like CF is now uncommon in most developed countries, but remains a problem in developing countries and in certain indigenous populations mainly due to tuberculosis and other lung infections. The nutritional management of bronchiectasis is similar to that of CF and COPD.

Chronic Lung Disease of Infancy

Chronic lung disease of infancy (CLDI) describes a heterogeneous group of pulmonary disorders that originate from an acute respiratory disorder during the neonatal period, which may lead to chronic lung disease in childhood and adult life. The majority of cases are attributable to bronchopulmonary dysplasia (BPD), which is common in very preterm infants with respiratory distress syndrome. BPD has recently been defined as the requirement of supplemental oxygen for 21 of the first 28 days of life and is further defined according to severity (Table 6).

Short-term vitamin A supplementation has been shown to reduce BPD at 36 postmenstrual age (postmenstrual age (weeks): gestational age plus chronological age) in extremely low birth weight infants (<1000 g), but the effects are similar to caffeine that is currently used, and the long-term risk-benefit ratio of vitamin A for neurodevelopment has not been fully established.

Growth failure is common in infants with BPD. They have increased metabolic demands and a relative state of protein-calorie malnutrition. Early growth restriction may increase long-term cardiovascular risks and have adverse effects on respiratory function. Currently, guidelines promote 'aggressive'

Table 6 Definition of bronchopulmonary dysplasia (BPD) according to severity (severity depends on the duration and level of supplemental oxygen and mechanical ventilatory support at 36 weeks postmenstrual age (PMA))

Gestational age	< 32 Weeks	≥ 32 Weeks
Mild BPD	Breathing room air at 36 weeks PMA	Breathing room air by 56 days postnatal age
Moderate BPD	Requirement for <30% oxygen at 36 weeks PMA	Requirement for <30% oxygen at 56 days postnatal age
Severe BPD	Requirement for ≥30% oxygen and/or positive pressure at 36 weeks PMA	Requirement for ≥30% oxygen and/or positive pressure at 56 days postnatal age

Source: Data extracted from Table 1 on Jobe AH and Bancalari E (2001) Bronchopulmonary dysplasia. *American Journal of Respiratory and Critical Care Medicine* 163: 1723–1729, with permission from American Thoracic Society.

nutrition for extremely preterm infants (born <28 weeks' gestation), which includes introducing parenteral amino acids from day one and lipids from day two, and using milk fortifiers to increase daily protein and caloric intakes to 4.4 g kg⁻¹ and 130–150 kcal kg⁻¹, respectively.

Respiratory Tract Infections and Micronutrients

Studies have shown that undernourished children have a relative risk of 1.2 for an increased incidence of any acute respiratory illness (ARI) and 1.9 for lower respiratory tract infections (LRTIs). Single and multiple micronutrient deficiencies as part of malnutrition may be associated with increased prevalence of respiratory disease (Table 7). However, supplementation trials with single and multiple micronutrients in the prevention and as treatment adjunctive of ARIs have so far mainly shown contradictory results and further research is required to make recommendations. There are suggestions that adjunct therapy with zinc during the treatment of acute pneumonia may reduce the length of symptoms and improve outcome. Similar suggestions have been made with regard to selenium supplementations.

Table 7 Micronutrients: evidence for the effect of deficiency and supplementation on acute respiratory infections

Micronutrient	Deficiency	Supplementation
Zinc	<ul style="list-style-type: none"> ● Increased prevalence and severity of LRTI 	<ul style="list-style-type: none"> ● Possible beneficial effects as adjunct therapy on length of symptoms and outcome in acute pneumonia ● Reduction in LRTI prevalence
Vitamin A	<ul style="list-style-type: none"> ● Increased risk of developing ARI 	<ul style="list-style-type: none"> ● No evidence as adjunct therapy during ARI ● No evidence as preventative supplementation^a
Vitamin D	<ul style="list-style-type: none"> ● Increased risk and severity of LRTI 	<ul style="list-style-type: none"> ● No evidence yet as adjunct therapy or preventative supplementation
Iron	<ul style="list-style-type: none"> ● Increased incidence of LRTI 	<ul style="list-style-type: none"> ● No overall effect on risk of LRTI
Selenium	<ul style="list-style-type: none"> ● Found in premature babies with RDS and associated with CLDI in low birth weight infants 	<ul style="list-style-type: none"> ● Possible benefits as adjunct therapy in ARI
Multiple micronutrients	<ul style="list-style-type: none"> ● Increased risk of LRTI 	<ul style="list-style-type: none"> ● Limited evidence. Suggested reduction in incident of LRTI similar to zinc supplementation alone

^aApart from in those who are malnourished.

For detailed discussion of nutrition and tuberculosis, see article Nutrition and Susceptibility to Tuberculosis (00268).

See also: Antioxidants. Appetite: Physiological and Neurobiological Aspects; Psychobiological and Behavioral Aspects. Breast Feeding. Cancer: Epidemiology of Lung Cancer. Cystic Fibrosis. Early Origins of Disease: Fetal; Non-Fetal. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Food Allergies: Diagnosis and Management. Food Intolerance. Infection: Nutritional Management in Adults. Low Birth Weight and Preterm Infants: Causes, Prevalence, and Prevention; Nutritional Management. Malnutrition: Secondary, Diagnosis and Management. Nutrition and Susceptibility to Tuberculosis. Nutritional Support: Adults, Enteral; In the Home Setting; Infants and Children, Parenteral. Obesity: Complications. Older People: Nutritional Requirements; Physiological Changes. Parenteral Nutrition. Salt: Epidemiology. Selenium. Sodium: Physiology. Supplementation: Developed Countries; Developing Countries; Dietary Supplements; Programmatic Issues. Tuberculosis: Nutritional Management. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. Vitamin D: Physiology, Dietary Sources, and Requirements. Vitamin E: Metabolism and Requirements; Physiology and Health Effects. Vitamin K

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LYCOPENES AND RELATED COMPOUNDS

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Introduction

Lycopene, the most abundant pigment in ripe red tomatoes and in a few other fruits, is one of the major carotenoid pigments that is widely present in the diet of the human population. **Figure 1** illustrates the chemical formula of selected carotenoids that occur widely both in human diets and in the noncellular fraction of human blood in most regions of the world. Carotenoids are yellow-to-red in color, with lycopene being nearer the red end of the carotenoid series. However, unlike the other carotenes and cryptoxanthins, it does not possess a beta-ionone ring structure at either end of the molecule, and this precludes it from becoming a precursor of vitamin A in humans and animals. Nevertheless, it is readily transformed from the all-*trans* form that is characteristic of most plants and plant foods for animals and humans, to a range of mono- and di-*cis* forms within the animal's body. In addition, oxidation to epoxides and hydroxylated derivatives occurs, although the control of these oxidation pathways and the nature of their products are not yet well understood or characterized.

In plant tissues, where it is synthesized, lycopene is thought to help protect vulnerable photosynthetic tissues from light- and oxygen-catalyzed damage. Its role in humans and other animals, which can only obtain the pigment from their diet, is less well understood. Indeed it remains unproven that there is an essential role for lycopene in animal tissues. Nevertheless, considerable research effort is currently being undertaken to test hypotheses that are attempting to link human dietary and tissue lycopene levels to the risk of degenerative diseases, such as vascular diseases, cancers, etc., especially in older people. As discussed in more detail below, this research is being performed in a wide range of tissue culture and animal model systems and human epidemiological studies.

In this article, some key aspects of the chemical and physical properties, the dietary sources, biochemical status indices, and biological significance of lycopene will be described.

Chemical and Physical Properties of Lycopene; its Food Sources and Enteric Absorption

Lycopene (molecule weight 536.9) is the most commonly encountered of that subgroup of the naturally occurring

carotenoids that have a straight-chain poly-isoprenoid molecule without any terminal β -ionone ring structures (**Figure 1**). The chain length and number of conjugated double bonds determine the absorption spectrum, which peaks at 472 nm with a molar extinction coefficient, $\epsilon^{1\%}$ of 3450. It is one of the most nonpolar members of the carotenoids, and in organic solution it is also one of the most easily oxidized and thus is easily destroyed, which necessitates the use of rigorous precautions against its oxidative destruction during its extraction and analysis from plants, foods, animal tissues, and body fluids. Currently, such analytical determination is usually based on high-performance liquid chromatography (HPLC), using either its characteristic light absorption property, or its natural fluorescence, or its redox character, for detection and quantitation by absorbance or fluorometric or electrochemical detection. The lycopene content of selected commonly consumed foods is listed in **Table 1**. The original information can be found in the US Department of Agriculture (USDA) ARS recent publication (2010), Nutrition Data Laboratory Home Page. It is interesting to find out that in south Asia, the fruit Gac (*Momordica cochinchinensis*) contains more lycopene, almost three-fold more, than does the tomato fruit. Another characteristic that greatly affects lycopene stability and the problems of its storage and analysis is the phenomenon of *cis-trans* isomerization. Naturally occurring lycopene in tomatoes, the major human food source of this carotenoid, is nearly 100% all-*trans* (**Figure 1**), but during the processing of food, and then during the processes of absorption and accumulation in animal tissues, there is a progressive increase in the proportion of a variety of *cis*-forms. Most of these *cis*-forms contain a single *cis*-bond (mono-*cis*-lycopene), and the 5-, 9-, 13-, and 15- mono-*cis*-lycopenes account for more than 50% of the total lycopene in human serum. Smaller quantities of di-*cis*-lycopenes are normally also present. Curiously, another food source of lycopene, red palm oil, has a much higher natural proportion of the *cis*-forms of the pigment. Isomerization is catalyzed by low pH; therefore, stomach acid is believed to be a major factor in the conversion of the all-*trans*-lycopene ingested from tomatoes and their products to a mixture of *cis*-forms in the digestive tract. There is also evidence that further isomerization occurs between the digestive tract and the portal lymphatic lipid micelles. The *cis*-isomers differ from the all-*trans* form in their absorption and inter tissue transportation properties, and also in their functional characteristics; for instance, they are more soluble in lipophilic solvents and structures are less likely to aggregate into

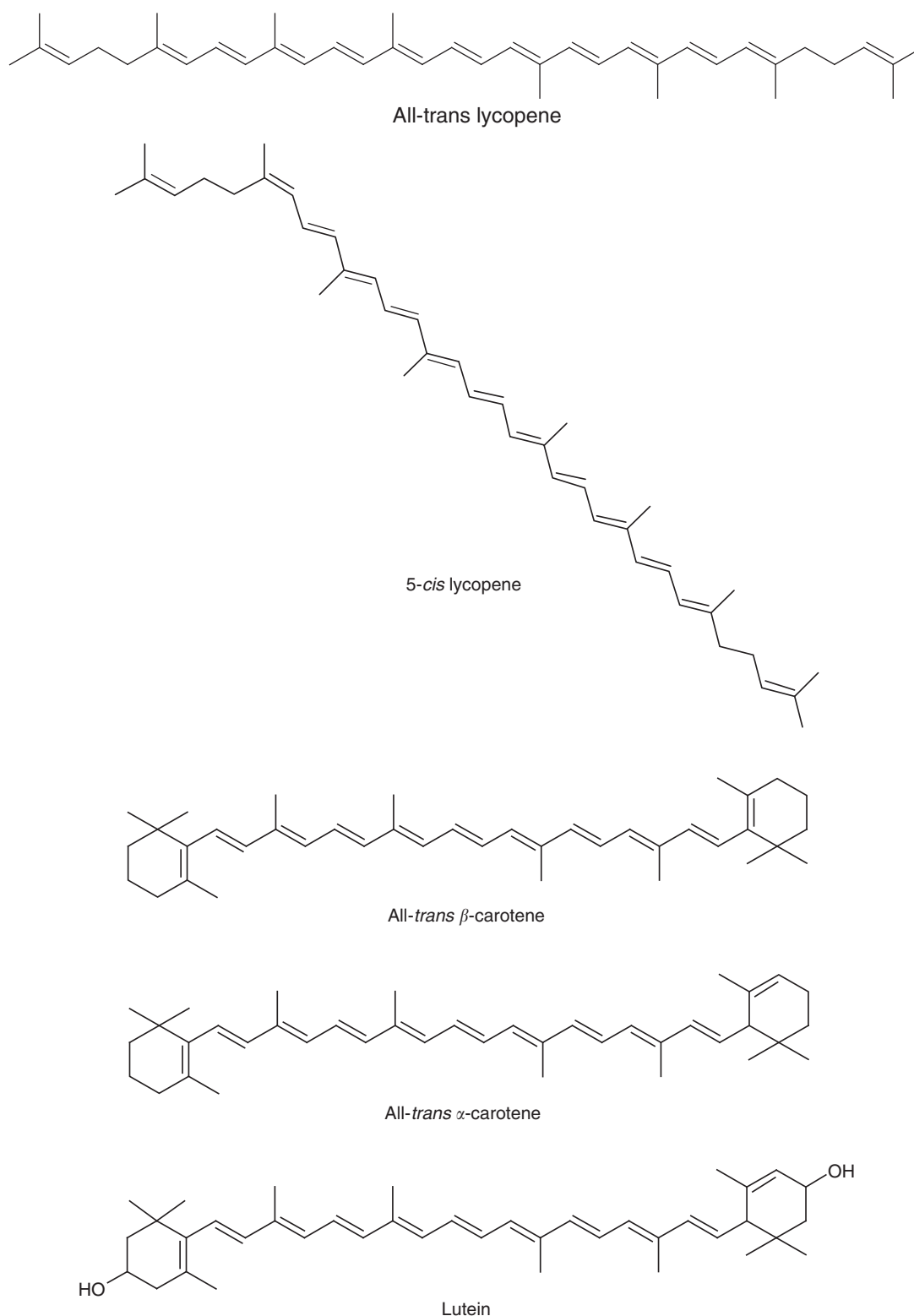


Figure 1 Structures of lycopene and certain other carotenoids found in human blood and tissues.

crystalline forms. More research data suggested that the greater bioaccessibility of *cis*-forms compared with all-*trans* form of lycopene was the reason for higher proportion of the *cis* form in tissues. A recent report showed that cholesterol membrane

transporter SR-B1 was involved in lycopene intestinal absorption. However, lycopene and its various forms in association of physicochemical differences and their related biological consequences have yet to be adequately explored.

Table 1 Lycopene (mg) content of selected foods, sorted by nutrient content collected from the USDA Nutrient Database for Standard Reference, Release 23

<i>Food description</i>	<i>Lycopene content (mg per 100 g)</i>
Tomatoes, sun-dried	45.9
Tomato products, canned, paste, without salt added	14–28.8
Sauce, pasta, spaghetti/marinara, barbecue, ready-to-serve	4.4–12.7
Catsup (Ketchup)	16.7
Sauce, salsa, ready-to-serve	10.5
Vegetable juice cocktail, canned	9.7
Tomato juice, canned, with salt added	9.0
Pasta with meatballs in tomato sauce, canned entree	7.2
Soup, minestrone, canned, reduced sodium, ready-to-serve or tomatoes, canned, stewed, prepared with equal volume water	4.1–6.4
Watermelon, raw	4.5
Tomatoes, red, ripe, canned, stewed	4.1
Spaghetti with meat sauce, frozen entree	3.2
Soup, clam chowder, manhattan, canned, prepared with equal volume water with bean, pork, beef noodle or chunky vegetable, etc.	2.9–4.3
Papayas, raw	1.8
Tomatoes, red, ripe, raw, year round average	2.6
Pizza, pepperoni, cheese, meat and vegetable topping, regular crust	1.8–2.0
Grapefruit, raw, pink and red, all areas	1.4
Fast foods, cheeseburger; single, regular patty, with condiments	1.0
Fast foods, hamburger; single, regular patty; with condiments	
Beans, baked, canned, with franks	0.4
Salad dressing, Russian dressing, Thousand Island dressing, French dressing, reduced fat, etc.	0.6–3.6

Source: <http://www.ars.usda.gov/ba/bhnrc/ndl>

Of all the most common naturally occurring carotenoids, lycopene is by far the most efficient in reacting with and quenching singlet oxygen, $^1\text{O}_2$, which is a non-free-radical excited and reactive form of oxygen. This form of oxygen reacts rapidly with lycopene to yield nonexcited triplet oxygen and excited triplet lycopene. The latter then dissipates its extra energy by solvent interactions, thus regenerating nonexcited lycopene and preserving its original structure by recycling. However, another of its chemical interactions with molecular oxygen appears to result in irreversible oxidation to yield one or more cyclic epoxides, which then probably undergo ring-opening. Nevertheless, there are many unresolved questions about the nature and importance of the many degradation pathways that are believed to result in the irreversible destruction of lycopene both *in vitro* and *in vivo*.

As a food component, consumption of lycopene in tomato is well tolerated and generally safe. The possible side effect of high consumption of tomato products would be mild digestive upset. Lycopene from tomatoes is permitted as a food color.

Lycopene is an essential intermediate in the pathway for synthesis of the β -ionone ring-containing carotenoids such as β -carotene in plant tissues, and in most plant tissues it is present in only minor amounts. However, in a few, including tomato fruit, watermelon, and red grapefruit, this conversion to the β -ionone ring products by the enzyme lycopene cyclase is hindered, so that the intermediate carotenoid forms, lycopene, phytoene, and phytofluene, accumulate instead.

In the US, tomato products provide more than 85% of the total quantity of lycopene consumed by the human population. Mean lycopene intakes in the US are considerably greater than they are in the UK, where the mean daily intake is thought to be less than one-third that in the US, while lycopene intakes in Far Eastern countries such as China and Thailand appear to be much lower still. Wild tomatoes originated in Central America and were introduced into Europe following the opening up of the New World, and were later introduced back into North America from Europe. Because tomatoes are the major source of dietary lycopene in many human populations, some epidemiological studies have been designed on the simplistic assumption that tomato consumption can be used as a general proxy for lycopene consumption, and that any disease associations with tomato consumption can be attributed to the biological effects of lycopene. However, tomatoes also contain significant amounts of other carotenoids, vitamin C, bioflavonoids such as naringenin, and phenolic acids such as chlorogenic acid. Much of the existing epidemiological evidence for possible beneficial effects of lycopene (see the following sections.) cannot distinguish unequivocally between the biological effects of lycopene and those of the many other bioactive constituents present in tomatoes.

The bioavailability of lycopene from raw tomatoes is low, but it is greatly increased by cooking or by commercial processing such as conversion to soup, sauce, ketchup (catsup), etc., and its availability is also increased by increasing the fat content of the food.

A survey conducted by National Health and Nutrition Examination Survey (NHANES) in years 2007–2008 on “what we eat in America”, showed that the average intake of lycopene from food was 5.5 mg per day per person, see **Table 2**. More information can be obtained from the USDAs web page.

Bioavailability of synthetic lycopene in an oil capsule was reported as better than from cooked and pureed tomatoes when taken by human subjects. Interactions with other carotenoids are complex and have only partly been studied, for instance β -carotene in the same dish seems to increase the absorption of lycopene, but large doses of β -carotene given separately seem to decrease the lycopene content in serum. The strength of the correlation between dietary lycopene intake and blood (serum or plasma) lycopene concentration varies greatly among studies and clearly depends on many factors, one of which is the degree of sophistication of the food table values, since subtle differences in food sources and meal composition affect its bioavailability very considerably.

Tissue Contents and Kinetics of Lycopene Turnover

Once absorbed, passively from lipid micelles by the enterocyte, lycopene enters the portal lymphatics and thence the liver, from which it enters the peripheral bloodstream, mainly

Table 2 Daily lycopene intake from food consumed by the US population – mean amount consumed per individual and percentages of lycopene consumed with meals and snacks by age and gender

Gender and age	Lycopene (SE) mg	Percentage of lycopene intake in meals and snacks
Males		
≥20 years	6.8 (0.3)	Breakfast, 7
Sample size,		Lunch, 31
n = 2662		Dinner, 51
		Snacks, 11
Females		
≥20 years	4.6 (0.3)	Breakfast, 4
Sample size,		Lunch, 27
n = 2758		Dinner, 57
		Snacks, 13
Males and females		
≥2 years	5.5 (0.2)	Breakfast, 5
Sample size,		Lunch, 30
n = 8529		Dinner, 53
		Snacks, 12

Abbreviation: SE, standard error.

Source: <http://www.ars.usda.gov/services/docs.htm?docid=18349>.

in association with the β -lipoproteins, in which it is transported to the peripheral tissues. Its half-life in plasma is of the order of 12–33 days; longer than that of β -carotene, which is less than 12 days. Clearly, many of these factors are interdependent, and there is a need for further clarification of the key independent determinants of lycopene status, and whether plasma levels can provide an adequate picture of tissue and whole body status.

Patients with alcoholic cirrhosis of the liver have greatly reduced hepatic lycopene concentrations; indeed, hepatic lycopene seems to offer a sensitive index of hepatic health. Studies of organ concentrations (Table 3), suggest a gradient from circulating levels in plasma to different ones in specific tissues. The different carotenoid ratios between organs (not shown) also indicate selective transport and accumulation. However, the mechanisms involved are poorly understood. No lycopene is detectable in the retina or lens of the eye, where lutein and zeaxanthin are found; however, lycopene is present in the ciliary body.

Functional Properties and Tissue Health

The capacity for quenching of singlet oxygen has been mentioned above; the exceptionally high rate constant, $K = 3.1 \times 10^{10} \text{ mol}^{-1} \text{ s}^{-1}$, renders it one of the most efficient of known quenchers of this powerful oxidant. In the plant, it probably protects chlorophyll, which produces singlet oxygen as a by-product of photosynthesis. In experiments with lymphoid cells, lycopene provided better protection against singlet oxygen damage than several other carotenoids tested. In skin exposed to UV light, lycopene disappears much more rapidly than β -carotene. Lycopene is also able, in model systems, to inhibit the peroxidation of polyunsaturated lipids and the oxidation of DNA bases to products such as 8-hydroxydeoxyguanosine

Table 3 Concentrations of lycopene reported in human tissues

Tissue	Range of mean or median (in <i>Italics</i>) lycopene concentrations (nmol per g weight)
Adrenal	1.9–21.6
Testis	4.3–21.4
Liver	0.6–5.7
Brain	2.5
Lung	0.2–0.6
Kidney	0.1–0.6
Stomach, colon	0.2–0.3
Breast, cervix	0.2–0.8
Skin	0.4
Adipose tissue	0.2–1.3
Prostate	0.1–0.6
Plasma	0.2–1.1

Note: Values were gathered from 12 publications, all based on HPLC analysis.

(8-OHdG). It can react directly with hydrogen peroxide and nitrogen dioxide. In addition, lycopene supplementation may modify DNA through DNA repair mechanisms.

Several studies in tissue culture have shown a reduction in the formation of oxidation damage products such as malondialdehyde, and have found less injury to cells exposed to oxidants such as carbon tetrachloride, if lycopene (or other carotenoids) is present.

Another characteristic of lycopene and other carotenoids that may be relevant to inhibition of cancer cell growth is the modulation of gap junction cell–cell communication processes. In particular, carotenoids including lycopene have been shown to enhance the efficacy of the protein, connexin43, which helps to ensure the maintenance of the differentiated state of cells and to reduce the probability of unregulated cell division, which is deficient in many tumors. They may also interact with and enhance the synthesis of binding proteins that down-regulate the receptor for the growth-promoting hormone insulin-like growth factor-1 (IGF-1). In one small clinical intervention with tomato drinks, it was reported that changes in circulating lycopene were inversely and significantly correlated with those of IGF-1.

In certain circumstances, lycopene can reduce low-density lipoprotein (LDL) -cholesterol levels, possibly by inhibiting hydroxymethylglutaryl CoA reductase (HMGCoA reductase), the rate-limiting enzyme for cholesterol synthesis (see the Section on Lycopene and Cardiovascular Disease). Lycopene was shown to have modest hypocholesterolemic properties in one small clinical trial.

Lycopene has shown significant antimigration and anti-invasion activity in association of its induction of nm23-H1 expression, a metastasis suppressor gene.

Health, Research Models, and Epidemiological Evidence

Table 4 summarizes the various types of evidence that have been used to test the hypothesis that lycopene may have health-promoting or protective properties in man. The ultimate proof of efficacy, which would be long-term controlled

intervention studies with clinical diseases or mortality as the end points, are extremely difficult, expensive, and time-consuming to obtain, and cannot address all possible benefits in a single intervention trial.

The two disease categories that have so far received most attention for possible long-term benefits of lycopene have been the amelioration of cancers and of heart disease. Both benefits are plausible in view of the physicochemical and biological properties of lycopene outlined above, because both categories of disease are characterized by tissue damage, which is thought to be induced or exacerbated by reactive oxygen species in the environment or those generated within the body.

Table 4 Types of evidence being sought to show that a nutrient such as lycopene may protect against oxidation-induced or other disease processes

1. Model *in vitro* systems, for example, oxygen-derived free-radical trapping in cell free chemical mixtures.
2. Tissue (cell and organ) cultures, for example, reduction of optical opacity development in cultured eye lenses; reduced growth rates or apoptosis in tumor cell cultures; and protection of key macromolecules, especially nucleic acids.
3. Animal studies demonstrating a reduction of oxidation-induced damage or disease with lycopene supplements or with lycopene-rich foods such as tomatoes or tomato products.
4. Human observation studies using intermediate biochemical markers: for example, inverse relationships between lycopene intakes or blood levels and biochemical markers, such as lipid or DNA oxidation products.
5. Studies using pathology-related intermediate markers, for example, arterial thickening or reduced arterial elasticity; precancerous polyposis, etc.
6. Relationships (without intervention) between tomato intakes or estimated lycopene intakes or lycopene contents of serum, plasma, or tissues (e.g., fat biopsies) and actual disease prevalence or incidence in human cross-sectional, case-control, or prospective epidemiological studies.
7. Intervention studies: lycopene supplements producing a reduction in biochemical markers of oxidation damage or in functional markers, or, eventually, in actual human disease incidence or progression.

Evidence for Possible Anticancer Protection by Lycopene

Most of the indications with respect to cancer come from human studies linking tomato intake, total estimated lycopene intake, and serum or plasma lycopene concentrations to the subsequent development of cancers (Table 5). There is a small amount of evidence from experimental animal studies, for instance, rat and mouse dimethylbenzanthracene-induced mammary tumor studies have supported the hypothesis, as has a model of spontaneous mammary tumor formation in one strain of mice, but many of the animal models of tumor promotion have been criticized as being too dissimilar from the likely processes of spontaneous tumor genesis in humans.

Partly for historical reasons, there has been a particular interest in prostate cancer (Table 5). A large and early trial in the US (US Health Professionals Follow-up Study) reported an impressive difference between groups with high and low intakes of tomatoes and hence of lycopene for subsequent prostate cancer development, which was not shared with other carotenoids. Plausibility was enhanced by the fact that although human prostate lycopene concentrations are not especially high on an absolute basis (Table 3), they are higher than those of other carotenoids in this tissue. Subsequent studies have had variable outcomes. A small pilot study reported that tomato oleoresin supplements given for a short period to prostate cancer sufferers who were due for radical prostatectomy resulted in smaller tumor size and other apparent benefits, but this trial now needs to be repeated on a larger scale. Although lycopene has shown protective effects against prostate cancer and inhibits the progression in patients with benign prostate hyperplasia, there is insufficient evidence to recommend the use of lycopene supplements for cancer patients.

Several studies have provided evidence for protection of certain regions of the digestive tract against tumor occurrence or growth. Two studies, one in Iran and another in Italy, found an inverse relationship between esophageal cancer and tomato consumption. Two Italian and one Japanese studies reported evidence for protection against gastric cancer, and two studies claimed a reduction in pancreatic cancer. A case-control study on histologically confirmed pancreatic cancer cases

Table 5 Summary of evidence for association of dietary intake of tomato, or serum or plasma concentrations of lycopene, with possible protection against prostate cancer

Number of studies	Locations	Total number of participants	Types of trial	Outcome conclusion
2	Greece, Canada	937	Case-control (intake of tomato or lycopene, or blood level)	Significant association
7	USA, UK, Canada, New Zealand	3824	As above	NS
3	USA	954	Prospective studies based on dietary estimates	Significant association
1	Netherlands		As above	NS
3	USA	723	Prospective studies based on serum or plasma lycopene	Inconclusive; one study found a marginal ($p = .05$) benefit versus aggressive cancer

Note: Significant association = $p < .05$ and NS = no significance ($p > .05$).

Table 6 Summary of evidence for association of relatively high serum or plasma lycopene with reduced risks of cardiovascular disease (CVD)

Study	Location	Sex (total participants)	Types of trial and outcome measures	Outcome conclusion
Euramic	Europe, multicenter	M (1379)	C-C, MI	Significant association with protection ^a
ARIC	USA	M + F (462)	C-C, IMT	NS
Street	USA	M + F (369)	NC-C, MI in smokers	NS
Rotterdam	Netherlands	M + F (216)	C-C, PC	Significant association with protection
Bruneck	Italy	M + F (392)	CS + PFU, PC	NS
Linköping-Vinus	Sweden and Lithuania	M (210)	CS, mortality from heart disease	NS
Kuopio (KHID)	Finland	M (725)	PFU, acute coronary event or stroke	Significant association with protection
Kuopio (ASP)	Finland	M + F (520)	IMT	Significant protection for males; not significant for Females
Physicians' Health Study	USA	M (case 499 + matched free CVD, 499)	C-C, CVD	NS
Women's Health Study	USA	F (case 483 + matched free CVD 483)	C-C, CVD	Significant association with protection

^aNo association with plasma b-carotene in this study.

Abbreviations: C-C, case-control study; NC-C, nested case-control study; CS, cross-sectional study; PFU, prospective follow-up study; MI, myocardial infarct; IMT, intima-media thickness estimate; PC, plaque count; NS, no significant evidence for protection.

Note: Significant association with protection is $p < .05$, generally after appropriate adjustment for other known CVD risk factors.

and population-based controls in eight Canadian provinces reported that dietary intake of lycopene, provided mainly by tomatoes, was associated with 31% reduction in pancreatic cancer risk among men when comparing the highest and lowest quartiles of intake.

Results with other cancers have been mixed and inconclusive.

Lycopene and Cardiovascular Disease

Table 6 summarizes the evidence. The European Multicentre Euramic Study reported that risk of developing myocardial infarct was inversely related to lycopene intake, after appropriate adjustment for other cardiovascular risk factors. Some Scandinavian studies have subsequently supported this claim; moreover, lycopene is capable of reducing LDL-cholesterol levels, possibly by inhibiting hydroxymethylglutaryl CoA reductase (HMGCoA reductase), the rate-limiting enzyme for cholesterol synthesis.

Other Disease-Related Investigations

In an organ culture model, some evidence for protection of rat lenses against induction of cataractogenesis has been reported. There is good reason to believe that carotenoids in general may play a role in the protection of ocular tissues against the damaging effects of UV light and of reactive oxygen substances, whose exposure to light carries some analogy with the known functions of carotenoids in plant tissues. A possible protective role in the ciliary body and iris has been proposed, but not yet tested.

Conclusions

Clearly lycopene possesses chemical and biological properties which make it a very attractive candidate for tissue protection and reduction of disease, especially degenerative diseases. Lycopene probably interacts more efficiently with one particular reactive oxygen species, singlet oxygen, than any other commonly occurring nutrient. It appears to share with several other carotenoids the capacity to reduce lipid peroxidation and DNA oxidative damage, and to enhance cell-cell gap junction communication and to protect normal IGF-1 function. It may reduce cholesterol formation and its tissue accumulation in some circumstances. Studies related to cancers and cardiovascular disease are ongoing and are attracting increased research interest.

See also: Antioxidants. Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Carotenoids: Chemistry, Sources and Physiology; Health Effects. Vitamin A: Deficiency and Interventions

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MAGNESIUM

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Glossary

Hypermagnesemia High levels of magnesium in blood or serum.

Hypermagnesuria High levels of magnesium in urine.

Magnesium An alkaline earth element with symbol Mg, atomic number 12, the seventh most abundant element in

the earth's crust and the 11th most abundant in the human body.

Reabsorption Movement of filtered substances (such as magnesium) and water from the kidney tubules back into the plasma.

Magnesium (Mg), the second intracellular cation after sodium, is an essential mineral. It is a critical cofactor in more than 300 enzymatic reactions. It may be required for substrate formation (Mg-ATP) and enzyme activation. It is critical for a great number of cellular functions, including oxidative phosphorylation, glycolysis, DNA transcription, and protein synthesis. It is involved in ion currents and membrane stabilization. Mg deficiency may be implicated in various metabolic disorders, including cardiovascular diseases, immune dysfunction, and free radical damage.

Magnesium Metabolism

Distribution of Mg within the Body

The normal adult body contains approximately 25 g of Mg, with more than 60% in bone tissue (**Table 1**). Only a fraction of bone Mg (at the surface of the bone crystal) is exchangeable with extracellular Mg. The muscle contains 25% of total body Mg, and extracellular Mg accounts for only 1%. Plasma Mg is approximately 0.8 mmol l^{-1} , half of which is ionized and active in physiological reactions half bound to proteins or complexed to anions. In cells, Mg is associated with various structures, such as the nucleus and intracellular organelles, and free Mg accounts for 1–5% of total cellular Mg. Intracellular free Mg is maintained at a relatively constant level, even if

extracellular Mg level varies. This phenomenon is due to the limited permeability of the plasma membrane to Mg and the existence of specific Mg transport systems that regulate the

Table 1 Magnesium in human tissues

	% distribution	Concentration
Bone	60–65	0.5% of bone ash
Muscle	27	6–10 mmol per kg wet weight
Other cells	6–7	6–10 mmol per kg wet weight
Extracellular	<1	
Erythrocytes		2.5 mmol l^{-1}
Serum		$0.7\text{--}1.1 \text{ mmol l}^{-1}$
Free	55	
Complexed	13	
Bound	32	
Mononuclear blood cells		$2.3\text{--}3.5 \text{ fmol per cell}$
Cerebrospinal fluid		1.25 mmol l^{-1}
Free	55	
Complexed	45	
Sweat		0.3 mmol l^{-1} (in hot environment)
Secretions		$0.3\text{--}0.7 \text{ mmol l}^{-1}$

Source: Reproduced from Vormann J (2003) Magnesium: Nutrition and metabolism. *Molecular Aspects of Medicine* 24: 27–37.

rates at which Mg is taken up by cells or extruded from cells. Mechanisms by which Mg is taken up by cells have not been completely elucidated, and Mg efflux particularly requires the antiport $\text{Na}^+/\text{Mg}^{2+}$. Various hormonal and pharmacological factors influence Mg transport, and it can be assumed that recent developments in molecular genetics will lead to the identification of proteins implicated in Mg transport.

Intestinal Absorption

Net Mg absorption results from dietary Mg absorption and Mg secretion into the intestinal tract via bile and gastric and pancreatic juice. In healthy adults, 30–50% of dietary Mg is absorbed. The secreted Mg is efficiently reabsorbed and endogenous fecal losses are only 20–50 mg day⁻¹. Mg absorption occurs along the entire intestinal tract, but the distal small intestine (jejunum and ileum) is the primary site. It is essentially a passive intercellular process by electrochemical gradient and solvent drag. The active transport occurs only for extremely low dietary Mg intake and its regulation is unknown. Mg uptake in the brush border may be mediated by an Mg/anion complex, and Mg efflux across the basolateral membrane may involve $\text{Na}^+/\text{Mg}^{2+}$ antiport systems. A gene implicated in Mg deficit in humans has been identified. It is expressed in intestine and kidney and appears to encode for a protein that combines Ca- and Mg-permeable channel properties with protein kinase activity. This gene may be implicated in Mg absorption. Because of the importance of the passive process, the quantity of Mg in the digestive tract is the major factor controlling the amount of Mg absorbed.

The possibility of an adaptive increase in the fraction of Mg absorbed as Mg intake is lowered is controversial. In fact, experimental studies indicate that fractional intestinal absorption of Mg is directly proportional to dietary Mg intake. Because only soluble Mg is absorbed, all the factors increasing Mg solubility increase its absorption while formation of insoluble complexes in the intestine may decrease Mg absorption. Most well-controlled studies indicate that high calcium intake does not affect intestinal Mg absorption in humans. In contrast, dietary phytate in excess impairs Mg absorption by formation of insoluble complexes in the intestinal tract. Negative effects of a high intake of dietary fiber have often been reported, but these actions have certainly been overestimated. In fact, only the impact of purified fiber was considered, but fiber-rich diets are a major source of Mg and roles of the intestinal fermentation and the large bowel in mineral absorption were neglected. It was demonstrated in animal models that fermentable carbohydrates (oligosaccharides and resistant starch) enhance Mg absorption in the large bowel and that a similar effect exists in humans. Other nutrients may influence Mg absorption but these effects are important only at low dietary Mg intake.

Urinary Excretion

Magnesium homeostasis is essentially regulated by a process of filtration–reabsorption in the kidney. Urinary Mg excretion increases when Mg intake is in excess, whereas the kidney conserves Mg in the case of Mg deprivation. Usually, 1000 mmol

per 24 h of Mg is filtered and only 3 mmol per 24 h is excreted in urine.

A total of 10–15% of the filtered Mg is reabsorbed in the proximal tubule by a passive process. The majority of filtered Mg (65%) is reabsorbed in the thick ascending loop of Henle. The reabsorption in this segment is mediated by a paracellular mechanism involving paracellin-1. It is also related to sodium transport by a dependence on the transepithelial potential generated by NaCl absorption. Thus, factors that impair NaCl reabsorption in the thick ascending loop of Henle, such as osmotic diuretics, loop diuretics, and extracellular fluid volume expansion, increase Mg excretion. At least 10–15% of the filtered Mg is reabsorbed in the distal tubule. The reabsorption occurs via an active transcellular mechanism and is under the control of special divalent cation-sensing receptors. Thus, elevated plasma Mg concentrations inhibit reabsorption of Mg from the distal tubule, leading to an increased magnesuria. Other active transport may also exist because some hormones (parathyroid hormone (PTH), glucagon, calcitonin, and insulin) may increase Mg reabsorption. Other factors may also influence Mg reabsorption, such as hypercalciuria or hypophosphatemia, which inhibit the tubular reabsorption of Mg. Metabolic alkalosis leads to renal Mg conservation, whereas metabolic acidosis is associated with urinary Mg wasting. Thus, the chronic low-grade metabolic acidosis in humans eating Western diets may contribute to decreased Mg status.

Dietary Sources of Magnesium

Mg is present in all foods, but the Mg content varies substantially (Table 2). Cereals and nuts have high Mg content. Vegetables are moderately rich in Mg, and meat, eggs, and milk are poor in Mg. A substantial amount of Mg may be lost during food processing, and refined foods generally have a low Mg content. In addition to Mg content, it is important to consider the Mg density of food (i.e., the quantity of Mg per unit of energy). Vegetables, legumes, and cereals thus contribute efficiently to daily Mg intake, whereas fat- and sugar-rich products have a minor contribution. Some water can also

Table 2 Mg density of foods

<i>Food</i>	<i>Magnesium density (mg MJ⁻¹)</i>
Vegetables (lettuce, broccoli)	211
Legumes (bean)	113
Whole cereal (wheat)	104
Nuts (almond)	105
Fruits (apple)	30
Fish (cod)	75
Meat (roast beef)	40
Whole milk	38
Cheese (camembert)	15
Eggs	18
Dessert	
Biscuit	10
Chocolate	52

Source: Reproduced from Répertoire Général des Aliments (1996) *Table de Composition Minérale*. Lavoisier, Paris: Tec & Doc.

be a substantial source of Mg, but it depends on the area from which the water derives.

Requirements

Assessment of Mg Status

Several potential markers for estimating daily Mg requirement have been suggested. Plasma Mg concentration is the most commonly used marker to assess Mg status. In healthy populations, the plasma Mg value is 0.86 mmol l^{-1} and the reference value is $0.75\text{--}0.96 \text{ mmol l}^{-1}$. A low plasma Mg value reflects Mg depletion, but a normal plasma Mg level may co-exist with low intracellular Mg. Thus, despite its interest, plasma Mg is not a good marker of Mg status.

Ion-specific electrodes have become available for determining ionized Mg in plasma, and this measurement may be a better marker of Mg status than total plasma Mg. However, further investigation is necessary to achieve a standardized procedure and to validate its use as an appropriate marker of Mg status.

Erythrocyte Mg level is also commonly used to assess Mg status, and the normal value is $2.06\text{--}2.54 \text{ mmol l}^{-1}$. However, erythrocyte Mg level is under genetic control, and numerous studies have shown no correlation between erythrocyte Mg and other tissue Mg.

The total Mg content of white blood cells has been proposed as an indicator of Mg status. However, lymphocytes, polymorphonuclear blood cells, and platelets may have protective mechanisms against intracellular Mg deficiency, and the determination of total Mg content in leukocytes and platelets to assess Mg status is of questionable usefulness.

Mg excretion determination is helpful for the diagnosis of Mg deficit when there is a hypomagnesemia. In healthy populations, the urinary Mg value is $4.32 \text{ mmol day}^{-1}$ and the reference value is $1.3\text{--}8.2 \text{ mmol day}^{-1}$. In the presence of hypomagnesemia, normal or high urinary Mg excretion is suggestive of renal wasting. On the contrary, Mg urinary excretion lower than normal values is a convincing evidence of Mg deficiency.

The parenteral loading test is probably the best available marker for the diagnosis of Mg deficiency. The Mg retention after parenteral administration of Mg seems to reflect the general intracellular Mg content, and an Mg retention more than 20% of the administered Mg suggests Mg deficiency. However, this test is not valid in the case of abnormal urinary Mg excretion and is contraindicated in renal failure.

Determination of exchangeable Mg pools using Mg stable isotopes is an interesting approach to evaluate Mg status. In fact, Mg exchangeable pool sizes vary with dietary Mg in animals. However, more studies are necessary to better appreciate the relationship between Mg status and exchangeable Mg pool size in humans.

Magnesium Deficit

Two types of Mg deficit must be differentiated. Dietary Mg deficiency results from an insufficient intake of Mg. Secondary Mg deficiency is related to dysregulation of the control mechanisms of Mg metabolism.

Dietary Mg Deficiency

Severe Mg deficiency is very rare, whereas marginal Mg deficiency is common in industrialized countries. Low dietary Mg intake may result from a low energy intake (reduction of energy output necessary for physical activity and thermoregulation, and thus of energy input) or from low Mg density of the diet (i.e., refined or processed foods). Moreover, in industrialized countries, diets are rich in animal source foods and low in vegetable foods. This leads to a dietary net acid load and thus a negative effect on Mg balance. In fact, animal source foods provide predominantly acid precursors (sulfur-containing amino acids), whereas fruits and vegetables have substantial amounts of base precursor (organic acids plus potassium salts). Acidosis increases Mg urinary excretion by decreasing Mg reabsorption in the loop of Henle and the distal tubule, and potassium depletion impairs Mg reabsorption. Mg deficiency treatment simply requires oral nutritional physiological Mg supplementation.

Secondary Mg Deficiency

Failure of the mechanisms that ensure Mg homeostasis, or endogenous or iatrogenic perturbing factors of Mg status, leads to secondary Mg deficit. Secondary Mg deficiency requires a more or less specific correction of its causal dysregulation.

Intestinal Mg absorption decreases in the case of malabsorption syndromes, such as chronic diarrhea, inflammatory enteropathy, intestinal resection, and biliary and intestinal fistulas.

Hypermagnesuria is encountered in the case of metabolic and iatrogenic disorders, such as primary and secondary hyperaldosteronism (extracellular volume expansion), hypercalcemia (competition Ca/Mg at the thick ascending loop of Henle), hyperparathyroidism, and phosphate or potassium depletion. Hypermagnesuria may also result from tubulopathy, as the selective defect of the Mg tubular reabsorption (chromosome 11q23), Bartter's syndrome (thick ascending loop of Henle), or Gitelman's syndrome (distal convoluted tubule).

Administration of medications can be a causal factor in the development of secondary Mg deficiency. Administration of diuretics is the main cause of iatrogenic deficit because it decreases NaCl reabsorption in the thick ascending loop of Henle and thus increases the fractional excretion of Mg.

Causes of Mg Deficit

Complex relations exist between Mg and carbohydrate metabolism. Diabetes is frequently associated with Mg deficit and insulin may play an important role in the regulation of intracellular Mg content by stimulating cellular Mg uptake. Hypomagnesemia is the most common ionic abnormality in alcoholism because of poor nutritional status and Mg malabsorption, alcoholic ketoacidosis, hypophosphatemia, and hyperaldosteronism secondary to liver disease.

Stress can contribute to Mg deficit by stimulating the production of hormones and thus increasing urinary Mg excretion and by impairing neurohormonal mechanisms that spare Mg.

Consequences of Mg Deficit and Implications in Various Metabolic Diseases

Mg deficit causes neuromuscular manifestations, including positive Chvostek and Trousseau signs, muscular fasciculations, tremor, tetany, nausea, and vomiting. The pathogenesis of the neuromuscular irritability is complex, and it implicates the central and peripheral nervous system, the neuromuscular junction, and muscle cells.

Mg deficit perturbs Ca homeostasis and hypocalcemia is a common manifestation of severe Mg deficit. Impaired release of PTH and skeletal end organ resistance to PTH appear to be the major factors implicated, probably by a decrease in adenylcyclase activity.

Perturbations in the action and metabolism of vitamin D may also occur in Mg deficit. Because Mg plays a key role in skeletal metabolism, Mg deficit may be a possible risk factor for osteoporosis. However, epidemiologic studies relating Mg intake to bone mass or rate of bone loss have been conflicting, and further investigation is necessary to clarify the role of Mg in bone metabolism and osteoporosis.

Hypokalemia is frequently encountered in Mg deficit. This is due to an inhibition of Na,K-ATPase activity that impairs K and Na transport in and out of the cell and to stimulation of renin and aldosterone secretion that increases K urinary excretion.

There is increasing evidence that Mg deficiency may be involved in the development of various pathologies. Mg deficit is frequent in diabetes and can be a factor in insulin resistance. It can modify insulin sensitivity, probably by influencing intracellular signaling and processing. Mg deficit has also been implicated in the development or progression of micro- and macroangiopathy and neuropathy.

Mg deficit appears to act as a cardiovascular risk factor. Experimental, clinical, and epidemiological evidence points to an important role of Mg in blood pressure regulation. Mg deficit can lead to cardiac arrhythmias and to increased sensitivity to cardiac glucosides. Mg deficit may also play a role in the development of atherosclerosis. In experimental animal models, dietary Mg deficiency results in dyslipidemia, increased sensitivity to oxidative stress, and a marked proinflammatory effect, thus accelerating atherogenesis. Macrophages and polynuclear neutrophils are activated and synthesize a variety of biological substances, some of which are powerful inducers of inflammatory events (cytokines, free radicals, and eicosanoids). The effect of Mg depletion or Mg supplementation may result in the ability of Mg to modulate intracellular calcium. Pharmacological doses of Mg may reduce morbidity and mortality in the period following infarction. The beneficial effect of Mg may result from calcium-antagonist action, decreased platelet aggregation, and decreased free radical damage.

Magnesium Excess

Magnesium overload can occur in individuals with impaired renal function or during massive intravenous administration of Mg. It is most often iatrogenic. Clinical symptoms such as drowsiness and hyporeflexia develop when plasma Mg is two- or three-fold higher than the normal value.

Recommended Dietary Allowances

The estimated average requirement (EAR) is the nutrient intake value that is estimated to meet the requirement of 50% of individuals in a life stage and a gender group. Balance studies and data on stable isotopes suggest an EAR of $5 \text{ mg kg}^{-1} \text{ day}^{-1}$ for males and females. This value is greater during growth in adolescents and is estimated to be $5.3 \text{ mg kg}^{-1} \text{ day}^{-1}$. The Mg requirement is also higher during pregnancy because of Mg transfer to the fetus in the last 3 months; therefore, an additional 35 mg day^{-1} is recommended.

In infants, the determination of the adequate intake (AI) is based on the Mg content of mother's milk and the progressive consumption of solid food. Thus, the AI is 30 mg day^{-1} during the first 6 months of life and 75 mg day^{-1} during the second 6 months of life.

The Recommended Dietary Allowance (RDA) is the average daily dietary intake that is sufficient to meet the nutrient requirement of 97.5% of individuals and is set at 20% above the $\text{EAR} + 2 \text{ CVs}$ where the CV is 10%. During recent years, dietary reference intakes for the US and Canada have been revised by the Institute of Medicine. The recommended intakes of Mg are given in Table 3. It is not known whether decreased urinary Mg and increased maternal bone resorption provide sufficient amounts of Mg to meet increased needs during lactation. Thus, the French Society for Nutrition suggests adding 30 mg day^{-1} to intake for lactation, whereas no increase is recommended during lactation for the US and Canada.

The intake of Mg has been determined in various populations. Evidence suggests that the occidental diet is relatively low in Mg compared to recommended intakes, whereas the vegetarian diet is rich in Mg. For instance, the mean Mg intake of the subjects in the French Supplementation with Antioxidant Vitamins and Minerals Study was estimated to be 369 mg day^{-1} in men and 280 mg day^{-1} in women. However, it is possible that the recommended intakes are set somewhat high, as clinical problems are uncommon when such intakes are not caused or accompanied by metabolic diseases such as diabetes and alcoholism.

Table 3 Recommended dietary allowances of Mg

Age	RDA (mg day^{-1})		AI (mg day^{-1})	
	Male	Female	Male	Female
0–6 months			30	30
6–12 months			75	75
1–3 years	80	80		
4–8 years	130	130		
9–13 years	240	240		
14–18 years	410	360		
19–30 years	400	310		
31–50 years	420	320		
51–70 years	420	320		
<70 years	420	320		
Pregnancy		+ 40		
Lactation		+ 0		

Source: Reproduced from Institute of Medicine (1997) *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D and Fluoride*. Washington, DC: National Academy Press.

Conclusion

Based on evidence of low Mg intake in industrialized countries, intervention studies are needed to test whether improving Mg status would improve health outcomes.

See also: Calcium. Cereal Grains

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MALABSORPTION SYNDROMES

Nutritional Management

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Glossary

Anthropometrics Body measurements used to determine body fat composition.

Bioelectrical impedance A method that uses a small electrical current to measure body composition.

DEXA scan A dual energy X-ray absorptiometry scan that uses X-ray beams to measure bone density and/or body composition.

Endoscopy A procedure that uses an instrument to look inside the body for diagnostic or therapeutic purposes.

Protein hydrolysate A type of formula in which proteins are broken down into smaller peptides by hydrolysis.

Introduction

The human gastrointestinal tract has an impressive capacity for water, electrolyte, and nutrient absorption. In some disease states, however, this excess capacity is outpaced by either intestinal secretion or inadequate absorption. Malabsorption is defined as the inability of the gastrointestinal tract to adequately absorb nutrients. Although strictly speaking, malabsorption is distinct and contrasted with maldigestion (inadequate breakdown of nutrients in the intestinal lumen); the therapeutic implications of these two conditions are often similar. Multiple causes of malabsorption exist and reviews of these individual diagnoses can be found in separate sections of this text (e.g., inflammatory bowel disease, cystic fibrosis, short bowel syndrome, etc.). The pathophysiology, symptoms, and nutritional therapies for common malabsorption syndromes have been reviewed.

Pathophysiology and Symptoms

Malabsorption can occur when any of the several steps in nutrient digestion, absorption, and/or assimilation are interrupted; see **Table 1** for a list of congenital defects in nutrient assimilation. Carbohydrate malabsorption can occur, for instance, when intestinal disaccharidases are reduced in concentration at the enterocyte. The brush border membrane produces four disaccharidases that are important in carbohydrate digestion. These enzymes are sucrase-isomaltase, maltase-glucoamylase, trehalase, and lactase-phlorizin hydrolase. Worldwide, lactase deficiency is the most common type of acquired disaccharidase deficiency. With lactase deficiency, malabsorbed carbohydrate remains in the intestinal lumen and exerts an osmotic pull on fluids and electrolytes, leading

to abdominal cramping and loose stools. Malabsorbed carbohydrate can be metabolized by gastrointestinal tract bacteria, and the fermented gas produced is associated with flatulence and bloating. Bacterial overgrowth of the small intestine, as seen with short bowel syndrome, can also be associated with carbohydrate malabsorption.

Steatorrhea, excessive fat in the stools, results from fat malabsorption or maldigestion and can have several causes, most notably pancreatic insufficiency due to cystic fibrosis, chronic pancreatitis, Shwachman-Diamond syndrome, and Johanson-Blizzard syndrome. Failure of pancreatic secretion of lipase, amylase, and other digestive enzymes leads to persistence of dietary fat in the intestinal lumen, causing bloating, abdominal pain, and bulky, foul-smelling, oily stools. The stools often float due to a high gas content and test positive for fat. Patients also complain of blunted appetite and nausea. Other causes of fat malabsorption include hepatobiliary disease with inadequate bile salt circulation, severe mucosal disease, and short bowel syndrome.

The most common cause of protein malabsorption is so-called protein-losing enteropathy. Etiologies include diffuse mucosal disease such as celiac disease or Crohn's disease, elevated right heart pressure with resultant dilatation of lymphatics and leakage of lymph into the lumen, and invasive enteropathies as seen with *Shigella* or *Salmonella* infections. Because protein is a relatively minor component of dietary energy compared with carbohydrate and fat, symptoms of protein malabsorption can sometimes be minimal. However, infectious colitis or exacerbations of inflammatory bowel disease often present with frequent loose stools, which may be bloody. Rarely, congenital etiologies of protein malabsorption include enterokinase and trypsinogen deficiencies (**Table 1**).

Finally, the malabsorption of various micronutrients can occur in conjunction with or separate from the macronutrient

Table 1 Congenital defects in nutrient assimilation. Included are congenital defects that are associated with gastrointestinal symptoms and/or nutritional deficiencies. Congenital defects not included here include multiple defects in amino acid absorption

<i>Disorder</i>	<i>Gene/protein affected</i>	<i>Symptoms</i>
<i>Carbohydrate digestion</i>		
Congenital lactase deficiency	Lactase	Lactose-induced diarrhea
Hypolactasia	Lactase	Lactose-induced diarrhea
Congenital sucrase-isomaltase deficiency	Sucrase-isomaltase	Sucrose-induced diarrhea
<i>Carbohydrate absorption</i>		
Glucose-galactose malabsorption	Sodium-glucose-co-transport (SGLT1); SLC5A1	Glucose-induced diarrhea
Fructose malabsorption	Facilitative fructose transport (GLUT5); SLC2A5	Fructose-induced diarrhea
Fanconi-Bickel syndrome	Facilitative glucose transport (GLUT2); SLC2A2	Diarrhea and nephropathy
<i>Protein digestion</i>		
Enterokinase deficiency	Serine protease 7	Diarrhea and edema
Trypsinogen deficiency	Trypsinogen	Diarrhea and edema
<i>Fat digestion</i>		
Pancreatic lipase deficiency	Pancreatic lipase	Steatorrhea
<i>Fat assimilation</i>		
Abetalipoproteinemia	Microsomal triglyceride transfer protein	Steatorrhea
Hypobetalipoproteinemia	Apolipoprotein B	Steatorrhea
Chylomicron retention disease	Sar1-ADP-ribosylation factor family GTPases	Steatorrhea
Primary bile acid malabsorption	Sodium-bile acid transporter; SLC10A2	Steatorrhea, bile acid diarrhea
Tangier disease	ATP-binding cassette transporter 1	Hepatosplenomegaly
Sitosterolemia	ATP-binding cassette, subfamily G, member 8; ABCG8	Atherosclerosis
<i>Ion and metal absorption</i>		
Congenital sodium diarrhea	Defective Na^+/H^+ exchange	Secretory diarrhea
Congenital chloride diarrhea	Defective $\text{Cl}^-/\text{HCO}_3^-$ exchange	Secretory diarrhea
Cystic fibrosis	CFTR	Pancreatic insufficiency, meconium ileus
Acrodermatitis enteropathica	Zinc and iron-regulated transport proteins; SLC39A4	Diarrhea and dermatitis
Menkes disease	Copper transporter	Developmental delay
Primary hypomagnesemia	Paracellin 1; claudin 16	Seizures, deafness and polyuria
Hemochromatosis	Hepcidin, others	Cirrhosis, cardiomyopathy, diabetes
<i>Vitamin absorption</i>		
Folate malabsorption	?	Macrocytic anemia, diarrhea, developmental delay
Congenital pernicious anemia	Intrinsic factor	Macrocytic anemia, developmental delay
Imerslund-Graesbeck syndrome	Cubilin, amnionless	Anemia, proteinuria
Congenital deficit of transcobalamin II	Transcobalamin II	Anemia, diarrhea, developmental delay
Thiamine-responsive megaloblastic anemia	Thiamine transport protein; SLC19A2	Anemia, diabetes, cranial nerve defects
Familial retinol binding protein (RBP) deficiency	Retinol-binding protein 4	Ophthalmologic problems
Selective vitamin E deficiency	Alpha-tocopherol transport protein	Vitamin E malabsorption

Source: Adapted from Martin M and Wright EM (2008) Congenital intestinal transport defects. In: Kleinman RE, Goulet O, Mieli-Vergani G, Sanderson IR, Sherman P, and Schneider B (eds.) *Walker's Pediatric Gastrointestinal Disease: Pathophysiology, Diagnosis, Management*, 5th edn., p. 290. Hamilton, Ontario: BC Decker.

malabsorption syndromes noted above. For instance, steatorrhea can be accompanied by excessive fecal losses of the fat soluble vitamins A, D, E, and K, as well as calcium and other minerals. Alternatively, atrophic gastritis or surgical resection of the terminal ileum can lead to Vitamin B₁₂ malabsorption in the absence of any symptoms of diarrhea. Proximal bowel resection can result in iron, zinc and calcium malabsorption. A rare cause of micronutrient inadequacy is abetalipoproteinemia in which fat soluble nutrients are normally digested and absorbed by the intestine but are not delivered to the circulation due to defective transepithelial transport. Other rare causes of micronutrient malabsorption are noted in [Table 1](#).

General Nutritional Management of Malabsorption

As with all nutritional disorders, a thorough nutritional assessment is needed to plan rational therapy of malabsorption. Important historical points to review include duration of symptoms, underlying etiology of malabsorption, ability to meet nutritional needs by mouth, presence of food allergies, and concurrent medical and surgical problems. The patient's nutritional status (weight, height, body mass index, and their respective percentiles) should be determined. Tests of body composition such as arm anthropometrics, bioelectrical impedance, or DEXA scan should be considered. If the

underlying cause of malabsorption is not known, diagnostic gastrointestinal endoscopy, laboratory studies, and/or imaging studies are indicated.

Specific Nutritional Management of Malabsorption

Fluids and Electrolytes

Diarrhea is usually the most distressing problem for patients with malabsorption and may cause dehydration. Care should be taken to correct fluid losses with appropriately designed oral rehydration solutions. Even in the setting of massive secretory diarrhea such as seen with cholera infections, oral rehydration solutions are effective at treating dehydration. Recent data have supported the safety and efficacy of oral rehydration solutions of reduced osmolality in children with dehydration from acute diarrhea. An oral rehydration solution with the following composition: glucose 75 mmol l⁻¹, sodium 75 mmol l⁻¹, potassium 20 mmol l⁻¹, base 30 mEq l⁻¹, and osmolality 245 mOsm l⁻¹ is well-suited for the rehydration and maintenance therapy during dehydration due to diarrhea.

In some cases of severe diarrhea, parenteral hydration is the mainstay of therapy. Examples include glucose–galactose malabsorption, congenital chloride diarrhea, microvillous inclusion disease, and tufting enteropathy. These cases, as well as other severe causes of more common malabsorptive syndromes, also frequently require the use of parenteral nutrition therapy.

Carbohydrate Malabsorption

Lactose Intolerance

Lactose intolerance is defined by the occurrence of symptoms after ingestion of lactose, the main carbohydrate in milk. These symptoms may include abdominal pain, bloating, diarrhea, or flatulence. Lactose intolerance is usually secondary to lactose malabsorption caused by a relative deficiency of the disaccharidase lactase, which reduces the ability to digest lactose. Primary lactase deficiency is a condition in which lactase activity falls after weaning around 2 years of age. Secondary lactose intolerance may be temporary and is usually due to mucosal injury associated with a condition or disease such as infectious diarrhea, Crohn's disease, or short bowel syndrome.

In addition to the presence or absence of the lactase enzyme, other factors determine whether a person will have symptoms of lactose malabsorption, including the amount of lactose in the diet, the mixture of lactose with other foods, gastric emptying rate, colonic scavenge of malabsorbed carbohydrate, ethnic origin and age. Although persons of Northern European ancestry commonly maintain the ability to digest lactose into adulthood, primary lactose intolerance is prevalent in African–American, Hispanic, Native American, and Asian populations.

Nutritional management of lactose intolerance consists largely of the removal of lactose from the diet. Lactose is a common ingredient in many foods, including breads, crackers, soups, cereals, cookies, and baked goods. Eliminating or reducing lactose-containing ingredients from one's diet is

Table 2 Commercial calcium supplements

Product	Manufacturer	Mg Calcium/ tablet	IU vitamin D
Citracal Regular	Bayer	500	400
OsCal 500 + D	GlaxoSmithKline	500	200
Tums	GlaxoSmithKline	500	0
Calcium Milk Free	Nature's Plus	250	50
Cal-citrate + D	Freeda	250	100
Caltrate 600 + D	Pfizer	600	400
Viactiv ^a	McNeil	500	500
Nutritionals			

^aThis product contains less than 0.5 mg lactose.

Source: Adapted from DiSanto C and Duggan C (2005) Gastrointestinal diseases. In: Hendricks KM and Duggan C (eds.) *Manual of Pediatric Nutrition*, 4th edn., p. 212. Hamilton, Ontario: BC Decker.

usually adequate to relieve symptoms. Individuals with primary lactose intolerance may require a permanent dietary change. Individuals with secondary lactose intolerance should eliminate all lactose from their diets for a short period of time ranging from 2 to 6 weeks. If symptoms resolve, lactose may be reintroduced slowly as tolerated by the individual. The amount of lactose that an individual can tolerate is highly variable. Many children can tolerate small amounts of lactose, particularly yogurt or hard cheese, without discomfort. Many adults who consider themselves lactose intolerant can actually tolerate moderate amounts of milk. Lactose intolerant individuals may also tolerate small amounts of lactose consumed over the course of the day better than a large dose all at once.

For individuals who choose to restrict lactose in their diets, a variety of lactose-free and low-lactose food choices are available. Lactose-reduced products, containing 70–100% less lactose than standard foods, are available commercially. Individuals may also choose to consume dairy products with concomitant administration of lactase enzyme tablets or drops.

Frequent consumption of milk and other dairy foods has been associated with better bone health in some studies, and a strict lactose-free diet may not contain adequate amounts of calcium and vitamin D. **Table 2** provides a list of some commercially available lactose-free and lactose-reduced calcium supplements.

Sucrose

Congenital sucrase-isomaltase deficiency (SID) is the most common congenital disaccharidase deficiency. Patients with this disorder lack functional sucrase, although isomaltase deficiency may be normal or absent. Symptoms of SID can include diarrhea, abdominal pain, and poor weight gain. Dietary avoidance of sucrose or table sugar helps relieve symptoms, and can sometimes help with the diagnosis. Sucraid[®], a sacrosidase produced from *Saccharomyces cerevisiae*, is an enzyme that can be given with meals and allows increased tolerance to sucrose.

Fat Malabsorption: Fat and Fat-Soluble Nutrients

Patients with pancreatic insufficiency are unable to produce and secrete enough enzymes to aid with the breakdown of fats

in the intestinal lumen. In studies of normal adults and those with pancreatic insufficiency, pancreatic enzyme secretion needs to be lower than 15% of normal levels before significant steatorrhea is seen (Figure 1). Once clinically significant steatorrhea is determined, recovery of pancreatic function is therefore unlikely.

Historically, patients with pancreatic insufficiency due to cystic fibrosis (CF) were told to minimize symptoms of steatorrhea by limiting dietary fat. However, epidemiologic studies confirmed that this advice led to negative energy balance, undernutrition, and higher mortality rates, compared to communities in which CF patients were treated with high-energy and high-fat diets. The introduction of effective pancreatic replacement therapy has been heralded as one of the most significant breakthroughs in the nutritional management of CF, responsible partly for the substantial increase in lifespan enjoyed by more recent generations of CF patients. In fact, the finding of a lower incidence of growth failure in CF patients diagnosed and treated with aggressive nutritional therapy early in infancy has been used as justification for neonatal screening of this condition.

Judicious use of pancreatic replacement enzymes is the hallmark of nutritional therapy of CF and other disorders of pancreatic insufficiency. Multiple commercial preparations of porcine pancreatic enzymes are available, most of which contain lipase, amylase, and protease enzymes. A nonporcine pancreatic enzyme is currently under development. The dose is usually titrated to the amount of steatorrhea. If meals take more than 30 min, the dose may be divided with half given before the meal and half given mid-way through the meal. Patients who cannot swallow pills may open the capsules and sprinkle the enzymes into acidic foods.

Another critical aspect of the nutritional management of fat malabsorption is routine supplementation with the

fat-soluble vitamins A, D, E, and K. Multiple studies have confirmed that patients with CF, Crohn's disease, and other malabsorptive disorders are prone to micronutrient deficiencies, and some literature suggests that dietary needs for these and other antioxidant nutrients may be increased in settings of infectious and catabolic stress often suffered by these patients. The contribution of fat malabsorption contributing to other important mineral malabsorption, as in the case of calcium or zinc, should also be recognized.

Routine supplementation of fat-soluble vitamins is indicated in patients with fat malabsorption. In addition, serial measurement of fat-soluble vitamin biochemical status is recommended. Because blood nutrient concentrations of these and other nutrients can vary with the concentration of transport proteins, correction for these can aid the interpretation of these lab findings. For instance, vitamin A toxicity should be suspected if the molar ratio of vitamin A: Retinol-binding protein exceeds 1. Vitamin E concentrations, for example, should be corrected for circulating lipids.

Some patients with pancreatic malabsorption may benefit from a diet enriched in medium chain triglycerides (MCTs). MCTs are absorbed directly into the portal circulation and therefore bypass the steps of intraluminal digestion, reesterification, and enterocyte uptake. Therefore, these fats may be a dietary source of fats more easily absorbed in settings of fat malabsorption due to either pancreatic insufficiency or mucosal disease. However, MCT oils are less energy dense than long-chain fats, are more expensive, and do not contain the essential fatty acids alpha-linoleic and linolenic acid.

Protein Malabsorption

Protein-losing enteropathy (PLE) can also be treated with a variety of nutritional interventions. PLE due to dilated lymphatics as with right heart failure results in leakage of lymphocytes, proteins, and fats into the intestinal lumen. As with fat malabsorption, MCT-supplemented foods and formulas are therefore indicated to allow improved fat absorption in PLE. Fat-soluble vitamin supplementation is indicated. In congenital protein malabsorption syndromes, peptide- or amino acid-based formulas are often helpful.

Mucosal disorders including inflammatory bowel disease, allergic diseases, and celiac disease are other examples of disorders causing protein malabsorption. Once intestinal inflammation is reduced with appropriate medical or nutritional therapy, absorption of protein is usually improved. In *Shigella* infections, some studies have suggested improved nutritional outcomes with a high-protein diet during recovery from the acute symptoms of diarrhea.

Route of Nutrition in Malabsorption

Several factors need to be considered when recommending whether oral, enteral, or parenteral nutrition should be used in providing nutrition to the patient with malabsorption. These factors include etiology of malabsorption, severity of gastrointestinal disease, and underlying nutritional and medical conditions. Oral nutrition using modified diets as noted above is, of course, the most customary and desirable by

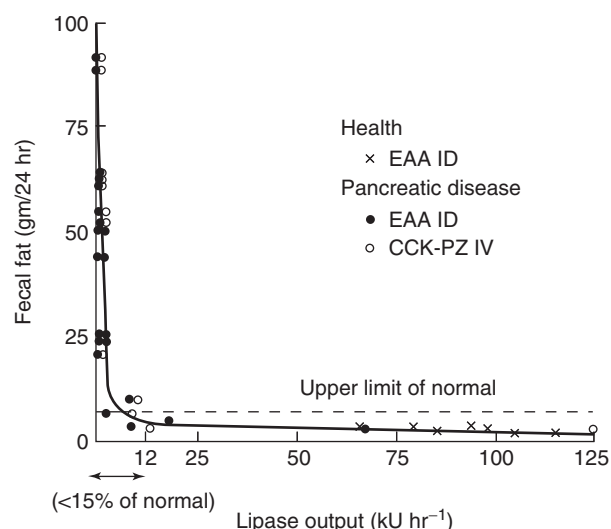


Figure 1 Pancreatic enzyme secretion and steatorrhea. Significant steatorrhea ensues when pancreatic function is less than 15% of normal. Reproduced with permission from DiMagno EP, Go VLW, and Summerskill WHJ (1973) Relations between pancreatic enzyme output and malabsorption in severe pancreatic insufficiency. *New England Journal of Medicine* 288: 814. Copyright © 1973 Massachusetts Medical Society. All rights reserved.

physician and patient alike. In cases of mild lactose malabsorption, for instance, modification of a regular, healthy diet to avoid foods high in lactose should be sufficient. In cases where widespread gastrointestinal disease is leading to severe malabsorption, enteral or 'tube' feeding is helpful for two main reasons: (1) use of proprietary formulas specially designed for malabsorption are often indicated, and these formulas may be unpalatable, and (2) enteral feedings, especially with slow continuous 'drip' feedings make efficient use of nutrient transport kinetics, thereby maximizing residual gastrointestinal absorptive function. In severe cases of malabsorption in which tube feedings are unable to achieve adequate nutritional intake, parenteral nutrition may be indicated. Emerging data suggest that serum citrulline, an amino acid synthesized principally by the enterocyte, is a reliable biomarker of mucosal mass and may help distinguish among patients who require parenteral versus enteral nutrition.

Selection of Enteral Formulas for Malabsorption

A number of commercially available formulas are designed for patients with malabsorption, and these differ with regard to energy density, macronutrient composition, and indicated age. Because infant formulas are often handled in a separate regulatory fashion by governments, infant formulas are usually considered separately from formulas designed for older children and adults. In addition, formulas are also conventionally categorized by the extent of the hydrolysis of their protein source. Categories include intact protein formulas, protein hydrolysate formulas, and amino acid-based formulas. Protein hydrolysate formulas are also sometimes referred to as 'semielemental' formulas, and amino-acid formulas are sometimes called 'elemental' formulas. However, these terms suffer from vagueness and inaccuracies because not all of their macronutrients are semi or completely elemental. Marketing strategies often compound the confusion with misleading formula names. These terms should be discouraged, and the terms that refer to the composition and/or biochemical processing should be used instead.

Patients who have carbohydrate malabsorption from lactose intolerance should use lactose-free formula. Fat malabsorption may call for MCT enriched formula. In cases of protein malabsorption or severe enteropathy, a formula that is a protein hydrolysate or amino acid based would be most appropriate. Because many malabsorption syndromes overlap in terms of the macronutrient affected, as in cases of severe mucosal disease, some formulas are designed for fat, protein, and carbohydrate malabsorption. For example, all formulas designed for use in adults are lactose free, and several formulas contain both hydrolyzed proteins and MCT oils.

Clinical Management of Malabsorption

Two of the most clinically challenging scenarios for the management of malabsorption are inflammatory bowel disease (especially Crohn's disease) and short bowel syndrome. Both are discussed in separate articles of the text, but are considered briefly below.

Inflammatory Bowel Disease

Patients with Crohn's disease have widespread and intermittent gastrointestinal inflammation. Some patients with inflammatory bowel disease may require complete bowel rest for several days or even a few weeks to allow time for mucosal healing. To provide nutrition during this period of time, parenteral nutrition may be needed.

Numerous studies have shown that patients with Crohn's disease may safely and effectively achieve clinical remission with primary nutritional therapy. Early literature in the field highlighted the use of protein hydrolysate formulas, which, due to unpalatability, often required administration via a nasogastric or gastrostomy tube. More recent data have confirmed that intact protein formulas, termed 'polymeric' formulas when describing formulas designed for adults, may work as well as protein hydrolysates, and these formulas can be feasibly given by mouth.

As patients are recovering from an exacerbation and begin advancing their diet, patients should temporarily minimize the amount of fiber ingested to decrease trauma to healing mucosa. Patients whose disease affects the small intestine often benefit from temporary avoidance of lactose products as the mucosa heals and brush border membrane enzyme production is restored.

Micronutrients are also needed in the nutritional management of inflammatory bowel disease. Iron supplementation is recommended for anemia due to acute or chronic blood loss. Treatment of inflammatory bowel disease frequently requires the use of steroids, which affects bone density. Calcium and vitamin D supplementation is commonly needed to minimize the osteopenic effects of steroid therapy and/or the effects of malabsorption and chronic inflammation.

Short Bowel Syndrome

Patients who have suffered acquired or congenital loss of small intestinal surface area that makes them dependent on specialized enteral or parenteral support are said to have short bowel syndrome (SBS). Patients with SBS often malabsorb carbohydrates, proteins, fat, as well as numerous micronutrients, depending on the extent and location of bowel resection, as well as the presence of mucosal disease in the nonresected bowel.

Special attention should be given to exactly what part of the intestine remains as well as the length of the remaining intestine. Some patients may have the terminal ileum removed and are unable to absorb vitamin B₁₂ and bile acids. Removal of the ileocecal valve increases the risk of bacterial overgrowth. Reduced length also means reduced surface area for the absorption of nutrients and decreased intestinal transit time.

In the immediate postoperative period, parenteral nutrition and gut rest should be used because significant stool output is the norm. Output should be quantified, and electrolytes must be carefully monitored in order to determine appropriate replacement fluids to make up for excess urine, stool, and ostomy losses. Replacement fluids should generally be given separately from standard parenteral nutrition so that

Table 3 Feeding advancement in short bowel syndrome

1. Stool output If $< 10 \text{ g kg}^{-1} \text{ d}^{-1}$ or < 10 stools d^{-1} If $10\text{--}20 \text{ g kg}^{-1} \text{ d}^{-1}$ or $10\text{--}12$ stools d^{-1} If $> 20 \text{ g kg}^{-1} \text{ d}^{-1}$ or > 12 stools d^{-1}	Advance rate by $10\text{--}20 \text{ ml kg d}^{-1}$ no change Reduce rate or hold feeds ^a
or 2. Ileostomy output If $< 2 \text{ g kg}^{-1} \text{ h}^{-1}$ If $2\text{--}3 \text{ g kg}^{-1} \text{ h}^{-1}$ If $> 3 \text{ g kg}^{-1} \text{ h}^{-1}$	Advance rate by $10\text{--}20 \text{ ml kg d}^{-1}$ no change Reduce rate or hold feeds ^a
3. Stool reducing substances If $< 1\%$ If $= 1\%$ If $> 1\%$	Advance feeds per stool or ostomy output no change Reduce rate or hold feeds ^a
4. Signs of dehydration If absent If present	Advance feeds per stool or ostomy output Reduce rate or hold feeds ^a
5. Gastric aspirates < four times previous hour's infusion > four times previous hour's infusion	Advance feeds Reduce rate or hold feeds ^a

^aFeeds should generally be held for 8 h, then restarted at 3/4 the previous rate.

Source: Adapted from Utter SL and Duggan C (2005) Short bowel syndrome. In: Hendricks KM and Duggan C (eds.) *Manual of Pediatric Nutrition*, 4th edn., pp. 728–729. Hamilton, Ontario: BC Decker.

they can be adjusted as needed to rapid shifts in fluid and electrolyte status.

As patients recover from surgery, every attempt should be made to feed them enterally as soon as is feasible. Enteral feeds facilitate growth and adaptation of the remaining bowel to allow partial compensation for the missing portion, and several studies have correlated early feeding with better long-term outcome. Attaining independence from parenteral nutrition may take weeks to months to years. Table 3 outlines an approach to determine feeding advancement in SBS. Although some patients are able to grow well or maintain their body weight with only enteral feeds, many are dependent on parenteral nutrition. Some patients with SBS also have oral feeding aversion due to prematurity, prolonged mechanical ventilation, and/or prolonged orogastric or nasogastric feeding. Gastrostomy tubes are particularly helpful in this regard.

In infants, breast milk should be used if available. The breast milk may need to be fortified to increase calories, protein, or fat. For older patients or infants who are not receiving breast milk, protein hydrolysates or amino acid-based formulas may be better tolerated because the residual bowel more easily absorbs these nutrients. Lactose-free and MCT-containing formulas are often used, as well. Formulas may need to be supplemented with oral rehydration solutions if electrolyte abnormalities persist, particularly with sodium losses through persistent high stool or ostomy output.

Because many patients with SBS are dependent on parenteral nutrition for prolonged periods of time, selenium, carnitine, copper, and zinc blood concentrations should be checked periodically and supplemented if needed. Parenteral nutrition should be cycled off for a few hours each day to help simulate more natural cyclic fluctuations of gastrointestinal hormones. These patients also often have poor absorption of calcium and need calcium supplements to prevent osteopenia, which increases the risk of fractures. Iron may also be needed in patients with anemia from decreased absorption secondary to resection of the duodenum or jejunum. Ultimately,

weaning from parenteral and enteral nutrition remain the goals of treatment, though lifelong dietary therapy is often needed.

Summary

Congenital or acquired diseases of the gastrointestinal tract can lead to life-threatening malabsorption of numerous macronutrients and micronutrients. Determining the type and etiology of malabsorption is essential to provide appropriate nutritional and medical therapy. Multiple formulas, supplements, and dietary regimens exist to target specific defects in the digestion, absorption, and assimilation of nutrients. In addition, many new nutrients are undergoing investigation that may become a standard part of care in the future, including probiotics, prebiotics, and various amino acids.

See also: Celiac Disease. Cystic Fibrosis. Lactose Intolerance

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Relevant Websites

- <http://www.nutrition.org/>
American Society for Nutrition.

MALNUTRITION

Secondary, Diagnosis and Management

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Glossary

Cachexia Loss of weight, and fat and muscle mass, caused by disease and loss of appetite in people who are not trying to lose weight.

Enteral feeding Delivery of nutrients, in the form of special solutions, to the stomach or small intestine through a tube, i.e. 'tube-feeding'.

Nosocomial pneumonia Pneumonia contracted while in hospital, 2–3 days after admission.

Parenteral nutrition Delivery of nutrients through a tube inserted into a vein, because the intestine is nonfunctional.

Pathognomonic Characteristic or diagnostic of a specific disease.

Percutaneous Delivery of nutrients into the stomach or intestine by a tube passed through the abdominal wall.

Primary malnutrition Malnutrition due to inadequate or excessive food intake.

Secondary malnutrition Malnutrition caused primarily by illness, infections, or disease.

Introduction

In its broadest context, malnutrition is a state of having an inappropriate nutritional status with respect to one or more macronutrients (water, electrolyte, protein, or fat) or micronutrient (vitamin or mineral) constituent of the body. This imbalance can be a deficit, leading to an insufficient supply or content of the nutrient (undernutrition), or an excess, leading to an excessive content or overloading of the organism with a nutrient (overnutrition). The six possible causes for all nutrient deficiencies have been summarized as: inadequate intake, impaired absorption, increased wastage, impaired utilization, increased destruction, and elevated requirements. Correspondingly, overnutrition and excesses can result from reciprocal defects, that is: Hyperphagia, hyperabsorption, increased retention; decreased destruction, and decreased requirements.

As discussed in the previous article, the term 'primary' malnutrition relates almost exclusively to the first of these mechanisms, that of inadequate or excessive ingestion of nutrients from the diet. It is concerned with food consumption and intake. Secondary malnutrition, in contrast, concerns the disturbed and disordered handling of nutrients. When diseases or abnormal physiological conditions interfere with the normal disposition of nutrients ingested from the diet, this is the basis of a situation of 'secondary' malnutrition.

Causes of Secondary Under- and Overnutrition

A representative, but not exhaustive, list of diseases and conditions producing secondary undernutrition is provided in [Table 1](#), and of secondary overnutrition in [Table 2](#).

The basis for suspecting secondary malnutrition is that there is evidence of deficiency or excess, but foods and nutrients are presumably being consumed in adequate amounts. Once the suspicion emerges, three distinct diagnostic principles need to be addressed: (1) the confirmation of dietary intake, and estimation of its adequacy; (2) the diagnosis and classification of abnormal nutritional status; and (3) the diagnosis of the functional, physiological, or pathological origins of disordered nutrient disposition. The evaluator must remain attuned to the nutritional status of patients, clients, or populations, and sensitive to the possibility of a nonprimary origin of any under- or overnutrition.

Coexistence of Primary and Secondary Malnutrition

It is important to recognize the potential for the simultaneous coexistence of primary and secondary malnutrition in the same individual. Primary malnutrition in the free-living populations can be associated with famine conditions (crop failure, conflict, natural disaster, and refugee crisis), in which sufficient food is simply not available. Alternatively, it can

Table 1 Diseases and conditions associated with secondary macronutrient or micronutrient undernutrition

<i>Inadequate nutrient absorption</i>
Gastric abnormalities: Gastric atrophy
Pernicious anemia
Intestinal abnormalities: Celiac disease
Inflammatory bowel disease
Intestinal cryptosporidiasis
Radiation enteritis
Chronic intestinal pseudo-obstruction HIV/AIDS
Hepatobiliary abnormalities: Cystic fibrosis
Biliary obstruction
Pancreatic insufficiency
<i>Increased nutrient excretion</i>
Gastric disorders: Gastric adenoma
Intestinal abnormalities: Laxative abuse
Peptic ulcer
Gastrointestinal fistula
Colonic adenoma
Amebiasis
Hookworm
HIV/AIDS
Schistosomiasis
Hepatic disorders: Hepatic cirrhosis
Endocrine disorders: Diabetes mellitus
Hypoadosteronism
Renal Disorders: Fanconi syndrome
Hemodialysis; peritoneal dialysis
<i>Increased destruction or use of nutrients</i>
Endocrine disorders: Hyperthyroidism
Chronic disease: Cardiac cachexia
Cancer cachexia
Cystic fibrosis
Bone marrow transplants
Infections: Pulmonary tuberculosis
HIV/AIDS
<i>Decreased utilization of nutrients</i>
Lead poisoning
Menkes' copper storage disease

Table 2 Diseases and conditions associated with secondary macronutrient or micronutrient excess (overnutrition)

<i>Increased nutrition absorption</i>
Wilson's disease
Hemochromatosis
<i>Increased nutrition retention</i>
Prader-Willi syndrome
Hypercorticotesteroidism
Hyperpituitarism
Acute tubular necrosis
Chronic renal failure
<i>Decreased destruction of nutrients</i>
Hypothyroidism

arise from the poverty of landlessness or urban margination, where food is not accessible given the household income. A large number of communicable diseases with consequences of poor nutrient absorption, retention or utilization, such as

parasitoses, tuberculosis or HIV/AIDS, are common in these situations of deprivation and misery. To the extent that a disease process produces anorexia or dysphagia, or even psychic depression, the net effect is to reduce total intake of dietary energy and nutrients. Whatever, malabsorptive or nutrient-wasting components of the underlying disorders will further compromise the nutritional state.

The Reverse Paradigm: Underlying Pathology Revealed by Detection of Abnormal Nutrition

In clinical medicine, a type of 'reversal of roles' often occurs. Rather than primarily recognizing the presentation of the underlying pathology, recognition of an abnormal nutritional status without a dietary explanation leads to the diagnosis of the underlying disorder before any specific (pathognomonic) sign or symptom has yet occurred. For instance, the Prader-Willi syndrome of pathological obesity would initially present as common obesity. Similarly, in hypercorticotesteroidism (Cushing's syndrome), abnormal fat deposition and weight gain can be the changes that lead to the recognition of the underlying pituitary or adrenal dysfunction.

Classically, in type 2 diabetes, unexplained weight loss is a presenting complaint when polyuria is mild or absent. Moreover, with common forms of childhood gastrointestinal disorders, such as celiac sprue or Crohn's disease, arrested linear growth is often the first clue that something is clinically awry. It provokes the diagnostic inquiry that leads to the recognition of the bowel pathology. In milder presentations of cystic fibrosis, a similar growth failure occurring in infancy can indicate an underlying pathological disorder.

In fact, the entire roster of conditions listed in **Tables 1** and **2**, as well as others of a similar nature, are subject to being diagnosed as the result of a secondary change in nutritional status. The practical message is that the nutritional specialist, physician or nonphysician, may be the first person to whom the secondarily malnourished patient is referred, and the acumen of recognizing a secondary causation will guide the case to an appropriate clinical diagnostic program to uncover (and hopefully address and remedy) the underlying medical or surgical problems. Overarching guideline principles for uncovering secondary malnutrition states are provided in **Table 3**.

Diagnosis of Secondary Malnutrition

In general terms, a common set of principles applies for assessment of nutrient status whether the bases are primary, secondary, or a combination of both. These principles include: body composition measures, hematological and biochemical (biomarker) values, functional variables, and clinical signs and symptoms. It is more productive to focus here on the nuances, caveats, and distinctions for the detection of altered nutrition due to background conditions beyond spontaneous food intake.

Caveats for the Diagnosis of Secondary Excess Nutriture

The conditions that cause increased retention of energy and hypometabolism are listed in **Table 2**. When it comes to

Table 3 Three diagnostic principles related to secondary malnutrition

Assessment of dietary and nutrient intake: A quantitative and qualitative evaluation of usual dietary intake by a nutritionally trained practitioner or clinical dietitians serves to exclude the possibility that the situation is not primary (low or excessive intake) in nature and suggests a secondary basis for the nutritional problem. Caveat: In certain situations, a combination of reduced intakes and nutritional stress due to poor absorption, retention, or utilization may coexist.

Assessment of nutritional status: This includes measures of anthropometry and body composition, hematological status, biomarkers, and functional indicators, as well as clinical (physical) evaluation.

Diagnosis of underlying cause(s) of secondary nutritional imbalance: It is important, where possible, to identify the underlying entities that are causing the nutritional problem, such as absorptive or hormonal problems, to enable (where possible) a direct remedial approach to the cause of malnutrition and to orient management based on any pathophysiological knowledge about the underlying disease.

overweight and obesity, the absence of clear-cut overeating (which can be difficult to detect) combined with other characteristic signs of the different entities should raise suspicion. Excesses of vitamins and minerals may not easily be detected because the homeostatic control of circulating concentrations confounds biochemical diagnosis. Excessive urinary excretion rates of the nutrients or their metabolites often provide better indications than blood levels when micronutrient overload is the issue.

Caveats for the Diagnosis of Secondary Undernutrition

Undernutrition due to disease and dysfunction obviously requires establishment of the following: (1) the existence of deficiencies; and (2) the factors other than underconsumption that are influencing the deficiency states. The cut-point for diagnosing macronutrient undernutrition is a body mass index (BMI) of $<18.5 \text{ kgm}^{-2}$. However, with the worldwide pandemic of overweight, recent weight loss of 10% or more of usual body weight may be a more sensitive and reliable indicator of an incipient undernutrition problem. Weight problems diagnosed in this manner would certainly be detectable well before the BMI will have fallen to the aforementioned criterion.

Ill patients with adequate or excessive body mass indices can manifest metabolic substrate metabolism reminiscent of the severe malnutrition syndromes of adult kwashiorkor or marasmus (inanition). Moreover, fluctuations in weight under acute or semiacute situations often reflect changes in fluid balance. This is also the situation in patients with end-stage renal failure undergoing chronic dialysis. Methods such as bioelectrical impedance, dual X-ray absorptiometry, or isotope dilution in association with indirect calorimetry can assess true lean- and fat-mass status of patients with apparently normal body mass.

Hematological evaluation is important in nutritional assessment. A low hemoglobin, hematocrit, or red cell count signifies anemia, but in individuals with associated diseases, anemia can have a series of origins (hemolytic, hypoproliferative) that are nonnutritional and will not respond to nutritional therapy.

Biochemical evaluation for nutrient deficiency status in patients with associated disease is fraught with caveats and limitations. Two classes of nutritional deficiency are sometimes defined: in type 1 deficiencies, nutritional desaturation of tissue stores occurs, circulating levels of nutrients reflect the total body nutrient status and specific nutrient deficiency syndromes manifest (e.g., iron deficiency and anemia); in type

2 deficiency, there is homeostatic conservation of tissue, circulating concentrations of nutrients, such that blood concentrations remain virtually unaltered in the face of depletion. Typically, type 2 deficiencies manifest with growth failure or general signs of undernutrition. Deficiencies of zinc and magnesium, among others, fall into this second category. Inflammation and infection are stimuli that directly alter the circulating concentrations of nutrient indicators. Ferritin and circulating copper are elevated, whereas zinc, iron, and vitamin A concentrations are depressed with activation of the acute-phase response to injury or inflammation. In liver disease, depressed production of binding proteins can alter the usual indicators of nutritional status as a consequence of hepatic pathophysiology itself, rather than preexisting secondary malnutrition. Finally, it almost goes without saying that attempting biochemical nutrient evaluations from blood samples taken during concurrent infusion of micronutrient solutions in parenteral nutrition regimens – especially without a period of distribution and equilibration – will not reflect the tissue stores and total body reserves of the respective nutrients of interest.

Functional indicators of nutritional status have been applied to the assessment of secondary malnutrition and have been plagued by pitfalls. This applies to tests of nitrogen status, immune function, and hepatic protein secretion. Tests such as creatinine excretion, white blood cell counts, and cutaneous delayed hypersensitivity anergy, as well as decreased serum albumin, transferrin, transthyretin (prealbumin), and retinol-binding protein concentrations are sensitive to alteration by stress and injury. Failure to recognize distortion from stress underlies an early fallacy in surgical nutrition, in which low values for albumin, lymphocyte counts, and prealbumin, together with anergy, predicted poor postoperative outcomes. This misconception justified aggressive preoperative parenteral nutrition and albumin infusions, with little impact on predicted outcomes. In these situations, it was the stress and injury of the underlying disease, rather than nutritional status that was producing the abnormal values of the biomarkers. Recently, insulin-like growth factor has been advanced as a sensitive indicator of protein status in older patients, but whether it is confounded by nonnutritional features of disease remains to be clarified.

Management of Secondary Malnutrition

Secondary malnutrition has many faces and facets. It may have to be addressed both in a public health sense for

communicable diseases such as parasitoses or HIV/AIDS, and in a medical care context for disorders that are particular and clinical in nature, such as hereditary or degenerative diseases.

Principles of Management

The first principle is to identify the underlying functional, physiological, or pathological cause of the malnourished state. If the condition is curable, then the management issues are simplified. For instance, if a person is dehydrated because of hyperglycemic diuresis in uncontrolled diabetes mellitus, the short-term management involves administration of exogenous intravenous fluids to restore normal hydration; however, restoring adequate diabetic control to the patient would be the long-term and definitive solution. The undernutrition and growth failure due to undetected celiac disease is easily eliminated by institution of a gluten-free diet. With inadequate nutrition in cystic fibrosis, adequate management of pulmonary problems and digestive-enzyme deficiencies should allow patients to recover and maintain normal nutrition on a balanced oral diet. Thus, medical or surgical address of the underlying disorder, where possible, is the primary tool for management of secondary undernutrition.

Public Health Approaches

The management of the secondary iron deficiency attributable to hookworm or schistosomiasis can be achieved both by anthelmintic medications or supplemental iron to compensate for parasite-induced losses. In countries where HIV/AIDS is rampant, efforts for its prevention are fundamental. A food-security crisis grips the whole society in AIDS endemic areas, and this must be relieved with food and economic assistance. The wasting syndromes produced by tuberculosis are best addressed proactively by prevention of transmission and early detection. However, when primary prevention fails, as in the aforementioned infections, efforts to enhance the enteral intake of infected members of the community are particularly essential for their comfort and well being.

Dietary Management of Secondary Overnutrition

The dietary management of secondary overnutrition would logically include restricted intake of the nutrients accruing in excess. This is not always facile or feasible, however, due to the intrinsic complexity of foods and beverages, where most are sources of multiple essential micronutrients. Marked reduction in total energy intake can also jeopardize the intake of proteins and essential fats. For the metal-storage afflictions such as Wilson's disease and hemochromatosis, removing copper and iron from the diet, respectively, are the fundamental elements of management. Some additional benefits can be gained by blocking the metals' absorption, as with high doses of zinc in Wilson's disease or with strong black tea (tannins) in hemochromatosis. Fundamentally, however, the management of metal-storage diseases requires some interventions to selectively remove the overload – by chelating agents in Wilson's disease and recurrent phlebotomy in hemochromatosis. In a related variant condition, African

hemosiderosis, common among Bantu in southern Africa, removing concentrated iron sources from the diet, specifically the iron-loaded traditional beers, provides effective long-term control.

Dietary and Nutritional Management of Secondary Undernutrition

The syllogism for dietary and nutritional management is to get enough nutrients into the body to restore nutritional adequacy and balance, taking any chronic barriers to uptake and retention into consideration. The blend of nutrients must be tailored to the specific absorptive or utilization problems, for example, compensatory fat-soluble vitamins in water-miscible forms with severe fat malabsorption, and extra doses of highly available iron with chronic blood loss. These can be delivered within a dietary context with supplements and fortified vehicles, in nonacute conditions. Even nondietary routes have been devised as in the treatment of vitamin D deficiency due to Crohn's disease with tanning bed ultraviolet B radiation.

When accumulated undernutrition is dangerously advanced, absorptive barriers are especially severe or nutrient losses are excessive, more concerted nutritional intervention is required. Intensive therapy can be delivered by three routes: orally, with special diets supplemented by liquid formulas; enterally, with liquid formulas perfused by intragastric or intrainestinal feeding tubes; and parenterally, with intravenous formulas infused into peripheral or central veins. Up to 50% of patients on dialysis have protein-energy malnutrition, which may continue undetected. For end-stage renal patients, intradialytic alimentation (adding nutrients to the dialysis fluids) has been used to reduce nutrient loss. Each approach has its distinct costs, special potential, and limitations and risks, and has been explored and refined in the context of age, physiological status, and specific disease states or surgical indications.

Tailoring of nutrient delivery is required with both enteral and parenteral nutrition, depending on the pathophysiology of the underlying conditions. Both hypo- and hypermetabolic states can occur; indirect calorimetry with metabolic carts is in vogue for prescribing energy delivery in intensive care. When pulmonary compromise is present, the balance among macronutrients is important to minimize carbon dioxide formation in metabolism.

Maintaining abundant amino acid supply promotes protein-sparing and prevents loss of lean tissue in catabolic states. Enrichment of enteral or parenteral regimens with branched-chain amino acids or keto-analog amino acids has been devised to compensate for the metabolic defects of nitrogen handling in hepatic or renal failure states. The objective of nutritional support in patients with liver failure is to provide adequate macronutrients to ensure the specific substrates for energy and protein synthesis and integrity of normal hepatic tissue function, without inducing or accentuating encephalopathy or otherwise aggravating hepatic insufficiency.

In juvenile cholestasis, large amounts of fat-soluble vitamin supplements and medium-chain triglycerides are usually required for optimum growth. With protracted secretory diarrheal diatheses, fluid and electrolyte balance may be the

primary concern, followed by macro- and micronutrient nutriture, invoking the institution of parenteral feeding. Cancer cachexia is a major secondary consequence of disseminated neoplasms. It is tempting to prescribe aggressive nutritional support, but a caveat is that certain nutrients acting with certain neoplasms favor the tumor's growth and dissemination. To the extent that various forms of cachexia are partly driven by catabolic responses mediated by proinflammatory cytokines, antagonists directed at counteracting their action hold promise for retarding the nutrient-wasting in various forms of cachexia.

With intensive nutrition, there are risks and adverse consequences intertwined with the benefits. A variation of the refeeding syndrome, that is, hyperalimentation complications from excessive energy substrate perfusion or infusion, can produce hypophosphatemic and hypokalemic episodes. Improper formulation of fluids or liquids with micronutrients can cause deficiency or toxicity states in chronic nutritional support. The hazards of indwelling catheters are multiple, from phlebitis of the veins to sudden dislocation or migration. Fluid overload and sepsis are the most troubling complications of intravenous parenteral nutrition.

For tube-feeding enteral alimentation, tube placement is the crucial element. With nasal placement of the tube, there is a finite risk of respiratory tract inflammation and infection from aspiration of formula and secretions. In hospital, enteral nutrition is a risk factor for nosocomial pneumonia. An alternative site for long-term administration of tube-feeds is percutaneous placement of an intragastric feeding tube under endoscopic control.

Aggressive nutritional support, with its attendant expense and potential morbidity, in critically ill patients remains controversial. In terms of cost-benefit analysis, the use of the intensive formats of enteral artificial nutrition seems to be cost effective to reduce post-hip-fracture hospital stay in underweight women and for preoperative nutritional support, if carried out at home. Preoperative parenteral nutrition has been judged as prohibitively expensive for the small reduction in postoperative morbidity that it produces.

Conclusions

Dietary intake is the most important determinant of over- or undernutrition, but it is not the only influence on an individual's nutritional status. A series of extrinsic environmental factors or intrinsic clinical or physiological disorders can alter the absorption, retention, utilization, and integrity of nutrients. These give origin to secondary malnutrition states. Primary (dietary origin) and secondary (environmental, pathological) factors often combine within the same individuals. From a public health perspective, the goal is to implement broad policies and programs that increase the availability of specific

nutrients imperiled by the local environmental problems, for example, iron in hookworm infested areas, while addressing the primary diseases. In the clinical setting, management requires diagnosing and managing the underlying pathological states interfering with nutritional health while providing compensatory measures to correct secondary nutritional imbalances.

See also: Cystic Fibrosis. Diabetes Mellitus: Etiology and Epidemiology. Liver Disorders: Nutritional Management. Nutritional Support: Adults, Enteral; Infants and Children, Parenteral. Parenteral Nutrition

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MANGANESE

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Chemical and Physical Properties

Manganese is the twelfth most abundant element in the Earth's crust and constitutes approximately 0.1% of it. Chemical forms of manganese in their natural deposits include oxides, sulfides, carbonates, and silicates. Anthropogenic sources of manganese are predominantly from the manufacturing of steel, alloys, and iron products. Manganese is widely used as an oxidizing agent, as a component of fertilizers and fungicides, and in dry cell batteries. Methylcyclopentadienyl manganese tricarbonyl (MMT) improves combustion in boilers and motors and can substitute for lead in gasoline as an antiknock agent. Concentrations of manganese in groundwater normally range between 1 and 100 $\mu\text{g l}^{-1}$, with most values being below 10 $\mu\text{g l}^{-1}$. Typical airborne levels of manganese (in the absence of excessive pollution) range from 10 to 70 ng m^{-3} .

Manganese is a transition element located in group VIIA of the periodic table. It occurs in 11 oxidation states ranging from -3 to $+7$, with the physiologically most important valences being $+2$ and $+3$. The $+2$ valence is the predominant form in biological systems and is the form that is thought to be maximally absorbed. The $+3$ valence is the form in which manganese is primarily transported in biological systems.

The solution chemistry of manganese is relatively simple. The aquo-ion is resistant to oxidation in acidic or neutral solutions. It does not begin to hydrolyze until pH 10, and therefore free Mn^{2+} can be present in neutral solutions at relatively high concentrations. Divalent manganese is a $3d^5$ ion and typically forms high-spin complexes lacking crystal field stabilization energies. The previous properties, as well as a large ionic radius and small charge-to-radius ratio, result in manganese tending to form weak complexes compared with other first-row divalent ions, such as Ni^{2+} and Cu^{2+} . Free Mn^{2+} has a strong isotropic electron paramagnetic resonance (EPR) signal that can be used to determine its concentration in the low micromolar range. Mn^{3+} is also critical in biological systems. For example, Mn^{3+} is the oxidative state of manganese in superoxide dismutase, is the form in which transferrin binds manganese, and is probably the form of manganese that interacts with Fe^{3+} . Given its smaller ionic radius, the chelation of Mn^{3+} in biological systems would be predicted to be more avid than that of Mn^{2+} . Cycling between Mn^{3+} and Mn^{2+} has been suggested to be deleterious to biological systems because it can generate free radicals. However, at low concentrations, Mn^{2+} can provide protection against free

radicals, and it appears to be associated with their clearance rather than their production.

Dietary Sources

Manganese concentrations in typical food products range from 0.4 $\mu\text{g g}^{-1}$ (meat, poultry, and fish) to 20 $\mu\text{g g}^{-1}$ (nuts, cereals, and dried fruit). Breast milk is exceptionally low in manganese, containing only 0.004 $\mu\text{g g}^{-1}$, whereas infant formula can contain up to 0.4 $\mu\text{g g}^{-1}$. Teas can be particularly rich in manganese, containing up to 900 $\mu\text{g g}^{-1}$ of the element. An important consideration with respect to food sources of manganese is the extent to which the manganese is available for absorption. For example, although tea contains high amounts of the element, the tannin in tea can bind a significant amount of manganese, reducing its absorption from the gastrointestinal tract. Similarly, the high content of phytates and fiber constituents in cereal grains may limit the absorption of manganese. Conversely, although meat products contain low concentrations of manganese, absorption and retention of manganese from them is relatively high. Based on studies utilizing whole body retention curves after dosing with ^{54}Mn , the estimated percentage absorption of 1 mg of manganese from a test meal was 1.35%, whereas that from green leafy vegetables (lettuce and spinach) was closer to 5%. Absorption from wheat and sunflower seed kernels was somewhat lower than that from the leafy greens at 1 or 2%, presumably due to a higher fiber content or to higher amounts of phytates and similar compounds in the wheat and sunflower seeds. The dephytinization of soy formula increased manganese absorption 2.3-fold from 0.7 to 1.6%.

Analysis

Although manganese is widely distributed in the biosphere, it occurs in only trace amounts in animal tissues. Serum concentrations can be as low as 20 nM and typical tissue concentrations are less than 4 $\mu\text{mol g}^{-1}$ wet weight; tissue concentrations of 4–8 $\mu\text{mol g}^{-1}$ wet weight are considered high. Because of the high environmental levels of manganese relative to its concentration in animal tissues, considerable effort must be made to minimize contamination of samples during their collection and handling.

The most common analytical methods that can sensitively measure manganese include neutron activation analysis, X-ray fluorescence, proton-induced X-ray emission, inductively coupled plasma emission, EPR, and flameless atomic absorption spectrophotometry (AAS). Currently, the most common method employed is flameless AAS. All of these methods, with the exception of EPR, measure the total concentration of manganese in the samples. EPR allows selective measurement of bound versus free manganese.

Physiological Role

Tissue Concentrations

The average human body contains between 200 and 400 μmol of manganese, which is fairly uniform in distribution throughout the body. There is relatively little variation among species with regard to tissue manganese concentrations. Manganese tends to be highest in tissues rich in mitochondria; its concentration in mitochondria is higher than in cytoplasm or other cell organelles. Hair can accumulate high concentrations of manganese, and it has been suggested that hair manganese concentrations may reflect manganese status. High concentrations of manganese are normally found in pigmented structures, such as retina, dark skin, and melanin granules. Bone, liver, pancreas, and kidney tend to have higher concentrations of manganese ($20\text{--}50\text{ nmol g}^{-1}$) than do other tissues. Concentrations of manganese in brain, heart, lung, and muscle are typically $<20\text{ nmol g}^{-1}$; blood and serum concentrations are approximately 200 and 20 nmol l^{-1} , respectively. Typical concentrations in cow milk are on the order of 800 nmol l^{-1} , whereas human milk contains 80 nmol l^{-1} . Bone can account for up to 25% of total body manganese because of its mass. Bone manganese concentrations can be raised or lowered by substantially varying dietary manganese intake over long periods of time, but bone manganese is not thought to be a readily mobilizable pool. The fetus does not accumulate liver manganese before birth, and fetal concentrations are significantly lesser than adult concentrations. This lack of fetal storage can be attributed to the apparent lack of storage proteins and the low prenatal expression of most manganese enzymes.

Absorption, Transport, and Storage

Absorption of manganese is thought to occur throughout the small intestine. Manganese absorption is not thought to be under homeostatic control. For adult humans, manganese absorption has been reported to range from 2 to 15% when ^{54}Mn -labeled test meals are used and to be 25% when balance studies are conducted; given the technical problems associated with balance studies, the ^{54}Mn data are probably more reflective of true absorption values. Data from balance studies indicate that manganese retention is very high during infancy, suggesting that neonates may be particularly susceptible to manganese toxicosis.

The higher retention of manganese in young animals relative to adults in part reflects an immaturity of manganese excretory pathways, particularly that of bile secretion, which is

very limited in early life. The avid retention of the small amount of manganese from milk and the postnatal changes in its excretory pattern underscore the considerable changes in manganese metabolism that occur during the neonatal period.

In experimental animals, high amounts of dietary calcium, phosphorus, fiber, and phytate increase the requirements for manganese; such interactions presumably occur via the formation of insoluble manganese complexes in the intestinal tract with a concomitant decrease in the soluble fraction available for absorption. The significance of these dietary factors with regard to human manganese requirements remains to be clarified. Studies in avian species have demonstrated that high dietary phosphorus intakes decrease manganese deposition in bone by approximately 50%. Given that the diet of many individuals may be marginal in manganese ($\leq 2\text{ mg day}^{-1}$ intake) whereas high in phosphorus ($\geq 2000\text{ mg day}^{-1}$ intake), this antagonism may have important implications for human health. For example, the low fractional absorption of manganese from soy formula has been related to its relatively high phytate content. The mechanism underlying this effect of soy protein on manganese absorption/retention has not been fully delineated. However, dephytinization of soy formula with microbial phytase can markedly enhance manganese absorption.

An interaction between iron and manganese has been demonstrated in experimental animals and humans. Manganese absorption increases under conditions of iron deficiency, whereas high amounts of dietary iron can accelerate the development of manganese deficiency. The chronic consumption of high levels of iron supplements ($>60\text{ mg Fe day}^{-1}$) can have a negative effect on manganese balance in adult women. The mechanisms underlying the interactions between iron and manganese have not been fully elucidated; however, they likely involve competition for either a transport site or a ligand. Both iron and manganese can utilize divalent metal transporter 1 (DMT1); however, the expression of DMT1 is regulated by iron status via the IRE/IRP system. Thus, during iron deficiency, DMT1 is upregulated causing an increase in manganese absorption. Rats fed iron-deficient diets accumulate manganese in several brain regions compared with rats fed control diets; the involvement of DMT1 in this accumulation of manganese is an area of active study. It should be noted that the interaction between manganese and iron can also affect the functions of some enzymes. For example, manganese can replace iron in the iron-sulfur center of cytosolic aconitase (IRP-1), resulting in an inhibition of the enzyme and an increase in iron regulatory protein (IRP) binding activity. Given the central role of IRPs in cellular iron metabolism, elevated cellular manganese concentration could in theory disrupt numerous translational events dependent on IRPs. That this in fact occurs is illustrated by the observation that following the addition of manganese to cells in culture, there can be sharp reductions in ferritin protein abundance, whereas there are increases in transferrin receptor abundance. This results in changes in intracellular iron metabolism, as reflected by decreases in mitochondrial aconitase (m-aconitase) abundance.

Manganese entering the portal blood from the gastrointestinal tract may remain free or become associated with α_2 -macroglobulin, which is subsequently taken up by the liver. A small fraction enters the systemic circulation, where it may

become oxidized to Mn^{3+} and bind to transferrin. Studies *in vivo* suggest that the Mn^{3+} complex forms very quickly in blood, in contrast to the slow oxidation of the Mn^{2+} -transferrin complex *in vitro*. Manganese uptake by the liver has been reported to occur by a unidirectional, saturable process with the properties of passive mediated transport. After entering the liver, manganese enters one of at least five metabolic pools. One pool represents manganese taken up by the lysosomes, from which it is transferred subsequently to the bile canaliculus. The regulation of manganese is maintained in part through biliary excretion of the element; up to 50% of manganese injected intravenously can be recovered in the feces within 24 h. A second pool of manganese is associated with the mitochondria. Mitochondria have a large capacity for manganese uptake, and the mitochondrial uptake and release of manganese and calcium are thought to be related. A third pool of manganese is found in the nuclear fraction of the cell; the roles of nuclear manganese have not been fully delineated, but one function may be to contribute to the stability of nucleosome structure. A fourth manganese pool is incorporated into newly synthesized manganese proteins; biological half-lives for these proteins have not been agreed upon. The fifth identified intracellular pool of manganese is free Mn^{2+} . Fluctuations in the free manganese pool may be an important regulator of cellular metabolic control in a manner analogous to those for free Ca^{2+} and Mg^{2+} . Consistent with this concept, in pancreatic islets manganese blocks glucose-induced insulin release by altering cellular calcium fluxes, and manganese directly augments contractions in smooth muscle by a mechanism comparable to that of calcium.

The mechanisms by which manganese is transported to, and taken up by, extrahepatic tissues have not been identified. Transferrin is the major manganese binding protein in plasma; however, it is not known to what extent transferrin facilitates the uptake of manganese by extrahepatic tissue. The concentration of manganese citrate in blood can be fairly high, and this complex may be important for manganese movement across the blood-brain barrier. DMT1 may be involved in manganese transport because it is expressed in discrete areas of the brain. Manganese uptake by extrahepatic tissue does not seem to be increased under conditions of manganese deficiency, suggesting that manganese, in marked contrast to iron, does not play a role in the induction (or suppression) of manganese transport proteins.

There is limited information concerning the hormonal regulation of manganese metabolism. Fluxes in the concentrations of adrenal, pancreatic, and pituitary-gonadal axis hormones affect tissue manganese concentrations; however, it is not clear to what extent hormone-induced changes in tissue manganese concentrations are due to alterations in cellular uptake of manganese-activated enzymes or metalloenzymes.

Metabolic Function and Essentiality

Manganese functions as a constituent of metalloenzymes and as an enzyme activator. Manganese-containing enzymes include arginase (EC 3.5.3.1), pyruvate carboxylase (EC 6.4.1.1), and manganese-superoxide dismutase (MnSOD) (EC 1.15.1.1). Arginase, the cytosolic enzyme responsible for urea formation,

contains 4 mol Mn^{2+} per mole of enzyme. Reductions in arginase activity resulting from manganese deficiency result in elevated plasma concentrations of ammonia and lowered plasma concentrations of urea. Reductions in arginase activity due to manganese deficiency may affect flux of arginine through the nitric oxide synthase (NOS) pathway, resulting in alterations in NO production. It has been suggested that arginase plays a regulatory role in NO production by competing with NOS for the same substrate, arginine. Rats fed manganese-deficient diets have shown effects indicative of increased NO production, such as increases in plasma and urinary nitrates plus nitrites and decreased blood pressure; however, neither NOS activity nor NO production have been measured directly. In addition, manganese binding by arginase is critical for the pH-sensing function of this enzyme in the ornithine cycle, suggesting that manganese plays a role in the regulation of body pH. With experimental diabetes, liver and kidney manganese concentrations and arginase activity can be markedly elevated. This manganese effect on arginase has been suggested to be due to an effect of Mn^{2+} on the conformational properties of the enzyme with a resultant modification of arginase activity. Whether this finding implies an increased manganese requirement for people with diabetes has not been determined.

Pyruvate carboxylase, the enzyme that catalyzes the first step of carbohydrate synthesis from pyruvate, also contains 4 mol Mn^{2+} per mole enzyme. Although the activity of this enzyme can be lower in manganese-deficient animals than in controls, gluconeogenesis has not been shown to be markedly inhibited in manganese-deficient animals.

MnSOD catalyzes the disproportionation of $^{\bullet}O_2$ to H_2O_2 and $^{\bullet}O_2$. The essential role of MnSOD in the normal biological function of tissues has been clearly demonstrated by the homozygous inactivation of the SOD2 gene for MnSOD in mice. Mice with this phenotype die within the first 10 days of life with a dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle, and metabolic acidosis. The activity of MnSOD in tissues of manganese-deficient rats can be significantly lower than in controls due to downregulation of MnSOD at the (pre)transcriptional level. That this reduction is functionally significant is suggested by the observation of higher than normal levels of hepatic mitochondrial lipid peroxidation in manganese-deficient rats. Tissue MnSOD activity can be increased by several diverse stressors, including alcohol, ozone, irradiation, interleukin-1, and tumor necrosis factor- α , presumably as a consequence of stressor-associated increases in cellular free radical (or oxidized target(s)) concentrations. Stressor-induced increases in MnSOD activity can be attenuated in manganese-deficient animals, potentially increasing their sensitivity to these insults. Transgenic mice have also been produced that overexpress MnSOD; a decreased severity of reperfusion injury has been noted in these animals, further supporting its physiological significance.

Considerable research is focused on the introduction of the human MnSOD gene into research animals utilizing viral vectors or plasmid/liposome delivery. This gene therapy has been shown to decrease radiation-induced injury, extend pancreatic islet transplant function, and slow the growth of malignant tumors in animal models via overexpression of the MnSOD protein. Another field of research that is rapidly advancing utilizes MnSOD mimetics for treatment of a variety of

diseases in which the native SOD enzyme has been found to be effective. These mimetics are small manganese-containing synthetic molecules that have catalytic activity equivalent or superior to the native enzyme. They possess the additional beneficial properties of being nonimmunogenic because they are nonpeptides, able to penetrate cells, selective for superoxide (they do not interact with biologically important molecules), stable *in vivo*, and not deactivated by the destructive free radical peroxynitrite, which is capable of deactivating native MnSOD via nitration of tyrosine. These mimetic compounds have been found to be protective in animal models of acute and chronic inflammation, reperfusion injury, shock, and radiation-induced injury. Both of these therapies, MnSOD gene delivery and MnSOD mimetics, hold promise for future treatments in human chronic and acute conditions.

Finally, further evidence for the biological and research relevance of MnSOD is that experiments have been undertaken on the International Space Station to improve three-dimensional growth of MnSOD crystals in order to develop a better understanding of the role of structure in the reaction mechanism of this enzyme.

In contrast to the relatively few manganese metalloenzymes, there are a large number of manganese-activated enzymes, including hydrolases, kinases, decarboxylases, and transferases. Manganese activation of these enzymes can occur as a direct consequence of the metal binding to the protein, causing a subsequent conformation change, or by binding to the substrate, such as adenosine triphosphate (ATP). Many of these metal activations are nonspecific in that other metal ions, particularly Mg^{2+} , can replace Mn^{2+} . An exception is the manganese-specific activation of glycosyltransferases. Several manganese deficiency-induced pathologies have been attributed to a low activity of this enzyme class. A second example of an enzyme that may be specifically activated by manganese is phosphoenolpyruvate carboxykinase (PEPCK; EC 4.1.1.49), the enzyme that catalyzes the conversion of oxaloacetate to phosphoenolpyruvate, GDP, and CO_2 . Although low activities of PEPCK can occur in manganese-deficient animals, the functional significance of this reduction is not clear.

A third example of a manganese-activated enzyme is glutamine synthetase (EC 6.3.1.2). This enzyme, found in high concentrations in the brain, catalyzes the reaction $NH_3 + \text{glutamate} + ATP \rightarrow \text{glutamine} + ADP + P_i$. Brain glutamine synthetase activity can be normal even in severely manganese-deficient animals, suggesting that the enzyme either has a high priority for this element or magnesium can act as a substitute when manganese is lacking. It should be noted that this enzyme can be inactivated by oxygen radicals; therefore, a manganese deficiency-induced reduction in MnSOD activity theoretically could act to depress further the activity of glutamine synthetase.

Manganese Deficiency

Manganese deficiency has been demonstrated in several species, including rats, mice, pigs, and cattle. Signs of manganese deficiency include impaired growth, skeletal abnormalities,

impaired reproductive performance, ataxia, and defects in lipid and carbohydrate metabolism.

The effects of manganese deficiency on bone development have been studied extensively. In most species, manganese deficiency can result in shortened and thickened limbs, curvature of the spine, and swollen and enlarged joints. The basic biochemical defect underlying the development of these bone defects is a reduction in the activities of glycosyltransferases; these enzymes are necessary for the synthesis of the chondroitin sulfate side chains of proteoglycan molecules. In addition, manganese deficiency in adult rats can result in an inhibition of both osteoblast and osteoclast activity. This observation is particularly noteworthy, given the reports that women with osteoporosis tend to have low blood manganese concentrations and that the provision of manganese supplements might be associated with an improvement in bone health in postmenopausal women.

One of the most striking effects of manganese deficiency occurs during pregnancy. When pregnant animals (rats, mice, guinea pigs, and mink) are deficient in manganese, their offspring exhibit a congenital, irreversible ataxia characterized by incoordination, lack of equilibrium, and retraction of the head. This condition is the result of impaired development of the otoliths, the calcified structures in the inner ear responsible for normal body-righting reflexes. The block in otolith development is secondary to depressed proteoglycan synthesis due to low activity of manganese-requiring glycosyltransferases.

Defects in carbohydrate metabolism, in addition to those described previously, have been shown in manganese-deficient rats and guinea pigs. In the guinea pig, perinatal manganese deficiency results in pancreatic pathology, with animals exhibiting aplasia or marked hypoplasia of all cellular components. Manganese-deficient guinea pigs and rats given a glucose challenge often respond with a diabetic-type glucose tolerance curve. In addition to its effect on pancreatic tissue integrity, manganese deficiency can directly impair pancreatic insulin synthesis and secretion as well as enhance intracellular insulin degradation. The mechanism(s) underlying the effects of manganese deficiency on pancreatic insulin metabolism have not been fully delineated, but they are thought to be multifactorial. For example, the flux of islet cell manganese from the cell surface to an intracellular pool may be a critical signal for insulin release. It is also known that insulin messenger ribonucleic acid levels are reduced in manganese-deficient animals, which is consistent with their depressed insulin synthesis. In addition, insulin sensitivity of adipose tissue is reduced in manganese-deficient rats, a phenomenon that may be related to fewer insulin receptors per adipose cell. Manganese deficiency may also affect glucose metabolism by means of a reduction in the number of glucose transporters in adipose tissue by an unidentified mechanism. Finally, the effect of manganese deficiency on insulin production may also be due to the destruction of pancreatic β cells. It is worth noting that constitutive pancreatic MnSOD activity is lower than in most tissues; this, coupled with the observation that most diabetogenic agents function via the production of free radicals with subsequent tissue damage, suggests that an additional mechanism underlying pancreatic dysfunction in manganese-deficient animals may be free radical mediated.

In addition to its effect on endocrine function, manganese deficiency can affect pancreatic exocrine function. For example, manganese-deficient rats can be characterized by an increase in pancreatic amylase content. The mechanism underlying this effect of manganese deficiency has not been delineated; however, it is thought to involve a shift in amylase synthesis or degradation because secretagogue-stimulated acinar secretion is comparable in control and manganese-deficient rats.

Although a majority of studies concerning the influence of manganese deficiency on carbohydrate metabolism have been conducted with experimental animals, there is one report in the literature of an insulin-resistant diabetic patient who responded to oral doses of manganese (doses ranged from 5 to 10 mg) with decreasing blood glucose concentrations. Although this is an intriguing case report, others have reported a lack of an effect of oral manganese supplements (up to 30 mg) in diabetic subjects, and low blood manganese concentrations have not been found to be a characteristic of diabetics.

Abnormal lipid metabolism is also characteristic of manganese deficiency. Specifically, a lipotrophic effect of manganese has been suggested in the literature. Severely manganese-deficient animals can be characterized by high liver fat, hypocholesterolemia, and low high-density lipoprotein (HDL) concentrations. Deficient animals can also be characterized by a shift to smaller plasma HDL particles, lower HDL apolipoprotein (apoE) concentrations, and higher apoC concentrations. As stated previously, tissue lipid peroxidation rates can be increased in manganese-deficient animals, possibly as a result of low tissue MnSOD activity.

There is considerable debate as to the extent to which manganese deficiency affects humans under free-living conditions. Manganese deficiency can be induced in humans under highly controlled experimental conditions. In one study, manganese deficiency was induced in adult male subjects by feeding a manganese-deficient diet ($0.1 \text{ mg Mn day}^{-1}$) for 39 days. The subjects developed temporary dermatitis, as well as increased serum calcium and phosphorus concentrations and increased alkaline phosphatase activity, suggestive of bone resorption. Since the late 1980s, several diseases have been reported to be characterized, in part, by low blood manganese concentrations. These diseases include epilepsy, Meleni disease, maple sirup urine disease and phenylketonuria, Down's syndrome, osteoporosis, and Perthes' disease. The finding of low blood manganese levels in subsets of individuals with the previously mentioned diseases is significant because blood manganese levels can reflect soft tissue manganese concentrations. The reports of low blood manganese concentrations in individuals with epilepsy are particularly intriguing, given the observations that manganese-deficient animals can show an increased susceptibility to drug and electroshock-induced seizures, and a genetic model for epilepsy in rats (the GEPR rat) is characterized by low blood manganese concentrations. It is evident that a deficiency of manganese may contribute to the pathology of epilepsy at multiple points, given that Mn^{2+} is implicated in activation of glutamine synthetase, a Mn^{2+} -specific brain ATPase; production of cyclic adenosine monophosphate (AMP); altered synaptosomal uptake of noradrenalin and serotonin; glutamate, GABA, and choline metabolism; and biosynthesis of acetylcholine receptors.

Evidence of widespread manganese deficiency in human populations is lacking. Typically, manganese intakes approximate the 2001 US Institute of Medicine's suggested adequate intakes as follows: $3 \mu\text{g day}^{-1}$ for infants 0–6 months old, 0.6 mg day^{-1} for infants 7–12 months old, $1.2\text{--}1.9 \text{ mg day}^{-1}$ for children 1–13 years old, $1.6\text{--}2.2 \text{ mg day}^{-1}$ for older children, and $1.8\text{--}2.6 \text{ mg day}^{-1}$ for adults. The Tolerable Upper Intake Level (UL) is the highest level of a daily nutrient intake that is likely to pose no risk of adverse health effects in almost all individuals. The Institute of Medicine's recommended intakes for manganese set ULs at 2, 3, and 6 mg day Upper Intake Level (UL) for children 1–3, 4–8, and 9–13 years old, respectively. Values were set at 9 mg day^{-1} for adolescents 14–18 years old and at 11 mg day^{-1} for adults.

Manganese Toxicity

In domestic animals, the major reported lesion associated with chronic manganese toxicity is iron deficiency, resulting from an inhibitory effect of manganese on iron absorption. Additional signs of manganese toxicity in domestic animals include depressed growth, depressed appetite, and altered brain function.

In humans, manganese toxicity represents a serious health hazard, resulting in severe pathologies of the central nervous system. In its most severe form, the toxicosis is manifested by a permanent crippling neurological disorder of the extrapyramidal system, which is similar to Parkinson's disease. In its milder form, the toxicity is expressed by hyperirritability, violent acts, hallucinations, disturbances of libido, and incoordination. The previous symptoms, once established, can persist even after the manganese body burden returns to normal. Although a majority of reported cases of manganese toxicity occur in individuals exposed to high concentrations of airborne manganese ($> 5 \text{ mg m}^{-3}$), subtle signs of manganese toxicity, including delayed reaction time, impaired motor coordination, and impaired memory, have been observed in workers exposed to airborne manganese concentrations less than 1 mg m^{-3} . Therefore, an inhalation reference concentration range for manganese has been established by the US Environmental Protection Agency to be between 0.09 and $0.2 \mu\text{g m}^{-3}$. Manganese toxicity has been reported in individuals who have consumed water containing high levels ($\geq 10 \text{ mg Mn}$) of manganese for long periods of time. Recently, there has been concern that the risk for manganese toxicity may be increasing in some areas because of the use of MMT in gasoline as an antiknock agent, although there is little evidence that air, water, or food manganese concentrations have increased where this fuel is used.

In addition to neural damage, reproductive and immune system dysfunction, nephritis, testicular damage, pancreatitis, lung disease, and hepatic damage can occur with manganese toxicity, but the frequency of these disorders is unknown. Although there is a limited body of epidemiological data that suggests that high levels of manganese can result in an increased risk for colorectal and digestive tract cancers, most investigators do not consider manganese to be a carcinogen. In contrast, both divalent (MnCl_2) and heptavalent forms (KMnO_4) of manganese are recognized to be strong clastogens

both *in vitro* and *in vivo*; exposure to high concentrations of either form results in chromosomal breaks, fragments, and exchanges. High concentrations of manganese can also induce forward and point mutations in mammalian cells. High levels of dietary manganese have not been reported to be teratogenic in the absence of overt signs of maternal toxicity. However, there are reports that exposure to high levels of manganese during prenatal development can result in behavioral abnormalities. High levels of brain manganese have been reported in subjects with amyotrophic lateral sclerosis, and it has been suggested that this increase may contribute to the progression of the disease. Similar to the cases in humans, chronic manganese toxicity in rhesus monkeys is characterized by muscular weakness, rigidity of the lower limbs, and neuron damage in the substantia nigra. Findings from a recent study suggest that iron and aluminum, which accumulate in the globus pallidus and the substantia nigra of these animals, induce tissue oxidation that may contribute to the damage associated with manganese toxicity. Neural toxicity is a consistent finding in rats exposed to chronic manganese toxicity. Significant manganese accumulation was accompanied by an increase in cholesterol content in the hippocampal region of manganese-treated rats, which was associated with impaired learning; this impairment was corrected by an inhibitor of cholesterol synthesis. The development of manganese toxicity in individuals with compromised liver function, or compromised biliary pathways, is well documented. Significantly, these individuals can have abnormal magnetic resonance imaging (MRI) patterns, which improve following the alleviation of the manganese toxicity. For example, in some cases improvements in brain function have been achieved after liver transplant. The mechanisms underlying the toxicity of manganese have not been agreed on but may involve multiple etiologies, including endocrinological dysfunction, excessive tissue oxidative damage, manganese-mediated disruptions in intracellular calcium and iron metabolism, and mitochondrial dysfunction caused by manganese inhibition of some pathways of the mitochondrial respiratory chain.

Severe cases of manganese toxicity in humans have been reported for adults, as well as isolated cases in other groups of individuals who are vulnerable, including children on long-term parenteral nutrition and parenteral nutrition patients who have cholestasis or other hepatic disease. In many cases, the previously mentioned groups of individuals have been reported to be characterized by high brain manganese concentrations based on MRI. Although no known cases have been reported, infants may be at a high risk for manganese toxicity due to a high absorptive capacity for the element or an immature excretory pathway for it. If manganese is taken up by extrahepatic tissues via the manganese-transferrin complex, the developing brain may be particularly sensitive to manganese toxicity due to the high number of transferrin receptors elaborated by neuronal cells during development, coupled with the putative need by neural cells for transferrin for their differentiation and proliferation. Newborn rats given daily doses of dietary manganese at a level equivalent to that of soy formula exhibited significant neurodevelopmental delays as assessed by several behavioral tests. It should be noted that the concentration of manganese in soy formula is relatively modest but approximately 60–100 times higher than

that of breast milk. Brain manganese concentration was increased and striatal dopamine concentrations were significantly decreased even 45 days after the supplementation ended, suggesting that the impact of manganese on the brain and behavior was irreversible. Thus, dietary exposure to high levels of manganese during infancy can be neurotoxic to rat pups and result in developmental deficits. Further studies on human infants fed diets with different levels of manganese are needed to assess whether there are any long-term consequences of early manganese exposure of newborns.

Another group of neuropathological conditions that has been associated with elevated levels of brain manganese is transmissible spongiform encephalopathies. These diseases found in animals and humans are also referred to as prion diseases. There is strong evidence that in their native state, prions are normal brain glycoproteins that bind copper and have an antioxidant function. However, it has been suggested that in the disease process an abnormal isoform of the protein is generated in which manganese is substituted for copper. This isoform is proteinase resistant, no longer has antioxidant activity, and may play a role in the etiology of these diseases. Indeed, elevated levels of brain manganese, along with lower than normal levels of brain copper, have been measured in patients with the prion disease, Creutzfeldt-Jakob disease. Whether the elevated levels of brain manganese observed in these patients as well as in animal models of these diseases play an important role in their pathogenesis or are secondary to other factors remains to be determined.

Assessment of Manganese Status

Reliable biomarkers for the assessment of manganese status have not been identified. Whole blood manganese concentrations are reflective of soft tissue manganese levels in rats; however, it is not known whether a similar relationship holds for humans. Plasma manganese concentrations decrease in individuals fed manganese-deficient diets and are slightly higher than normal in individuals consuming manganese supplements. Lymphocyte MnSOD activity and blood arginase activity are increased in individuals who consume manganese supplements; however, their value as biomarkers for manganese status may be complicated due to the number of cytokines and disease states that may also increase their expression. Urinary manganese excretion has not been found to be sensitive to dietary manganese intake. With respect to the diagnosis of manganese toxicosis, the use of MRI appears to be promising because the images associated with manganese toxicity are relatively specific. Whole blood manganese concentrations can be correlated with MRI intensity and T₁ values in the globus pallidus even in the absence of symptoms of neurological damage. Thus, although it is relatively expensive, MRI may be particularly useful as a means of identifying susceptible individuals in, or around, manganese-emitting factories. In addition, the method may be useful in the evaluation of patients with liver failure.

See also: Carbohydrates: Regulation of Metabolism. Cofactors: Inorganic

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MEAL SIZE AND FREQUENCY

Effect on Absorption and Metabolism

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Abbreviations

AMP	adenosine monophosphate	DIT	dietary induced thermogenesis
AMPK	adenosine monophosphate-activated protein kinase	GIP	glucose dependent insulotropic polypeptide
BMR	basal metabolic rate	GLP	glucagon-like peptide
CCK	cholecystokinin	HmG-CoA	hydroxymethylglutaryl-coenzyme A
CCK-8	octapeptide of cholecystokinin	reductase	hydroxymethylglutaryl-coenzyme A reductase
CoA	coenzyme A	NPY	neuropeptide-Y
		PYY	peptide YY

Introduction

Eating habits are changing. Terms such as “super-sizing,” “portion distortion,” “grazing,” and “snacking” have appeared in the contemporary vernacular as a result. Therefore, a better understanding of meal size and frequency is particularly topical, not least when considering the potential role which these eating patterns may be playing in the dramatic rise of noncommunicable diseases such as obesity, diabetes, and cardiovascular disease.

The principal consequences that variations in meal size and frequency have on the body relate to the absorption and metabolism of that meal. There are several factors in addition to meal size and frequency which influence absorption and metabolism, such as macronutrient composition, the energy density of the diet, and the physical volume of the meal. However, the particular contribution which variations in meal size and frequency have made to the dramatic change in society's eating patterns makes them worthy of special attention.

The Effect of Meal Size on Absorption

When a meal of mixed macronutrient composition is consumed, the rate at which the carbohydrate, protein, and fat in that meal is absorbed differs. Carbohydrate in the form of glucose, and protein in the form of amino acids enter the portal vein within 30 min of meal ingestion and later appear in the general circulation. As the glucose concentration in the portal vein rises there is an increase in the uptake of glucose into the hepatocytes. Pancreatic islet cells react to the increase in blood glucose and secrete insulin, among other hormones, into the circulation. As a result of this increase in circulating glucose and insulin there is a drop in the release of non-esterified fatty acids/free fatty acids from the adipose tissue.

Fatty acid oxidation in the skeletal muscle tissue decreases and, as glucose uptake takes place, the muscle cells increase the rate at which glucose is oxidized. Glycogen synthesis in the muscle and liver cells is increased and the uptake of amino acids by muscle tissue may also occur. Up to 4 h after the ingestion of the meal, fat in the form of chylomicron-triacylglycerol enters the circulation via the lymphatic system. The action of the hormone lipoprotein lipase in the adipose tissue has by now increased, which promotes the storage of fatty acids as triacylglycerol in adipocytes. This synopsis indicates that following the ingestion of a meal, there is a marked increase in glucose oxidation with a corresponding decrease in fat utilization resulting in the storage of fat.

It is therefore fair to say that the larger a meal that is consumed, the more pronounced the responses described above will be. If a large meal is eaten, for example, the plasma glucose concentration may remain elevated for up to 4 h following ingestion. Conversely, the smaller a meal, the more subtle the effect. This indicates that meal size does indeed influence absorption. However, in order for the relationship between meal size and absorption to be fully understood, the role which absorption itself plays in determining meal size needs to be considered. The following section will therefore focus on the process of absorption and the systems which are in place to control the amount of food eaten. These systems appear not to be working optimally in many cases leading to a state of positive energy balance, which is the cause of the worldwide increase in the incidence of obesity.

The Regulation of Meal Size by Gut-Derived Satiety Peptides and Adiposity Signals

The signaling systems that underlie the regulation of appetite, dietary intake, and therefore meal size are complex. These include signals from the gastrointestinal (GI) tract such as ghrelin, CCK, and GLP-1, and from adipose tissue such as

leptin and adiponectin. This information is routed to the hypothalamus and brain stem where neuronal networks are activated which signal the commencement or conclusion of a meal.

Ghrelin is an orexigenic hormone whereas CCK and GLP-1 both promote satiety. Serum levels of ghrelin fall on commencement of eating whereas CCK is secreted from duodenal mucosal epithelial cells and stimulates the delivery of digestive enzymes from the pancreas, as well as bile from the gall-bladder, into the small intestine. In addition, CCK is produced by neurons in the enteric nervous system and is widely distributed in the brain. Although CCK (and other GI satiety signals) acts to limit meal size, it is important to note that CCK concentration post-meal is not affected by body-fat stores, meaning that it does not take into consideration the existing energy depot of the individual when signaling the onset of satiety. Adiposity signals must therefore be considered in parallel, as they also play a part in determining the size of a meal consumed.

Until quite recently, it was thought that adipose tissue was an inert storage depot but it is now widely recognized as an active endocrine organ. Leptin and adiponectin are two adipose-derived hormones which play a role in the regulation of appetite and therefore meal size. The treatment of obese, hyperphagic, leptin-deficient individuals with exogenous leptin resulting in consumption of more appropriately sized meals has demonstrated that leptin is intimately involved in the regulation of food intake. Leptin however seems to be an overall 'caretaker' of energy intake rather than responding acutely to individual meals. When adipose mass increases, circulating leptin concentrations increase in turn suppressing appetite until which time adipose mass is lost. The mechanism by which leptin can help to reduce food intake is not fully understood although it appears to activate pathways in the brain that reduce food intake while inhibiting pathways that activate food intake.

Adiponectin is an adipokine with cardio-protective and antidiabetic properties and it is hypothesized that adiponectin regulates food intake in conjunction with leptin. When fasting, there is an increase in the adiponectin signal in the hypothalamus leading to an increase in the activity of AMP-activated protein kinase and a subsequent stimulation of food intake. The leptin signal in the hypothalamus is regulated inversely in relation to the adiponectin signal in the brain; therefore adiponectin enhances hypothalamic AMPK activity and food intake, as opposed to the action of leptin.

To conclude, the size of a meal that an individual consumes is determined by a number of physiological, behavioral, and societal factors that interact and play a critical role in the regulation of dietary intake.

The Effect of Meal Size on Metabolism

Energy homeostasis, achieved by matching energy intake with energy expenditure, is partially dependent on the regulation of meal size consumed. In order for meal size to have an effect on energy metabolism, it must affect either or both components that are involved in the regulation of energy balance, namely energy intake and energy expenditure. Energy balance is the difference between energy ingested and energy expended over a given period of time. Consequently, energy storage is equal to intake minus expenditure. The following sections examine the effect of meal size on the two components of the energy balance equation.

The Effect of Meal Size on Energy Intake

Meal portion sizes have been growing steadily since the 1970s, and have been doing so in parallel with the increasing prevalence of obesity. It is known that portion and meal sizes vary depending on the food source, location of consumption, and number of people eating a meal together. Not surprisingly, the largest portions consumed in terms of energy are generally those obtained at fast food restaurants, although the portion sizes of home-cooked meals have been growing steadily as well. Meal size may thus be contributing to the problem of obesity by leading to a daily total energy intake which is greater than the daily total energy expenditure resulting in a positive energy balance.

The Effect of Meal Size on Energy Expenditure

Total energy expenditure can, as shown in [Table 1](#), generally be divided into three major components: BMR, thermogenesis, and physical activity. In order for meal size to have an effect on the energy expenditure side of the energy balance equation, it must have an effect on one or more of these three components. There is no evidence to suggest that meal size has an effect on BMR, which refers to the energy expended to run the body on a day-to-day basis. Thermogenesis broadly refers to the body's production of heat, which is divided into three categories: dietary, thermoregulatory, and adaptive. It is the dietary category, commonly known as DIT, which is of greatest relevance to the current discussion of the effect of meal size on energy expenditure. It refers to the heat lost by the body as a result of the absorption and metabolism of a recently ingested meal. DIT represents approximately 10% of energy intake, and therefore the energy expended on DIT increases and decreases in relation to the size of the meal and, more importantly, the energy value of the meal consumed. The larger a meal,

Table 1 Major components of energy expenditure

Component	Total energy expenditure (%)	Represents
Basal metabolic rate (BMR)	60–75	Day-to-day running costs of an individual, for example, circulation
Thermogenesis	10–20	Heat produced by the body through dietary, adaptive, and thermoregulatory processes
Physical activity	100 minus (BMR + thermogenesis)	The sum of work carried out by an individual

the more energy will be expended to absorb, transport, and metabolize the nutrients consumed in that meal. For example, in the case of a meal containing 2000 kJ of energy, approximately 200 kJ will be expended on DIT alone. It is in the physical activity component of energy expenditure that the greatest variation between individuals can be observed, as physical activity levels (and therefore the energy expended on activity) in the population are contingent on lifestyle choices such as employment and leisure time activities. The effect which meal size could have on physical activity is somewhat difficult to quantify. Meal size is perhaps more important to elite athletes whose energy expenditure is two to three times greater than untrained weight-matched athletes with up to 40% of their energy expenditure being the cost of training.

The Effect of Meal Frequency on Absorption

Humans are eating more often than before with an average eating frequency among US adults of five eating occasions per day. The perceived health advantages of increased meal frequency (as opposed to eating larger, infrequent meals) have been interesting researchers since the 1930s. It has been suggested that frequent eating increases metabolism, reduces food cravings, improves insulin and glucose control, and reduces body weight. However, eating frequently may actually cause an increased exposure to energy-dense foods, leading to increased energy intake and weight gain as opposed to weight loss.

The benefits of a frequent eating approach were originally made apparent by the discovery that insulin requirements in diabetics could be decreased in a frequent meal regime. In a series of case reports on patients taking high insulin doses, it was demonstrated that improved glycemic control and decreased insulin requirements can be achieved when glucose is sipped at hourly intervals throughout the day. Similarly, in healthy individuals a diet composed of many small meals compared with an isoenergetic one composed of larger meals results in decreased insulin and glucose fluctuations.

Meal frequency not only affects insulin and glucose levels but also influences an individual's circulating lipids. An inverse relationship exists between meal frequency and lipid levels, suggesting that infrequent feeding leads to an increased risk of cardiovascular disease due to large fluctuations in circulating lipids. Increased meal frequency, however, is associated with several benefits such as decreased serum cholesterol levels, decreased total : high density lipoprotein cholesterol (HDL-C) ratio, decreased esterified fatty acids and decreased enzyme levels in adipose tissue associated with fatty acid storage. Paradoxically, individuals who report that they eat more frequently not only have lower total and low density lipoprotein cholesterol (LDL-C), but also have a greater intake of energy, total fat, and saturated fatty acids. Considering that some of these results were found in a free-living population, it is possible that dietary mis-reporting, a common occurrence in an overweight population, could be to blame for this inconsistency.

Mechanisms Underlying the Metabolic Effect of Meal Frequency

The mechanisms underlying beneficial responses to frequent feeding as opposed to an infrequent meal pattern are not fully understood. Frequent feeding has been shown to elicit lower plasma glucose fluctuations than does a more infrequent eating pattern. The absolute amount of carbohydrate eaten at each episode of ingestion in a frequent feeding pattern is simply not great enough to elevate glucose to the same extent as more infrequent eating. Small elevations in plasma insulin seen with frequent feeding are most likely to have been in response to minimal fluctuations in glucose. The mechanisms responsible for the effect of an increased frequency of meal eating on lipid metabolism are not as clear cut. The lower serum cholesterol levels observed during frequent feeding may be related to lower serum insulin levels. Insulin appears to have a key role in enhancing the hepatic synthesis of cholesterol through its ability to stimulate HmG-CoA reductase, the rate limiting enzyme in hepatic cholesterologenesis. Exogenous insulin quickly increases HMG-CoA reductase activity in rats with diabetes and raises levels of the enzyme in animals without the disorder. It is possible that the reduction of serum cholesterol during a diet of habitual frequent feeding in normal healthy individuals may result from a reduction in hepatic cholesterol synthesis, secondary to the maintenance of euglycemia at lower serum insulin levels. A reduction in cholesterol synthesis would result in an increase in LDL receptors, further lowering total and LDL-cholesterol levels.

Alternatively, or in addition, the benefits associated with an increased feeding regimen may reflect unintentional or uncontrolled changes in dietary energy and fat intake that may occur when an individual's meal frequency is altered. It is not clear whether a diet of frequent eating results in any adaptational responses of enzymes or hormones which in turn may be providing additional benefit to the individual.

Much of the research in which these benefits were uncovered is difficult to interpret due to the variety of methods used, the lack of information available regarding the foods consumed and the exact nature of the dietary intervention. The majority of measurements are made on fasted blood samples, when in fact most individuals are in a postprandial state for the greater part of every 24-h period. The results of such research must be interpreted with a degree of caution for a number of reasons such as the small sample size used and the interactions with other factors which may prolong absorption time (e.g., soluble fiber, low-glycemic index foods, and the administration of alpha-glycosides).

As discussed, frequent feeding has been demonstrated to lower circulating plasma glucose, insulin, and lipids in both healthy and diabetic subjects in the short term. In addition to the lack of clarity on the mechanisms involved, further research is needed to investigate any medium- and long-term benefits of frequent feeding. It is important that, if deemed desirable in terms of metabolic control, increasing the number of periods of feeding encourages the desired dietary pattern and mix of macronutrients and micronutrients and is not offset by the failure to decrease the size of the meals.

The Effect of Meal Frequency on Metabolism

The same maxim which was earlier applied to the study of meal size, namely that it can only influence energy metabolism if it affects energy intake and energy expenditure, is applicable to meal frequency. The following sections will focus on energy intake and energy expenditure, respectively.

The Effect of Meal Frequency on Energy Intake

It has long been argued that the frequency of meal intake may have an effect on body weight regulation. It has been suggested that there is an inverse association between meal frequency and body weight in individuals. However, there are a number of flaws in the design of many of the research studies from which these data have been derived, and great caution is required in the interpretation of the results. Design flaws include (1) dietary under-reporting of the number of eating occasions and of energy intake, (2) reverse causality, which refers to the possibility that people abstain from eating meals when they become overweight in an attempt to lose weight or to prevent further weight gain, (3) lack of measurement of physical activity or energy expenditure, and (4) inclusion of people in a diseased state. These important confounding factors may help to resolve the contradictory results of many research trials. Erroneous conclusions have been drawn from the misinterpretation of such results because these studies are extremely vulnerable to methodological errors that may generate spurious relationships which may not actually exist. A recently published review article examined almost 200 abstracts and articles pertaining to eating frequency and body weight which ranged from one to nine eating occasions per day over intervention periods of 2–8 weeks in duration. No association between eating frequency and health was observed and the authors concluded that a number of methodological shortcomings made it difficult to fully confirm this finding. Studies of longer duration with sufficient sample size are warranted which could include the use of dietary biomarkers to validate both reported eating frequency and energy intake.

There appears to be very little direct empirical evidence in humans to suggest that frequent feeding *per se* affects appetite and energy intake. Individuals who eat frequently seem to exhibit a greater capacity to compensate for changes in the energy content of specific meals, relative to those individuals who derive most of their energy intake from fewer larger meals. Over very short periods, and under highly controlled experimental conditions, frequent feeding can decrease energy intake at a subsequent meal, which may in turn have an effect on appetite regulation. It remains to be seen however, whether the same would occur in free-living conditions. One final consideration is that of reduced eating frequency, that is less than three meals per day, and data indicate that this pattern negatively affects appetite control. It is important to note however that many of the controlled studies that were designed to investigate the effect of meal frequency on energy intake were conducted whereby meal patterns were enforced onto individuals irrespective of the habitual eating frequency of those individuals. There may also be differences in the

short-term compensation of energy intake depending on an individual's habitual eating pattern.

Mechanisms by which Meal Frequency may Influence Energy Intake

Although the evidence is inconclusive, there is a suggestion that feeding frequency may have an impact on appetite and hence affect energy intake. The control of appetite is a very complex issue which is determined by a number of factors as discussed previously. However, the question remains as to whether the frequency of feeding elicits effects on any of these factors, in turn affecting appetite and possibly body weight. It is noteworthy that rapid declines in blood glucose concentration are associated with hunger in addition to the initiation of feeding in humans.

Although difficult to measure, frequency of feeding may affect the release of appetite regulatory hormones including neuroendocrine hormones such as NPY, galanin, orexin, and melanocortins from the hypothalamus. The release of such hormones may either be stimulated or suppressed during frequent feeding, leading to either higher or lower than normal hormone levels, which may in turn have knock-on effects on energy intake and/or expenditure. When subjective appetite sensations were measured using visual analog scales, increased eating frequency tended to lead to lower peaks in perceived appetite and PYY responses compared with decreased eating frequency although this effect disappeared when investigated over a 24-h period. The release of other gut hormones such as CCK, GLP, and GIP may be altered in relation to feeding frequency. In rats, the infusion of the sulfated octapeptide of CCK (CCK-8) causes a significant reduction in meal size as previously mentioned, whereas meal frequency is increased to compensate for the small meals. However, little is known about the effects of meal pattern on CCK in animals or humans. It is possible that frequent feeding may affect CCK-release in one of two ways: (1) Frequent feeding may cause the regular release of the hormone in response to each feed, persistently alerting the brain that the individual is satiated; or (2) CCK may be released into the circulation in such small amounts in response to frequent feeding that it is not recognized by the brain and the individual continues to eat. Similar effects may occur with GLP and GIP. It is notable that the rate of gastric emptying is also unaffected by antecedent eating frequency.

The Effect of Meal Frequency on Energy Expenditure

As detailed in Table 1, the three components of energy expenditure are BMR, thermogenesis, and physical activity. For meal frequency to have an influence on energy expenditure, it must affect one or more of these components. The first component, BMR (which represents 60–75% of energy expenditure in sedentary individuals), is not known to be influenced by meal frequency. Much the same can be said for thermogenesis, an area where extensive research has failed to demonstrate a link between feeding frequency and DIT. It is reasonable and logical to expect that any difference between frequent and infrequent meal-eating patterns would be seen most clearly during the postprandial period when food has

just been eaten, where the rate of ingestion of nutrients may alter EE and fuel storage.

Although much research has been carried out on the effects of meal frequency on total energy expenditure, little of it has isolated the physical activity component *per se*. The one area where greater attention has been paid to the relationship between meal frequency and physical activity is that of the performance of elite athletes, as the manipulation of the meal pattern can potentially be used as a tool to achieve optimal performance. Because carbohydrate requirements of elite athletes are high and endogenous glycogen reserves are limited, athletes undertaking prolonged strenuous exercise seek to maximize carbohydrate availability at all times.

Irrespective of the above, the key determinant of feeding frequency's overall effect on energy balance is whether it has an impact on 24-h energy expenditure, where energy intake is fixed in content and composition, and where physical activity is kept constant. Although many of these studies have demonstrated differences in carbohydrate, fat, and protein oxidation rates in response to a gorging versus a nibbling eating pattern, each study has reached the same conclusion, namely that no relationship exists between frequency of feeding and overall 24-h energy expenditure. The majority of these studies used either direct or indirect calorimetry or doubly labeled water in their measurements, each of which are highly reliable energy expenditure measurement techniques.

In conclusion, the contemporary terminology referring to the tendency to increase the amount of food eaten at a meal and the greater frequency at which food is eaten which has appeared in our vocabulary recently demonstrates the importance of a clear understanding of the consequences of meal size and frequency on health. We know that satiety peptides and adiposity hormones attempt to control the size of a meal eaten, and that increased meal frequency, within the constraints of energy balance, has been found to have beneficial effects attenuating circulating substrates. However, in order to elucidate the influence that meal size and frequency has on absorption and metabolism, and to clarify whether this increase in the volume of food eaten at a meal and the greater frequency at which food is eaten have a direct effect on health, further research within the free-living population is required.

See also: Adipose Tissue: Structure, Function and Metabolism. Appetite: Physiological and Neurobiological Aspects; Psychobiological and Behavioral Aspects. Energy Metabolism

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MEAT, POULTRY, AND MEAT PRODUCTS

Nutritional Value

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Glossary

Glycerides Glycerol with fatty acids attached.

Lipids Fats, glycerides, fatty acids, cholesterol, sterols.

Muscle foods Meat and meat products.

Introduction

Animal source foods are major contributors to the nutrients in the food supply in many countries. Of these foods, animal muscle (or meat) foods and products are excellent examples of nutrient-dense, naturally nutrient-rich foods, which provide a relatively large amount of many nutrients per the amount of calories provided in a typical serving. For the purposes of this review, discussion will be limited to the muscle foods: beef, pork, lamb, veal, poultry, and some of the processed products made from these muscle species. Fish/seafood will be covered in a separate article. Milk and dairy products, eggs, and major nonmuscle animal source foods, will also be covered separately.

For meat and meat products there are extensive and comprehensive nutrient databases available for reference for particular products of interest. Thus, this review will provide a sampling of the data available for representative meats and meat products.

One of the best and most comprehensive listings of the nutrient values of all meat, poultry, and other meat products is the extensive nutrient database developed and maintained by the United States Department of Agriculture. In this database complete nutrient profiles are listed for more than 700 beef, 200 pork, 195 lamb, 85 veal, 140 poultry, and 130 turkey products. This database can be accessed and searched on-line from the link/website: <http://www.ars.usda.gov/ba/bhnrc/ndl> and continues to be updated as new data become available for various food products. The most recent version of this database is USDA National Nutrient Database for Standard Reference, Release 23, published 2010.

For another extensive listing of the nutrient values of many meat and meat products, including some by brand name, the reader is referred to Bowes & Church's Food Values of Portions Commonly Used, 19th edn. This reference, although not as extensive in terms of products listed, does provide data directly on common serving sizes and on some additional nutrient and nutrient-related components of meat products (e.g.,

omega-3 and *trans* fatty acids, glutathione, vitamin D activity, and other vitamin-like compounds).

Nutritional Value

The nutritional value of foods, including meat and meat products, can be defined in a number of different ways, from simply listing the quantities of various nutrients contained in the foods, to considering biological factors that affect the utilization of these nutrients by the body. Some foods may contain nutrients in forms that the body cannot readily utilize. Thus, nutrient bioavailability, or availability becomes important. Other articles and sections of this book will discuss some of these bioavailability issues and subsequent metabolism of food and nutrient sources.

The nutritional value of meat and meat products is related to the quantity and utilization of nutrients and the potential for these products to either enhance or restrict nutrient utilization by the body. There are five major classes of nutrients: proteins, lipids, carbohydrates, vitamins, and minerals.

The nutrient content of meat (muscle foods) is fairly similar among the various mammals, birds, and fish. However, differences in the levels of the various nutrients may result from differences in the carcass composition among species and within species due to differences in the ratio of fat to muscle in the edible portion. As fat percentage increases, nutrient concentration of the muscle portion decreases. Also, to a certain extent, the fat profile/composition and other nutrient content levels may be modified/affected by the animal's diet and genetic makeup.

In general, cooking or heat processing has only minimal effects on the nutritional value of muscle foods. In most cases, cooking usually decreases moisture content and concentrates other nutrients, including fat content, especially in lower fat products. This is due to moisture loss. However, in some intensely heated meat products fat content may also be reduced significantly with negligible loss of other nutrients.

Classes of Nutrients and Meat Products

Protein

Proteins comprise the structural unit of all muscle cells and connective tissues. As such, meat and meat products (muscle foods) are major protein sources. Further, muscle foods, as a group, are excellent sources of high-quality protein that supplies all the essential amino acids in desirable proportions for human consumption. Amino acids are the building blocks of protein and those provided by meat match or exceed the profile required by humans.

The protein content of most muscle foods, on a wet basis, is between 15% and 35%. This figure will change due to the moisture and lipid content of the specific product. On a raw weight basis as purchased at the store, the protein content is generally less than 20%. However, people do not eat muscle foods raw and visible fat in red meat products and skin in poultry products is usually trimmed away. Therefore muscle foods, as consumed, have a much higher protein content, in the range of 30%.

Lipids

The lipid component of meat and meat products includes a diverse group of substances such as glycerides (glycerol with fatty acids attached), phospholipids, and sterols. The basic component of most meat lipids is the fatty acids, which can be saturated, monounsaturated, or polyunsaturated.

The relative amount of lipid in muscle foods is probably the most variable aspect of the nutritional profile. Within the lipid components, the relative amount of the different forms of fatty acids present is another variable among meat products. Despite the common reference to animal fats (and especially meat and meat products) as 'saturated', less than half of all the fatty acids of meats are saturated. The largest proportions of fatty acids in meats are monounsaturated, followed by saturated and then polyunsaturated fatty acids. Among meat products, poultry has a higher proportion of polyunsaturated fatty acids and slightly less saturated fatty acids.

The fat in meat products provides much of the flavor associated with these foods and also contributes to the palatability and overall acceptability by consumers. In addition, the fats in meat and meat products also contain several essential fatty acids (linoleic and linolenic acids) and the fat-soluble vitamins A, D, E, and K.

Carbohydrates

Meat and meat products are not significant sources of dietary carbohydrates. Almost all dietary carbohydrates come from plant sources. The only naturally occurring carbohydrate in muscle foods is glycogen. In some processed meat products, such as those that are 'sugar-cured', there may be additional sucrose or glucose added.

Vitamins

Meat and meat products are especially good sources of most of the water-soluble vitamins. In general, meat is the major

dietary source of vitamin B₁₂ and is an excellent source of many of the other B-vitamins, such as pyridoxine (B₆), biotin, niacin, pantothenic acid, riboflavin, and thiamin. For vitamin B₁₂, red meat products such as beef and lamb are especially good sources. Pork products are one of the very best sources of thiamin. Although present in muscle foods, the fat-soluble vitamins are less abundant than in plant foods. Vitamins E and K are present, but at lower levels.

Vitamin D activity may be present in some meat products, but at low levels. This is reflected in the latest update to the USDA Nutrient database (<http://www.ars.usda.gov/ba/bhnrc/ndl>), where vitamin D activity is listed for some beef, pork, lamb, veal, chicken/turkey, and processed meat products; however, such data is not consistently available. In recent years there has been production research with beef, pork, and lamb to determine if added vitamin D₃ or its metabolites, fed to the animal for a brief period of time before slaughter, can result in improved meat tenderness. Although the results are inconsistent, and commercial application is premature, there is some indication that tenderness may be improved with relatively low levels of vitamin D supplementation, but these seem to leave very little residual vitamin D₃ or its metabolites in the muscle. Research in Denmark found that the more biologically active 25-OH D is present at low levels in meat; however there is as yet no consensus on the conversion factor for 25-OH D to calculate vitamin D activity. Also, there is currently very little data on the vitamin D and 25-OH D levels in many meat products. This represents a potential future area of research regarding the nutrient composition of meat. Meat products are a very good source of choline, second only to whole eggs. In recent years a database for the choline content of common foods has been updated and expanded, <http://www.ars.usda.gov/Services/docs.htm?docid=6232>.

Minerals

Meat and meat products are good to excellent sources of most minerals. Among the macrominerals, calcium is not high in muscle foods although phosphorus and potassium are prominent. In natural meat products, sodium is present, but not a significant contributor to the diet. However, processed meat products may contain significantly higher levels of sodium (added as part of curing, preserving, or flavor-enhancing ingredients). Some of the microminerals (trace elements) are especially abundant in meat and meat products. Iron is of the greatest significance from meat sources because it is present in the heme form, which is more bioavailable than the nonheme form. Of meat products, beef is an especially rich source of iron in this bioavailable form.

Muscle tissue is an especially rich source of minerals such as phosphorus, potassium, magnesium, iron, copper, zinc, and selenium. For instance, pork, poultry, and beef are especially good sources of selenium.

Bioavailability of Nutrients and Efficiency for Child Development

Muscle foods have been shown to contain "intrinsic" factors that improve the bioavailability of a variety of nutrients.

Moreover, the bioavailability of these nutrients from muscle foods is high; often exceeding the availability of the same nutrients in foods derived from plants. Heme iron is one example. Zinc and copper have been shown to be more available from meat sources than from plant sources. Several of the B-vitamins may also be more bioavailable from meat sources than from plant sources.

Another interesting aspect of meat products is the ability to promote the bioavailability of nutrients in nonmuscle foods when the two are eaten together. This has been referred to as the 'meat factor'. Perhaps the best example of this is the positive effect of meat in the diet on nonheme iron sources, also in the diet.

The efficacy of meat in the diet for its benefits for child growth and development and micronutrient status continues to be demonstrated. Studies with school children in Kenya have shown that animal-source foods, including meat, improve dietary quality, micronutrient status, growth, and cognitive function. In the US, studies with breastfed infants have shown that feeding meat as an early complementary food is feasible and associated with improved zinc status. On a larger scale, a multi-site, multi-national study is underway to further determine the impact of a daily intake of 1/2 oz of meat in 6–18-month-old infants on linear growth, zinc and iron status, brain growth and neurocognitive development, and infectious disease morbidity. This is being done in populations traditionally dependent on nonmicronutrient fortified plant foods for complementary feeding.

Nutrient Density of Meat and Meat Products

The nutrient density of meat is high. Muscle foods have high levels of essential nutrients per unit of weight and per amount of calories provided. Meat and meat products (muscle foods) provide significant amounts of essential nutrients at levels/concentrations higher than from most other foods relative to the calorie content also provided. The United States Food and Drug Administration (US FDA) food labeling guidelines allow a food to be designated a 'good' source of a nutrient if it contributes 10% or more of the Daily Value (DV), and an 'excellent' source, if it contributes 20% or more of the DV, for that nutrient, per 3 oz serving. Most meat products are good or excellent sources of many nutrients. It is generally recognized that in diets that lack muscle foods, greater care is required in diet/menu selection to ensure that adequate levels of essential nutrients are present and bioavailable.

Meat Sources and Nutritional Values

Beef

Beef is an excellent source of high-quality protein, along with significant contributions of many B-vitamins and minerals. In macronutrient terms, the lean to fat ratio of the particular beef product influences the calorie and nutrient composition. In general, as the fat content decreases the concentration of other nutrients (especially protein, B-vitamins, and minerals) in beef tend to increase. Most beef products available to the consumer are much leaner than they were 20 or 30 years ago.

This is a result of changes due to feeding and genetics to produce leaner animals, and also due to closer trim levels on the products that consumers see in the meat case. Whereas in the past, beef cuts with 1/4" of fat trim were common, now the same products have only 1/8" fat trim, or in some cases even 0" fat trim. In the case of ground beef products, 10 or 20 years ago, 17% fat ground beef was considered as 'extra lean'. Now ground beef is commonly available at fat levels as low as 5% or 10%. Other common fat levels for ground beef are 15%, 20%, and 25%; however, a large proportion of current ground beef sales are now in the 5–15% fat level range.

The fat content of beef contains a varied fatty acid profile, with the largest proportion being contributed by mono-unsaturated fat, followed by saturated fat and polyunsaturated fatty acids. In addition, being a ruminant product, beef is an excellent source of the naturally occurring fatty acid, conjugated linoleic acid (CLA), which has been demonstrated to provide anticarcinogenic properties among other health benefits.

Table 1 provides the energy, protein, and lipid profile of beef along with other meat sources. For a comparison of the mineral composition of beef products versus that of other common meat sources, see Table 2. For a comparison of the vitamin composition of beef products versus that of other common meat sources, see Table 3.

Pork

Pork, like beef, is an excellent source of high-quality protein and contributes significant amounts of many B-vitamins and minerals. As for other muscle foods, pork's nutrient composition is greatly affected by its fat and water content. As fat percentage decreases, the concentration of other nutrients increases. In addition, as pork is cooked, and moisture is removed, the concentration of nutrients also increases. Pork is an excellent source of minerals, such as selenium, iron, zinc, phosphorus, and potassium. Compared to other muscle foods, the contribution of pork to selenium in the food supply is especially significant.

In terms of the B-vitamins, pork is an excellent source. Pork is an especially good source of thiamin (vitamin B₁), being the single best source of this vitamin among commonly eaten foods. The fat profile of pork can be influenced by feeding regimes such that it is more, or less, saturated or firm. However, overall the fatty acid profile of pork is largely mono-unsaturated, followed by saturated and then polyunsaturated fatty acids.

Table 1 provides the energy, protein, and lipid profile of pork along with other meat sources. For a comparison of the mineral composition of pork products versus that of other common meat sources, see Table 2. For a comparison of the vitamin composition of pork products versus that of other common meat sources, see Table 3.

Lamb

Although representing a smaller portion of overall muscle food consumption, lamb still provides a nutrient profile with significant benefits for the human diet. As a source of

Table 1 Energy, protein, and lipid profile of meats and meat products^a (amount per 3 oz/85 g, lean only, cooked, except as noted)

Meat species/cut ^b	Serving size (g)	Energy (kcal kj ⁻¹)	Total protein (g)	Total fat (g)	Total SFA (g)	Total MUFA (g)	Total PUFA (g)	Total cholesterol (mg)
Beef								
Composite, Ln 0", ckd, all grades	85	179/751	25.4	7.9	3.01	3.32	0.27	73
Top Round, Ln 0", brld, all grades	85	158/662	27.0	4.8	1.67	2.02	0.18	65
Top Loin, Ln 0", brld, all grades	85	155/649	24.9	5.4	2.06	2.16	0.20	54
Shoulder Pot Roast, Ln 0", brsd, all grades	85	167/697	26.8	6.6	2.21	2.84	0.40	83
95% Ln Ground Beef, brld	85	145/609	22.4	5.6	2.53	2.31	0.28	65
Pork								
Composite, fresh, Ln, ckd	85	171/713	23.4	7.8	2.63	3.32	0.73	71
Tenderloin, fresh, Ln, rstd	85	122/510	22.2	3.0	1.02	1.13	0.43	62
Center Loin Chop, fresh, Ln, pan-fried	85	190/796	23.5	10.0	3.66	4.51	1.27	60
Shoulder, blade steak, fresh, Ln, brld	85	193/808	22.7	10.7	3.78	4.79	0.92	80
Ham, fresh, Ln, rstd	85	179/751	25.0	8.0	2.80	3.78	0.72	80
Lamb								
Composite, Australian, Ln 1/8", ckd	85	171/715	22.7	8.2	3.44	3.28	0.36	74
Loin, Australian, Ln 1/8", brld	85	163/683	22.6	7.4	3.13	2.97	0.31	69
Leg, Australian, Ln 1/8", rstd	85	162/676	23.2	6.9	2.80	2.81	0.32	76
Foreshank, Australian, Ln 1/8", brsd	85	140/586	23.4	4.4	1.60	1.99	0.25	78
Composite, New Zealand, Ln, ckd	85	175/733	25.2	7.5	3.28	2.96	0.44	93
Composite, US Domestic, Ln 1/4", ckd	85	175/733	24.0	8.1	2.89	3.54	0.53	78
Veal								
Composite, Ln, ckd	85	167/697	27.1	5.6	1.56	2.00	0.50	100
Cutlet, leg top round, Ln, pan-fried	85	156/651	28.2	3.9	1.10	1.40	0.35	91
Loin chops, Ln, rstd	85	149/622	22.4	5.9	2.19	2.12	0.48	90
Shoulder, blade, Ln, brsd	85	168/704	27.8	5.5	1.54	1.96	0.49	134
Chicken/turkey								
Broilers, meat only, rstd	85	162/676	24.6	6.3	1.73	2.26	1.44	76
Broilers, Lt meat only, rstd	85	147/615	26.3	3.8	1.08	1.31	0.83	72
Broilers, Dk meat only, rstd	85	174/729	23.3	8.3	2.26	3.03	1.92	79
Turkey, all classes, meat only, rstd	85	145/604	24.9	4.2	1.39	0.88	1.22	65
Turkey, all classes, Lt meat only, rstd	85	133/558	25.4	2.7	0.88	0.48	0.73	59
Turkey, all classes, Dk meat only, rstd	85	159/665	24.3	6.1	2.06	1.39	1.84	72
Processed meats								
Bacon, pork, cured, pan-fried, 1 slice	7.9	42/176	3.0	3.2	1.05	1.42	0.35	9
Sausage, pork, fresh, ckd, 2 links	48	163/680	9.3	13.6	4.38	5.94	1.79	40
Bologna, beef & pork, low fat, 1 slice	28	64/269	3.2	5.4	2.05	2.56	0.46	11
Salami, beef, ckd, 1 slice	26	68/284	3.3	5.8	2.56	2.77	0.27	18

^aUSDA, ARS (2010) USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, <http://www.ars.usda.gov/ba/bhnrc/ndl>^bbrld, broiled; brsd, braised; ckd, cooked; Dk, Dark; Ln, lean and trim level; Lt, Light; rstd, roasted; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2 Mineral composition of meats and meat products^a (amount per 3 oz/85 g, lean only, cooked, except as noted)

<i>Meat species/cut^b</i>	<i>Serving size (g)</i>	<i>Ca (mg)</i>	<i>Fe (mg)</i>	<i>Mg (mg)</i>	<i>P (mg)</i>	<i>K (mg)</i>	<i>Na (mg)</i>	<i>Zn (mg)</i>	<i>Cu (mg)</i>	<i>Mn (mg)</i>	<i>Se (mcg)</i>
Beef											
Composite, Ln 0", ckd, all grades	85	7	2.54	22	196	302	56	5.76	0.11	0.02	18.1
Top Round, Ln 0", brld, all grades	85	6	2.28	18	172	223	36	4.67	0.07	0.01	30.8
Top Loin, Ln 0", brld, all grades	85	16	1.56	21	195	314	51	4.56	0.07	0.01	28.6
Shoulder Pot Roast, Ln 0", brsd, all grades	85	11	3.06	21	199	305	52	8.02	0.11	0.01	32.4
95% Ln Ground Beef, brld	85	6	2.41	19	175	296	55	5.47	0.08	0.01	18.4
Pork											
Composite, fresh, Ln, ckd	85	15	0.85	21	196	303	47	2.46	0.07	0.01	37.6
Tenderloin, fresh, Ln, rstd	85	5	0.98	25	227	358	48	2.06	0.09	0.01	32.5
Center Loin Chop, fresh, Ln, pan-fried	85	4	0.66	23	201	386	44	1.82	0.06	0.01	38.6
Shoulder, blade steak, fresh, Ln, brld	85	28	1.33	20	187	292	63	4.27	0.05	0.01	33.4
Ham, fresh, Ln, rstd	85	6	0.95	21	239	317	54	2.77	0.09	0.03	42.4
Lamb											
Composite, Australian, Ln 1/8", ckd	85	14	1.74	20	176	270	68	4.37	0.13	0.01	9.3
Loin, Australian, Ln 1/8", brld	85	18	1.85	22	187	289	68	2.96	0.13	0.01	8.8
Leg, Australian, Ln 1/8", rstd	85	8	1.83	21	182	277	61	4.11	0.13	0.01	5.0
Fore Shank, Australian, Ln 1/8", brsd	85	12	1.62	19	150	217	85	6.74	0.11	0.01	7.7
Composite, New Zealand, Ln, ckd	85	11	2.00	19	209	160	42	3.65	0.10	0.03	1.7
Composite, US Domestic, Ln 1/4", ckd	85	13	1.74	22	178	292	65	4.48	0.11	0.02	22.2
Veal											
Composite, Ln, ckd	85	20	0.99	24	212	287	76	4.33	0.10	0.03	11.1
Cutlet, leg top round, Ln, pan-fried	85	6	0.74	27	246	376	65	2.87	0.05	0.03	8.8
Loin chops, Ln, rstd	85	18	0.72	22	189	289	82	2.75	0.10	0.03	9.9
Shoulder, blade, Ln, brsd	85	34	1.25	24	214	259	86	6.28	0.15	0.03	12.3
Chicken/Turkey											
Broilers, meat only, rstd	85	13	1.03	21	166	207	73	1.78	0.06	0.02	18.7
Broilers, Lt meat only, rstd	85	13	0.90	23	184	210	65	1.05	0.04	0.01	20.7
Broilers, Dk meat only, rstd	85	13	1.13	20	152	204	79	2.38	0.07	0.02	15.3
Turkey, all classes, meat only, rstd	85	21	1.51	22	181	253	60	2.63	0.08	0.02	31.3
Turkey, all classes, Lt meat only, rstd	85	16	1.15	24	186	259	54	1.73	0.04	0.02	27.3
Turkey, all classes, Dk meat only, rstd	85	27	1.98	20	173	247	67	3.79	0.14	0.02	34.8
Processed meats											
Bacon, pork, cured, pan-fried, 1 slice	7.9	1	0.11	3	44	47	192	0.29	0.01	0.00	5.1
Sausage, pork, fresh, ckd, 2 links	48	6	0.65	8	78	141	360	1.00	0.04	0.00	0.0
Bologna, beef & pork, low fat, 1 slice	28	3	0.18	3	51	44	310	0.42	0.02	0.00	3.1
Salami, beef, ckd, 1 slice	26	2	0.57	3	53	49	296	0.46	0.05	0.01	3.8

^aUSDA, ARS (2010) USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, <http://www.ars.usda.gov/bz/bhmrc/ndi>^bbrld, brolied; brsd, braised; ckd, cooked; Dk, Dark; Ln, lean and/or firm level; Lt, Light; rstd, roasted.

Table 3 Vitamin composition of meats and meat products^a (amount per 3 oz/85 g, lean only, cooked, except as noted)

<i>Meat species/cut^b</i>	<i>Serving size (g)</i>	<i>Thiamin (mg)</i>	<i>Riboflavin (mg)</i>	<i>Niacin (mg)</i>	<i>Pantothenic acid (mg)</i>	<i>Vit. B₆ (mg)</i>	<i>Folate (mg)</i>	<i>Vit. B₁₂ (mg)</i>	<i>Vit. E (mg)</i>	<i>Vit. K (mcg)</i>
Beef										
Composite, Ln 0", ckd, all grades	85	0.08	0.20	3.40	0.33	0.30	7	2.64	0.14	1.50
Top Round, Ln 0", brld, all grades	85	0.06	0.15	4.84	0.53	0.36	9	1.49	0.34	1.30
Top Loin, Ln 0", brld, all grades	85	0.07	0.13	7.12	0.49	0.53	8	1.39	0.32	1.20
Shoulder Pot Roast, Ln 0", brsd, all grades	85	0.07	0.23	4.01	0.70	0.46	7	2.88	0.09	1.36
95% Ln Ground Beef, brld	85	0.04	0.15	5.05	0.55	0.35	6	2.10	0.31	1.10
Pork										
Composite, fresh, Ln, ckd	85	0.57	0.26	5.53	0.62	0.49	3	0.58	0.09	0.00
Tenderloin, fresh, Ln, rstd	85	0.81	0.33	6.32	0.86	0.63	0	0.48	0.07	0.00
Center Loin Chop, fresh, Ln, pan-fried	85	0.65	0.29	4.35	0.66	0.33	7	0.52	0.19	0.00
Shoulder, blade steak, fresh, Ln, brld	85	0.64	0.37	3.66	0.69	0.26	4	0.96	0.23	0.00
Ham, fresh, Ln, rstd	85	0.59	0.30	4.20	0.57	0.38	10	0.61	0.22	0.00
Lamb										
Composite, Australian, Ln 1/8", ckd	85	0.11	0.31	4.94	0.75	0.34	c	2.56	c	c
Loin, Australian, Ln 1/8", brld	85	0.15	0.28	6.93	0.71	0.44	c	1.71	c	c
Leg, Australian, Ln 1/8", rstd	85	0.12	0.36	4.87	0.84	0.39	c	2.71	c	c
Foreshank, Australian, Ln 1/8", brsd	85	0.08	0.24	4.58	0.56	0.22	c	2.72	c	c
Composite, New Zealand, Ln, ckd	85	0.11	0.43	6.53	0.49	0.12	0	2.51	0.16	c
Composite, US Domestic, Ln 1/4", ckd	85	0.09	0.24	5.37	0.59	0.14	20	2.22	0.16	c
Veal										
Composite, Ln, ckd	85	0.05	0.29	7.16	1.13	0.28	14	1.40	0.36	5.60
Cutlet, leg top round, Ln, pan-fried	85	0.06	0.32	10.74	1.04	0.43	14	1.28	0.36	4.20
Loin chops, Ln, rstd	85	0.05	0.26	8.04	1.08	0.32	14	1.11	0.42	4.70
Shoulder, blade, Ln, brsd	85	0.05	0.31	4.83	1.35	0.21	13	1.71	0.38	5.80
Chicken/turkey										
Broilers, meat only, rstd	85	0.06	0.15	7.80	0.94	0.40	5	0.28	0.23	2.00
Broilers, Lt meat only, rstd	85	0.06	0.10	10.56	0.83	0.51	3	0.29	0.23	0.30
Broilers, Dk meat only, rstd	85	0.06	0.19	5.57	1.03	0.31	7	0.27	0.23	3.30
Turkey, all classes, meat only, rstd	85	0.05	0.16	4.63	0.80	0.39	6	0.31	0.28	3.10
Turkey, all classes, Lt meat only, rstd	85	0.05	0.11	5.81	0.58	0.46	5	0.31	0.08	0.00
Turkey, all classes, Dk meat only, rstd	85	0.05	0.21	3.10	1.09	0.31	8	0.31	0.54	3.30
Processed meats										
Bacon, pork, cured, pan-fried, 1 slice	7.9	0.04	0.02	0.91	0.10	0.03	0	0.10	0.02	0.00
Sausage, pork, fresh, ckd, 2 links	48	0.14	0.10	3.00	0.35	0.16	1	0.57	0.26	0.20
Bologna, beef & pork, low fat, 1 slice	28	0.05	0.04	0.71	c	0.05	1	0.37	0.06	0.10
Salami, beef, ckd, 1 slice	26	0.03	0.05	0.84	0.25	0.05	1	0.80	0.05	0.30

^aUSDA, ARS (2010) USDA National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory Home Page, <http://www.ars.usda.gov/ba/bhmrc/nd/>^bbrld, broiled; brsd, braised; ckd, cooked; Dk, Dark Ln, lean and trim level; Lt, Light; rstd, roasted.^cComparable data not available.

high-quality protein, lamb is also a good source of many minerals and B-vitamins. Vitamin B₁₂ is especially abundant in lamb. It is also a good source of the minerals iron and zinc.

In addition, as a ruminant, lamb is another naturally occurring dietary source of CLA, a unique fatty acid with anticarcinogenic and other health benefits (from animal model studies).

Table 1 provides the energy, protein, and lipid profile of lamb along with other meat sources. For a comparison of the mineral composition of lamb products versus that of other common meat sources, see **Table 2**. For a comparison of the vitamin composition of lamb products versus that of other common meat sources, see **Table 3**.

Veal

Although representing a smaller proportion of overall meat consumption, veal still provides a nutrient profile that is very beneficial. As with all meat sources, veal provides high-quality protein in a product that may be slightly leaner (in terms of fat) than other red meat sources. Compared to other meat sources, veal would have a lower iron content.

Table 1 provides the energy, protein, and lipid profile of veal along with other meat sources. For a comparison of the mineral composition of veal products versus that of other common meat sources, see **Table 2**. For a comparison of the vitamin composition of veal products versus that of other common meat sources, see **Table 3**.

Poultry

The nutrient composition of poultry (chicken and turkey) is similar to that of red meat animals (beef, pork, lamb, veal) with a few exceptions. Poultry is lower in iron content, and thus heme iron, than beef. Turkey is slightly higher in several minerals (Ca, Fe, P, K, Zn, and Cu) than chicken. As in red meats, there are significant amounts of several B-vitamins (e.g., niacin, B₆, pantothenic acid) compared to other meat sources, and these are not significantly reduced during cooking.

The fat content of poultry is predominantly monounsaturated fat, followed by saturated fat and polyunsaturated fat. Poultry fat, like pork fat, is somewhat more unsaturated than beef fat. Poultry is significantly higher in polyunsaturated fat compared to beef, pork, lamb, and veal.

Table 1 provides the energy, protein, and lipid profile of chicken and turkey along with other meat sources. For a comparison of the mineral composition of chicken and turkey products versus that of other common meat sources, see **Table 2**, and of the vitamin composition of chicken and turkey products versus that of other common meat sources, see **Table 3**.

Processed Meats

Processed meats represent a diverse array of products that have undergone additional treatment from the fresh meat form to the point of consumption. Some of these processing/treatments might include curing with other ingredients added, addition of salt or other flavor or preservative mixtures, etc. Also, these products often represent combined meat sources.

Table 1 provides the energy, protein, and lipid profile of several processed meat products along with other meat sources. For a comparison of the mineral composition of these processed products versus that of other common meat sources, see **Table 2**, and for the vitamin composition of these processed products versus that of other common meat sources, see **Table 3**.

Summary

Muscle foods (meat and meat products) provide significant amounts of essential nutrients at levels/concentrations higher than from most other foods relative to the calories provided. Most of all the essential nutrients are present in muscle foods at some level. Furthermore, muscle foods provide nutrients in a form that enhances the bioavailability of nutrients from both the meat itself and from other dietary sources. It is generally recognized that in diets that lack muscle foods, greater care is required in diet/menu selection to ensure that adequate levels of essential nutrients are present and bioavailable.

See also: Amino Acids: Chemistry and Classification; Metabolism; Specific Functions. Bioavailability. Biotin: Physiology, Dietary Sources, and Requirements. Carbohydrates: Chemistry and Classification; Regulation of Metabolism; Requirements and Dietary Importance. Cholesterol: Factors Determining Blood Levels; Sources, Absorption, Function, and Metabolism. Choline and Phosphatidylcholine. Copper. Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Eggs. Energy: Adaptation; Balance. Energy Metabolism. Energy Requirements. Fats and Oils. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids; Metabolism. Fish and Seafood: Nutritional Value. Folic Acid. Food Composition Data. Iron: Physiology, Dietary Sources, and Requirements. Magnesium. Manganese. Niacin and Pellagra. Nuts and Seeds. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Pantothenic Acid. Phosphorus: Physiology, Dietary Sources, and Requirements. Potassium. Protein: Quality and Sources; Requirements and Role in Diet; Synthesis and Turnover. Protein Deficiency. Protein Digestion and Bioavailability. Riboflavin. Salt: Epidemiology. Selenium. Sodium: Physiology. Thiamin: Beriberi; Physiology. Trans-Fatty Acids: Health Effects, Recommendations, and Regulations. Ultratrace Elements. Vegetarian Diets. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. Vitamin B₆: Physiology. Vitamin E: Metabolism and Requirements; Physiology and Health Effects. Vitamin K. Zinc: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements

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MICROBIOTA OF THE INTESTINE

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Glossary

Bifidogenic A compound, typically a prebiotic food ingredient, that promotes the growth of *Bifidobacteria* in the intestine; also known as Bifidus Factor.

Fructan A polymer of fructose found naturally in fruit, grains, and vegetables, and added as a food ingredient. Inulin and fructo-oligosaccharide are two common fructans.

Galactan A polymer composed of galactose residues found naturally in human milk and some plant foods. Galacto-oligosaccharide is a common galactan.

Microflora Microorganisms that collectively inhabit a bodily organ or part; those within the gastrointestinal (GI) tract are referred to as GI microflora or GI microbiota.

Oligosaccharide (From the Greek oligos, *a few*, and sacchar, *sugar*) A carbohydrate polymer containing a small number (usually 2–10) monosaccharides.

Prebiotic Nondigestible food ingredients that stimulate the growth and activity of bacteria in the digestive system in ways claimed to be beneficial to health.

Introduction

The gastrointestinal (GI) system in humans comprises the largest surface area of any organ in the body. The system provides us with the ability to assimilate and selectively process nutrients, offers a vehicle for excretion of waste, and at the same time provides a first line of defense against pathogens and other noxa. The indigenous ecosystem of bacteria that inhabit the GI tract (gut microbiota) mediates the interaction between the external environment and the host. The basic development and composition of the human intestinal microbiota, its metabolic activity and interactions, and immune functional effects on the host are presented below. The importance and clinical benefits of maintaining balance of this ecosystem, including the ingestion of prebiotics – substances that foster the growth of beneficial bacteria, are further explored.

Intestinal Microbiota in Healthy Individuals

The Human Microbiome Project, commenced in 2007, aimed to collect and identify genomic information from many diverse human microbiomes (bacterial genes in the microbiota), and results from these studies, along with those from recent large-scale surveys, have extended our knowledge of

commonalities among healthy individual's microbiota. Although the majority of all species isolated from adult microbiota samples belong to only four bacterial divisions (phylum) (e.g., Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria), hundreds-to-thousands of phylotypes at the species level have been suggested. A number of represented sequences are from species that have yet to be cultivated.

There is no clear consensus of what constitutes a “normal” microbiota, or the ratio and locations of specific bacterial species within the GI tract necessary to maintain health. Despite this, there is agreement that the intestinal microbiota of healthy humans is comprised of more than 500 currently recognized species of bacteria with a population of 10^{12} – 10^{14} colony-forming units (CFU) per gram, of which greater than 98% are resident in the colon. It is estimated that this bacterial population exceeds the population of human cells by nearly 10 fold. The microbiota is composed of both aerobic, and predominantly anaerobic, microorganisms that when equilibrium within an individual is maintained, confers nutritional and immune benefits. The interaction of the mucosal surface with the luminal microbiota is critical in modulating gut associated lymphoid tissue (GALT) response and thus, GI, as well as systemic, immunity.

The presence of microorganisms in different segments of the GI tract varies both qualitatively and quantitatively.

Bacteria from the mouth, predominately anaerobes, including *Streptococci*, *Bacteroides*, *Lactobacilli*, and some yeasts, are washed down to the stomach with the intake of food and swallowing. In the stomach, the acid environment destroys most of the oral and food ingested microorganisms, resulting in mostly gram positive and aerobic bacterium at very low levels (10^3 CFU ml⁻¹). Concentrations of bacteria found in the small intestine are also comparatively limited, ranging between 10^3 and 10^4 CFU ml⁻¹; both facultative anaerobes and aerobic bacteria, including *Lactobacilli*, *Streptococcus* and *Bacteriodes* are present. The microbiota of the colon dramatically increases to concentrations of 10^{11} – 10^{12} CFU g⁻¹. This bacterial load accounts for up to 50% of the volume of colonic content. Although the bacterial species are too numerous to count, the colonic microbiota are predominately anaerobic including *Bacteroides*, *Fusobacterium*, *Bifidobacterium*, *Lactobacilli*, *Enterobacter*, coliforms and other facultative anaerobes (*Staphylococcus* and *Candida* species). Owing to difficulty in obtaining samples from distinct regions within the GI tract of healthy individuals, most data on the identification and concentration of bacteria within the GI tract is from fecal samples.

Development of Intestinal Microbiota

An infant's GI tract is essentially sterile at birth and begins colonization on ingestion of bacteria from the environment. Progression of colonization of bacteria in the newborn GI tract is initially fast, followed by a gradual modification over the first few years of life (Figure 1). As the baby passes through the birth canal, *Bifidobacteria* and *Lactobacilli* are typically acquired and rapid colonization of mainly *Enterobacteria* occurs. The type of delivery, the type of feeding, and the immediate environment following birth, such as that of the hospital or home, or neonatal care setting can affect the early colonization

of the intestine after birth. Vaginal birth permits the transfer of bacteria from the mother as the infant passes through the birth canal. However, with cesarean delivery, this transfer is absent and colonization can be more significantly affected by the hospital or other immediate environmental bacterium. In these infants, colonization with anaerobic bacteria, especially *Bacteroides*, occurs later than for vaginally delivered infants. Infants born via cesarean section will generally have a less numerous and less diverse microbiota.

Within the first few days after birth and with introduction of feeding, the newborn intestine (through oxidation–reduction) promotes the establishment of aerobic bacteria, predominantly *Enterobacteria*, *Enterococci* and *Staphylococci* and anaerobic bacteria, *Bifidobacteria*, *Bacteroides* and *Clostridia*. As the aerobic bacteria consume oxygen, the intestinal milieu becomes more amenable to anaerobic bacteria and aerobic bacteria in turn decline. In breast fed infants, *Bifidobacteria* counts increase dramatically and will account for up to 80–90% of the total fecal bacteria. *Lactobacilli* and *Bacteroides* also increase, but to a lesser extent, whereas counts of *Enterobacteria* decrease. In formula fed babies, *Enterococcus* is the predominant bacteria isolated in stool, with significantly less *Bifidobacteria* and *Bacteroides* identified than in stool samples from breast fed infants. The differences in intestinal microbiota between breast fed and formula fed infants is likely due to a response from multiple factors, only some of which are elucidated.

Breast milk itself contains bacteria in amounts that may be physiologically or clinically relevant. Bacterial species described in breast milk exceed those that would typically be expected from only skin bacteria contamination, and include species from *Bifidobacterium*, such as *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium bifidum*, and others, as well as *Lactobacillus reuteri*, *Lactobacillus rhamnoses*, *Lactobacillus*

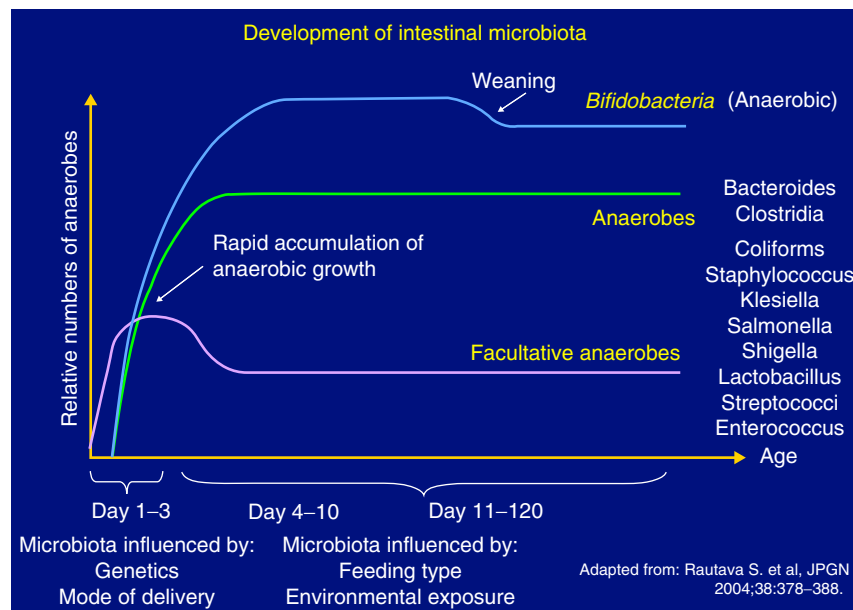


Figure 1 Development of the intestinal microbiota. Reproduced from Rautava S, Ruuskanen O, Ouwehand A, Salminen S, and Isolauri E (2004) The hygiene hypothesis of atopic disease - an extended version. *Journal of Pediatric Gastroenterology and Nutrition* 38: 378–388, with permission from LWW.

fermentum, and other *Lactobacilli* species. Counts of total bacteria within healthy women's breast milk samples may reach 10^6 CFU ml⁻¹, with lactic acid bacterial species contributing up to 10^5 CFU ml⁻¹, of which up to 10^3 CFU ml⁻¹ of specific *Bifidobacterium* species have been identified. The difference in microbiota among breast fed, compared to formula fed infants, resulting from the presence of *Bifidobacteria*, oligosaccharides and other bifidogenic factors in breast milk likely confers a protective effect to the infant against infection, particularly against diarrheal disease.

With the introduction of weaning foods, the fecal bacteria of babies begins to change, resembling that of adults by 1 year of age. Concentrations of aerobes decrease; *Streptococci*, *Enterobacter*, *E. coli* and anaerobes (*Bacteroides* and *Lactobacillus*) predominate by 1–2 years of life. *Bifidobacteria* concentration also decreases, but is generally maintained throughout adulthood (Figure 1). Once well established, the microbiota is unique to each individual and maintained fairly undisturbed throughout the adult life. Changes in general health and wellness, exposure to toxins in the food supply, utilization of medications, particularly antibiotics, all may transiently alter the colonic microbiota, often profoundly. When disequilibrium of this complex microbiota system transpires, the host is potentially compromised. However, recovery to the original state of colonization usually occurs on removal of the altering factors.

Metabolic Activity of the Microbiota

The structure and function of the GI tract is influenced greatly by the presence and composition of indigenous microbiota. In germ-free animal models, lack of microbiota leads to a thinner, less cellular intestinal wall; villi are thinner, crypts shallower, and mucosal surface area is decreased. Intestinal permeability (leakiness of the gut) is also greater in germ-free animal models. Impairment of immune system function and normal nutrient handling processes result. The intestinal microbiota is also responsible for production of some micronutrients, and bacterial activity in the GI tract is responsible for fermentation of carbohydrates which result in the production of short-chain fatty acids (SCFA) (acetate, propionate, and butyrate). These end products are known to be active in the regeneration and health of the mucosal cellular make-up, and inhibit the growth of coliforms and potential pathogens. Additionally, the microbiota modulates the release of peptides and some proteins from the endocrine cells in the mucosa of the GI tract.

Microbiota–Nutrient Interactions

A complex interaction between food and microbiota exists in a feedback like system within the intestinal lumen. Different types of diets can lead to changes in fecal bacteria and its resultant metabolic activity can be altered. In healthy individuals, sucrose, lactose, and maltose rarely reach the colon. However, when intestinal brush border enzyme concentration is absent, or insufficient, disaccharides not absorbed in the small bowel ultimately reach the colon where they interact

with the abundant colonic bacteria. Subsequent fermentation may cause a significant osmotic imbalance, pulling water into the lumen, resulting in diarrhea. Moreover, rapid production of SCFA from the fermentation process changes the fecal pH, of which can further irritate the colonic mucosa when an individual faces a compromised situation from osmotic diarrhea.

Complex carbohydrates, such as some dietary fibers, are poorly absorbed, and therefore become fermentable substrate for colonic bacteria. As mentioned above, predominant by products include SCFA, and in lesser amounts carbon dioxide, hydrogen, methane, and water. Slow and regular generation of SCFA provide an energy source that helps serve as trophic factors toward the regeneration of colonic mucosa as well as other benefits within the healthy individual. Specific types of fermentable carbohydrates in the diet are also an important determinant of the composition of the intestinal bacterial population. The presence of particular galacto- or fructo-oligosaccharides, for example, can preferentially support growth, of certain GI species over others. The use of these dietary sugars, to modulate the composition of luminal bacteria, has given rise to the concept of prebiotics, which is described in a subsequent section below.

Generally, fermentation of dietary fiber provides a preferential energy source for gut bacteria in order to spare the host from fermenting dietary or indigenous protein metabolic products (e.g., epithelial cells and proteolytic enzymes). Bacterial microbiota of both the small and large bowel also synthesizes a number of essential vitamins. Most importantly, vitamin K production by the liver is dependent on the metabolic activity of bacteria in the ileum. In addition, Vitamin B₁₂ is synthesized from microbiota in animals. Additionally, biotin and other B complex vitamins (folic acid and thiamine) are synthesized by microbiota.

Varying levels of bacteria throughout the GI tract have inherent benefit to the function of each portion of the GI tract. Thus, selective discouragement of colonization is necessary. For example, the low number of bacteria in the small bowel allows the function of nutrient breakdown and absorption. Intrinsically, the small bowel limits the levels of bacteria through antegrade peristalsis, bactericidal action of the gastric acid and biliary enzymes of the liver. The ileocecal valve at the terminal end of the small bowel functions as a gate deterring the entrance or proliferation, of colonic bacteria in the small bowel. Bacterial overgrowth syndrome, due to anatomical and physiologic alterations of the small bowel may cause proliferation of bacteria in the upper GI tract. This abnormal presence of higher concentrations of certain colonic bacteria causes mucosal inflammation and villous atrophy, ultimately interfering in its function.

Effect of Intestinal Microbiota on Intestinal Barrier Function

Colonization resistance, mucin production and intestinal impermeability are all protective barrier factors which result from the presence of gut luminal microbes.

Colonization resistance: Microbial diversity in the healthy GI tract is an important component of host protection. The large numbers of species, which compete for the luminal

environment, are in itself a modulating factor in the predominance of potentially pathogenic organisms. The specific composition of the microbiota can play a protective role. For example, resident *Bifidobacteria* and *Lactobacilli* in the gut can offer resistance (by competition or inhibition) to colonization by other potentially pathogenic microbes, thereby functioning as part of the gut defense barrier. In addition, certain *Lactobacilli* and *Bifidobacterium* have also been associated with the secretion of substrates that have antimicrobial properties, termed bacteriocins.

Mucus/mucin glycoproteins: Intestinal mucus production is an important component of gut barrier function, and bacteria are the primary stimulus for secretion by gut mucosa. Mucus is continuously produced by goblet cells to lubricate and protect the GI epithelium and forms a viscous gel that coats the epithelial surface of the intestine protecting it from chemical and mechanical stress. Coating of the epithelia denies pathogenic bacteria the opportunity to adhere, preventing infections and inflammatory response, thus providing a major line of defense of the intestine against pathogenic microbes.

Intestinal permeability: The intestinal mucosa needs to maintain a very selective permeability to the large number of antigens, toxins, and other noxa in the GI lumen. Tight junctions between cells of the epithelium are an important layer of barrier function, and their activity is significantly affected by the presence of bacteria in the gut lumen. In the absence of microbiota, the intestinal permeability dramatically increases, leaving the host vulnerable to antigens, infections agents, and toxins.

Effect of Intestinal Microbiota on Immune Response

One of the most important functions of the intestinal microbiota is activation of the mucosal immune response. The absence of microbes in the GI tract (as in germ-free reared animals) leads to an atrophic mucosa and an underdeveloped cellular and humoral immune response. Thus, the intestinal microbiota profoundly influences the development of specific and nonspecific host immune response. This microbial–host interaction is underscored by the fact that approximately 80% of all immunologically active cells of the body are in GALT, which depend on microbial interaction for normal development and function. Signaling through specific receptors, particularly toll-like receptors, intestinal bacteria activate and modulate GALT, which responds with activation of humoral response (antibody production), as well as with T cell differentiation, which regulates cellular immune response. Thus, intestinal bacteria are primary determinants of immune related host defense mechanisms. Alterations of bacterial populations in the GI lumen can also lead to overexpression of inflammatory immune responses, which may not always be beneficial.

One major effect of intestinal bacteria is the enhancement of secretory immune function and intestinal bacteria. Secretory immunoglobulin A (sIgA), the most important and predominant immunoglobulin in mucosal surfaces, provides protection against antigens, potential pathogens, toxins, and virulence factors. Intestinal sIgA synthesis is stimulated by the presence of microbes. The development of IgA producing plasmablasts, in the intestinal mucosa, precursors for sIgA, are

influenced greatly by intestinal bacteria. In this way, intestinal bacteria, including ingested bacteria, can help decrease risk of intestinal infections, such as acute diarrhea due to rotavirus infection. Certain bacterial species will also have a significant effect on mucosal T cell response. In the infant, intestinal colonization induces modulation of the ratio of T helper type 2 (Th2-pro allergic) to T helper type 1 (Th1-suppressive) responses, which decrease the chances for immune hyper reactivity, such as in allergic disease later in life. Thus, inadequate bacterial colonization, such as in infants born by cesarean section, or receiving antibiotics repeatedly early in life, can increase propensity for allergic conditions.

Although many bacterial species will have varying effects over GALT development and response, some, such as *Bifidobacteria* and *Lactobacilli* can do so in ways that may benefit the host, whilst not being pathogenic. This has led to the rather arbitrary, but practical concern that certain components of the intestinal bacteria as *Lactobacilli* and *Bifidobacteria* are 'beneficial', whereas others, such as pathogenic *Clostridia* or *Bacteroides* are less so.

Altering Gut Microbiota

The concept of manipulating microbiota to enhance their positive aspects on the GI tract has become a more focused endeavor. One approach has been that of consuming oral 'beneficial bacteria' to 'help balance' the intestinal bacteria. This concept is not new. The beginning recognition of fermented foods offering health benefits dates back to the early 1900s. Eli Metchnikoff first recognized this benefit as he observed the long lives and good health of the Bulgarian peasants and associated this with the great amounts of milk, soured with lactic acid bacteria, they consumed.

Since then, much study of the health benefits from the introduction of orally supplemented beneficial bacteria has taken place. This concept has been termed probiotics, and is defined as the consumption of microbes which confer a positive effect on the host in prevention and treatment of specific pathologic conditions. *Bifidobacteria*, *Lactobacilli* and *S. thermophilus* have been the most recognized and studied probiotics because of their ability to survive the upper GI tract and proliferate the colonization, although transiently, in the colon. The health benefits that these and other probiotics have purported include prevention and treatment of diarrhea (particularly rotaviral and antibiotic associated), improved lactose digestion, enhanced gut immune function, and most recently, prevention and treatment of food allergy and its systemic effects (atopic dermatitis and possibly GI allergic disease). Probiotics, as a way to beneficially alter intestinal microbiota composition and its clinical effects, will be elaborated in a separate chapter.

Another way to modulate intestinal bacteria, particularly the native colonic bacteria, is by providing a substrate for gut bacterial growth through fermentation. Certain dietary carbohydrates and fibers which escape digestion of the upper GI tract are ideal for this action. The recognition of dietary factors that can preferentially support the growth of specific, and 'more desirable' or 'beneficial' bacterial species, particularly *Bifidobacteria*, in the human GI tract, has led to the concept of prebiotics.

Prebiotics

Definition

A prebiotic is generally accepted as a nondigestible food ingredient, selectively fermented in the GI tract, that result in specific changes, in the composition or activity of the GI microbiota, which may result in benefits on the health of the host. Generally accepted characteristics of a substance to be considered a prebiotic include:

- Have the ability to pass through the upper GI tract. This implies resistance to gastric acidity, resistance to hydrolysis by intestinal enzymes, and no intestinal absorption.
- Are fermentable by colonic bacteria.
- Selectively stimulate the growth and activity of intestinal bacteria associated with health and well-being, particularly *Bifidobacteria* and *Lactobacilli*.
- Are able to alter the colonic microbiota to a more beneficial spectrum of bacteria.

Classifications

The majority of data on prebiotic effects have been obtained from studies with food ingredients or supplements from two groups, namely dietary fructans, such as inulin or fructo-oligosaccharide (FOS) and galactans, such as galacto-oligosaccharide (GOS). However, prebiotic effects for other carbohydrates such as lactulose, isomaltuligosaccharides, lactosucrose, and polydextrose have been suggested, and the list of carbohydrate derived compounds meeting the classification for prebiotic status continues to expand.

Food ingredients that have been frequently studied in healthy populations with consensus that they possess attributes of a prebiotic are dietary fructans, and galactans. Dietary fructans can be either derived from naturally occurring oligosaccharides or artificially synthesized. These carbohydrates contain one or more fructosyl-fructose links that make up the majority of osidic bonds. They are linear or branched fructose polymers with either β -2-1 linked inulins or β -2-6 linked levans. The degree of polymerization (DP) distinguishes the fructans. FOS are β -D-fructans with DP between 2 and 10 whereas inulin has DP 10 to greater than 60. Essentially, they are sucrose molecules with 1-3 fructose units linked by a β -(2,1)-glycosidic bond. Oligofructose is a form synthesized from sucrose by β -fructofuranosidase linking fructose monomers to sucrose. Oligofructose and FOS are considered synonymous terms to describe small oligomers with $DP < (10)$.

Mammalian digestive enzymes cannot hydrolyze β -glycosidic bonds. Thus, both inulin derived FOS and synthesized FOS resist digestion in the upper GI tract. Ninety percent of consumed inulin and FOS is excreted at the terminal ileum of adult ileostomy patients. Furthermore, the undigested oligosaccharides are not recovered in the fecal mass, indicating they are completely fermented in the colon. Short-chain oligosaccharides are more actively fermented in the proximal colon, whereas longer chain molecules (inulin) tend to be more actively fermented in the distal colon. In many ways, prebiotics behave as a form of dietary fiber which have specific effects on colonic microbiota.

Dietary galactans can be either derived from naturally occurring oligosaccharides or artificially synthesized. Synthesized GOS are usually 2–3 monomer chains. Human milk, however, is a naturally occurring source of complex dietary galactans and contains high concentrations of carbohydrates and glycoconjugates that fall under the general category of prebiotic food substances. The monomers of breast milk include D-glucose, D-galactose, L-fucose, sialic acid, and N-acetylglucosamine. Oligosaccharides in human milk range from smaller molecules, with chains of 3–10 monomers, to more complex branched molecules, with the majority having lactose at the reducing end and a fucose or sialic acid at the non-reducing end. More than 130 varieties of oligosaccharides have been identified in human milk, including GOS. Several of these are considered 'bifidus factors' or 'bifidogenic', which increase the growth and establishment of *Bifidobacteria* in the intestine of the breast fed infant. Human milk oligosaccharides also appear to prevent attachment of pathogenic microorganisms by competing with epithelial ligands for bacterial binding sites. For example, sialylated oligosaccharides inhibit attachment of *Pneumococci*, and influenza viruses, whereas human milk GOS and fucosylated oligosaccharides can inhibit *E. coli* attachment. The bifidogenic effects, as well as those of direct interaction with the intestinal mucosa are considered to be some of the mechanisms by which these agents are protective for the lactating infant.

Dietary Intake

Oligosaccharides exist naturally in many plants including onions, garlic, the roots of Jerusalem artichoke, asparagus root, chicory root, and wheat. Inulin is extractable from root plants, particularly Jerusalem artichoke and chicory, whereas FOS is a hydrolysis from inulin yielding a shorter chain sugar.

Food composition databases are largely incomplete with values of fructan (Table 1) and even less so with galactan concentrations. Average dietary consumption as part of a normal adult diet has been estimated to be 1–4 g day⁻¹ in the US. Limited data from European diets suggest a higher intake, from 3 to 10 g day⁻¹. Oligosaccharide concentration in mature human milk ranges from 5 to 10 g l⁻¹. Owing to the nondigestible nature of FOS and GOS, the nutritional value in terms of calories is negligible. The actual energy produced by these carbohydrates relates to the byproducts of fermentation, specifically SCFA and lactate.

Based on available food composition data and dietary intake estimates, it is unlikely that most adults would consume a dietary quantity necessary to recognize a clinical effect of prebiotics with the consumption of whole foods alone. Thus, many products worldwide, including some infant formulas, are produced with supplemental oligosaccharides for purposes of providing a beneficial prebiotic effect to consumers.

Clinical Effects of Prebiotics

Effect in the Proximal GI Tract

From the dietary point of view, oligosaccharides meet the criteria to be considered a dietary fiber, and are highly

Table 1 Fructan (fructo-oligosaccharide and inulin) concentration of selected fruits, vegetables, and grains and grain products

Food type	Fructan concentration (mg per 100 g fresh wt)	Food type	Fructan concentration (mg per 100 g as eaten)
<i>Fruits</i>		<i>Grains and grain products</i>	
Cantaloupe	160	Corn flake cereal	1070
Grapefruit	230	Couscous	730
Honeydew melon	210	Muesli cereal	1260
Nectarine	210	Oats, dry	320
Peach, white	400	Pasta, wheat	340
Watermelon, seedless	320	Pretzels	1400
		Rye bread	1050
<i>Vegetables</i>		Rice cakes, plain	780
Artichoke, globe	1 200	White bread	680
Artichoke, Jerusalem	12 200	Whole wheat bread	690
Beet, root	400		
Brussel sprouts	270		
Chickpeas, canned	160		
Garlic	17 400		
Red kidney beans, boiled	540		
Shallot	8 900		
Spinach, baby	140		
Zucchini	290		

Source: Adapted from Biesiekierski JR, Rosella O, Rose R, *et al.* (2011) Quantification of fructans, galacto-oligosaccharides and other short-chain carbohydrates in processed grains and cereals. *Journal of Human Nutrition and Dietetics* 24: 154–176, and Muir JG, Shepherd SJ, Rosella O, Rose R, Barrett JS, and Gibson PR (2007) Fructan and free fructose content of common Australian vegetables and fruit. *Journal of Agricultural and Food Chemistry* 55: 6619–6627, with permission from Wiley and ACS.

fermentable. Although dietary fructans have limited viscosity and gel forming ability, they are soluble in water and may exert some effect on the upper GI tract by slowing gastric and small bowel transit time, thereby increasing satiety, altering glucose metabolism and increase sensitivity to insulin. Increased intestinal mucosal endocrine cell secretion of anorexigenic peptides involved in energy regulation (e.g., glucagon like peptide (GLP), peptide YY, and others) have been postulated as an explanatory mechanism as to why decreased food intake has resulted with feeding of prebiotic ingredients in multiple animal, and some human, trials. Increased GLP-1 has also been implicated in the improved glucose control noted in several trials with animals that were supplemented with prebiotic ingredients. Limited controlled clinical trials with humans are available; however, decreased hepatic glucose production and decreased postprandial glucose responses have been demonstrated with prebiotic feeding. Altered fat metabolism by the binding of bile acids, thus decreasing serum cholesterol and triglyceride levels has been reported with oligosaccharide supplementation in hypercholesterolemic patients. Decreased insulinemia after prebiotic supplementation, could explain, in part, findings of reduced hepatic lipogenesis.

There is a strong link to oligosaccharide consumption on the integrity of the GI mucosa. A trophic effect of the mucosa and hyperplasia of the epithelial cells, resulting in enhancement of the absorptive area may increase the absorption capacity for such minerals as calcium, magnesium, iron, and zinc. Of particular interest is the effect of oligosaccharides on calcium absorption. Recent studies have demonstrated increased calcium absorption in older women and with teenage girls consuming prebiotics. A minimal intake of $\sim 8 \text{ g day}^{-1}$ appears necessary to improve calcium absorption in adults, and a combination of prebiotic ingredients (e.g., those with various chain lengths) seems to enhance the calcium

absorption effect. It is proposed that the SCFA produced from fermentation lowers luminal pH, which in turn, increases solubility and absorption of calcium. Improved calcium absorption via a cation exchange mechanism or active calcium transcellular transport within the mucosal epithelial cells have also been proposed to explain the prebiotic effect of increased calcium absorption.

Effects in the Colon

The main effect of oligosaccharides in the colon is directly related to fermentation. The process of fermentation from innate bacteria produces SCFA and lactate. Increase in biomass contributes to the bulking effect that oligosaccharides have on stool. Additionally, fecal pH is decreased due to the suppression of the production of putrefactive substances. However, the greatest value of oligosaccharides is their role in stimulating the growth of specific innate microbes in the colon.

Inulin and FOS selectively promote proliferation of *Bifidobacteria* and to a lesser degree, *Lactobacilli*. In adult studies, FOS and inulin given in doses of 10 g day^{-1} resulted in increased levels of *Bifidobacteria* and decreases in *Enterobacter* and *Enterococci* without GI side effects. In establishing a predominant microbial environment of *Bifidobacteria*, epithelial adherence of pathogenic bacteria may be deterred.

It is generally assumed that the immunologic effects seen with probiotic consumption (*Bifidobacteria*, *Lactobacilli*) would apply with the altered microbial balance with prebiotics. And some initial scientific work suggests that foods and ingredients with prebiotic effect can modulate specific markers of immune function. Composition of gut microbiota has been implicated in pathophysiology of several GI diseases and syndromes (e.g.,

irritable bowel, ulcerative colitis), yet few controlled trials with humans have documented beneficial effects on patient symptoms or resolution of disease with increased dietary intake of prebiotic supplements. Numerous animal studies have reported decreased colon tumor incidence after feeding prebiotic ingredients. However, nonprebiotic dietary carbohydrates have also been associated with beneficial colon cancer prevention effects.

Safety and Tolerance

Dietary fructans and galactans have been thoroughly studied in animal and human trials. They are used as feed additives, approved as a food ingredients, and many specific prebiotics have been granted generally regarded as safe (GRAS) status. However, due to carbon dioxide, hydrogen, and water produced during the fermentation process, disagreeable side effects may result when given in high doses. These symptoms occur in a dose-dependent fashion. Abdominal cramping, increased flatulence and bloating have been shown to occur significantly more in studies of which adults received 15 or more grams per day of FOS and inulin as compared to a placebo group. Prebiotic (synthetic GOS) supplemented infant formula is well tolerated by full-term infants at intakes up to 5 g l⁻¹.

Conclusion

The intestinal microbiota plays a critical role in intestinal and systemic health, particularly supporting gut barrier function and immune response. The composition of this microbiota can be modified toward a more favorable pattern via dietary means. Prebiotic supplementation assists in promoting growth of indigenous beneficial microbiota, specifically *Bifidobacteria* and *Lactobacilli* by acting as a selective substrate for fermentation. Clinical benefits associated with dietary supplementation of prebiotic fructans and galactans in the upper and lower GI tract have been demonstrated, including their

bifidogenic effects. Additional studies are needed to identify further benefits of these dietary ingredients.

See also: Carbohydrates: Chemistry and Classification; Requirements and Dietary Importance. Fiber: Resistant Starch and Oligosaccharides. Microbiota of the Intestine: Role and Effects of Probiotics

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Probiotics

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Glossary

Healthy microbiota Normal individual microbiota of an individual, which both preserves and promotes well-being and absence of disease.

History of safe use of microorganisms Traditional use of microorganisms without any deleterious effects on human health.

Intestinal microbiota Microbes inhabiting the human gastrointestinal tract, barrier against harmful environmental exposures.

Metagenomics Study of the microbial genomes present in an environment by direct sequencing and sequence-based analysis.

Probiotic Live microorganisms, which when administered in adequate amounts confer a benefit to human health.

Introduction

The human gastrointestinal tract (GIT) harbors a complex collection of microorganisms. The individual digestive system contains approximately 1.5 kg of viable (live) bacteria, made up of more than 1000 different identified microbial species, of which a minority are culturable and the rest have been identified using molecular methods of analysis. Indeed, the total number of bacteria in the gut amounts to more than 10 times that of eucaryotic cells in the human body, and this bacterial biomass can constitute up to 60% of fecal weight. The gene set of this intestinal microbial ecosystem is approximately 150 times larger than that of the human genome. This complex microbiological community is called intestinal microbiota. Although most people are familiar with the side-effects of some members of it (e.g., diarrhea), the beneficial effects in stabilizing gut well-being and general health are less well known. The complex composition includes both beneficial and potentially detrimental microbes. Both types are naturally present in the GIT as part of the normal healthy intestinal microbiota and ensure the balance, which creates a healthy individual microbiota. Beneficial microbes (sometimes termed 'friendly bacteria') constitute the main source of potential probiotics used to improve intestinal and host health. The normal healthy microbiota cannot be easily defined by microbiological terms. However, one could assume that a microbiota is balanced and potentially healthy when the host feels well and there is long-term absence of disease. In early infancy, a model of healthy gut microbiota is that of a healthy breast-fed infant who remains healthy. Members of the gut microbiota in healthy breast-fed infants formed the original basis of the probiotic concept.

Fermented products containing living microorganisms have been used for centuries in the human diet providing an intake of a variety of potentially health-promoting bacteria. They have also been used to restore gut health. Such utilization of live microorganisms to improve host health forms the second basis of the probiotic concept.

Usually probiotics are taken in the form of dairy products, drinks, or supplements, but in African countries they have traditionally also been ingested in fermented cereal and in fermented

vegetables in Asian countries. The claimed benefits of traditional fermented foods range from treatment of diarrheal diseases to alleviation of the side-effects of antibiotics to the prevention of a number of other health problems. In some countries fermented foods have even been associated with benefits to the skin.

Probiotics

Probiotics have been defined as 'live microorganisms which when administered in adequate amounts confer a benefit on the host'. According to this definition the safety and efficacy of probiotics must be scientifically demonstrated. However, as different probiotics may interact with the host in different manners, their properties and characteristics should be well defined. It is understood that probiotic strains, independent of genera and species, are unique and that the properties and human health effects of each strain must be assessed in a case-by-case manner. Most probiotics are currently either lactic acid bacteria or bifidobacteria, but new species and genera are being assessed for future use. The probiotic bacteria in current use have been isolated from the intestinal microbiota of healthy human subjects of long-standing good health and thus most of them are also members of the healthy intestinal microbiota.

It has been demonstrated that probiotics have specific properties and targets in the human intestinal tract and that they are able to modulate the intestinal microbiota.

Intestinal Microbiota

Composition of the Intestinal Microbiota

The human gastrointestinal tract hosts a very rich and complex microbiota, which is specific for each person depending on environmental and genetic factors. Different bacterial groups and levels are found throughout the gastrointestinal tract, as corresponds to the different ecological niches present from mouth to colon. The stomach and the upper bowel are sparsely populated regions (10^3 – 10^4 CFU g⁻¹ contents)

whereas the colon is heavily populated (10^{11} – 10^{12} CFU g⁻¹ contents). In the small intestine genera such as *Lactobacillus* and *Bacteriodes* are usually found, whereas those considered predominant in the large bowel include *Bacteriodes*, *Bifidobacterium*, *Eubacterium*, *Clostridium*, *Fusobacterium*, and *Ruminococcus* among others. Several health-promoting properties have been attributed to defined members of the intestinal microbiota such as lactobacilli and bifidobacteria. A balanced microbiota provides a barrier against harmful food components and pathogenic bacteria and has a direct impact on the morphology of the gut. Hence, the intestinal microbiota constitutes an important factor for the health and well-being of the human host and a healthy stable microbiota affords a potential source of future probiotics.

Development and Succession of Microbiota During Life-time

Traditionally the human fetus has been considered sterile with the maternal vaginal microbiota being the first inoculum of microbes that a neonate receives. However, some recent studies are starting to challenge this traditional thinking and suggest that colonization of the infants starts prenatally. Then, the indigenous intestinal microbiota develops over time, determined by an interplay between genetic factors, mode of delivery, contact with the initial surrounding environment, diet, and disease. As a result, every individual has a unique characteristic microbiota. As a defined entity the human intestinal microbiota does not thus exist; this population comprises a dynamic mixture of microbes in each individual.

The establishment of the gut microbiota provides an early and massive source of microbial stimuli to the immune system, and may consequently be a good candidate 'infection'. This step-wise succession begins with facultative anaerobics such as the enterobacteria, coliforms, and lactobacilli first colonizing the intestine, rapidly succeeded by bifidobacteria and lactic acid bacteria. The indigenous gut microbiota plays an important role in the generation of an immuno-physiological regulation of the gut, providing key signals for the development of the immune system in infancy and also interfering with and actively controlling the gut-associated immunological homeostasis later in life. A healthy microbiota can thus be defined as the normal individual microbiota of a child, which both preserves and promotes well-being and absence of disease, especially in the gastrointestinal tract, but also beyond it. It provides the first step in long-term well-being for later life and the basis for this development lies in early infancy. Failure in the establishment of a healthy microbiota has been linked to the risk of infectious, inflammatory, and allergic diseases later in life. Demonstration of this has stimulated researchers to elucidate the composition and function of the intestinal microbiota.

Microbiota Research

Until recently our knowledge on microbiota development and characterization rested largely on the culture-based assessment pioneered by Japanese researchers. The identification of different microbial species and strains has been dependent on microbial characterization – which is usually based on limited

phenotypic properties and the metabolic activity of the microbes, for example, sugar fermentation profiles. The recent development of molecular culture-independent techniques has demonstrated that there are several bacteria, which are not culturable and cannot be isolated or identified by the traditional methods. The culture technique as used in microbial assessments of feces is also hindered by the fact that microbes in the feces will mainly represent the microbiota in the lumen of the sigmoid colon, whereas the composition of the intestinal microbiota differs both along the gastrointestinal tract and between the lumen and the mucosa. For more accurate information on the population elsewhere in the intestine, samples should thus be taken by endoscopy or during surgery. Most of our microbiota data today are derived from results obtained from fecal samples and culturing. These data indicate that there are several successive phases in microbiota development related to age (Figure 1). In early infancy the microbiota is scant and simple, consisting mainly of bifidobacteria. During breast feeding it remains so, but following weaning its complexity increases, reaching the state observed in adults where the microbiota is specific to each person. Ageing is related to further changes and the diversity is again decreased. The microbiota becomes more unstable and vulnerable to diseases, for example, diarrheal diseases caused by intestinal pathogens.

Current research efforts focus on extensive DNA sequencing, so called metagenomics, of the gut microbiota and of genomes from both probiotic microorganisms and intestinal commensals. This has provided information indicating that gut commensals not only derive food and other benefits from the intestinal contents but also have a role in influencing the human host by providing maturational signals for the developing infant and child and later signals providing for alteration to gut barrier mechanisms.

Metagenomic approaches have increased enormously our knowledge on the gut microbiome composition with over 3 million genes from intestinal microorganisms sequenced so far. On the other hand, the genomic data on, for instance, *Bifidobacterium longum*, and *Bacteroides thetaiotaomicron*, both important members of the human intestinal microbiota, give indication as to how specific bacteria are adapted to the development of the gut by specific genes enabling the use of intestinal mucins and breast milk oligosaccharides as main sources of nutrients. *Bifidobacterium dentium*, on the other hand, has been associated with dental caries and the oral mucosal surfaces, indicating that not all bifidobacteria are beneficial to health.

Genomic information on *B. longum* also gives insight into the adhesive mechanisms, which comprise a basis both for populating the infant gut and for communicating developmental signals to specific areas and sites of the gut mucosa. Furthermore, a large part (> 8.5%) of the *B. longum* genome is devoted to carbohydrate transport and metabolism, indicating a versatile metabolism well adapted to life in the intestine and making it very different from, for instance, *Lactobacillus johnsonii*.

B. thetaiotaomicron has also been shown to modulate glycosylation of the intestinal mucus and to induce angiogenesis expression, revealing proposed mechanisms whereby intestinal microbes may influence the gut microecology and shape

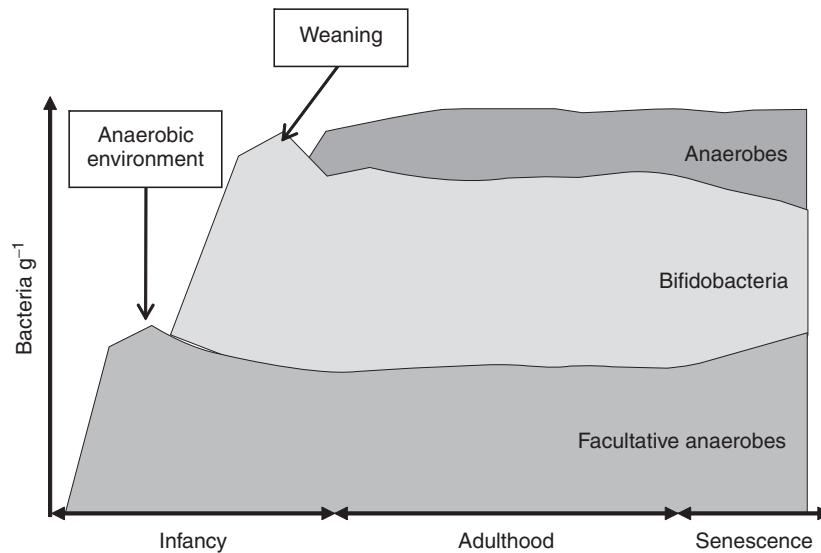


Figure 1 Development of microbiota throughout life.

the immune system. Incorporating such information with host gene expression data from the exposed mucosal sites and beyond them will enable us to understand the role of both microbial transfer and succession and microbe–microbe and host–microbe interactions. Recent information demonstrates that the vast community of indigenous microbes colonizing the human gut also shapes our development and biology.

Role of Microbiota in Health and Disease

Major dysfunctions of the gastrointestinal tract are thought to be related to disturbances or aberrations of the intestinal microbiota. Recent findings confirm that aberrations can be documented and related to disease risk. The microorganisms present in our gastrointestinal tract have thus a significant influence on our health and well-being.

Specific variations in the intestinal microbiota may predispose to disease. Such aberrations have been identified in infants at risk of diarrhea or allergic disease and the deviations often include decreased numbers of bifidobacteria and an atypical composition of bifidobacterial microbiota. Also differences in *Clostridium* content and composition have been reported to be important. Similar predisposing factors may also exist in the case of microbiota and both inflammatory gut diseases and rotavirus diarrhea. Microbiota differences have also been reported in rheumatoid arthritis, juvenile chronic arthritis, and irritable bowel syndrome (IBS) patients. A thorough knowledge of the intestinal microbiota composition will offer a basis for future probiotic development and the search for new strains for human use. Many diseases and their prevention can be linked to the microbiota and microbiota deviations in the gut.

Modulation by Probiotics

In general, probiotic bacteria do not colonize the human intestinal tract permanently, but specific strains are able to

transiently colonize or persist for some time in the intestine and may modulate the indigenous microbiota.

The rationale for modulating the gut microbiota by means of probiotics derives from the demonstration that this microbiota is important to the health of the host. Specific probiotics have been shown temporarily to colonize the human intestinal tract, thereby modulating the intestinal microbiota both locally and at the commensal level. Such modification has not been reported to be permanent; rather it is related to a balancing of aberrant or disturbed microbiota to assist it to return to normal metabolic and physiological activities. Such modulation and restoration of the normal state of the microbiota activity is a key target for probiotic action. However, the state of the microbiota should be well characterized to enable the selection of specific probiotics to counteract the disturbance in question.

Specific probiotic bacteria can modulate both the intestinal microbiota and local and systemic immune responses. Activation of immunological cells and tissues requires close contact of the probiotic with the immune cells and tissue on the intestinal surface. Interestingly, both lactobacilli and bifidobacteria, which colonize mainly the small and large intestine, respectively, when given as probiotic supplements were able to modify immunological reactions related to allergic inflammation, whereas lactobacilli were ineffective in protection against cow milk allergy. In this respect, preferential binding of probiotics on the specific antigen-processing cells (macrophages, dendritic, and epithelial cells) may be even more important than the location of adhesion. It is also known that the cytokine stimulation profiles of different *Bifidobacterium* strains vary and that strains isolated from healthy infants stimulate mainly noninflammatory cytokines.

Results of an increasing number of clinical and experimental studies demonstrate the importance of constituents within the intestinal lumen, in particular the resident microbiota, in regulating inflammatory responses. Probiotic bacteria may counteract inflammatory processes by stabilizing the disturbed gut microbial environment, forming a stable healthy

microbiota and thus improving the intestine's permeability barrier. Another mode of action comprises enhancing the degradation of enteral antigens and altering their immunogenicity. Yet another mechanism for the gut-stabilizing effect could be improvement of the intestine's immunological barrier, particularly intestinal IgA responses. Probiotic effects may also be mediated *via* control of the balance between pro- and anti-inflammatory cytokines. Such effects may be mediated through changes in the intestinal microbiota, especially by modulation of the bifidobacteria microbiota.

Probiotic Effects

Living microorganisms have long been used as supplements to restore gut health at times of dysfunction. It is clear that different strains from a given microbial group may possess different properties. It is thus important to establish which specific microbial strain may have a beneficial effect on the host; even closely related strains can have significantly different or even counteracting effects. Their properties and characteristics should thus be well defined and studies using closely related strains cannot be extrapolated to support each other. This phenomenon is also verified in the rapidly increasing studies on genomic information on specific probiotics.

Working hypotheses can be supported by studies carried out *in vitro* using cell culture models or *in vivo* using animal models. However, the studies most important for efficacy assessment are carefully planned and monitored clinical studies in humans.

In summary, well-designed human studies are required to demonstrate health benefits. Using the criteria thus obtained it can be concluded that certain specific probiotics have scientifically proven benefits, which can be attributed to specific products (*see* Scientifically Documented Effects). Other reported probiotic health-related effects require more data from larger double-blind placebo-controlled studies before reaching firm conclusions.

Scientifically Documented Effects

Reducing Duration/Severity of Diarrhea

The mechanisms by which probiotics prevent or ameliorate diarrhea may involve stimulation of the immune system, competition for binding sites on intestinal epithelial cells (Figure 2) or the elaboration of bacteriocins or binding of virus particles in the gut contents. These and other mechanisms are thought to be dependent on the type of diarrhea being investigated, and may therefore differ between viral diarrhea, antibiotic-associated diarrhea, or traveler's diarrhea.

Viral diarrhea. Shortening of the duration of rotavirus diarrhea using *Lactobacillus* GG (LGG) is perhaps the best documented probiotic effect. A reduction in the duration of diarrhea was first shown in several studies and also in a multicenter European study. Other investigators demonstrated that supplementation with a combination of *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduces the incidence of diarrhea and shortens the duration of rotavirus shedding in

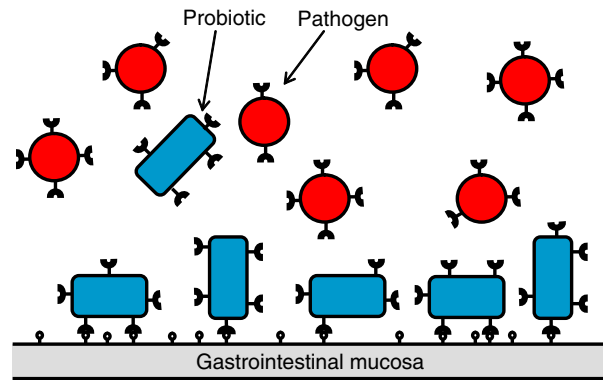


Figure 2 Probiotic adhesion and replacement of pathogenic bacteria.

chronically hospitalized children. On average, the duration of diarrhea was shortened by one day in both hospitalized children and those treated at home.

Other investigators have studied the immune modulating effects of probiotics as a means of reducing diarrhea, suggesting that the humoral immune system plays a significant role in the probiotics' effect.

From these numerous studies it is clear that probiotics do indeed play a therapeutic role in viral diarrhea. Meta-analyses have also been conducted in this area, showing that therapy with specific probiotics (e.g., *L. rhamnosus* GG or *Saccharomyces boulardii*) shortens the duration of acute diarrhea in children.

Antibiotic-associated diarrhea. The incidence of antibiotic-associated diarrhea is between 5 and 30%. The success of probiotics in reducing or preventing this form has been convincing, and includes a number of probiotics as well as various antibiotics.

LGG has been shown to prevent antibiotic-associated diarrhea when consumed in both yoghurt form or as a freeze-dried product. Also *S. boulardii* has been found effective in preventing antibiotic-associated diarrhea.

Currently, there are evidence-based guidelines for probiotics and acute gastroenteritis prevention in infants. There is also a convincing body of evidence on the efficacy of specific probiotics in preventing necrotizing enterocolitis in infants.

Reducing the Risk and Alleviation of Symptoms of Allergic Disease

It has been shown that differences in intestinal microbiota composition precede the development of some allergic diseases, indicating a potential area for probiotic application. LGG given prenatally to mothers, and during the first months to infants with a high risk of atopic disease has reduced the prevalence of atopic eczema to approximately half in the infants receiving the strain. Other studies appear to support the use of probiotics in reducing eczema risk, but also studies with no clinical effect have been reported. It is important to clarify the role and characteristics of specific probiotic strains in at risk populations. For some strains, recommendations have already been made for use in infant populations.

On the other hand, extensively hydrolyzed whey formula supplemented with LGG or *Bifidobacterium lactis* Bb-12 is more effective than unsupplemented formula in eczema alleviation

in infants with atopic eczema. These results indicate a high potential for probiotic application in the treatment and reduction of risk of allergic diseases.

Decreasing Symptoms of Lactose Intolerance

Several studies have shown that lactose-intolerant individuals suffer fewer symptoms if milk in the diet is replaced with fermented dairy products. The mechanisms of action of lactic acid bacteria and fermented dairy products include the following: lower lactose concentration in the fermented product due to lactose hydrolysis during fermentation, high lactase activity of bacterial preparations used in the production, increased active lactase enzyme entering the small intestine with the fermented product or within the viable bacteria, and modulation of gut microbiota composition and activity.

The bacterial enzyme beta-galactosidase, which can be detected in the duodenum and terminal ileum after consumption of viable yoghurt, is thought to be the major factor improving digestibility by the hydrolysis of lactose, mainly in the terminal ileum. Another factor suggested to influence lactose digestion is the slower gastric emptying of semi-solid milk products such as yoghurt.

In conclusion, there is good scientific evidence to demonstrate the alleviation of lactose intolerance symptoms by specific probiotic lactic acid bacteria. However, the strain-specific lactase activities may vary and, therefore, different products may have varying lactose contents and individual strains, when released into the duodenum, vary in their lactase activity.

Incidence and Severity of Necrotizing Enterocolitis (NEC)

Recent studies suggest that specific probiotics can reduce the severity and incidence of NEC in preterm neonates. A recent meta-analysis, concluded that probiotics appear effective in reducing the risk of NEC and associated death.

Irritable Bowel Syndrome

There is a rationale for investigating the effect of probiotics in the treatment of this common disorder where intestinal motility and dysfunctions in the intestinal microbiota are important factors to consider. In a recent study using *Lactobacillus plantarum* 299v, a reduction of symptoms has been reported. *Enterococcus faecium* preparations have also been evaluated for the treatment of patients with IBS, showing that although patient-recorded symptoms did not show significant differences, the physician's subjective clinical evaluation revealed an improvement. Recent studies suggest that specific probiotic combinations may be effective in alleviating IBS like symptoms in the general population.

Inflammatory Bowel Disease

IBD comprises a heterogeneous group of diseases of unknown etiology (Crohn's ulcerative colitis and pouchitis), but factors related to the intestinal microbiota seem to be involved again, providing a rationale for the application of probiotics. Reviewing studies made of the use of probiotics in IBD it can be concluded that, although there are some promising preliminary findings, more well-planned long-term studies are needed before drawing any firm conclusions.

Traveler's Diarrhea

There are a few studies on the prevention of traveler's diarrhea and these show a positive outcome for LGG and a combination of *Lactobacillus acidophilus* LA5 with *B. lactis* Bb-12. The results offer some indication of beneficial effects, whereas some studies yielded no reported effects, but information from good and extensive human studies using defined stains for traveler's diarrhea is still largely lacking. Perhaps due to the many different organisms causing diarrhea, the current information on traveler's diarrhea show no scientifically proven effects for any strains used. More studies are required for efficacy assessment.

Helicobacter pylori Eradication

Specific strains of lactic acid bacteria have been reported to inhibit a wide range of intestinal pathogens including, *Helicobacter pylori*. Lactic acid bacteria are often able to survive acidic gastric conditions and it has therefore been proposed that they may have a beneficial influence during the eradication of *H. pylori*, which is involved in the process of gastric ulcer development. It has been reported that both the inhibitory substances and the specific strains may influence the survival of *Helicobacter*, and studies have been conducted especially with a *L. johnsonii* strain. It has been shown that there is good *in vitro* inhibition and that fermented milk containing the strain has a positive effect when consumed during *Helicobacter* eradication therapy. In a multicentre study a *Lactobacillus casei* strain was reported to increase eradication efficacy of the standard treatment in children. In spite of the positive findings, additional controlled human studies in different populations are required to verify this effect.

Intestinal Microecology and Cancer

A number of studies have focused on the impact of probiotics on intestinal microecology and cancer. *L. acidophilus*, *L. casei* Shirota strain, and LGG have been shown to have inhibitory effects on chemically induced tumors in animals. Some specific strains of probiotic bacteria are able to bind carcinogens and to downregulate some microbial carcinogenic enzymatic activities. This phenomenon may then reduce carcinogen production and exert a beneficial effect in the colon, the urinary tract, and the bladder.

The most interesting documentation is that concerning on *L. casei* Shirota. There have been several mechanistic studies on the effects of the strain reporting decreased mutagen excretion, and some human clinical studies have been conducted using this strain. In clinical and multicentre studies carried out in Japan, prophylactic effects of oral administration of *L. casei* Shirota on the recurrence of superficial bladder cancer have been reported. Recently, a large Japanese case control study has been conducted on the habitual intake of lactic acid bacteria and risk reduction of bladder cancer. Results suggested that the habitual intake of fermented milk with the strain reduces the risk of bladder cancer in the Japanese population. More studies, and especially human studies also in other countries, are needed before the establishment of firm conclusions.

Safety

Safety assessment is an essential phase in the development of any new food. Although few probiotic strains or prebiotic compounds have been specifically tested for safety, the long history of safe consumption of some probiotic strains could be considered the best proof of their safety. Although some lactobacilli and bifidobacteria have been associated with rare cases of bacteremia, usually in patients with severe underlying diseases, the safety of the members of these genera is generally recognized due to their long history of safe use and their lack of toxicity. On the other hand, the low incidence of infections attributable to these microorganisms, together with a recent study showing that there is no increase in the incidence of bacteremia due to lactobacilli in Finland despite the increased consumption of probiotic lactobacilli, support this hypothesis. With regard to other bacteria such as enterococci, *S. boulardii*, *Clostridium butyricum* or some members of the genus *Bacillus*, which have been used as probiotics, the situation is more complicated even when they have been used for some time.

In addition to the possibility of infection there are other risks, which must be taken into account (Table 1). These include those associated with the metabolic properties of the strain, (capacity for deconjugation/dehydroxylation of bile salts, production of enzymes favouring the invasion/translocation through the epithelium, etc.), risks associated with the presence of active substances in the probiotic or product (immunoactive substances, toxic compounds, etc.) or with antibiotic resistance. It is clear that strains harboring transferable antibiotic resistance genes should not be used. In this context the specific risks related to each probiotic strain must be carefully identified.

Guidelines are needed to test the safety of probiotics. However, taking into account the great diversity of probiotic microorganisms, it is necessary to identify the specific risks associated with the respective strains, as well as the risk factors associated with the host and the possible interactions between probiotic–host–food components in order to assess the safety of these products. Additional epidemiological surveillance and follow-up of novel strains should be conducted. In this context, the specific risks related to each probiotic strain must be

carefully identified. With regard to this, knowledge of mechanisms is a key factor not only for the assessment of health effects but also the safety aspects of probiotics.

Future Challenges

The authors have currently several clear indications for probiotic use and such applications have also been included in therapeutic recommendations and evidence-based guidelines for reducing the risk of specific diseases or alleviating symptoms as part of nutritional treatment of diseases. Many of the claimed beneficial effects of probiotics are backed by good clinical studies. These include, for example, the following: treatment and prevention of acute gastroenteritis in infants, alleviation and reduction of risk of antibiotic side-effects, prevention of NEC in infants. This demonstrated that there is a significant life-saving factor associated with probiotics and NEC.

Protocols for human clinical intervention studies need to be developed for probiotics. In some cases, even post-marketing surveillance studies on intakes and long-term effects are desirable. Such studies have in fact also been used for the safety assessment of current probiotics.

Also the assessment of potential probiotic strains must be based on a valid scientific hypothesis and realistic studies supporting it. In this respect, knowledge of mechanisms of action may be a key factor for hypothesis formulation. The selection of biomarkers or risk factors to assess effects on the human health and well-being is crucial. Thus we need to improve our knowledge of the mechanisms involved and take into account that probiotic mechanisms of action are multifactorial and that each probiotic may have specific functions affecting the host.

It is also of key interest to increase our knowledge of intestinal microbiota composition and activity and to understand its role in health and disease, identifying those microorganisms related to the health status of the host. These factors are required in order to select probiotic strains able to modulate the intestinal microbiota in a beneficial manner.

Knowledge accrued regarding the intestinal microbiota, nutrition, immunity, mechanisms of action, and specific diseases should be carefully combined with genomic data to allow the development of a second generation of probiotics; strains for site- and disease-specific action. At the end, also meta-analyses and evidence based guidelines are needed in the future to better characterize both effective and noneffective probiotics and to apply them into the nutrition of both special target groups and the general population.

Conclusions

In this review of the human intestinal microbiota in health and disease and the use of probiotics in clinical diseases, the following critical points are of key importance:

1. The intestinal microbiota forms the basis for human health and well-being.

Table 1 Probiotic action: Potential benefits and risks

Action mechanisms	Potential risks
Improvement of gut barrier (immunologic, nonimmunologic)	Proinflammatory effects
Modulation of aberrant gut microbiota	Adverse effects on innate immunity
Modulation of inflammatory response	Opportunistic infection
Degradation of antigens	Production of harmful substances
Binding/inhibition of carcinogens, pathogens, and viruses	Antibiotic resistance
Direct interaction with epithelial and immune cells in the gut	(Specific risks related to host, strain characteristics, or interactions)

2. The development of the intestinal microbiota during infancy is a strong modulating factor, which is influenced by genetic background, mode of delivery, breast-feeding, diet and environmental conditions.
3. Probiotics are viable microbial food supplements with a documented beneficial effect on human health.
4. Probiotic effects can be mediated *via* the intestinal microbiota but probiotics can also have direct effects on gut epithelium with health effects beyond the gut.
5. There is a strong suggestion that specific probiotics can be effective in combating the increasing incidence of bacterial resistance to antibiotics and food-borne infection.
6. Many probiotic effects are now well documented in human intervention studies.
7. Current probiotics have a long history of safe use in foods.
8. Probiotic effects are always strain-specific and properties unique to each strain and this is documented also by genomic studies on specific strains.
9. Even closely related strains may have different and at times counteractive effects on human health.
10. The mechanisms of probiotic action should be further characterized to identify new strains for specific uses.
11. More human intervention studies are required to prove the full spectrum of potential probiotic effects.

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NIACIN AND PELLAGRA

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Glossary

B-vitamins There are seven B-group vitamins that are all essential components of human diets. All are either enzyme cofactors *per se* or are converted in the body to enzyme cofactors (which are small molecules that form part of the active site of specific enzymes and participate in their catalytic reactions). B-vitamins are efficiently absorbed in the gastrointestinal tract and are resistant to urinary excretion, unless intakes and blood concentrations exceed tissue requirements.

Clinical deficiency Often results from severe prolonged tissue (nutrient) deficiency, which may in turn result from inadequate (nutrient) intake, but may also arise from impaired absorption, increased turnover, and excretion, increased tissue demand, etc. Deficiency signs are directly observable; symptoms can be elicited by testing, and the response of these to interventions (e.g., nutrient supplementation) can help to confirm a diagnosis of deficiency.

Estimated Average Requirement (EAR) Similar to RDA and RNI, except that this is the mean (i.e., average) nutrient requirement of the individuals in a defined population group (USA and UK).

Nutrient (e.g., vitamin) status Is commonly assessed by measuring the concentration of the nutrient or a derivative in an accessible body fluid such as serum or urine or else the functionality of an enzyme or a biochemical pathway (functional status). Published 'normal ranges' enable the result to be classified as, for example, deficient, low, normal, or high.

Recommended Dietary Allowance (RDA) The amount of a nutrient (per day) that covers the needs of the majority (usually approximately 97.5%) of the individuals in a defined population group (e.g., adult males) in the USA. The term 'Reference Nutrient Intake' (RNI) is used for a similar concept in the UK.

History, Signs and Symptoms of Deficiency, and Antimetabolites

Pellagra (meaning 'rough' or 'raw' skin) was common in western Europe (e.g., France, Italy) and especially in the southern half of the USA, up to the early twentieth century: it caused approximately 10 000 deaths in the USA in 1929 alone. The typical signs and symptoms of human pellagra are listed in **Table 1** and the history of its recognition and causation in **Table 2**.

By 1810, a European source concluded that the disease was neither contagious nor hereditary but probably caused by poor diets, especially those in which grains such as corn (i.e., maize) were the principal staple; a good hospital diet was curative. By 1860, it was known that poor Mexican peasants, whose diet was mainly corn-based but roasted the corn with

lime did not exhibit pellagra. However, for approximately the next 50 years, the 'toxin' theory of pellagra-causation held sway. In Europe, the prevalence of pellagra declined markedly, just as it was beginning to emerge as a major scourge in the southern USA. Poor sanitation, infection, insect-borne disease, and toxins from bacteria or molds were variously blamed – until Joseph Goldberger, from 1914 until his death in 1929, carried out controlled feeding studies in human convicts and an animal model ('blacktongue', a corn diet-induced condition in dogs (**Table 1**)). The important outcome was that pellagra was now seen not as an infectious disease, but as primarily diet related.

Funk's newly formulated hypothesis of essential dietary vitamins and Gowland Hopkins' concept of 'accessory food factors' then initiated a search for curative organic substance(s). Goldberger classified foods according to their

'pellagra-preventative'(PP) properties, and found that both dried yeast and a water-soluble extract from yeast were curative in small quantities. During the 1930s, and following recognition of the role of pyridine nucleotide coenzymes in food energy release, the central roles of nicotinic acid and nicotinamide were elucidated and equated with the 'PP' factor. The

term 'niacin' was then coined, because nicotinic acid was associated with tobacco and hence considered unsuitable for an essential dietary factor.

Although pellagra in dogs and humans responds well to supplements of pure niacin, there were further strands to the story. For instance, the niacin content of foods, as measured by chemical analysis, was not always a good guide to their pellagra-producing or -preventing properties (as in [Table 3](#)). During the 1940s, it was shown that in rats (which respond to pellagragenic diets by a reduced growth rate but not by skin lesions), high dietary tryptophan levels reduced the requirement for niacin. Tryptophan was then shown to be effective in humans in reducing the pellagragenic properties of diets, and the metabolic pathway linking tryptophan to niacin and to the pyridine nucleotide coenzymes was elucidated ([Figure 1](#)). Between 34 and 86 mg tryptophan in human diets is now considered to be equivalent to 1 mg niacin, with a mean conversion ratio of 60 mg tryptophan per milligram of niacin, now the basis of niacin equivalents (NEs) value of a diet. This is a much better index of antipellagra potency than niacin content alone ([Table 3](#)).

The causes of pellagra in human populations, and indeed in Goldberger's experimental studies, are a complex mixture of B-vitamin deficiencies, of which niacin and tryptophan are the dominant effectors, but riboflavin is also important, followed by thiamin, vitamin B₆, and other nutrients. Several bacterial, fungal, and other toxins can deplete the cellular levels of nicotinamide adenine dinucleotide phosphate (NAD(P)), and niacin in corn and other grains is often chemically bound into a macromolecular complex, niacytin, from which niacin cannot be released by digestive enzymes in the gastrointestinal tract, but that requires heat and alkali treatment (as in the preparation of Mexican tortillas) to make it bioavailable.

In India, the millet staple 'jowar' is frequently associated with pellagra signs and symptoms, even though it is apparently a reasonably good source of available niacin and tryptophan. Local studies suggested an association with jowar's high leucine content, which may impair the conversion of tryptophan to niacin coenzymes, but this remains

Table 1 Signs and symptoms of niacin deficiency in humans and animals

1. <i>Human deficiency</i>
Loss of appetite and weight
Dermatosis (hyperpigmentation, hyperkeratosis, desquamation of the epidermis, especially where frequently exposed to strong sunlight)
Anorexia
Achlorhydria
Angular stomatitis, cheilosis, magenta tongue
Diarrhea
Anemia
Neuropathy (headache, dizziness, tremor, neurosis, apathy)
Death in severe and prolonged cases
2. <i>Blacktongue in dogs and cats</i>
Pustules in mouth and excessive salivation, darkening, and necrosis of the tongue
Diarrhea
3. <i>Pigs</i>
Neurological lesions affecting ganglion cells; histopathology of nerves
Anemia
Degeneration of intestinal mucosa and diarrhea
4. <i>Rats</i>
Reduced growth rate
Alopecia (roughened skin)
Damage to peripheral nerves (cells and axons)
5. <i>Birds (e.g., chickens, ducks)</i>
Inflammation of the upper gastrointestinal tract
Dermatitis
Diarrhea
Poor growth of feathers; bowed and weakened legs

Table 2 History of pellagra, and recognition of its causes, in human populations

1. A poorly understood disease (dermatitis, gastrointestinal, and mental signs/symptoms) appeared in Europe in the eighteenth and nineteenth centuries, and then in southern USA in the first decade of the twentieth century and was named 'pellagra' (meaning raw/rough skin).
2. Favored causal hypotheses included infection, moldy grain, and insects.
3. 1914–1916, Joseph Goldberger disproved the infection hypothesis: by self-experimentation and then producing pellagra in prisoners fed mainly corn diets.
4. 1920s, Joseph Goldberger developed the 'blacktongue' model of pellagra in dogs, with corn diets.
5. 1930s, nicotinic acid was isolated as a pure water-soluble compound (vitamin) of known structure, from yeast and liver extracts, able to cure pellagra and blacktongue.
6. Mid-twentieth century, unavailable niacin is bound in the complex niacytin in corn. Heating in an alkaline environment (as in Mexican tortillas) can release it.
7. Niacin can be produced from tryptophan in the body. Pellagragenic diets are low in tryptophan as well as in niacin. The concept of 'niacin equivalents' (milligram niacin plus one-sixtieth of milligram tryptophan) in food developed. Human requirements estimated.
8. The Indian cereal 'jowar' is shown to be pellagragenic. Some, but not all, studies have implicated its high leucine content.
9. Niacin and riboflavin deficiencies often coexist; the signs and symptoms of pellagra-like disease are often attributable to multiple nutrient deficiencies.
10. Certain inborn errors of metabolism (genetic defects), or iatrogenic effects of drugs, can mimic the signs, symptoms, and metabolic defects of pellagra.

Table 3 Niacin equivalents in selected foods

	<i>Niacin equivalents from preformed niacin (mg per 100 g)</i>	<i>Niacin equivalents from tryptophan (mg per 100 g)</i>	<i>Total Niacin equivalents (mg per 100 g)/(mg per MJ)</i>
Milk	0.2	0.6	0.8/2.9
Raw beef	5.0	4.7	9.7/18
Raw white fish	2.4	3.4	5.8/17
Raw eggs	0.1	3.7	3.8/6.1
Raw potatoes	0.6	0.5	1.1/3.4
Raw peas	2.5	1.1	3.6/10.5
Raw peanuts	13.8	2.9	19.3/8.2
White bread	1.6	2.0	3.6/2.5
Rice, uncooked	4.2	1.6	5.8/3.6
Maize (sweetcorn)	2.0	0.5	2.5/5.3
Cornflakes (fortified)	15.0	0.9	15.9/10.0
Coffee	24.8	2.9	27.7/87

Note: Coffee is a good source of niacin, because it is released from the bound form, trigonelline, by roasting. Much of the niacin in maize and part of that in other cereals, including unfortified bread, is unavailable for use by the body, being bound in large molecular complexes.

On average, 60 mg tryptophan yields 1 mg NE.

controversial: balance studies in humans have failed to show a consistent effect of leucine on excretion of tryptophan metabolites, a sensitive test for impairment of tryptophan-conversion pathways. In parts of South Africa, iron overload has been reported to complicate the metabolic effects of low niacin intakes.

Estrogenic hormones affect the conversion of tryptophan to niacin coenzymes, hence women (except during pregnancy) are more susceptible to pellagra than men. Several drugs have antiniacin (iatrogenic) effects. Thus isoniazid, commonly used in the treatment of tuberculosis, inhibits kynureninase (an enzyme in the tryptophan conversion pathway) activity by inactivating the enzyme cofactor, pyridoxal phosphate. Any interference with vitamin B₆ metabolism is also likely to affect niacin economy as well. Because *ca.* 60% of Indians are genetically slow acetylators (i.e., deactivators) of isoniazid, this drug is especially likely to cause pellagra. Anti-Parkinsonism drugs Carbidopa and Benseride can cause pellagra symptoms and reduce the excretion of *N*-methylnicotinamide. Some antineoplastic drugs, for example 6-dimethylaminonicotinamide and 6-aminonicotinamide, inhibit key enzymes whose substrates are nicotinamide adenine dinucleotide (NAD) or NADP by being converted *in vivo* to analogs of the coenzymes. 3-Acetyl-pyridine, which also forms an analog of NAD, has either antagonistic or niacin-replacing properties, depending on the dose used. Metronidazole is a niacin antagonist, as is 2-amino,1,3,4-triazole.

Some inborn errors of metabolism can result in pellagra-like symptoms in humans. Thus in Hartnup disease, an autosomal recessive condition in which the cellular transport of tryptophan (and other neutral amino acids) is impaired, tryptophan is lost in the urine through a failure of renal tubular reabsorption. Supplementation with niacin or with tryptophan peptides (but not free tryptophan) can be palliative. Another genetic disease that may respond to niacin supplements is Fredrikson type I familial hypercholesterolemia; nicotinic acid lowers the raised blood cholesterol levels that are associated with it.

Other inborn errors of tryptophan economy include xanthurenic aciduria, hydroxykynurenuria, tryptophanuria (i.e.,

tryptophan dioxygenase deficiency), and excessive picolinate carboxylase activity. Tumors of the enterochromaffin cells, which synthesize excessive amounts of 5-hydroxytryptophan and 5-hydroxytryptamine, can also result in pellagra, because hyperactivity of this pathway results in the diversion of tryptophan.

Chronic alcoholics represent a high-risk for the development of pellagra in western society, having poor diets and liver damage due to alcohol abuse. Certain psychoses, including depression and schizophrenia, are associated with abnormalities of tryptophan metabolism pathways, including those involved in the formation of 5-hydroxytryptamine (serotonin) and 5-hydroxytryptophan in the central nervous system. Some patients may benefit from the modulation of these pathways by drugs and supplements. People with AIDS exhibit impairment of NAD production, which can respond to niacin supplements, and high-dose nicotinic acid is being used as part of the treatment of cardiovascular diseases (see the Section on Dietary Sources and High Intakes below). Diet-associated pellagra is still prevalent in some African countries, e.g., refugees in Malawi, and in parts of India and China.

Absorption, Transport, and Storage

Preformed niacin (formerly vitamin B₃) occurs in foods either as nicotinamide (niacinamide) or as the pyridine (i.e., nicotinic acid) nucleotide coenzymes derived from it, or as nicotinic acid, which lacks the amide nitrogen, and is the form known as niacin in North America. Both nicotinamide and nicotinic acid are equally effective in vitamin potency, but in large doses, they exert markedly different pharmacological effects; thus, it is important, at least in that context, to make the distinction.

The most important sources of preformed niacin in most foods, particularly animal foods, are the pyridine (i.e., nicotinamide) nucleotides, NAD(H) and NADP(H). Hydrolases and pyrophosphatases in biological tissues convert these coenzymes to tissue sources of the vitamin. NAD glycohydrolase and pyrophosphatase enzymes are present in the gut mucosa,

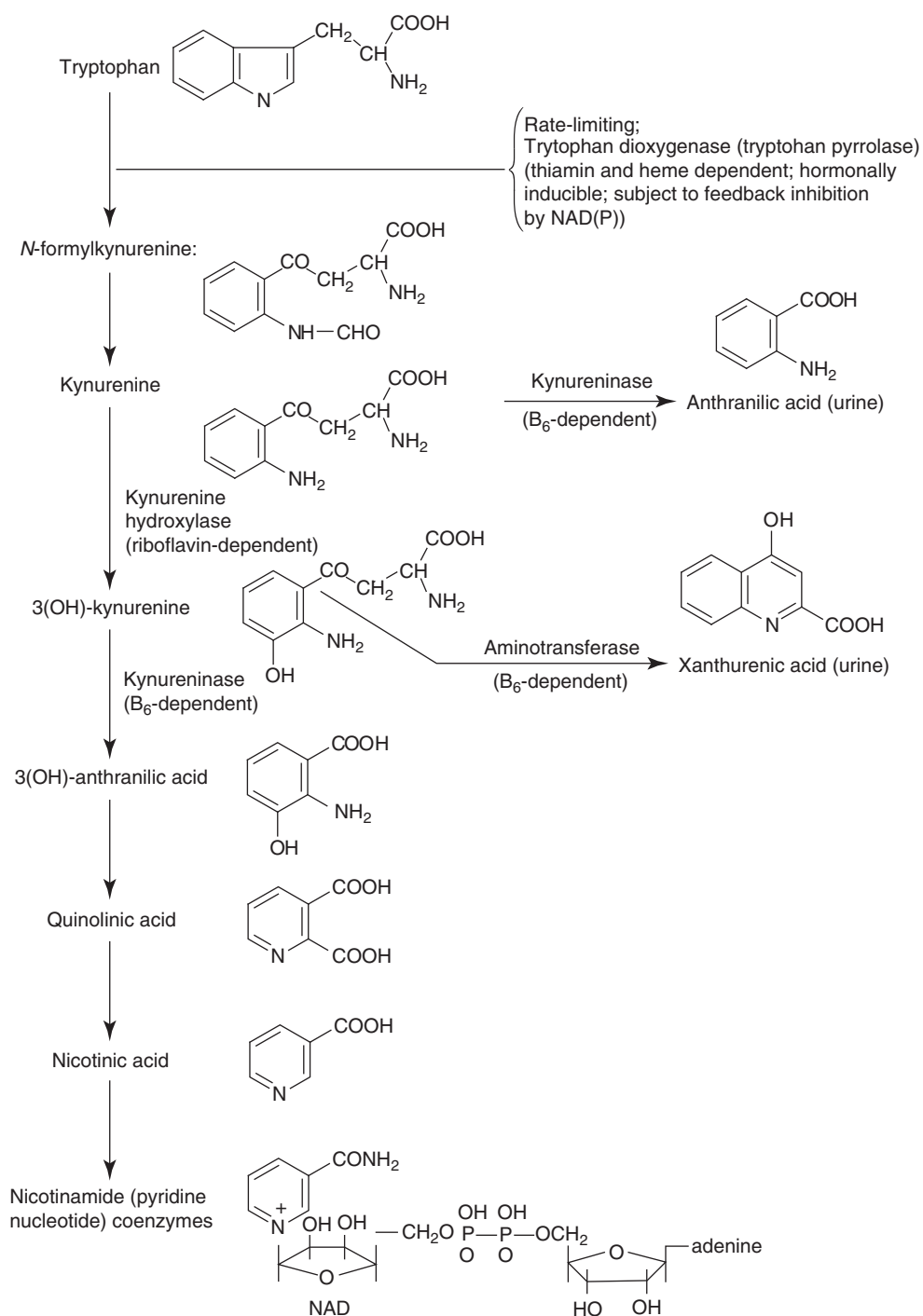


Figure 1 *In vivo* conversion of tryptophan to nicotinic acid and NAD.

and absorption of nicotinamide or nicotinic acid by the mammalian intestine consists of a saturable transport system, dominant at low intakes and dependent on sodium, energy, and pH, plus a nonsaturable component that is dominant at high doses or intakes. Absorption is efficient even at high doses of 3 g or more: as much as 85% then being excreted in the urine. Absorption of test niacin doses introduced directly into the human upper ileum is rapid, peak levels appearing in blood plasma within 5–10 min. Absorption also occurs in the

large intestine, making niacin from gut bacteria available to the intestinal cells.

Transport of niacin between the liver and the intestine can occur *in vivo*, as indicated by radioactive probes in animals, and the liver is a major site of conversion of niacin to its functional products: the nicotinamide–nucleotide coenzymes. Nicotinamide can pass readily between the cerebrospinal fluid and the plasma, thus ensuring a sufficient supply to the brain and the spinal cord. The liver contains greater niacin

coenzyme concentrations than most other tissues, but all metabolically active tissues contain these essential coenzymes. As in the gut, both facilitated diffusion (which is sodium- and energy-dependent and saturable) and passive diffusion (which is nonsaturable) contribute to tissue uptake from the bloodstream. Except for muscle, brain, and testis, nicotinic acid is a better precursor of coenzymes than nicotinamide. The liver is the most important site of conversion of tryptophan to the nicotinamide coenzymes.

NAD is present mainly in an oxidized form in the tissues, whereas NADP is principally present in the reduced form, NADPH. There are important homeostatic regulation mechanisms that maintain appropriate ratios of the oxidized and reduced forms in healthy tissues. Once converted to coenzymes within the cells, the niacin is effectively trapped and can only diffuse out again after degradation into smaller molecules. This implies, of course, that the synthesis of coenzyme nucleotides must occur within each tissue and cell type, each of which must possess the enzymatic apparatus for their synthesis from niacin. Loss of nicotinamide and nicotinic acid into the urine is minimized (except when the intake exceeds requirements) by efficient reabsorption from glomerular filtrate.

Metabolism and Excretion

The conversion pathway of tryptophan to nicotinic acid *in vivo* is shown in **Figure 1**. The rate of conversion of tryptophan to niacin and the pyridine nucleotides is controlled by the activities of tryptophan dioxygenase (alternatively known as tryptophan pyrrolase), kynurenine hydroxylase, and kynureninase enzymes. These depend on other B-vitamins, glucagon, glucocorticoid hormones, and estrogen metabolites; moreover, competing pathways can affect the rate of conversion. The conversion may be increased three-fold in pregnant women and in women taking oral contraceptives. Thus, a variety of nutrient deficiencies, toxins, genetic and metabolic abnormalities, etc. all influence the niacin status and requirements.

The two pyridine (i.e., nicotinamide) nucleotide coenzymes, formerly known as 'coenzymes I and II', then for a period as 'DPN and TPN', but now as 'NAD' and 'NADP' are involved in hundreds of enzyme-catalyzed redox reactions *in vivo*. Although a few enzymes can use either cofactor, most are highly specific for one or the other.

Catabolism of the pyridine nucleotide coenzymes *in vivo* is achieved by four enzymes: NAD glycohydrolase, ADP ribosyl transferase, and poly (ADP ribose) synthetase (acting in sequence to liberate nicotinamide), and NAD pyrophosphatase (which releases nicotinamide mononucleotide for hydrolysis to nicotinamide). Turnover of nicotinamide then results in the formation of 1-methylnicotinamide (N^1 -methyl nicotinamide or NMN), which is excreted into urine by the kidney, together with 1-methyl-2-pyridone-5-carboxamide and 1-methyl-4-pyridone-3-carboxamide (referred to as 2-pyridone and 4-pyridone, respectively). The concentrations of these excretory products can be used as indicators of whole-body niacin status (see the Section on Assessment of Niacin Status and Requirements below). At high intakes of niacin, as much as 85%

may be excreted unchanged; however, the excretion of nicotinamide always predominates over that of nicotinic acid.

Other urinary excretion products of niacin include nicotinic acid (nicotinoyl glycine), nicotinamide *N*-oxide, and trigonelline (N^1 -methyl nicotinic acid); the latter may arise from bacterial action in the gut or from its absorption from foods. The pattern of metabolites varies between species and between diets (depending partly on the ratio of nicotinamide to nicotinic acid) and with niacin status, indicating complex regulatory mechanisms.

Hydrolysis of hepatic NAD to nicotinamide allows the release of niacin for other tissues. Protection of the coenzyme content of key enzymes such as glyceraldehyde 3-phosphate dehydrogenase confers protection on key metabolic pathways. In contrast, enzymes that catalyze pyridine nucleotide turnover may be hyperactivated in cells damaged by carcinogens, such as mycotoxins, thus starving them of essential cofactors and causing their death. This may help explain why moldy grain in the diet can increase the risk of pellagra when niacin and tryptophan intakes are marginal. In normal healthy cells, the compartmentalization of hydrolytic enzymes limits coenzyme turnover, but this is impaired in damaged cells.

Metabolic Function and Essentiality

The key functions of niacin involve its coenzymes, NAD and NADP, in the hydrogen/electron-transfer redox reactions in living cells. Like most B-vitamins, niacin is not extensively stored. Inadequate dietary intake leads to tissue depletion within 1–2 months and then successively to biochemical abnormalities, clinical signs of deficiency, and eventually death. As with other B-vitamins, rates of turnover and hence rates of excretion of breakdown products decline progressively as dietary deficiency becomes more severe. Thus the tissue coenzymes are relatively spared.

NAD is responsible for the release of energy during the oxidation of energy-producing fuels. NADP, however, functions mainly in the reductive reactions of lipid biosynthesis, and the reduced form of this coenzyme is generated via the pentose phosphate cycle. NAD is essential for the synthesis and repair of DNA and for supplying adenosine diphosphate (ADP) ribose ligands to lysine, arginine, and asparagine residues in proteins such as histones, DNA lyase II, and DNA-dependent RNA polymerase, and to polypeptides such as bacterial (e.g., diphtheria and cholera) toxins. In the nucleus, poly (ADP ribose) synthetase is activated by binding to DNA breakage points and is involved in DNA repair. It is also concerned with condensation and expansion of chromatin during the cell cycle and in DNA replication. It regulates the fidelity of DNA transcription, and some inflammatory and immune responses. Three different enzyme classes catalyze ADP-ribose transfer: (1) poly-ADP-ribose polymerases (PARP), (2) mono-ADP-ribosyl transferases (which modify G-proteins that regulate cell signalling and glucose-regulated proteins such as GRP78, which is a molecular chaperone, regulating protein-folding in the endoplasmic reticulum), and (3) enzymes that form cyclic ADP-ribose, which mobilizes calcium from intracellular stores. Nicotinic acid-ADP (NAADP), formed by desamidation of NADP, is also a calcium transport regulator.

Table 4 Recommended and reference intakes of niacin equivalents^a

Age group	UK ^b		USA ^c
	LRNI (mg Niacin equivalents per day)	RNI	RDA (mg Niacin equivalents per day ⁻¹)
0–6 months	2	3	2
6–12 months	3–4	4–5	4
12 months–13 years	5–10	8–15	6–12
Adult	8–12	13–18	14–16
Lactation	10	15	17

This table provides a simplified summary of the published values.

^aOne niacin equivalent (mg NE) is equivalent to 1 mg niacin or one-sixtieth of the milligram tryptophan consumed.

^bUK values are calculated on the basis of Lower Reference Nutrient Intake (LRNI) of 4.4 mg NE per 1000 kcal food energy, and Reference Nutrient Intake (RNI) of 6.6 mg NE per 1000 kcal, both of which are constant for all the population groups. The LRNI is intended to cover the needs of the lower 2.5% of a healthy population, whereas the RNI is intended to cover 97.5% of a healthy population.

^cThe US Recommended Dietary Allowances (RDAs) are intended to cover the needs of 97.5% of a healthy population.

NAD is also required for the activity of silent information regulators (SIR or siruin enzymes), which are protein deacetylases for transcription regulation, genome stability, neuronal protection, and longevity. One of their substrates is p53, a protein of genome stability, DNA repair, and apoptosis. Niacin status affects the level of ADP ribosylation of these and other proteins, and may affect (1) chronic degenerative diseases like cancer, diabetes, and dementia, and (2) acute inflammatory conditions such as septic shock or stroke or myocardial infarction, with complex outcomes that are difficult to predict precisely. Nicotinic acid is part of the chromium-containing glucose-tolerance factor, whose functions are still being studied. A high level of poly (ADP ribose) synthetase activity, which is found in some tumors, can result in lower levels of NAD.

Because the electron-transport functions of NAD frequently involve flavin coenzymes, and because both flavin coenzymes and vitamin B₆ coenzymes are involved in the conversion of tryptophan to niacin *in vivo*, there are important metabolic interactions between these B-vitamins. Their clinical deficiency signs also converge, sometimes making it difficult to distinguish between deficiencies of different B-vitamins.

The body's need for niacin can be met completely by dietary tryptophan; thus, it is not, strictly speaking, an essential vitamin. In this respect, it resembles carnitine, which can be synthesized entirely from lysine, but for which, under some circumstances, a dietary requirement arises. Traditionally, however, niacin is classified as an essential vitamin, because some human diets lack both niacin and its precursor, tryptophan. Some animals such as sheep and cattle can synthesize sufficient niacin from tryptophan and thus do not need preformed niacin.

Assessment of Niacin Status and Requirements

Although the measurement of B-vitamin status is usually performed in blood samples, blood-based tests for niacin status are poorly developed. Some studies have suggested that erythrocyte concentrations of NAD or a reduction in the ratio of NAD to NADP may provide evidence of deficiency. The 95% range for healthy US adults for the NAD/NADP ratio was

found to be 127–223, with a wide variation between different populations. The niacin coenzymes can be quantified either by enzyme-linked reactions or by their natural fluorescence in an alkaline solution.

At present, however, niacin status is most commonly assessed by assaying its breakdown products in urine. Of these, N¹-methyl nicotinamide (NMN) is the easiest to measure, by conversion *in vitro* to a fluorescent product. However, more reliable information can be obtained by measurement of urinary NMN plus the urinary pyridones (N¹-methyl-2-pyridone-5-carboxamide and N¹-methyl-4-pyridone-3-carboxamide), all of which can be detected and quantified by UV absorption following high-pressure liquid chromatography. The Interdepartmental Committee on Nutrition for National Defense (USA) has recommended an NMN excretion rate criterion of less than 5.8 μmol (0.8 mg) NMN per day in 24-h urine samples as evidence of biochemical deficiency.

The requirement for niacin to prevent or reverse the human deficiency signs is not known very precisely and depends on ancillary dietary deficiencies or other insults and pathologies. To estimate niacin requirements for dietary reference values, restoration of urinary excretion of NMN during controlled human depletion–repletion studies has been used, and on this basis, the average adult requirement has been estimated as 5.5 mg (45 μmol) of NEs per 1000 kcal (4200 kJ). After adding a 20% allowance for individual variation, this becomes 6.6 mg (54 μmol) per 1000 kcal (4200 kJ), which is the current reference nutrient intake in the UK (Table 4). Niacin requirements used to be expressed as a ratio to energy expenditure.

The average content of niacin in human breast milk is 8 mg (65.6 μmol) per 1000 kcal (4200 kJ), and this content forms the basis for intake recommendations (and dietary reference values) for infants up to 6 months of age. In the UK, the Reference Nutrient Intake niacin increment during pregnancy is nil, but during lactation, it is 2 mg day⁻¹.

Dietary Sources and High Intakes

From Table 3, different foods differ considerably, not only in their total contribution to niacin equivalents but also in the

ratio of the contribution from preformed niacin and from tryptophan. In a typical Western diet, preformed niacin provides approximately 50% of the niacin supply in the diet. As for other B-vitamins, meat, poultry, and fish are excellent sources of niacin equivalents, followed by dairy and grain products, but certain grains such as maize and highly polished rice are very poor sources. Both nicotinamide and nicotinic acid have potentially useful pharmacological properties at high intakes.

Nicotinic acid, which has marked antihyperlipidemic properties at daily doses of 2–6 g, is of greatest interest in pharmacological terms. Large doses of nicotinic acid reduce the mobilization of fatty acids from adipose tissue by inhibiting the breakdown of triacylglycerols through lipolysis and by inhibiting hepatic triacylglycerol synthesis (by inhibiting hepatic diacylglycerol acyltransferase 2), thus limiting the assembly and secretion of very low-density lipoproteins from the liver and reducing serum cholesterol. It inhibits high-density lipoprotein (HDL) catabolism by decreasing the surface expression of the hepatic ATP synthase beta chain, thereby increasing HDL levels, and it may increase the ratio of HDL₂ to HDL₃, together with a reduced rate of synthesis of apolipoprotein A-II and a transfer of some apolipoprotein A-I from HDL₃ to HDL₂. Such blood lipid effects appear to be due to binding of nicotinic acid to a receptor called a 'niacin receptor' or HM74A, linked to an inhibitory G-protein, and leading to reduced cyclic-AMP levels and inhibition of a hormone-sensitive lipase. Nicotinic acid and niacin can also increase the redox potential in arterial cells. The unwanted side-effect of skin flushing is linked to prostaglandin D₂ and E₂ formation by macrophages; this can now be reduced by a prostaglandin (DP1) receptor antagonist such as laropiprant. These effects of high-dose nicotinic acid may be beneficial in managing dyslipidemias and in reducing the risk of cardiovascular disease, and it has recently been successfully combined with statin therapy plus laropiprant. If given intravenously, large doses of nicotinic acid can, however, produce undesirable side-effects such as vasodilatation, hypotension, nausea, vomiting, diarrhea, and gastrointestinal disturbance, headache, fatigue, difficulty in focusing, skin discoloration, dry hair, sore throat, jaundice, changes in liver function tests, changes in carbohydrate tolerance, and changes in uric acid metabolism including hyperuricemia. Hyperuricemia may result from effects on intestinal bacteria and enzymes, or on renal tubular function, and is especially severe if sustained-release preparations of nicotinic acid are used. Except under medical supervision, the upper tolerable limit for niacin intake has been set at 35 mg NE per day for adults and 10–30 mg day⁻¹ for children and adolescents (1–18 years), and is linked to the skin flushing reaction to nicotinic acid supplements.

A large trial for the secondary prevention of myocardial infarction by high-dose nicotinic acid, with a 15-year follow-up, has produced convincing evidence for moderate but significant protection against mortality, which was attributed to the cholesterol-lowering effect or reduction of reinfarction.

Nicotinamide does not share these effects of nicotinic acid on lipid metabolism or its associated toxicity. However, it has been shown to act as an inhibitor of poly (ADP ribose)

synthetase in pancreatic β -cells in animal studies, thereby protecting them from chemically-induced diabetes, albeit sometimes complicated by beta-cell tumor formation. Studies on human diabetes have so far yielded inconsistent results.

Because niacin deficiency or dependency can exacerbate some types of mental illness such as depression or dementia, and because the correction of depressed brain levels of serotonin would be advantageous, there have been attempts to treat depression with tryptophan or niacin; however, these have so far had only limited success. Schizophrenic patients have been treated with nicotinic acid, because the synthesis of NAD is impaired in some parts of their brains. Recent research has indicated that nicotinamide riboside uniquely supports neuronal NAD synthesis, thereby deserving future therapeutic studies.

In conclusion, niacin and some of its related compounds have many wide-ranging metabolic effects, and their medical significance has attracted renewed interest in recent years.

See also: Amino acids: Metabolism. Bioavailability. Hyperlipidemia: Prevention and Management; Overview. Riboflavin. Vitamin B₆: Physiology

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NUCLEIC ACIDS, PURINE, AND PYRIMIDINE NUCLEOTIDES AND NUCleosIDES

Physiology, Toxicology, and Dietary Sources

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Introduction

Nucleic acids, vital components of all living cells, were isolated in 1869 from the nuclei of pus cells and the spermatozoa of Rhine salmon. Later, it was shown that the major constituents of nucleic acids are sugars, phosphate groups, and the characteristic purine and pyrimidine bases. The chemical structures of the purine bases, including uric acid – the end (waste) product of purine metabolism in humans – were established at the end of the 19th century. The role of the nucleic acids in storing and translating the genetic information in the cells was elucidated in the 20th century. Although their caloric contribution to the diet is trivial, nucleotides, nucleosides, and bases have essential roles in metabolism and signaling within the cell and organism.

This article outlines the structure of nucleic acids and gives a brief overview of the physiological functions of nucleosides, nucleotides, and nucleic acids. It describes the toxicity that may arise from degradation of both endogenous and dietary (exogenous) nucleic acids in humans and contains a summary of the nucleic acid content of foods.

Physiology

Structure

The hereditary material in the nucleus of human cells is packed into 46 chromosomes and additional DNA is found in the mitochondria. The capacity of DNA to be copied into two complementary strands arises from the well-known double-helical structure and underlies the transfer of genetic information in all living organisms. Interactions between the DNA and transcription factors determine the time and place in the body where genes are transcribed, controlling development and metabolism.

RNA molecules are synthesized initially on a DNA template by a DNA-dependent RNA polymerase in a process called transcription, where ribonucleotides complementary to the bases of one strand of DNA are joined by 3'–5' phosphodiester bonds (Figure 1(a)).

[†]Deceased.

Nucleic Acid Biosynthesis in Humans

The first step in nucleic acid synthesis involves the formation of the purine and pyrimidine ribonucleotides. There are two endogenous routes: either the energetically expensive *de novo* route from small molecules such as carbon dioxide, amino acids, and ribose sugars, or the energetically less expensive 'salvage' pathway. Purine bases and pyrimidine nucleosides from the breakdown of nucleic acids and nucleotide cofactors are salvaged within the cells, generating nucleotides that can be incorporated into nucleic acids. In most cells, salvage processes are more important, and the ribonucleotides recycled in this way exert feedback control on the *de novo* routes.

Metabolic Roles of Nucleotides

The most abundant ribonucleotide in the body is adenosine 5'-triphosphate (ATP), which is the universal energy carrier in living organisms (Figure 1(b)). In addition, nucleotides are precursors of several coenzymes, used in many reactions including the conversion of food into energy. Within cells adenosine and guanosine nucleotides also have roles in the transduction of external signals into cellular responses, and in the translation and synthesis of proteins. Pyrimidine nucleotides are present at much lower concentrations in cells but also fulfill diverse functions. Uridine diphosphate (UDP)-glucose and Cytidine diphosphate (CDP)-lipids are active intermediates in the synthesis of glycogen and membranes, respectively, and sugars linked to UDP or GDP are used in the glycosylation of proteins. UDP-glucuronic acid is an essential component of the pathways that convert exogenous molecules and endogenous steroids into soluble forms for disposal from the body.

The free deoxyribonucleotides are very scarce in the normal cell because they are used exclusively for the synthesis of DNA.

Synthesis of Nucleic Acids

Synthesis of both DNA and RNA is prominent in cells and tissues with high turnover or metabolism (e.g., liver, gut epithelium, skin, dividing lymphocytes, bone marrow, and hair follicles). Different complements of enzymes

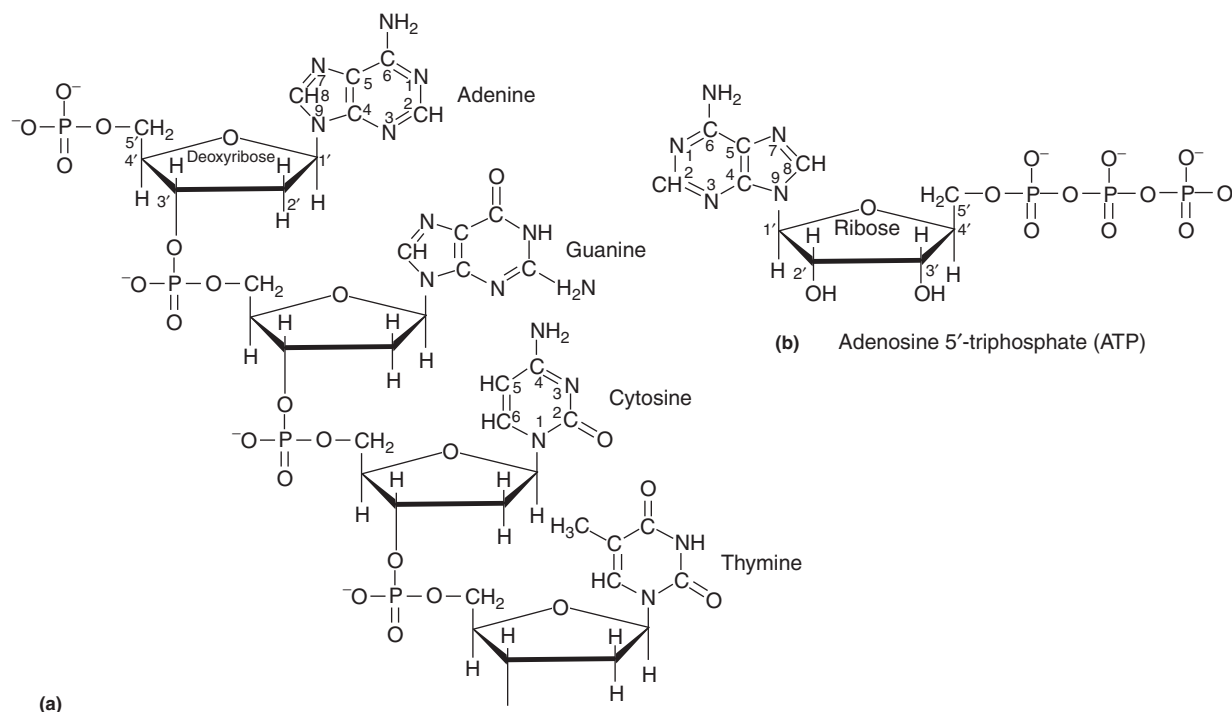


Figure 1 (a) Schematic representation of part of a DNA strand showing the structural formulae of the four constituent bases, adenine, guanine, cytosine, and thymine, linked via the 3'-OH group of the deoxyribose moiety to the 5'-phosphate group of the next nucleotide. Also shown is the numbering of the atoms in the deoxyribose, as well as the pyrimidine and purine rings. The bases are adenine (A), guanine (G), cytosine (C), and thymine (T). Two strands are wound in opposing chemical directions to allow the well-known double-helix structure, with hydrogen-bonding between complementary nucleotides (i.e., A-T and G-C), to form. The deoxyribose and phosphate groups form the outer sides of the 'ladder'. The RNA molecule is single-stranded, but double-helical regions arise when stretches of complementary sequences allow hairpin loops to form. In addition, the base uracil (U) is found instead of thymine, and the pentose is ribose. (b) Structural formula of ATP indicating that the ribose, as distinct from deoxyribose, has an OH group at the 2' position on the pentose ring. When a nucleoside triphosphate (NTP) is linked through the 5' phosphate groups to the 3' position of the previous residue on the growing chain, the chemical energy for the polymerization is provided by the removal of the second and third phosphate groups.

are expressed in each cell type, and therefore tissues have characteristic profiles of internal metabolites, including nucleotides and nucleosides. For example, in cells that do not continuously divide, such as heart and muscle, nucleotide profiles are relatively simple, relating to the major requirement to sustain high levels of ATP and cofactors. Contrastingly, rapidly dividing cells in liver and intestine show a complex nucleotide pattern, identifying these organs as major sites of nucleic acid metabolism. The gut is particularly important in this respect. The rate of cell turnover in the luminal villi is high, and it has been calculated that in rats approximately 30 mg of endogenous nucleic acid derived from dead cells enters the gut lumen daily. This means that nucleic acid synthesis in liver and intestine is much higher than in tissues such as muscle.

Metabolism of Endogenous Nucleic Acids and Excretion of Metabolic End Products

There is a considerable daily turnover of endogenous nucleic acids and ribonucleotides during muscle work, wound healing, erythrocyte senescence, mounting an immune response, etc. However, only a small fraction of these vital endogenous compounds is degraded and lost from the body. Because

de novo purine and pyrimidine synthesis is energetically expensive the contents of dead cells are normally used by other cells, and degraded RNA or cofactors are recycled within living cells using active 'salvage' routes.

Breakdown products of DNA and RNA enter the salvage pathways in the form of the purine bases hypoxanthine (Hx) and guanine or the pyrimidine bases uracil and thymine (Figure 2).

Any pyrimidine bases that are not salvaged are then further catabolized in a series of steps to β -amino acids, which are soluble and readily excreted. There is thus normally no measurable toxic pyrimidine end product from endogenous or dietary nucleic acids, except in the case of a small number of very rare metabolic disorders (Figure 3).

The purine base Hx is converted in the liver to the insoluble metabolites xanthine and then uric acid by the enzyme xanthine oxidase/xanthine dehydrogenase (XDH) in man. Urate can normally be disposed of in the urine, but high concentrations can crystallize and form kidney stones or deposits in the joints and under the skin. Some rare genetic disorders can remove feedback regulation of purine biosynthesis, or excessive breakdown of cells may overload the salvage system, each resulting in very high endogenous levels of uric acid. However most other animal species (with the notable exception of some dog varieties) possess an additional catabolic enzyme, uricase,

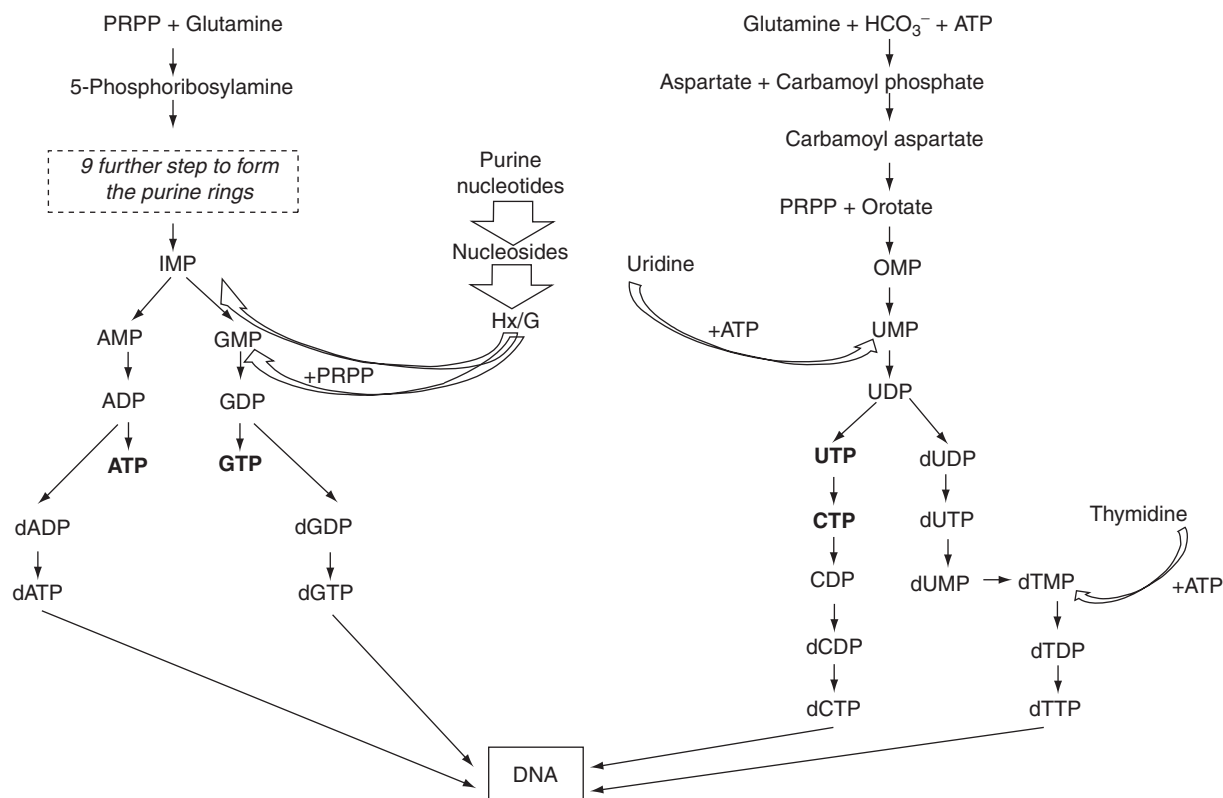


Figure 2 *De novo* synthesis of ribonucleotides uses small molecules and amino acids. The purine base of IMP is built up in several steps on a ribose phosphate molecule, and separate pathways generate ATP and GTP. In pyrimidine biosynthesis, the six-membered ring is formed before addition of the ribose phosphate to orotate, and decarboxylation to form UMP. Ribonucleotides (ATP, GTP, UTP, and CTP, shown in bold) are used in the synthesis of RNA, whereas DNA is synthesized after conversion of the ribose to deoxyribose, and of dUMP to dTMP. Salvage pathways (shown with open arrows) all follow two patterns: purine nucleotides and nucleosides, entering the cells or derived from hydrolysis of cofactors or nucleic acids, are converted to the free bases hypoxanthine or guanine and then converted by a specific phosphoribosyltransferase enzyme to the nucleoside; in contrast pyrimidines are salvaged from the nucleosides uridine or thymidine using specific kinase enzymes. It should be noted that PRPP (phosphoribosyl pyrophosphate) is essential for both *de novo* pathways and the purine salvage pathways.

which cleaves the purine ring of uric acid forming the readily soluble allantoin. This compound in turn may be degraded to ammonia in water-dwelling species such as fish.

Metabolism of Dietary Nucleic Acids in Humans

The human diet is naturally rich in nucleic acids because food is derived from once-living organisms. Because, as already described, nucleotides and nucleosides can be synthesized *de novo* and nucleobases liberated during catabolism can be salvaged, they are often considered to be dispensable nutrients in food. The metabolism of these exogenous nucleic acids follows a similar pattern to the intracellular process described above, but the bacterial flora of the intestine are the first point of degradation. This digestion is rapid. Studies in both pigs and humans demonstrated that up to 50% of dietary purine was degraded to carbon dioxide within 30 min, 43% was recovered in the urine and 5% excreted in the feces. Less than 2% of dietary purines is incorporated into nucleic acids.

Humans thus have no apparent essential requirement for purines from the diet and the intestinal mucosa provides an

effective barrier to their uptake through a battery of enzymes that can rapidly degrade purine nucleotides, nucleosides, and bases especially unusual purines found in plant materials. Because of this enzyme activity, and the rapid turnover of intestinal mucosa, approximately 200 mg of urate is excreted daily in the feces. This phenomenon ensures that levels of ATP do not fluctuate in concert with the dietary intake of purines, or may represent an important evolutionary development to protect the integrity of cellular DNA.

On the other hand, pyrimidine ribonucleoside monophosphates (NMPs) and ribonucleosides are absorbed readily from the intestine and utilized for nucleic acid synthesis. This has been demonstrated by studies of humans with hereditary orotic aciduria, a rare defect in conversion of orotic acid to uridine monophosphate (UMP) in *de novo* pyrimidine synthesis. Such patients have severe megaloblastic anemia. They have been sustained on oral uridine, indicating that the dietary pyrimidine nucleoside can compensate totally for lack of *de novo* synthesis in humans. Studies using radiolabelled purines and pyrimidines in mice provided further evidence for the incorporation of dietary pyrimidine nucleosides, but not purine nucleosides, into hepatic RNA.

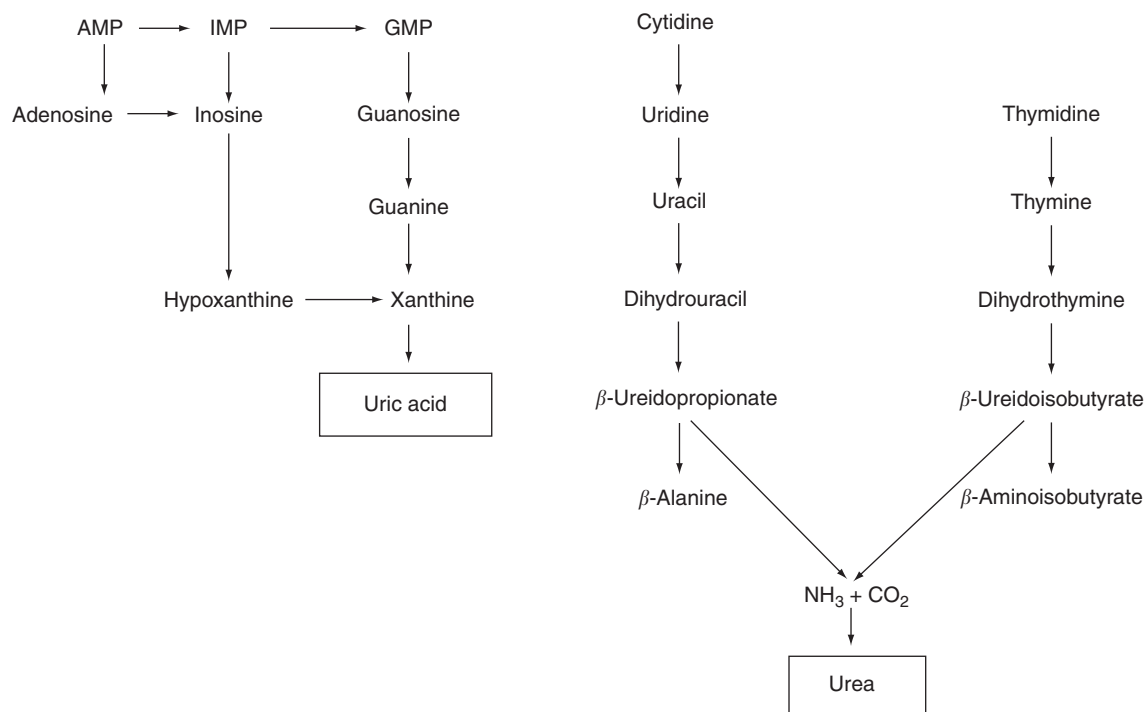


Figure 3 Breakdown of DNA and RNA begins when the molecules are degraded by nuclease enzymes to liberate nucleotides. The next step, degradation by specific 5′-nucleotidases (removing the phosphate groups) to nucleosides or deoxynucleosides, is essentially irreversible. Nucleoside phosphorylases generate (a) the purine bases hypoxanthine and xanthine, which are converted to uric acid or (b) the pyrimidine bases uracil and thymine, which are converted to β-amino acids, ammonia, and CO₂, and thus to urea, a solute in urine.

Nucleic Acid Content of Foods

The nucleic acid content of foodstuffs is expressed generally in terms of purine equivalents or ‘total potentially available nucleosides (TPAN)’ released from food by hydrolysis with sodium hydroxide or hydrochloric acid and enzymes. The data for purines are thus derived by analysis of the resultant constituent bases. Analysis by Robert McCance, Elsie Widdowson, and colleagues from the 1930s onward forms the basis of tables listing the composition of foodstuffs although, with a few exceptions, this demanding work has not been repeated using more modern analytical methods such as liquid chromatography–mass spectrometry (LC–MS).

Foods can be classified into three groups; high, low, or essentially free of purines (and hence of pyrimidines too) (Tables 1 and 2). As a general rule growing organisms such as yeast, or rapidly metabolizing tissues such as liver, will be rich in both nucleic acids. Seeds and grain are good sources of the genetic material, DNA, as well as free nucleotides, which are stored for use in germination. Muscle tissue is an excellent source of the nucleotide ATP and the nucleic acids in mitochondria. Offal is also metabolically very active so is usually high in free nucleotides as well as nucleic acids. Fish and shellfish that are eaten ‘whole’ and fish eggs and roe are also high in nucleic acids. Extracts of meat and yeast e.g., Bovril, Marmite, Vegemite, have very high purine contents, as do supplements such as Spirulina for sale in ‘Health Food’ shops, but are usually eaten in small quantities.

Fats, white flour, sugar, and fruit juices have been separated from the ‘living’ part of the food and so they are poor sources of nucleic acids.

Effect of Cooking on Nucleotide Content of the Diet

Nucleic acids are relatively resistant to hydrolysis at the moderate temperatures and short periods of time associated with cooking in water or frying in oil. On the other hand nucleoside triphosphates (NTPs) and nucleoside diphosphates (NDPs) break down readily during boiling in water forming first their related NMP and then their base. The rate of hydrolysis is significantly increased in acidic solutions. The rate of degradation is enhanced if any enzyme activity is still present.

The levels of nucleic acids and NTPs are well maintained during prolonged storage at −20 °C or below.

Nucleic Acid and Related Compounds in Beverages

Tea, coffee, and cola drinks contain a number of unusual nucleobases based on xanthine (Figure 4). Caffeine (1,3,7-trimethylxanthine) and theobromine are the best known. Caffeine is found in various quantities in the beans, leaves, and fruit of many plants. It is mainly consumed by humans in infusions extracted from the coffee bean and leaves of the tea bush. A cup of coffee can contain up to 175 mg of caffeine whereas a cup of tea contains approximately 40 mg.

Table 1 A quick reference guide to the purine (nucleic acid) content of foods

<i>Foods and Beverages Rich in Nucleic Acids/Purines</i>
Offal: sweetbreads, liver, kidney, heart, and paté
Wild or farmed game meats (venison, pheasant, rabbit, hare)
Seafoods: sardines, sprats, herring, bloaters, anchovies, fish roe, caviar, taramasalata, trout or salmon; lobster, crab, prawns
Vegetables: asparagus, avocado pears, peas, spinach, mushrooms, broad beans, cauliflower
Pulses and grains: legumes, pulses and soya products such as bean curd, tofu, Quorn
Cereals: all bran, oat, rye or wheat cereals and products; wholemeal, rye and brown breads
Other: beer and yeast extracts/tablets (Barmene™, Tastex™). Meat or vegetable extracts (Marmite™, Vegemite™, Bovril™, Oxo™)
Blue-green alga extracts (Spirulina)
<i>Foods that are Moderate or Low Sources of Purines</i>
Beef, lamb, pork (steak or chops), bacon, ham, sausages, some poultry, tongue (all should be eaten in moderation)
Carrots, parsnip, other root vegetables, potatoes, lettuce, leeks, cabbage, sprouts, marrow, squash, courgettes
Peanuts, cashew nuts
Breakfast cereals
Some fish (see Table 2)
<i>Foods and Beverages that are Essentially Purine-Free</i>
Milk, cheese, eggs, butter, margarine, cream, ice cream
White bread or flour, cakes, scones, biscuits
Sugar, jam, marmalade, honey, and sweets
Cucumber, tomato, onions, pumpkin
Fresh, cooked or tinned fruits, nuts
Puddings, custards, yogurt
Fruit juices, soft drinks

Table 2 Concentrations of purines in some common foods; results are recorded relative to 100 g of food for purine and for protein, although serving size for each ingredient may be larger or smaller than 100 g based on Diem and Lentner (1970).

Food	Purine (mg per 100 g)	Protein (g per 100 g)
<i>Meat</i>		
Beef liver	333	19.7
Beef kidney	285	15.4
Beef heart	285	16.8
Beef tongue	167	16.4
Beef steak	151	19.5
Calf liver	348	19
Sweetbreads	1212	19.6
Veal cutlet	152	19.2
Sheep kidney	312	16.8
Lamb chop	196	14.9
Pork liver	289	22
Pork cutlet	164	16.4
Bacon	85	9.1
Ham	136	19.5
Sausage (beef)	79	13.8
Sausage (pork)	66	11.5
Rabbit	118	20.4
Venison	156	20

(Continued)

Table 2 Continued

Food	Purine (mg per 100 g)	Protein (g per 100 g)
<i>Vegetables</i>		
Asparagus	32	2.1
Cauliflower	32	2.1
Celery	20	1.1
Kohlrabi	44	2.1
Mushrooms	72	3.5
Peas	72	6.7
Spinach	96	2.2
<i>Dried Legumes</i>		
Split peas	195	21
Red bean	162	20
Lentils	222	28
Haricot beans	230	22
Lima bean	149	21
<i>Other</i>		
Bovril™	340	18
Marmite™	356	2
Oxo™ cubes	236	10
Yeast extracts	2257	46
<i>Poultry</i>		
Chicken flesh	181	20.6
Chicken liver	372	22.1
Chicken heart	223	18
Duck	181	16
Goose	177	16.4
Turkey	239	20.1
<i>Fish, Seafoods</i>		
Anchovies	411	20
Bass	73	19.5
Bloaters	133	22.6
Bream	72	19.7
Cod	62	18
Crab	61	19.2
Clams	136	17
Eel	108	18.6
Fish cakes	36	12.1
Herring	378	17
Kippers	91	21.2
Lobster	100	20
Lemon sole	54	19.9
Mackerel	246	29
Plaice	53	18.1
Salmon	250	23
Sardines	345	23
Scallops	117	22.3
Sprats	250	25.1
Squid	135	15
Trout	92	19.2
<i>Canned Seafoods</i>		
Anchovies	321	30
Herring	378	17
Mackerel	246	26
Oysters	116	6
Salmon	88	26
Sardines	399	24
Shrimp	231	22
Tuna	142	29

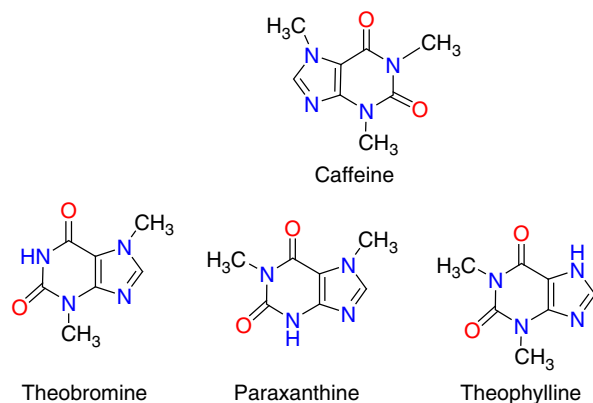


Figure 4 Chemical structures of unusual nucleobases derived from xanthine, and found in plant tissues such as cocoa beans, coffee beans, and tea leaves.

Many popular soft drinks, e.g., cola, may contain up to 300 mg per can. Theobromine (dimethylxanthine) occurs naturally in cocoa beans (20 mg g⁻¹ of cocoa powder) and is therefore present in chocolate. Theophylline (1,3-dimethylxanthine) occurs at trace levels in tea leaves and is now chemically synthesized for use as a drug treatment for asthma.

Caffeine is rapidly absorbed by the stomach and small intestine and is distributed throughout total body water. It is rapidly metabolized in the liver to paraxanthine (84%), theobromine (12%), and theophylline (4%).

In man, caffeine acts as a central nervous system stimulant, temporarily warding off fatigue and restoring alertness: it readily enters the brain and acts as a nonselective antagonist of purinergic adenosine receptors. Caffeine is the most widely consumed psychoactive substance; in North America, 90% of adults consume caffeine beverages daily. 'Energy drinks' contain very high levels of caffeine and some also contain alcohol, so over-use can lead to a 'wide-awake but drunken' state. The US Food and Drug Administration (FDA) lists caffeine as a 'multiple purpose drug generally recognized as safe food substance'.

Because beers and related drinks are produced by fermentation of grains with yeasts, the process is associated with vast increases in cell numbers and this leads to drinks with a considerable nucleic acid and nucleotide content, even if the yeasts are removed by filtration. The economic importance of brewing, as well as the clinical relevance of beer and wine in gout, means that there is considerable literature on the composition of beers and wines including their purine levels. A traditional British beer (ale or bitter) can contain up to 25 mg purine per litre (250 $\mu\text{mol l}^{-1}$) but lager beers have up to 20 mg l⁻¹. Ciders contain <1 mg l⁻¹ of purine. Wine also contains significant amounts of purines. Some low alcohol beers may contain three times these levels of purines. Spirits contain very little in the way of purines because these compounds are removed in the distillation step.

Nucleotides in Human Breast Milk and Infant Formula Milks

Human breast milk contains nucleic acids, nucleotides, and nucleosides, particularly cytidine and uridine, with a profile

that reflects the diet of the mother. Average TPAN concentrations in human milk are 172–222 $\mu\text{mol l}^{-1}$ (59–76 mg l⁻¹) at all stages of lactation. The content derived from cells is approximately 18% of TPAN during the first few days of lactation but drops to less than 10% later. Proportions of nucleosides derived from RNA (43–48%); free nucleotides (36–40%); free nucleosides (6.6–8%); and nucleotide adducts (9–10%) are similar in milk from women of several races. It is not known if all of these compounds in human milk are used by the breast-fed infant. However there is also a movement to supplement formula milks (based on cow's milk) with ribonucleotides derived from hydrolyzed RNA. Cow's milk contains lower levels of nucleotides, with a different profile from the human, but significant levels of the *de novo* pyrimidine intermediate orotic acid, which is low in humans (raised in milk from mothers who smoke).

In the late 1990s the FDA agreed to the nucleotide supplementation of infant formula based on cow's milk, but at lower concentrations than in human milk. The EU Food Committee recommended in 2003 (updated advice in 2007) that the content of nucleotides, if added to infant formulae and in follow-on formulae, should not exceed 5 mg per 100 kcal. If added the maximum nucleotide contents should be: cytidine monophosphate (CMP) 2.5 mg per 100 kcal, uridine monophosphate (UMP) 1.75 mg per 100 kcal, adenosine monophosphate (AMP) 1.5 mg per 100 kcal, guanosine monophosphate (GMP) 0.5 mg per 100 kcal, inosine monophosphate (IMP) 1 mg per 100 kcal.

Several trials have evaluated the effects of nucleotide addition to formula milk in infants but only two of the trials have studied formulae with 'human' nucleotide levels of 72 mg l⁻¹. Thus, there is no adequate scientific basis at present to conclude that the addition of nucleotides in higher concentrations than presently permitted for infant formula would provide additional benefits. Formula milks based on soy protein have a high natural content of nucleotides and are therefore not supplemented.

Beneficial Effects of Dietary Nucleosides and Nucleotides

In healthy adults, a normal varied diet is a good source of nucleic acids, nucleotides, and nucleosides, and supplementation is thought to be unnecessary.

There is substantial evidence (principally from research in animal models) that the presence of nucleotides or nucleosides in the diet helps cellular proliferation in the gut, in postoperative trauma, and in the development of the immune response in infants. A medical food supplement containing arginine, glutamine, nucleotides, and omega-3 fatty acids, demonstrates a better clinical outcome for (adult) surgical patients, and a 30% reduction in risk of infection. It should be noted that glutamine is a precursor for *de novo* synthesis of nucleosides as well as a source of energy for proliferating cells.

Dietary nucleotides have been shown to promote the incorporation of essential fatty acids into membrane lipids in healthy new-born babies, and to enhance the integrity and maturation of the intestine and of the immune system, and thus may contribute to the improved immunity seen in breast-fed infants. Studies in lower socioeconomic groups have found that supplementation of formula with 14.2 mg free

nucleotides per 100 g milk powder resulted in a significant reduction of first episodes of diarrhea. This may be linked to an alteration in the bowel flora, leading to a predominance of lactobacilli as seen in breast-fed babies.

An extract from sugar cane (trade name NucleomaxX), containing 17% nucleosides, is used in the HIV-positive community to counteract the unpleasant side-effects of HIV drugs that inhibit the formation of mitochondrial DNA and hence energy-producing processes. The use of oral uridine in metabolic disorders is described later.

Nucleotides based on both adenosine and uridine can activate the purinergic receptors on a wide range of cell types. Nucleotides influence the transcription of several genes in intestinal cells, and have been shown to improve growth and maturation of the gut in weanling rats.

In lymphocytes and other cells, synthesis of nucleotides *de novo* is expanded dramatically when a signal for proliferation is received; the rate of pyrimidine biosynthesis increases more than purine biosynthesis. Thus nucleotides are now considered to be 'conditionally essential' because their provision in the diet may provide help through the salvage system where cells are dividing rapidly or where other nutrients, used as precursors, are scarce.

Purine Ribonucleotides as Flavor-Enhancing Additives

The purine 5'-nucleoside monophosphates IMP and GMP, derived from degradation of RNA, have received much attention as the taste-active components in a variety of seafoods and meat. These purine 5'-nucleotides, but not the pyrimidine nucleotides CMP and UMP, enhance the savory flavor generated by monosodium glutamate (MSG), by interaction with receptors on the specific *umami* taste buds in the mouth. Because ATP is the major free nucleotide in muscle cells, its breakdown into the flavor-enhancing IMP provides a scientific rationale for the improved palatability of meat or game birds that have been hung for several days after slaughter. Similarly, the distinctive flavors of several cheeses are related to the metabolism, by bacteria, of the characteristic range of nucleotides present in the original milk.

Purine Ribonucleosides and Bases as Markers of Food Quality

Related to the above topic is the role of hypoxanthine (Hx) in the determination of food quality. As described earlier, when an animal is killed the tissues become ischemic and the intracellular ATP starts to degrade, forming first AMP and then Hx. At room temperature the majority of ATP will have degraded within 24 h. The Hx level will be maximal at approximately 2 weeks in meat stored at 4 °C. The change in Hx content of the food alters the sensory perception of the food, with higher Hx levels causing a bitterness in the taste of meat. This aspect of purine catabolism has been particularly well documented in seafood, which is perhaps the most perishable of foods. Hx in fish and fish products such as fish fingers increases linearly with storage time, and measurement of the Hx levels has been recommended as a marker of fish spoilage.

Toxicology

Pharmacological Uses for Nucleosides and Nucleotides

Rare genetic disorders highlight the sensitivity of lymphocytes to the efficient removal of waste from DNA catabolism, and in fact have provided the basis for development of novel immunosuppressant drugs which inhibit enzyme activities crucial to removal of purine nucleotides, and lead to mis-incorporation of nucleotides in DNA synthesis. On the other hand, the use of certain nucleotide analogs as drugs depends on their incorporation into DNA – for example, analogs used in HIV therapy are incorporated by the reverse transcriptase of the virus, and bring the reaction to a halt. Toxicity associated with several analogs is now known to arise from erroneous incorporation into the patient's mitochondrial DNA, because of less stringent proof-reading by the mitochondrial DNA polymerase enzyme. Azidothymidine (AZT) remains one of the most effective and least toxic drugs for AIDS, albeit now usually taken in triple therapy.

Nucleotide analogs have been used to inhibit the *de novo* pathways for the synthesis of the precursor nucleosides and nucleotides, leading to depletion of metabolites and imbalance of dNTPs, and hence to mis-incorporation of nucleotides in RNA or DNA, respectively. Malaria and other parasites rely exclusively on *de novo* pyrimidine biosynthesis, thus they may be susceptible at drug doses that do not affect the host, because the human body can obtain nucleotides from the salvage pathway. Similarly, because of the increased requirement for nucleotides in rapidly proliferating cells, almost all the enzyme reactions (Figure 2) have been investigated as potential targets for treatment of cancer, inflammation, or to prevent rejection of transplanted organs. Once again, combinations of drugs with different modes of action have often proved most effective.

Oral uridine, as described earlier, can be used where *de novo* biosynthesis of pyrimidines is defective, and it may be useful in reversing some effects of mitochondrial dysfunction, and to minimize the toxic effect of the antitumor drug 5-fluorouracil. Oral administration of compounds such as benzylacyclouridine, or 2'3'5' tri-O-acetyl uridine (PN401), inhibits the degradative processes in the liver and delivers more uridine into the circulation than oral uridine alone. Uridine is also a precursor for UDP-glucose, essential for the deposition of glycogen in the liver, and UDP-glucose has been proposed as a dietary supplement.

Oral CDP-choline is rapidly converted to its components, CDP (which can be recycled to uridine) and choline, an essential component of lipid membranes. Each molecule can then cross the blood-brain barrier where CDP-choline is used in regeneration of membranes within and around nerve cells, and its pharmacological effects may extend to protection against dementia, memory loss, visual degeneration, and to recovery from ischemic strokes.

Toxicity of Exogenous Nucleic Acids to Humans

The potential toxicity of dietary nucleic acids to humans usually arises from their metabolic end products (principally uric acid). Many investigators have shown that when normal

subjects are fed RNA, the increase in the urate excretion is dramatic, but there is only a modest rise in plasma urate concentrations.

The body pool of urate, and hence the plasma urate concentration, is the result of a balance between production, ingestion, and excretion. If kidney function is normal, the chief causes of high plasma uric acid concentrations are either a high intake of exogenous nucleic acid in the diet or overproduction of endogenous purine. A low-purine (low-nucleic acid) diet containing less meat, seafood, and other purine-rich foods and beverages (Tables 1 and 2) leads to a lower risk of gout symptoms. In contrast, subjects with genetic defects that remove the usual controls on purine biosynthesis may have overwhelmingly high endogenous levels of the waste product, uric acid.

The contribution of the two sources can be assessed by placing the subject on a purine-free diet for a week, and measuring the total urinary uric acid. In this way fewer than 5% of patients with gout are found to excrete abnormally large amounts of urate (>3 millimoles per day) derived from endogenous purines. In these cases, overproduction of purine nucleotides leading to excess uric acid can be traced to a genetic defect. Two such sex-linked disorders are hypoxanthine-guanine phosphoribosyltransferase (HPRT) deficiency and phosphoribosyltransferase superactivity (PRPS). Boys presenting in infancy usually have severe and eventually fatal neurological deficits. In those presenting as adolescents, neurological problems are milder or absent, and only gout may be evident.

Urolithiasis and Other Kidney Stones

Although modest overindulgence in purine-rich food does not precipitate gout in normal subjects, it can predispose to uric acid lithiasis. Uric acid stones are relatively common in countries where the consumption of nucleic acid-rich beverages and food is high, and in hot climates if insufficient fluids are consumed.

A number of compounds, such as vitamin C, increase uric acid clearance and thus can precipitate urolithiasis. Perhaps not so well recognized is the uricosuric effect of a high-protein diet and the fact that purine-rich foods also predispose to renal calcium stones. This may be because many purine-rich foods such as spinach are equally rich in calcium oxalate. Some vegetables may provoke gout attacks by virtue of their oxalic acid content rather than of purines, but legumes, fast-growing parts of brassicas and asparagus tips may also have significant nucleic acid content. Pulses and grains have a particularly high nucleic acid content. Approximately 25% of vitamin C intake is also excreted as oxalate, which can compound the problem.

The solubility of uric acid is very sensitive to the pH of the urine, which in turn may be made more acidic by a high-protein diet. The solubility of uric acid in urine at pH 5.0 is low (approximately 1 mmol l⁻¹), but it can be increased

12-fold by alkalinizing regimens such as sodium bicarbonate or potassium citrate, which raise the pH to 8.0.

Excess uric acid from dietary purines can also precipitate symptoms that may draw attention to endogenous uric acid accumulating in adults with milder forms of HPRT deficiency or PRPS superactivity, or to a defect leading to raised levels of a uric acid analog related to adenine. The ideal diet for subjects at risk of gout or of uric acid lithiasis is no more than one meat meal per day, using only the low-purine meat and vegetables indicated, and treatment with allopurinol.

The most common and effective treatment for gout is the drug allopurinol, which prevents conversion of xanthine to uric acid by inhibiting the enzyme xanthine oxidase. Although the uricase gene appears to be present in human cells, the promoter is not activated, so no enzyme activity is detected in the liver. Biochemical drugs using recombinant uricase are effective in refractory gout.

See also: Caffeine. Choline and Phosphatidylcholine. Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements

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NUTRIENT–GENE INTERACTIONS

Contents

Health Implications

Molecular Aspects

Health Implications

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Glossary

Acrodermatitis enteropathica Genetically determined impaired zinc absorption.

Hemochromatosis Excess iron uptake.

Menkes disease Genetically determined impaired copper absorption.

Mutation A deviation in base sequence in the DNA that results in a deviant gene product.

Myoblast Precursor muscle cell.

Pleiotropic effect The effect of a single factor on many different targets.

Polymorphism A deviation in base sequence in the DNA that does not result in a deviant gene product.

Wilson's disease Genetically determined impaired copper use.

Nutrient–gene interactions have health implications with respect to a variety of diseases including chronic diseases such as obesity, heart disease, and diabetes. Indeed the susceptibility of the individual to almost any disease is a reflection of both the environment (of which nutrition is but a part) and the genetic makeup of that individual. Even the simple nutrient deficiency diseases are manifestations of these interactions. In fact, when the first recommendations for nutrient intakes were devised, the scientists reviewing the data from the human studies on each of the required nutrients realized that there could be variability in nutrient need. It was this recognition that prompted these scientists to recommend an intake level that would satisfy the nutrient need of at least 90% of the population. As more studies were conducted, these recommendations changed, but the recognition of individual variation was always present. For most of the required nutrients, the intake recommendations are more than adequate to fulfill the body's needs; however, for a few nutrients, there is concern that there may be an upper limit to what should be consumed. This is especially true for the energy intake recommendation. What might be an adequate energy intake for one person might be too much for another and yet too little for a third person. Even allowing for differences in activity and for differences in age, gender, and life stage, individual differences in energy need are present and we do not know the reasons why individuals vary so much. If the individual cannot maintain his/her energy intake then weight will be lost and bodily function compromised; if the energy intake greatly exceeds

need then the excess will be stored and, again, bodily function will be compromised. For some individuals there are mutations in one or more of the genes that encode elements of the food intake regulatory system. In other individuals it is not a mutation per se but a polymorphism(s) in these codes that increases susceptibility to overeating and hence excess fat stores. Still others have mutation(s) in genes that encode the elements of energy conservation. These persons may be unable to dissipate their excess energy intake as heat or, in contrast, may be inefficient energy conservers. All of these instances will result in individuals who are unable to maintain an appropriate energy balance, and in the case of the former may be overly fat or in the case of the latter, overly thin with a voracious appetite. Other mutations and polymorphisms can also occur that affect energy balance. Mutations in genes for hormone receptors, in the hormone release system, in the sensors for hormones, and so forth, can affect energy balance and the development of or lack of development of appropriate fat stores.

The interaction of genetic background and nutritional status is a very active area of research as scientists have begun to explore the interaction of certain polymorphisms with specific nutrient exposures in the development of certain disease risks. Some of these are listed in [Table 1](#). As described in the preceding article, polymorphisms are base sequences that are not considered mutations and are not considered pathogenic. They are base substitutions that have been identified as different and yet not directly associated with a single gene

Table 1 Polymorphisms and nutrient response

<i>Gene</i>	<i>Polymorphism</i>	<i>Diet variable</i>	<i>Result</i>
MTHFR 677	C→T	Folacin intake	T genotypes had higher homocysteine levels than C genotypes
IL2	330 A→C	Vitamin E	Respiratory tract (RI) infection was lower in C
IL10	819 G→C	Vitamin E	RI was lower in C
	1982 C→T	Vitamin E	RI was lower in T
ANGPTL4	A→G	Fat intake	A had higher HDL-C and lower TG
		Carbohydrate	A had an inverse relationship between carbohydrate intake and serum lipids

HDL-C, high density lipoprotein cholesterol; TG, triglyceride.

product defect. Polymorphisms can be used in a variety of ways. They can indicate family relationships, and in this use, population geneticists can track families and migration patterns over time. Polymorphisms can also be used to identify members of a single family as is needed to establish paternity or maternity.

In contrast, we have a number of disorders that can be traced to a single base change that in turn results in a single gene product defect. The function of the gene product is compromised and a specific pathology develops. Acrodermatitis enteropathica is an example of this. In this disorder, the affected individual has an impaired zinc uptake mechanism. A mutation has occurred that results in a defective zinc transporter. Another example is Menkes disease. In this disease, copper absorption is affected. There is an X-linked mutation (a mutation carried by the female but expressed in the male progeny) in the gene that encodes a protein needed to release absorbed copper from the enterocytes into the circulation. Persons with this disorder develop symptoms of copper deficiency even though they consume adequate amounts of copper. Another genetic disorder in copper status is Wilson's disease. This condition is also associated with premature death and is due to an impaired incorporation of copper into ceruloplasmin and decreased biliary excretion of copper. This results in an accumulation of copper in the liver and brain. Early signs of Wilson's disease include liver dysfunction, neurological disease, and deposits of copper in the cornea manifested as a ring that looks like a halo around the pupil. This lesion is called the Kayser–Fleischer ring. Renal stones, renal aciduria, neurological deficits, and osteoporosis also characterize Wilson's disease. Periodic bleeding that removes some of the excess copper can be helpful in managing Wilson's disease as can treatment with copper-chelating agents such as D-penicillamine and increasing the intake of zinc which interferes with copper absorption.

Iron nutriture can be affected by genetics. The disorder hemochromatosis results when iron uptake by the enterocytes is uncontrolled. Usually, iron absorption efficiency is down regulated when stores are adequate and intake meets need. In hemochromatosis, downregulation does not occur and toxic iron levels build up. The condition is caused by a mutation in the HFE gene. Although mutation in both alleles of the gene is rare, it has been estimated that 10% of some population groups may be carriers and should two carriers mate, their children have a strong chance of developing the disease. Even being a carrier has a risk. Carriers have a greater than normal iron absorption efficiency than noncarriers. Heterozygotes (carriers) are at a greater risk for developing diabetes, liver

Table 2 Genetic disorders amenable to dietary management

<i>Disorder</i>	<i>Nutrition strategy</i>
Acrodermatitis enteropathica	Increase zinc intake
Fructosemia	Avoid fructose-containing foods
Galactosemia	Avoid lactose-containing foods
Hemochromatosis	Limit iron intake
Lactase deficiency	Avoid lactose-containing foods
Methylmalonemia	Have monthly B ₁₂ injections
Obesity	Limit energy intake; increase exercise
Phenylketonemia	Limit phenylalanine intake to just meet need; avoid excess intake; supplement diet with tyrosine
Type 2 diabetes mellitus	Limit energy intake to avoid obesity; increase exercise

disease, and heart disease as well as some other chronic conditions. Obviously, if these carriers can be identified a good nutrition strategy might be to consume an iron-poor diet. There are other single gene mutations that affect health as well and these are listed in **Table 2**. Included in this table are nutrition strategies that might prove helpful in suppressing the phenotypic expression of these genotypes. That nutritional strategies are useful has been known for some time. For example, it has long been known that twice as many persons have a diabetes genotype (there are more than 300 of these) than there are persons with the disease. Some persons with diabetes of the noninsulin-dependent type (called type 2 diabetes) can manage their impaired glucose homeostasis by diet and exercise for many years. This implies that diet choice and activity level could well determine the phenotypic expression of the diabetes genotype. This may apply to other genetic conditions whose expression may be avoided or postponed by dietary means and exercise. These examples of single gene mutations affecting the use of a single nutrient are fairly straight forward. There are others that are more complex.

It is interesting to note that for some genes involved in nutrient use, no clinical syndromes have been identified that are linked to mutation in these genes. The explanation for this has come from animal studies. Mice have been developed that have had single nutrient important genes deleted. These are called knock-out mice. In one study, the MTF-1 gene was deleted. MTF-1 is a transcription factor that regulates the expression of the gene for the zinc transporter and thus determines zinc transport into the circulation from the enterocytes. There are no homozygotes for this knockout. Only heterozygotes are born. Apparently, an MTF-1 knockout is lethal to the embryo.

Mutations in genes encoding transcription factors often have pleiotropic effects because these factors regulate a number of different genes and so, too, are the effects of nutrients, which are required components of these transcription factors. An example is the series of genes that encode the enzymes needed for the conversion of a fibroblast to a myocyte. The mammalian skeletal muscle cell is very large and multinucleated. It is formed by the fusion of myoblasts (myocyte precursor cells) and contains characteristic structural proteins as well as a number of other proteins that function in energy metabolism and nerve–muscle signaling. When muscle is being synthesized, all of these proteins must be synthesized at the same time. In proliferating myoblasts, very few of these proteins are present; yet, as these myoblasts fuse, the messenger RNAs for these proteins increase as does the synthesis of the proteins. This indicates that the expression of the genes for muscle protein synthesis is responding to a single regulatory DNA-binding protein. This protein (Myo D1) has been isolated and identified and occurs only in muscle cells. Should this protein be inserted into some other cell type such as a skin cell or an adipocyte, for example, the same expression will occur. That is, the skin or fat cell will resemble a muscle cell. It will take on the characteristics of a myoblast and become a myocyte.

Mutations in genes that encode any one of these transcription factors could result in disease. Of interest is the fact that although all of the genes needed for synthesis in the myocyte and its master controller are present, synthesis will not occur or will occur at a very limited rate if one or more of the essential amino acids needed for this synthesis are absent or deficient in the diet. Here is an example of a gene–nutrient interaction that has control properties with respect to muscle development and this interaction ultimately affects the overall process of growth. Turning this situation around, if the master regulator Myo D1 is aberrant, or if one or more of the genes that encode the enzymes needed for protein synthesis in the myocyte have mutated such that the enzyme in question is nonfunctional or only partly functional, muscle development will cease or be retarded. In either instance, abnormal growth will result.

Effects of Diet on Genetic Diseases

Some genetic diseases can be treated by dietary choices. For example, mutations of the biotinidase gene in humans are associated with decreased recycling of the vitamin biotin in human tissues, leading to increased urinary excretion of biotin metabolites and, hence, biotin deficiency. Afflicted individuals can be treated by lifelong supplementation with pharmacological doses of biotin. A similar example is lactose intolerance, which is caused by absence of the enzyme lactase, leading to decreased tolerance to dietary lactose ('milk sugar') in afflicted individuals. Lactose intolerance (gastrointestinal discomfort, gas, diarrhea in response to lactose feeding) can be observed in many ethnic groups that do not consume milk or milk products after weaning. Persons with lactose intolerance are symptom-free as long as their diet does not contain significant amounts of milk or milk products. In fermented milk products (e.g., hard cheeses, yoghurt, etc.), most

of the lactose has been metabolized by microbial cultures used to make the milk product, hence these products are tolerated reasonably well by most persons with lactose intolerance.

In contrast to biotinidase deficiency and lactose intolerance, many genetic diseases cannot be influenced by diet. There are a number of reasons for this. First, some gene mutations are lethal to embryos and are associated with spontaneous abortions. For example, there is not one reported case of a living patient with a complete absence of holocarboxylase synthetase, a gene that controls crucial steps in biotin metabolism. Second, some genes control essential steps in intermediary metabolism, and the expression of these genes cannot be sufficiently modulated by diet.

Genetic Diseases of Interest to Nutrition

If there is a mutation in the genetic code for a given protein, the amino acid sequence generated for that protein might be incorrect. Some mutations or base substitutions are such that the amino acid sequence of the resultant protein is unaffected. This happens because some amino acids have more than one codon and if the base substitution stipulates a base that is in one of these codons, the same amino acid will be encoded and used. In other instances, the substitution of one amino acid for another in the protein being generated may or may not affect the functionality of the protein being generated. The effect depends entirely on the amino acid in question, its position in the amino acid chain and the protein being synthesized. Some amino acids can be replaced without affecting the secondary, tertiary, or quaternary structures of the protein (and hence, its chemical and physical properties) whereas others cannot. The replaced amino acid might have similar chemical characteristics, for example, or may be in a part of the protein that is not involved in its functional properties. In addition, genetic errors in amino acid sequence may pose no threat to the individual if the protein in question is of little importance in the maintenance of health and well being, or it can have major effects on health if the protein is a critical one. An example of the former is the pentose dehydrogenase enzyme needed for the metabolism of the five carbon sugars present in cherries and plums. Although aberrant and nonfunctional, humans do not consume cherries and plums in large amounts daily and so not being able to metabolize the pentose contained by these fruits is not a health concern. The unmetabolized pentose is excreted in their urine with little untoward effect. An example of the latter concerns the synthesis of the important protein, hemoglobin; if the genetic code calls for the use of valine instead of the usual glutamic acid in the synthesis of the beta chain in the hemoglobin molecule, the resulting protein is less able to carry oxygen. This amino acid substitution not only affects the oxygen-carrying capacity of the red blood cell but also affects the solubility of the hemoglobin in red blood cells. The decreased solubility of the hemoglobin can be understood if one remembers the relative polarity of the glutamic acid and valine molecules. The glutamic acid side chain is more ionic and thus contributes more to the solubility of the protein than the nonpolar carbon chain of valine. This change in pH decreases its solubility in water, and, of course, a change in solubility

leads to an increased viscosity of the blood as the red cells rupture, spilling their contents into the blood stream. This disorder is called sickle cell disease.

The amino acid sequence within a given species for a given protein is usually similar. However, some individual variation does occur. An example of an acceptable amino acid substitution would be some of those that account for the species differences in the amino acid sequence of the hormone, insulin. As a hormone, it serves a variety of important functions in the regulation of carbohydrate, lipid, and protein metabolism. Yet, even though there are species differences in the amino acid sequence of this protein, insulin from one species can be given to another species and be functionally active. Obviously, the species differences in the amino acid sequence of this protein are not at the locations in the chains which determine the biological function of this hormone in the body.

There are a number of metabolic diseases affecting nutrient metabolism that are genetically determined. Most of these are quite rare and most are recessive disorders. There is a long list of these disorders and their associated mutations. For some of the disorders, there is more than one mutation associated with the disease. For example, there are a number of genetic mutations in the code for red cell glucose-6-phosphate dehydrogenase. The code is carried as a recessive trait on the X chromosome and thus only males are affected. These mutations are usually silent. That is, the male, having a defective red cell glucose-6-phosphate dehydrogenase, does not know he has the problem unless his cells are tested or unless he is given a drug such as quinine or one of the sulfur antibiotics that increases the oxidation of $\text{NADPH}^+ \text{H}^+$ by the red blood cell. When this happens, $\text{NADPH}^+ \text{H}^+$ is depleted and is not available to reduce oxidized glutathione. In turn, the red cell ruptures and hemolytic anemia results. In almost all cases the affected male has sufficient enzyme activity to meet the normal demands for $\text{NADPH}^+ \text{H}^+$. It is only when stressed by these drugs that a problem develops.

Another example of a silent genetic disease is that of McCord's disease. In this disease the individual is intolerant of exercise. This occurs because the individual is unable to use the glycogen in their muscles for fuel. Unless forced to exercise, these persons might not be aware of their metabolic defect. They may have adopted a very sedentary lifestyle by unconscious realization of their intolerance.

Unconscious food selection has been observed in children with some of the macronutrient intolerances. Children who are lactose intolerant may refuse to consume milk; those who are gluten intolerant may avoid wheat-containing products; and so on. There may be an instinctive avoidance that helps the individual enjoy their food without serious consequences.

Many genetic disorders have no cure, and many are characterized by a shortened life span. However, for some, there are nutrition strategies that may be helpful. Diseases associated with the malabsorption of carbohydrate, that is, lactose intolerance, galactose intolerance, etc. can be managed by the exclusion of these carbohydrates from the diet. Some of the amino acid disorders can be managed by the reduction of the dietary intakes of the particular amino acid in question. For example, phenylketonuria can be managed by a

reduction in the phenylalanine content of the diet. This is rather tricky because enough of this essential amino acid must be provided but not too much so that there is a surplus that cannot be appropriately metabolized. In addition, because phenylalanine is used to make tyrosine, this amino acid must then be provided in the diet in sufficient amounts to meet the need.

Vitamin D and the uptake of calcium and phosphate are linked. In the absence of active vitamin D, rickets develops. Some individuals are unable to activate the vitamin. That is, they do not have a normal activity of the renal enzyme $25(\text{OH})_2\text{D}$ hydroxylase. As a result, these individuals develop vitamin D rickets. The bones are not appropriately mineralized because there is a deficiency of the active vitamin. This can be managed by providing the active form of the vitamin. In addition, there is also a resistance to the activity of active vitamin D. The genetic problem here is a deficiency in the receptor for the vitamin. Five different mutations have been reported that account for this problem. Patients with these genetic problems also develop rickets and are collectively referred to as having vitamin D-resistant rickets. Lastly, there are a couple of complex disorders that affect more pathways in addition to those of vitamin D. These include the Franconi syndrome, X-linked hypophosphatemia and pseudohypoparathyroidism. In each of these, there is a disturbed mineralization of the skeletal system as well as disturbed regulation of calcium status, phosphorus status, and soft tissue mineralization.

Lastly, discussion of the health implications of nutrient–gene interactions would not be complete if a discussion of the role of diet in protecting the genetic material from damage due to free radicals was not included. DNA can be mutated by exposure to free radicals. A few or many bases in DNA can be destroyed by free radicals leaving the cell unable to faithfully produce one or more gene products. It should be noted that if this damage affects a single cell or a single mitochondrion, the event is not a lethal event except for that cell or mitochondrion. It becomes a problem when large numbers of cells are affected and the loss of cell function is significant. In many instances, the damage can be repaired with little long-lasting damage to gene product function. Free radical damage to DNA can be cumulative and it has been suggested that the loss in function that characterizes aging is due to the cumulative effects of free radical damage to DNA.

Table 3 Nutrients that have a role in free radical suppression

<i>Nutrient</i>	<i>Role</i>
Vitamin E	Quenches free radicals as they form via conversion of tocopherol to tocopheroxyl radical which is then converted to a quinone
Vitamin K	Serves as a H^+/e^- donor acceptor
Carotene	Serves as a H^+/e^- donor acceptor
Ascorbic acid	Serves as a H^+/e^- donor acceptor
Selenium	Essential part of glutathione peroxidase
Copper	Essential cofactor for cytosolic superoxide dismutase
Zinc	Essential cofactor for cytosolic superoxide dismutase
Manganese	Essential cofactor for mitochondrial superoxide dismutase

Nutrients can prevent a great amount of this damage through the free radical suppression system. This prevention is an extension of the discussion of nutrient–gene interactions. Such nutrients as vitamin E, ascorbic acid, carotene (provitamin A), selenium, and other nutrients serve to suppress free radical formation and thereby serve to protect the genetic material. **Table 3** lists the nutrients and their roles in free radical suppression.

Throughout this article, examples have been provided to illustrate how nutrients and the genetic material interact and how these interactions can affect the health and well being of the individual. The ultimate goal of understanding such interactions is to use this information to develop nutrient intake recommendations that will potentiate the expression of genes related to good health while suppressing the expression of genes associated with disease. This is a very active area of nutrition research and considerable progress has been made over the past decade.

See also: Aging. Antioxidants. Biotin: Physiology, Dietary Sources, and Requirements. Iron: Physiology, Dietary Sources, and Requirements. Magnesium. Nutritional Problems of Pre-School Children. Zinc: Physiology, Dietary Sources, and Requirements

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Molecular Aspects

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Glossary

Chromatin A protein-rich material that surrounds nuclear DNA. The proteins are both histone and nonhistone proteins.

Deoxyribonucleic acid (DNA) A series of bases (adenine, guanine, thymine, and cytosine) linked together by phosphodiester bonds through deoxyribose.

Epigenetics The study of changes in gene expression that do not involve change in the base sequence of the gene.

mRNA A short-lived RNA species that serves to transmit information from the nucleus to the ribosomes.

Ribonucleic acid (RNA) A series of bases (adenine, cytosine, uracil, and guanine) linked together by phosphodiester bonds through ribose.

Ribosomes An organelle on which proteins are synthesized as dictated by the sequence of bases in the messenger RNA.

Transcription Synthesis of messenger RNA.

Transcription factors Materials that influence the transcription of messenger RNA.

Translation Synthesis of specific proteins using the base sequence of the messenger RNA as the code for the sequence of amino acids joined together to generate the protein.

Gene Expression

The characteristics of an organelle, a cell, an organ, or tissue, and indeed the whole body within a species, are vested in the genetic material, DNA. Most of this DNA is found in the nucleus whereas a small amount is found in the mitochondria. DNA holds the code for the amino acid sequence of the many proteins that confer a cell or tissue or organ, and indeed the whole organism, with its particular characteristics. Should there be a change in the sequence of the nucleotides within the DNA, the sequence of amino acids in the resultant protein could be different. In some instances, there may be no outward effects of these amino acid changes if the amino acid change is not in the active area of the protein or if it does not change the shape of the protein it encodes, or if the change occurs in a protein of little significance in the overall metabolic profile of the individual.

DNA is composed of four bases: adenine, guanine, thymine, and cytosine. The bases are assigned a single-letter abbreviation: A for adenine, C for cytosine, T for thymine, and G for guanine. These bases are condensed to form the DNA chain in a process analogous to the condensation of amino acids that serve as the primary structure of a protein. Species vary in the percent distribution of these bases in their DNA. The chain of nucleotides that makes up the DNA is formed by joining adenine, guanine, thymine, and cytosine through phosphodiester bonds. The phosphodiester linkage is between the 5' phosphate group of one nucleotide and the 3' hydroxyl group of the adjacent nucleotide. This provides a direction (5'-to-3') to the chain. A typical segment of the chain is illustrated in **Figure 1**. The hydrophobic properties of the bases plus the strong charges of the polar groups within each of the component units are responsible for the helical structure of the DNA chain. Hydrogen bonding between the bases stabilizes this conformation. The

bases themselves interact so that, in the nucleus, the two chains are intertwined. Hence, the term 'double helix' applies to nuclear DNA. In the mitochondria, the DNA is also a double strand but is arranged as a double circle with connections between the light and the heavy strands. The synthesis of the bases that make up the DNA is dependent on the micronutrients as well as on energy. Folic acid, pyridoxine, Vitamin B₁₂, iron, copper, sulfur, zinc, magnesium, and phosphorus are all needed to synthesize these purines and pyrimidines. Indeed, should any of these nutrients be in short supply, cells with short half-lives such as epithelial cells and blood cells will not be replaced as readily. Thus, such micronutrient deficiencies are often characterized by skin lesions or anemia.

Gene Structure

The sequence of amino acids in each protein synthesized by the body is determined from a subunit of the DNA molecule known as the gene. It consists of several thousand bases (abbreviated as kb). Each protein has a unique base sequence in its cognate gene. Although only four bases are used for the DNA, the combinations and sequences of the combinations provide a specific code for each and every protein and peptide. It also functions to transmit genetic information from one generation to the next in a given species. Thus, DNA has a broad spectrum of functions – it ensures the identity of both specific cell types and specific species. Changes in the normal sequence of bases in the DNA can occur. If the change results in an aberrant gene product (the protein it encodes), then it is called a 'mutation.' If the change has little or no effect on the gene product, then it is called a polymorphism. There are polymorphisms that have long-term effects on the responses of the individual to changes in

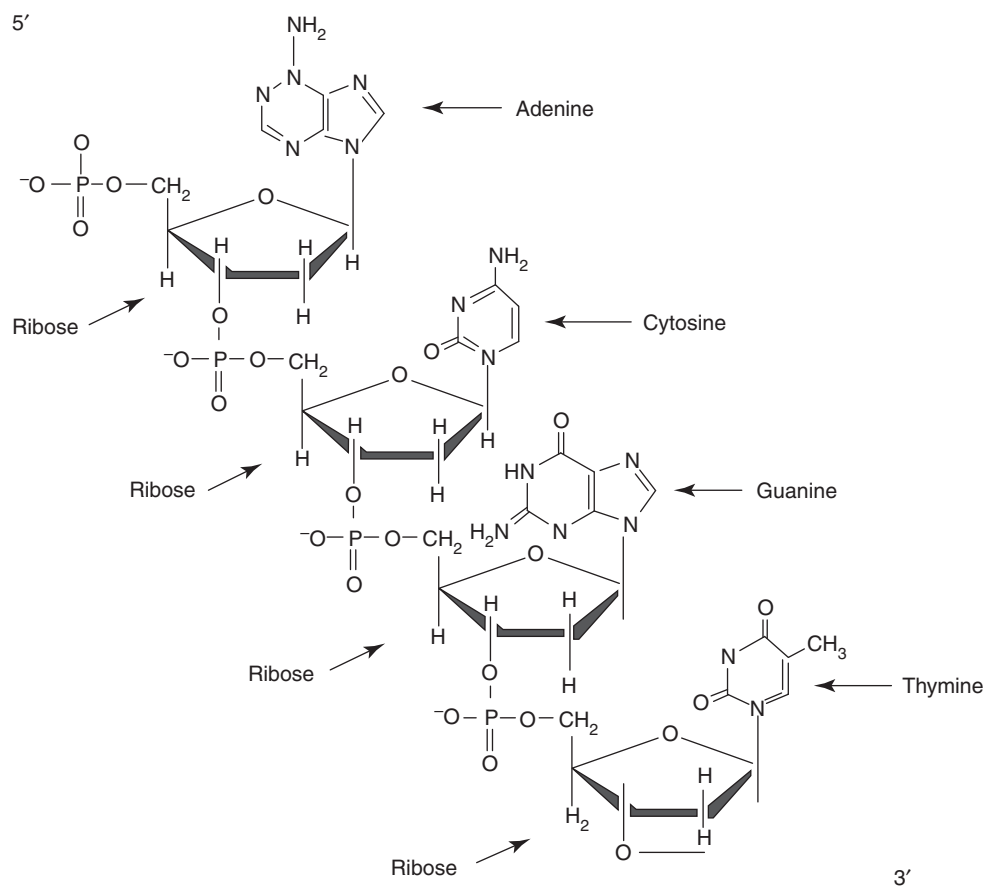


Figure 1 The bases that make up the DNA polynucleotide chain are joined together by phosphodiester bonds using ribose as the common link between the bases.

nutrient intake. A mutation can be spontaneous or one induced by drugs, a virus, or any one of a number of external variants that target the genetic material of the cell. Mutations can be base substitutions, base rearrangements, base deletions, or base additions. In most cases of DNA damage, the damage is random. That is, it does not occur in the same place in every cell and not every cell is affected. However, there are some places in the DNA that are more vulnerable to damage. These are places where there are direct repeats of bases. DNA damage, whether spontaneous or caused by external agents, can be repaired in the nucleus but the mitochondrial DNA has little self-repair. Sometimes repair takes place but is inaccurate. That is, the repair does not fully restore the base sequence to its predamaged state. In this scenario, a base addition or a different base substitution or mismatch repair could occur. This mutation will then become part of the genetic information transmitted to the next generation. Some of the base substitutions have no effect on the gene product. This is because some amino acids have more than one combination of bases in the messenger RNA (mRNA) that stipulate a particular amino acid in the gene product. For example, phenylalanine is encoded by UUU and also by UUC; leucine is encoded by CUU, CUC, CUA, and CUG. These codons in the mRNA mirror those in the nuclear DNA, except that uracil is used instead of thymine and the codons have U instead of T in their triplets.

In the nucleus, the DNA is found in the chromosomal chromatin. Chromatin contains very long double strands of DNA and a nearly equal mass of histone and nonhistone proteins. Histones are highly basic proteins varying in molecular weight from approximately 11 000 to 21 000. As a result of their high content of basic amino acids, histones serve to interact with the polyanionic phosphate backbone of the DNA so as to produce uncharged nucleoproteins. The histones also serve to maintain the DNA in a very compact form and also serve to protect this DNA from damage by external agents. In mammals, the mitochondrial DNA does not have this protective histone coat. It is 'naked' and much more vulnerable to damage. This damage can be quite severe and yet, because there are so many copies (8–10 copies per mitochondrion) of the mitochondrial DNA and so many mitochondria (200–2000) in a cell, the effects of this damage might not be apparent.

Epigenetics

Heritable changes in gene expression can occur without a change in the sequence of the DNA. Changes in the chromatin can result in changes in gene expression. This area of research is called epigenetics. Chromatin consists of DNA, histones (H1, H2A, H2B, H3, and H4), and some nonhistone proteins.

DNA and histones form repetitive nucleoprotein core particles. Each particle consists of 146 base pairs of DNA wrapped around an octamer of core histones (one H3–H3–H4–H4 tetramer and two H2A–H2B dimers). The DNA located between nucleosomal core particles is associated with histone H1. This 11-nm histone fiber is then further packed into an irregular 30-nm chromatin fiber structure, which is coiled into even more complex structures to eventually assemble the chromosome.

The amino terminal tails of histones protrude from the nucleosomal surface; covalent modifications of these tails alter chromatin structure, which in turn alter gene expression. The amino acid residues in the histone tails are modified by covalent acetylation, biotinylation, methylation, phosphorylation, and ubiquitination. When any of these modifications occur, there are effects on gene transcription, mitotic condensation of chromatin, and DNA repair. These modifications are deciphered by proteins containing motifs that target them to chromatin. For example, some transcription factors contain bromodomains that have an affinity for acetylated histones, which in turn can increase gene transcription. If there are modifications of the amino acid residues in the histones, there can be modification in the expression of particular genes. Methylation of lysine in a given histone can either increase or silence the expression of the gene(s) the histone surrounds. Covalent modifications of histones can be reversed by a large variety of enzymatic processes. Both DNA methylation and histone modification can occur as a result of environmental change that can include changes in nutrition status. Several vitamin-dependent modifications of histones have been identified. These include the binding of biotin to lysine residues in histones (see Biotin) that stabilizes the genome; the folate-dependent methylation of lysine residues (see Folate); and the niacin-dependent poly (ADP-ribosylation) of glutamate residues (see Niacin). The acetylation of histones also represents a vitamin-dependent form of chromatin structure regulation, based on the fact that pantothenate-derived coenzyme A is a building block in the formation of acetyl-CoA, which is the substrate for the acetylation of histones (see pantothenic acid). Another reaction of the histones is that of methylation. Methylation of cytosine residues occurring in CG dinucleotides is associated with transcription repression. This methylation of histones can alter acetylation patterns, and the deacetylation of histones is dependent on dietary status. The covalent binding of methyl groups (derived from folate) that produces 5-methylcytosine in mammalian DNA is an epigenetic event. This modification of DNA also depends on a number of other nutrients, including S-adenosyl methionine, vitamin B₁₂, pyridoxine, methionine, betaine, riboflavin, zinc, and choline. It is obvious that nutritional status can exert epigenetic effects on gene expression. Examples of this include caloric restriction, starvation, nutrient deficiency, and effects of aging on nutrient use.

Transcription

Having the codes in the nucleus for the synthesis of protein in the cytoplasm implies a communication between the cytoplasm and the nucleus and vice versa. Signals are sent to the

nucleus that ‘inform’ this organelle of the need to synthesize certain proteins. Signals include substrates for the needed proteins, nutrients, hormones, and other signaling compounds, some of which have yet to be identified. The nucleus directs protein synthesis in the cytoplasm by sending mRNA to the cytoplasmic compartment. This process is called ‘transcription’. The mRNA is similar to the DNA. It is an unbranched linear polymer in which the monomeric subunits are the ribonucleoside 5′monophosphates. The bases are the purines (adenine and guanine) and the pyrimidines (uracil and cytosine). Note that thymine is not used in mRNA. The mRNA is a much smaller molecule than is DNA. It also has a much shorter half-life. It needs to exist only long enough to carry its message to the ribosomes, where it can be translated into the gene product. Because the mRNA is so short lived, its component bases must be resynthesized. This resynthesis requires energy and the micronutrients cited above for the synthesis of DNA. This is another example of the influence of nutrients on gene expression.

Transcription occurs in several stages: initiation, elongation, editing, and termination as illustrated in **Figure 2**. Each gene will have its own unique signals for the initiation of transcription. Some signals are very strong whereas others are less so. Transcription does not occur spontaneously and it follows that nuclear DNA is quite stable unless signaled to transcribe. When DNA is destabilized, transcription is initiated. Unwinding a small portion of the DNA is a necessary step in the initiation of transcription and occurs when the stabilizing factors are perturbed and signals are sent to the nucleus that transcription of a specific gene should begin. Unwinding exposes a small (approximately 17 kb) segment of the DNA (the gene), allowing its base sequence to be available for complementary base pairing for the synthesis of mRNA. One of the two strands of the DNA serves as the template for the synthesis of mRNA. The segment that is exposed contains not only the portion of the gene that codes for the corresponding mRNA (‘coding region’) that encodes the structure of the gene product but also a sequence called the ‘promoter’ region. The promoter region precedes the start site of the coding region and this is said to be ‘upstream’ of the structural gene. Sequences of bases that bind specific proteins are called ‘elements’. The proteins that bind to these elements are receptors that in turn bind substances that affect transcription. Some of these substances are nutrients whereas others may be hormones or other gene activators, enhancers, or silencers. Those bases following the start site are ‘downstream’. The nucleotides that code for a specific protein or that provide elements of the promoter region may not be adjacent to each other on the DNA strand but may be located nearby on either the same strand or the noncoding DNA strand.

In the initiation phase, basal transcription factors recognize and bind to DNA. These factors form a complex with RNA polymerase II. Most of the gene expression can be defined as transacting factors (proteins) binding to cis-acting elements (base sequences) in the promoter regions of genes. Within the promoter, approximately 25 base pairs upstream of the start site is a consensus sequence called the TATA box, which contain A–T base pairs. One of the basal transcription factors, the TATA binding protein (TBP), recognizes this sequence of DNA and binds there. This begins the process of transcription

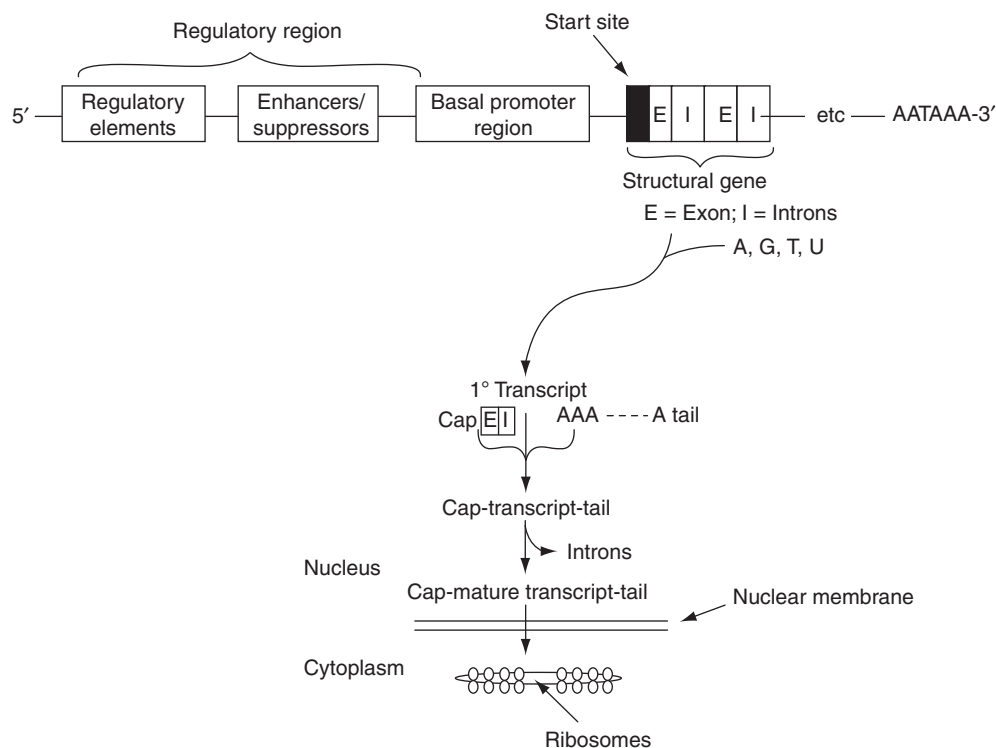


Figure 2 Transcription: The synthesis of messenger RNA and its migration into the cytosol.

initiation. The transacting TBP binds to the cis-acting TATA box and to a large complex of basal transcription factors, plus RNA polymerase II. Polymerase II is the enzyme that catalyzes the formation of mRNA. Some of the transcription factors bind specific nutrients and it is here that some nutrients play a role in gene expression. Elongation is the actual process of RNA formation through the use of a DNA template in the 5'-to-3' direction. After elongation, the 5'-end of mRNA is capped by 7-methylguanosine triphosphate. This cap stabilizes the mRNA and is necessary for processing and translation. After the processing and editing step, the mRNA chain is terminated.

The mRNA is much larger when it is synthesized than when it migrates out into the cytosol. This is because the initial messenger also contains bases that correspond to the noncoding regions of the DNA (the introns) and these have to be removed. The removal of these segments is a cut-and-splice process, whereby the intron is cut at its 5' end, pulled out of the way, and cut again at its 3' end; at the same time, the two exons are joined. This cut-and-splice routine is continued until all the introns are removed and the exons are joined. Some editing of the RNA also occurs, with base substitutions made as appropriate. In addition, not all of the mRNA is needed to code for new protein synthesis. The extra mRNA is degraded and its bases are either degraded or recycled for other use. Finally, a 3' terminal poly A tail is added.

This outline of transcription has omitted a number of important details with respect to transcription control. The regulation of transcription often occurs through the regulation of transcription factors. These factors can be regulated by the rates of their synthesis or degradation or by phosphorylation/

dephosphorylation or by ligand binding as well as by cleavage of a protranscription factor or by release of an inhibitor. For example, the regulation of the transcription of certain genes is exerted by a group of proteins that determine which region of the DNA is to be transcribed. Cells contain a variety of sequence-specific DNA-binding proteins. Nutrients can bind to these proteins and exert their effect in this way. These proteins are of low abundance and they function by binding to specific regions (elements) on the DNA. The regions are variable in size but are usually between eight and 15 nucleotides. Depending on the binding protein and the nutrient bound to it, transcription is either enhanced or inhibited and indeed cell types may differ because of these proteins. Because all cells contain the same DNA, gene expression in discrete cell types is controlled at this point simply by the binding of these very specific DNA-binding proteins. Thus, genes for the synthesis and release of insulin, for example, could be turned on in the pancreatic β cell, but not in the myocyte, simply because the β cell has the required specific DNA-binding protein(s) or regulatory protein that the myocyte lacks. At some point in differentiation, the myocyte did not acquire this regulatory factor and thus cannot synthesize and release insulin.

In many instances, specific DNA-binding proteins contain zinc bound to four cysteine residues. The zinc causes a folding of the protein around the DNA strand. These portions of the zinc-containing protein are referred to as zinc fingers. Gene expression is regulated by the formation of these zinc fingers, and yet they are only a part of this regulation. Most genes are regulated by a combination of regulatory factors. There may be a 'master' regulatory protein that serves to coordinate the

binding of several 'lesser' proteins. This is important for the coordinate expression of genes in a single pathway, as occurs, for example, in the expression of the four genes that encode the four enzyme complex, fatty acid synthetase. As mentioned, transcription is regulated by both the nearby upstream promoter region and the distant enhancer elements. The upstream enhancer element can include a TATA box that extends for approximately 100 bp. Enhancer fragments further upstream can bind multiple proteins, which, in turn, can influence transcription. These factors are proteins and are labeled JUN, AP2, ATF, CREB, SP1, OTF1, CTF, NF1, steroid response element (SRE), and others.

One well-studied group of DNA-binding proteins is that which binds the steroid hormones and related compounds. These proteins are members of the steroid super family of receptor proteins. They bind to specific base sequences called SREs. Included in this super family are receptors for retinoic acid (the gene active form of vitamin A), fatty acids, vitamin D, the glucocorticoids, the sex hormones, and the thyroid hormones. These receptors can bind together (called dimerization) to regulate gene expression and their activities are regulated by ligand binding. The ligands are the above-named compounds. All members of this family of DNA-binding proteins contain two zinc fingers in their DNA-binding domains. Each receptor protein consists of approximately 100 amino acids and zinc. As mentioned, they recognize a specific DNA sequence. For this family of proteins, the transcription-enhancing domain is localized at the amino terminus of the polypeptide chain. This is the part of the molecule that binds to the promoter region of the DNA and gives the protein gene specificity. At the carboxy terminus is the binding site for the ligand. Members of this family of compounds exert their effects through binding to their cognate receptors that in turn bind to the hormone response element on the DNA. Although zinc is an essential part of the receptor protein, it does not play a regulatory role. There are other transcription factors in which zinc does play a regulatory role. One of these is the metal response protein metal-binding transcription factor-1 (MRE-1) that binds to the metal response element (MRE). In this situation, MRE-1 increases in response to increases in zinc concentrations within the cell by translocating to the nucleus and activating the transcription of genes containing MREs in their promoter region. One well-described protein is the metallothionein that binds zinc and that plays an important role in zinc homeostasis.

There are other examples of effects of nutrients on gene transcription. The direct binding of a nutrient to a transcription factor is a simpler way to explain how nutrients can affect

gene expression. There are other less direct but equally important mechanisms. For example, it has been shown that supplementation with the vitamin biotin increases the synthesis of transcription factors Sp1 and Sp3, enhancing the transcriptional activity of Sp1/Sp3-dependent genes. Another mechanism of biotin-dependent gene expression is increased phosphorylation of 'inhibitor of NF- κ B' in the cytoplasm. Phosphorylation of inhibitor of NF- κ B causes its dissociation from NF- κ B in the cytoplasm. Subsequently, NF- κ B is shuttled to the cell nucleus, where it binds to regulatory regions in genes, mediating the transcriptional activation of these genes. Other examples are listed in [Table 1](#).

The metabolism and availability of macronutrients also influence gene transcription. Promoter elements have been described that allow a response to glucose (a carbohydrate response element). There is also an element that responds to cholesterol. Genes involved in cholesterol homeostasis are characterized by a cholesterol response element in their promoter regions that interacts with a sterol response-binding protein (SREBP). This protein is synthesized as a large precursor molecule that is stored in the endoplasmic reticulum membrane. It is unavailable to function in gene regulation until it is cleaved and released. Limited cholesterol availability results in cleavage and release from this membrane and translocation to the nucleus. Once in the nucleus, it activates the transcription of genes that encode the enzymes for cholesterol synthesis and for the LDL receptor. When there is a mutation in these genes (one or more), hypercholesterolemia results. The liver is unable to remove cholesterol from the circulation and continues to synthesize it as the SREBP remains active.

Nutrients can also affect gene expression indirectly by influencing the release of hormones that are gene active. Again, glucose serves as an example. It acts not only through its binding to the carbohydrate response protein binding to the carbohydrate response element but also through the stimulation of the release of insulin. Insulin in turn exerts effects on the transcription of a variety of genes.

Posttranscriptional regulation of gene expression is the next stage of control. As mentioned above, newly formed mRNA is edited before leaving the nucleus. RNA transcription can be terminated prematurely with the result of a smaller than expected gene product. A single mRNA can be translated into several different gene products, usually peptides. These proteins or peptides may have comparable or opposing functions depending on the products in question. As described, mRNA is edited and processed such that only approximately 5–10% of this RNA leaves the nucleus. The RNA that leaves the nucleus does so through pores in the nuclear

Table 1 Examples of nutrient effects on gene expression

<i>Nutrient</i>	<i>Intermediary protein</i>	<i>Gene/gene product</i>	<i>Effect</i>
Cholesterol	SREBP	LDL receptor	Suppresses transcription
Fatty acid	Peroxisome proliferator activated receptor	Fatty acid-binding protein	Increases transcription
Iron	Iron-regulatory protein	Ferritin	Increases transcription
Vitamin A	Retinoic acid receptor	Collagenase	Decreases transcription
Vitamin D	Vitamin D receptor	Calcium-binding proteins	Increases transcription
Vitamin K	Prothrombin	Carboxylated prothrombin	Increases calcium binding

membrane. Not all of the mRNA that exits the nucleus is immediately translated into protein.

Mitochondrial Gene Expression

Mitochondrial gene expression is similar to nuclear expression in several respects. Transcription is responsive to some of the same nutrients and hormones that affect nuclear transcription. Both genomes require binding proteins but it seems that each compartment has its preferred binding form. Unlike the nuclear genome, where each gene has its own promoter region, all of the genes in the mitochondrial genome have a common promoter found in the D-loop. In contrast to nuclear gene transcription and translation that involves both the nuclear and the cytoplasmic compartments, mitochondrial transcription and translation occur totally within the mitochondrial compartment. It is affected by a wide variety of nuclear-encoded transcription factors and nutrients.

Translation

Once the mRNA has migrated from the nucleus to the cytoplasm and attaches to ribosomes, translation is ready to begin. Translation is the synthesis of the protein using the order of the assemblage of constituent amino acids as governed by the mRNA. All of the amino acids needed for the protein being synthesized must be present and attached to a transfer RNA (tRNA). These tRNA–amino acids dock on the mRNA again, using base pairing, and the amino acids are joined to one another via the peptide bond. The newly synthesized protein is released as it is made on the ribosome and changes to its conformation and structure occur. These changes depend on the constituent amino acids and their sequence.

Translation is also influenced by specific nutrients. The translation of the ferritin gene, for example, is influenced by the amount of iron available in the cell. In iron deficiency, the mRNA start site for ferritin translation is covered up by an iron-responsive protein. This protein binds the 3′-untranslated region (UTR) and inhibits the movement of the 40 s ribosome from the cap to the translation start site. When iron status is improved, the start site is uncovered and translation can proceed. The actual site of translation is on the ribosomes; some ribosomes are located on the membrane of the endoplasmic reticulum and some are free in the cell matrix. Ribosomes consist almost entirely of ribosomal RNA and ribosomal protein. RNA is synthesized via RNA polymerase I in the cell nucleus as a large molecule; in this location, this RNA molecule is cleaved and leaves the nucleus as two subunits: a large one and a small one. The ribosome is reformed in the cytoplasm by the reassociation of the two subunits; the subunits, however, are not necessarily derived from the same precursor.

Ribosomal RNA makes up a large fraction of total cellular RNA. It serves as the ‘docking’ point for the activated amino acids bound to the tRNA and the mRNA that governs the amino acid polymerization sequence. Transfer RNA is used to bring an amino acid to the polysome, the site of protein synthesis. Polysomes are clusters of ribosomes that are

associated with the endoplasmic reticulum. Each amino acid has at least one specific tRNA. Each tRNA molecule is thought to have a cloverleaf arrangement of nucleotides. With this arrangement of nucleotides, there is the opportunity for the maximum number of hydrogen bonds to form between base pairs. A molecule that has many hydrogen bonds is very stable. Transfer RNA also contains a triplet of bases known, in this instance, as the ‘anticodon.’ The amino acid carried by tRNA is identified by the codon of mRNA through its anticodon; the amino acid itself is not involved in this identification.

Translation takes place in four stages, as illustrated in **Figure 3**. Each stage requires specific cofactors and enzymes. In the first stage, which occurs in the cytosol, the amino acids are activated by esterifying each one to its specific tRNA. This requires a molecule of ATP. In addition to a specific tRNA, each amino acid requires a specific enzyme for this reaction.

During the second stage, the initiation of the synthesis of the polypeptide chain occurs. Initiation requires that mRNA binds to the ribosome. An initiation complex is formed by the binding of the mRNA cap and the first activated amino acid–tRNA complex to the small ribosomal subunit. The ribosome finds the correct reading frame on the mRNA by ‘scanning’ for an AUG codon. The large ribosomal unit then attaches, thus forming a functional ribosome. A number of specific protein initiation factors (eIFs) are involved in this step.

In the third stage of protein synthesis, the peptide chain is elongated by the sequential addition of amino acids from the tRNA complexes. The amino acid is recognized by base pairing of the codon of mRNA to the bases found in the anticodon of tRNA and a peptide bond is formed between the peptide chain and the newly arrived amino acid. The ribosome then moves along the mRNA; this brings the next codon in the proper position for attachment of the next activated amino acyl–tRNA complex. The mRNA and nascent polypeptide appear to ‘track’ through a groove in the ribosomal subunits. This protects them from attack by enzymes in the surrounding environment.

The final stage of protein synthesis is the termination of the chain. The termination is signaled by one of three special codons (stop codons) in the mRNA. After the carboxy terminal amino acid is attached to the peptide chain, it is still covalently attached to tRNA, which is, in turn, bonded to the ribosome. A protein-release factor promotes the hydrolysis of the ester link between the tRNA and the amino acid. Once the polypeptide chain is generated and free of the ribosome, it assumes its characteristic three-dimensional structure. At this point, some posttranslational modifications can occur.

If, during the course of translation, there is any interference in the continuity of a supply of the needed amino acids, translation is stopped. Because protein biosynthesis is very costly in terms of its energy requirement, synthesis is severely inhibited by starvation or energy restriction. In experimental animals, it has been shown that starvation inhibits the polymerization of mRNA units, thus significantly reducing the activity of the transcription process. Other studies have shown that animals starved and then refed ‘overcompensate’ for this period of reduced mRNA synthesis by markedly increasing mRNA synthesis above normal during the period of

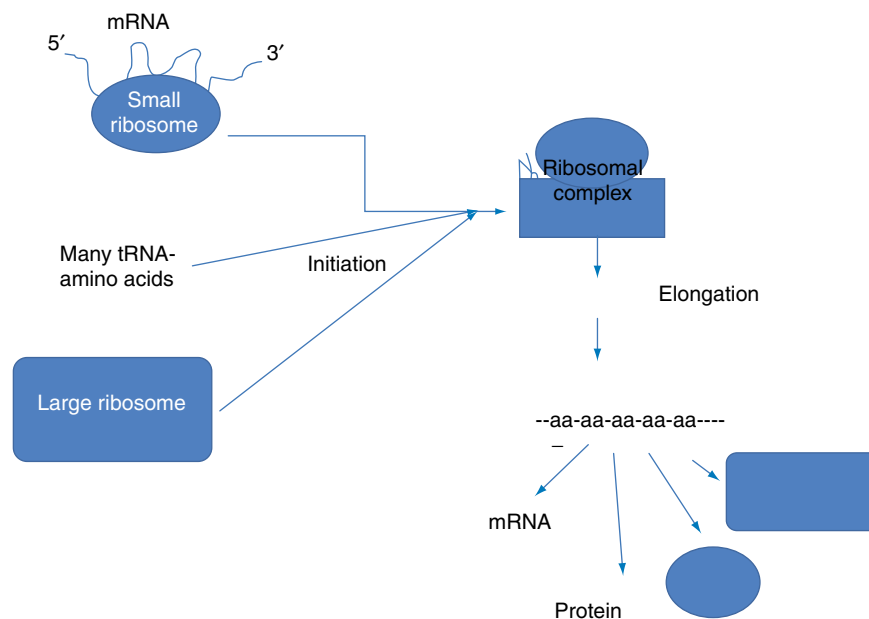


Figure 3 Translation uses mRNA as a template for the sequence of amino acids in the synthesis of the designated protein.

realimentation after the starvation period. This 'starvation-refeeding'-induced increase in mRNA is manifested as an increase in the synthesis of enzymes necessary for the metabolism of the various ingredients in the diet used for realimentation. The signal(s) for the release of the starvation-induced inhibition of mRNA and enzyme synthesis include the macronutrients in the diet as well as hormones such as the glucocorticoids, thyroid hormone, insulin, and others.

Posttranslational Protein Modification

After translation, the primary amino acid sequence is complete. The secondary and tertiary structure of the protein evolves via numerous interactions between the amino acids via hydrogen bonding, disulfide bridges, and ionic bonds. The newly synthesized proteins can be further modified via post-translation reactions. Posttranslational protein modification includes the association of the various subunits of an enzyme for example. Another example is the cleavage of a leader sequence of a mitochondrial protein that allows it to migrate into the mitochondria. This leader is removed as the oxidative phosphorylation system is assembled or as this protein functions within the mitochondrial compartment. A third example is the posttranslational carboxylation of prothrombin. This protein contains a large number of glutamic acid residues. In the presence of vitamin K, these residues are carboxylated, and this posttranslational change results in a drastic increase in the calcium-binding capacity of the resultant protein. Unless prothrombin can bind calcium, it cannot function in the clotting process. This is another example of how a nutrient can affect gene expression: in this instance, the expression

of functional prothrombin. The site of the nutritional effect is that of posttranslational protein modification.

As can be seen from the foregoing, nutrients affect gene expression in a variety of ways. Simply knowing the gene sequence is not enough to predict how a specific nutrient or a nutritional state can affect its expression. This is a complicated process, the details of which are gradually being elucidated.

See also: Biotin: Physiology, Dietary Sources, and Requirements. Carbohydrates: Regulation of Metabolism. Cholesterol: Factors Determining Blood Levels. Fatty Acids: Metabolism. Folic Acid. Iron: Physiology, Dietary Sources, and Requirements. Nutrient–Gene Interactions: Health Implications. Pantothenic Acid. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. Vitamin D: Physiology, Dietary Sources, and Requirements. Vitamin K. Zinc: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements

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NUTRIENT REQUIREMENTS

International Harmonization

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Glossary

Acceptable Macronutrient Distribution Range

(AMDR) Reference values or ranges for macronutrients; upper, lower, and/or mean percentages of energy from individual macronutrients as a percent of total energy intake.

Adequate Intake (AI) A value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of healthy people.

Dietary exposure A quantitative estimate of the intake of a nutrient or chemical present in an individual's diet by determining through dietary records or direct observation the amount of the foodstuff consumed and, based on the analyzed content of the nutrient or chemical in a sample of the foodstuff, estimating the total amount, usually reported as an amount per day. This is the same as Dietary Intake.

Dietary Reference Intake (DRI) A set of nutrient-based reference values, each of which has special uses.

Essential or indispensable nutrients Those constituents in food without which an individual or organism cannot function – death ensues.

Estimated Average Requirement (EAR) The daily intake value that is estimated to meet the requirement, as defined by the specified indicator or criterion of adequacy, of half of the apparently healthy individuals in a life stage or gender group.

Lipid-soluble A method by which a foodstuff can be prepared for chemical analysis; the food is placed in a solution with a chemical (such as ethanol) which can dissolve fats and oils (lipids) and separate them from the

water-soluble fraction and dry matter for subsequent analysis.

Recommended Dietary Allowance (RDA) The average daily dietary intake level that is sufficient to meet the nutrient requirements of nearly all (97–98 percent) healthy individuals in a particular life stage and gender group.

Reference value A quantitative amount of a daily intake of a nutrient or food component that is related to a specific aspect of nutritional status; includes values that are goals for intake as well as amounts to not exceed.

School lunch requirements Many national programs which provide food or money to purchase food have some type of nutritional guidelines or standards for the foods provided that must be met in order to sell or obtain the food; a major program in the USA is the Child Nutrition Programs, which include school breakfast and school lunch.

Standard deviation An estimate of the variability of a factor or item from other units – the greater the deviation, the greater the variability in response or items.

Statistical independence The characterization of one factor has no relation to or influence on the characterization of a second factor. Studies have shown that people eat when hungry (thus dependent), but do not chose a balanced diet when in a blinded study (independent).

Tolerable Upper Intake Level (UL) Highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all apparently healthy individuals in the specified life stage group. As intake increases above the UL, the potential risk of adverse effects may increase.

Determining human requirements for nutrients has been a major activity for nutritionists, biochemists, and physiologists for over a century since the advent of methods that have allowed for their isolation, quantification in food, and determination of their function in cell and whole body metabolism. Although initial efforts focused on identifying constituents in food required to maintain life and promote growth and thus considered essential or indispensable, research over the last few decades has increasingly focused on other bioactive components found in foods, elucidating the

specific roles each play in health, and then quantifying, through experimentation and study of healthy populations, the amounts needed on a daily basis to provide for optimal health and prevent disease. This process of estimating requirements for an individual with any level of precision is still in the early stages of development. Nevertheless, many facets of maintaining and improving the health of the public hinge on knowing how much is needed of which nutrients or chemical components of food, and how this differs at different stages of growth and development.

Multiple terms have been adopted to define nutrient requirements, allowances, or standards (Table 1). They have been established or adopted by various countries and then used for the major functions of planning food programs or of assessing diets for adequacy or excess (Figure 1). Major efforts over the last three decades by nutrition scientists throughout the world have resulted in a shift from establishing and periodically revising nutrient allowances or recommendations based on general consensus of adequate levels (such as the Recommended Dietary Allowances (RDAs) of the Food and Nutrition Board of USA, the Recommended Nutrient Intakes of Canada and UK, or the Safe Levels of Intake derived by the Expert Groups convened by the World Health Organization (WHO) and Food and Agriculture Organization (FAO) of the United Nations) to more definitively anchoring the reference values to specific, well-described scientific studies so that when new information becomes available from research, it is clear that new evaluations need to be undertaken. For example, the

RDAs in the USA have been used as the reference values in many situations, from setting the standards for nutrient content in programs that provide single meals, such as in school lunch programs, to the basis for government reimbursement for costs of care in skilled nursing homes (Table 2). It is not surprising that one reference value or number, even when adjusted for age or body size and based on scientific studies, is at times not appropriate for the situation in which it is used.

What is a Nutrient?

The traditional approach to establishing the human essentiality of a nutrient is to show that it can be chemically isolated from foods and can improve or remove a deficiency sign resulting from its lack in the diet. The number of required or essential nutrients defined in this way has grown over the years (Table 3).

Table 1 Definitions of reference nutrient values used by selected countries and groups

- **Overall term:** Used to describe a set of reference values (countries/groups listed in order of adoption):
 Dietary Reference Values (DRV): United Kingdom and European Community
 Dietary Reference Intakes (DRI): USA and Canada, South Korea, and Japan
 Nutrient Reference Values, Valores nutrimentales de referencia (NRV): FAO, Australia and New Zealand, Mexico, and Codex
 Reference Values for Nutrient Intake (RVNI): Germanic countries and Slovenia
 Nutrient Intake Values (NIV): 2005 UNU/FAO/WHO harmonization
- **Average (Median) Requirement:** The daily intake value that is estimated to meet the requirement, as defined by the specified indicator or criterion of adequacy, of half of the apparently healthy individuals in a life stage or gender group
 Average Requirement (AR): European Community
 Estimated Average Requirement (EAR): UK, USA and Canada, Chinese Nutrition Society, South Korea, Australia and New Zealand, and Japan
 Recommended Daily Nutrient Intake (RDNI): Nordic Countries
 Promedio de los requerimientos nutrimentales (Nutrient Requirement for 50% of individuals) (RN₅₀): Mexico
 Average Nutrient Requirement (ANR): 2005 UNU/FAO/WHO harmonization
- **Recommended Intake for an Individual:** Expected to meet the needs of almost all (97% or 98%) individuals in the specified population group: based on the average (median) requirement plus twice the standard deviation of the requirement (=AR + 2SD)
 Recommended Dietary Allowance (RDA): Canada and USA, Southeast Asia, and Japan
 Population Reference Intake (PRI): European Community
 Recommended Nutrient Intake (RNI): Chinese Nutrition Society, 2002 WHO/FAO Expert Consultation on Vitamins & Minerals, and Philippines
 Reference Nutrient Intake (RNI): UK
 Recommended Daily Intake (RDI): Australia and New Zealand
 Recommended Intake (RI): South Korea
 Ingestión diaria recomendada (Daily Recommended Intake) (IDR): Mexico
 Individual Nutrient Level_x (INL_x; where X=97.5%): 2005 UNU/FAO/WHO harmonization
- **Reference Intake for an Individual when Data Inadequate:** provided if accurate data or evidence is not available for distribution of nutrient requirements to obtain median and standards deviation, and therefore, the recommended intake for an individual cannot be calculated
 Adequate Intake (AI): A value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of healthy people. The AI for children and adults is expected to meet or exceed the amount needed to maintain a defined nutrition state or criterion of adequacy in essentially all members of a specific apparently healthy population:
 Canada and USA, Chinese Nutrition Society, South Korea, Australia and New Zealand, Japan, European Union
 The Netherlands: An amount of the nutrient that provides for the needs of almost all those in the group
 Ingestión diaria sugerida (Daily Suggested Intake) (IDS): Mexico
 Estimated Values (Schatzwerte; for known nutrients): Germanic Language countries and Slovenia
 Safe Intake (A level or range of intakes below which there is a risk of undesirable effects): UK; 2005 UNU/FAO/WHO harmonization
- **Lower Reference Intake:** An amount of the nutrient that is enough for only the few people in a group who have low needs
 Lower Reference Nutrient Intake (LRNI): UK
 Lower Threshold Intake (LTI): European Union
- **Upper Level of Intake:** The maximum level of chronic intake of a nutrient judged to be unlikely to pose a risk of adverse health effects to humans
 Tolerable Upper Intake Level (UL): Canada and USA, Chinese Nutrition Society, South Korea, Japan, Southeast Asia, European Union, The Netherlands
 Safe Upper Level (SUL): UK (an intake level that can be consumed daily over a lifetime without significant risk to health on the basis of available evidence)
 Limite superior de consumo (Upper Limit of Consumption) (LSC): Mexico
 Upper Nutrient Level (UNL): 2005 UNU/FAO/WHO harmonization

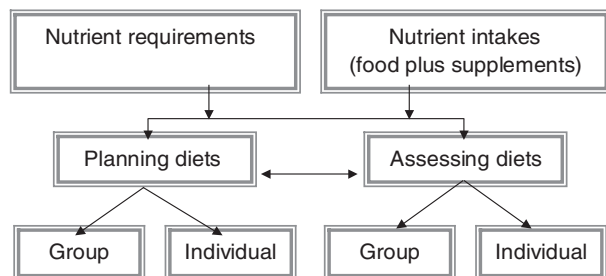
(Continued)

Table 1 Continued

- **Reference Values or Ranges for Macronutrients:** Upper, lower, and/or mean percentages of energy from individual macronutrients as a percent of total energy intake, representing dependence on other energy sources as well as the total energy requirement of the individual, to decrease risk of chronic disease
- Acceptable Macronutrient Distribution Range (AMDR): UK; Canada and USA; South Korea; Australia and New Zealand
- Adequate Intake to Promote Health (AI): Chinese Nutrition Society
- Reference Intake Range (RI): European Union
- Population Goal (Mean intake for the population as a percent of energy): WHO, 2003
- Dietary Goal (DG: quantity for primary prevention of lifestyle-related diseases): Japan

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**Figure 1** Uses of reference intakes in planning and assessing diets.

During the last three decades, as a result of scientific inquiry and experimentation, the line between nutrients that might be considered essential *versus* nonessential has blurred. There are few new food components or chemicals in foods that, when identified, have been shown to cause severe dysfunction or death when removed from the diet in a similar manner to many of those listed in [Table 3](#). However, many chemical constituents of food have been shown to contribute to health; while controversy exists on whether such dietary components are required nutrients, some are gaining increased recognition as playing important roles in health – for example, choline. The major difference brought on by modern scientific techniques is the ability now to detect finer gradations of inadequacy tied to intake and metabolism of specific

Table 2 Pre-1997 uses of RDAs in USA

- Planning for feeding groups of healthy people (school lunch; elderly feeding programs)
- Nutrient goals for healthy individuals
- Basis for foods provided in supplemental feeding programs (e.g., WIC in USA)
- Procurement of and purchasing food supplies for groups of healthy persons
- Reference point for evaluating the dietary intake of population subgroups
- Nutrient targets in intervention programs
- Basis of food groups in food and nutrition education programs
- Reference point for the nutrition labeling of food and dietary supplements
- Basis for fortification of food products
- Basis for formulating dietary supplements and special dietary foods
- Standards for menu planning for hospitals, correctional facilities, military operations, institutional feeding settings

food components resulting in not necessarily death or severe organ dysfunction but decline in health status or inability to function optimally. It could be said that there is merely a longer latency period than with typical nutrient deficiencies or excesses before the effect becomes manifested; such a situation may well characterize the typical diet-related chronic disease – most chronic diseases are multifactorial, in which other nutrients, genetics, and environment play major roles. An

Table 3 Nutrients for which quantitative RDAs and recommended intakes or ranges (in parentheses) have been established in the USA since 1941

Nutrient	1941	1989	1997–2004
Calories	X	X	X
Protein	X	X	X
Calcium	X	X	X
Iron	X	X	X
Vitamin A	X	X	X
Thiamin	X	X	X
Riboflavin	X	X	X
Nicotinic acid	X	X	X
Ascorbic acid	X	X	X
Vitamin D	X	X	X
Vitamin K		X	X
Vitamin B ₆		X	X
Vitamin B ₁₂		X	X
Folate		X	X
Pantothenic Acid		(X)	X
Biotin		(X)	X
Choline			X
Chromium		(X)	X
Copper		(X)	X
Fluoride		(X)	X
Iodine		X	X
Magnesium		X	X
Manganese		(X)	X
Molybdenum		(X)	X
Phosphorus		X	X
Selenium		X	X
Zinc		X	X
Potassium		(X)	X
Sodium			X
Chloride			X
Total water			X
Carbohydrate			X
Total fiber			X
Linoleic acid (<i>n</i> -6)			X
α -Linolenic acid (<i>n</i> -3)			X

example of this is the role of vitamin E in decreasing onset of cardiovascular disease. Demonstrated to be effective in animal studies, large-scale studies in humans have so far not documented the expected positive effects of vitamin E supplementation on primary prevention of the specific chronic disease.

Scientific Basis for Establishing Recommended Intakes

Since the initial development of quantitative recommended intakes of nutrients in the 1930s and 1940s, new approaches have provided a stronger scientific base to the reference values so established. Early development of recommended intakes usually involved convening a group of scientists who considered the available literature and, based on their expert judgment, developed quantitative estimates of requirements for specific subpopulation groups by age and gender. Newer

statistically supported methods now allow for a more science-based approach to such deliberations to achieve consensus.

A number of factors must be present before quantitative requirements for nutrients can be made most useful to those who use such estimates for program planning and evaluation.

- There must be some understanding of the chemical. For example, in early work on vitamins, an isolated fraction of cod liver oil was determined to be required for normal eye growth and bone development, and was named ‘vitamin A.’ Subsequent isolation and characterization allowed the isolated mixture to be further separated into what was called at the time the fat-soluble factor for bone growth compared with another required for sight. Thus, vitamin A was differentiated from vitamin D in the lipid-soluble fraction.
- There must be data on how much is present in the diet. To obtain these data, the content of the nutrient or food component in multiple samples of typical foods must be analyzed, which thus allows the data to be used to estimate intake or exposure.
- There should be some idea of intake among the population groups of interest. Studies in which known amounts of the nutrient are consumed at varying levels and evidence of inadequacy detected should be conducted. This is typically done first with animal models, followed by human clinical trials or metabolic studies, which include at least one level of intake at which effects of inadequacy are observed that can be linked directly to the nutrient under study. Frequently, it is not possible to remove or add some nutrients to a diet without altering the content of other nutrients; this is particularly true for energy-yielding nutrients such as ω -3 fatty acids, or substances such as fiber. This makes the interpretation of the resulting data less clear.

Adequate for What?

Usually, once these data are known or have been estimated, it becomes possible to establish an intake recommendation, initially based on observations of how much appears to prevent the deficiency and how much is in the diet of those not demonstrating the symptoms or signs (indicators) of inadequacy. Many of the earlier recommended intakes were established on this basis, which is why, in many cases, the values may vary greatly across expert groups and countries. As additional data derived from experiments, observations of intake, and consequences of inadequacy of a nutrient in the diet are generated, periodic updates of nutrient requirements and recommended intakes are released (Table 4). Changing recommendations often result in the need to make changes to programs and activities, such as school lunch requirements or food labeling. Of great importance from a programmatic perspective in evaluating the need for change is the specific statement of the goal of the derived reference value: Will the reference value provide guidance for minimizing overt deficiencies in a vulnerable population group usually by providing enough to prevent a known deficiency sign or symptom, or is it set at a dietary level required to maintain a

Table 4 Changing US recommendations for nutrients: RDAs for vitamins (adult males, moderately active)

Vitamin	1941	1943	1945	1948	1953	1958	1968	1976	1980	1989	1997–2010
Vitamin A (mg RE)	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	900 ^a
Vitamin D	400 IU ^b	400 IU ^b	^c	^c	^c	^c	400 IU	400 IU	5 µg	5 µg	10 µg
Vitamin E							30 IU	15 IU	10 IU	10 mg	15 mg ^d
Vitamin K (µg)										80	120 ^e
Vitamin C (mg)	75	75	75	75	75	75	60	45	60	60	90
Thiamin (mg)	1.8	1.8	1.5	1.5	1.5	1.6	1.3	1.4	1.4	1.5	1.2
Riboflavin (mg)	2.7	2.7	2.0	1.8	1.6	1.8	1.7	1.6	1.6	1.7	1.3
Niacin (mg)	18	18	15	15	15	21	17	18	18	19	16
Vitamin B ₆ (mg)						1–2 ^f	2.0	2.0	2.2	2.0	1.3
Pantothenic acid (mg)										4–7 ^f	5 ^e
Biotin (mg)										0.03–0.1 ^f	0.03 ^e
Folate (µg)						500 ^f	400	400	400	200	400 ^g
Vitamin B ₁₂ (µg)							3.0	3.0	5.0	2.0	2.4

^aUnit changed from RE (Retinol Equivalent) to RAE (Retinol Activity Equivalent).^bWhen not exposed to sunshine (400 IU ≈ 10 µg).^cSmall amount needed when not exposed to sunshine.^dAs α-tocopherol only.^eAdequate Intake (AI); not RDA.^fEstimate or range, no recommendation made.^gAs Dietary Folate Equivalents (DFE).**Table 5** Possible indicators or criteria that could be used to evaluate adequacy of iron intakes

Erythrocyte indexes
Erythrocyte protoporphyrin levels
Factorial modeling
Hemoglobin concentration and hematocrit
Iron balance studies ^a
Plasma total iron-binding capacity
Serum ferritin concentration
Soluble serum transferrin receptor levels
Serum transferrin saturation

^aBalance studies measure or estimate total excretion of a nutrient at different levels of intake and determine the lowest level at which intake = excretion.

blood concentration or function that might represent storage or a reserve, and thus be available in times of stress?

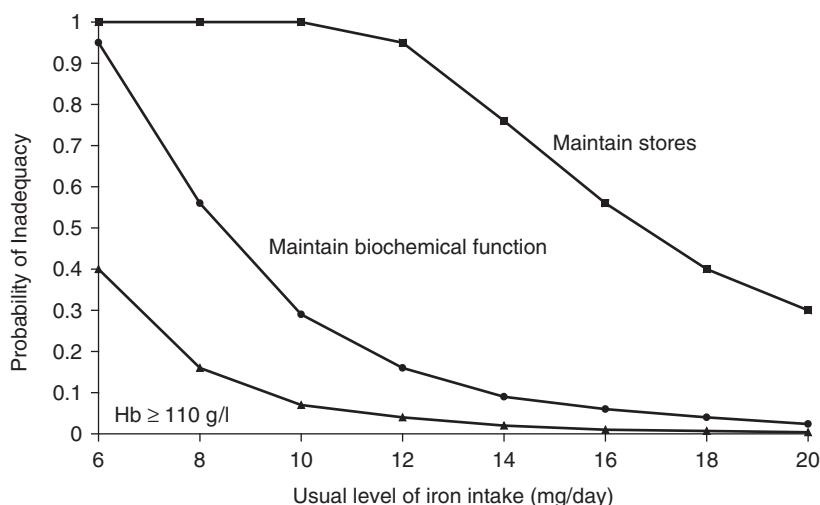
For example, the prevention of scorbutic gums, one of the signs of overt vitamin C deficiency, requires far less vitamin C on a daily basis than the amount needed to maintain 70% saturation of white blood cell ascorbate (vitamin C) levels to counteract potential oxidative stress and damage at a cellular level. Generally, for a nutrient, there exists a growing list of possible indicators or outcomes that could be used to estimate requirements (Table 5), and for each, a different amount may be needed daily for the specific indicator to meet the body's need and thus demonstrate adequacy.

There is usually a continuum of benefits that occurs as the level of intake increases. It becomes very important to define what the criterion(ia) is that has been used to establish the quantitative level of intake recommended. Figure 2 shows data relating iron intake to three possible criteria or indicators that could be used to determine adequate intakes (AIs) for women from a national survey in the Netherlands and analyzed by George Beaton. The data show that as the level of iron intake decreases, the number of individuals (or percentage of

the population group of women in this age group) who would have their needs met as documented by a given indicator of adequacy decreases. Thus, if prevention of anemia is used as the criterion (in this case, hemoglobin < 110 g l⁻¹), an individual whose intake averaged 6 mg day⁻¹ would have a 40% probability that the individual would be inadequate (i.e., the individual's hemoglobin value would be below the adequacy cutoff). However, if a biochemical marker of function of iron (e.g., total iron-binding capacity) were used, the level of intake needed for a 40% probability of being inadequate using that criterion would be approximately 9 mg day⁻¹. Finally, if the goal were to maintain a level of storage, such as ferritin concentration, the dietary level would need to approximate 18 or 19 mg day⁻¹. Thus, when comparing recommended intakes, it is critical to know specifically the criterion or criteria used in setting the recommended intake and evaluating adequacy.

Role of Estimates of Average (Median) Requirements

For many of the uses given for reference values (Table 2), it becomes important statistically to not depend on an allowance that would cover the needs of everyone and thus might include a safety factor added to some adequate level of intake but, rather, to apply estimates of the average requirement for the group of interest. For most nutrients, with iron a notable exception, it can be assumed that nutrient requirements are symmetrically distributed in a population of similar people (Figure 3). This means that some will have higher requirements than other similar individuals due to genetics and other factors. From research data on intake and the criterion used for adequacy, a median requirement intake level can be determined, such that consumption of a nutrient at that level would be adequate for half of the individuals in the group, but inadequate for the other half using the specific criterion chosen. When this distribution of requirements is



G. Beaton, 1994

Figure 2 Probability that specified usual iron intake would be inadequate to meet the needs of a randomly selected menstruating woman.

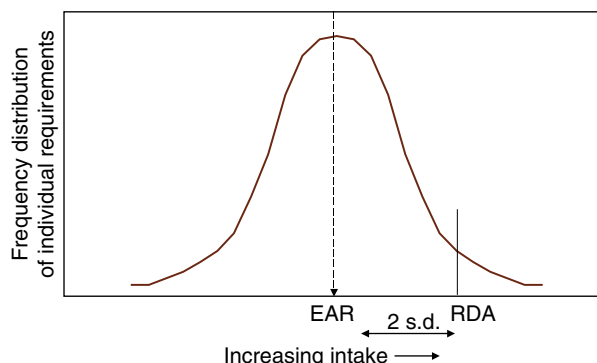


Figure 3 Probability distribution of individual nutrient requirements.

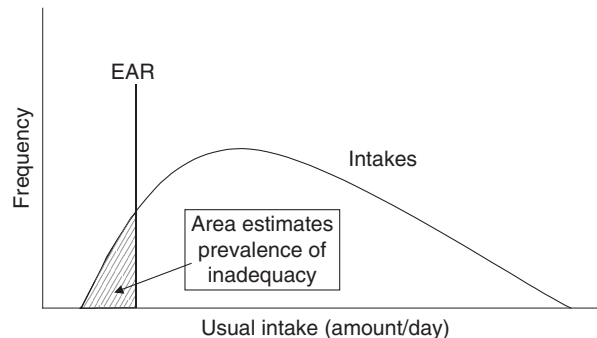


Figure 4 Using the EAR to estimate prevalence of inadequacy in a population from the distribution of nutrient intakes.

symmetrical, the median and the mean requirement are the same.

Why Have an Estimated Average Requirement?

There are two main reasons to determine an Estimated Average Requirement (EAR): To use as the basis for establishing the recommended intake for an individual and to assess the adequacy of intakes of similar population groups. The concept of establishing an average requirement, and assuming that the requirements of individuals in a population of similar people are symmetrically (or normally) distributed, is not new. Conceptually, it has served as the ideal basis for recommended intakes in most countries over the last few decades. However, it was rigorously used on only rare occasions. The RDA has been conceptually defined in the USA over the last few decades as the lowest amount of a nutrient that, in the judgment of the Food and Nutrition Board, meets the known nutritional needs of almost all of the population (subgroup), and it was also more mathematically defined as the mean requirement plus two standard deviations (SDs), which would equal an amount

required by 97% or 98% of the population to whom it is applied.

The Dietary Reference Intake (DRI) process – a joint effort of USA and Canada – retained the term RDA, limiting its use to serving as the goal for intake when planning diets for individuals and standardizing the method by which it is established. It is defined as follows: $RDA = EAR + 2SD_{EAR}$. When data on variation in requirements of a specific nutrient are lacking, it is assumed that the SD (variation) in requirements is approximately 10%. This variation in requirements (10%) is derived from the variation seen in basal metabolic rate in individuals and the variation seen in protein requirements, with protein being the nutrient whose variability has been most studied.

It has been demonstrated statistically that the prevalence of inadequacy in a population whose requirements are symmetrically distributed can be estimated by comparing its intake with the EAR for that nutrient in the same (or a similar) population (Figure 4). Thus, in the DRI process, when evaluating vitamin C requirements, experimental data from a clinical study indicated that the average intake for men needed to achieve 70% white blood cell ascorbate saturation (the

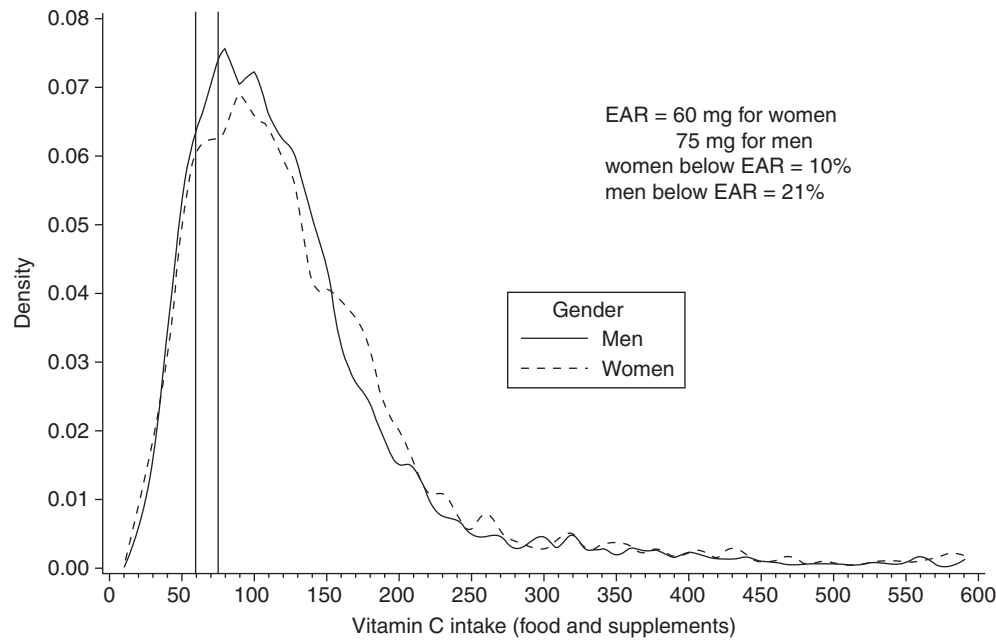


Figure 5 Vitamin C intake data from NHANES III for men and women; using the EAR to determine expected prevalence of inadequacy.

chosen indicator) was $\sim 75 \text{ mg day}^{-1}$, so the EAR was set at 75 mg day^{-1} . This is a value that can then be applied to the intakes of other similar populations of men who have similar characteristics to determine the percentage of the population who may be inadequate based on this criterion of adequacy (Figure 5).

To use this method to assess adequacy of other similar population groups, there are other basic statistical assumptions that should be met. First, the requirement for a nutrient of an individual in the population must be statistically independent of his or her intake for that nutrient (this does not hold for nutrients such as total energy or water – people eat or drink because they know they need energy or water). Second, the amount of variation (the distribution) in the nutrient intake levels in the population group must be greater than the variation in the group of the requirements for the nutrient (this is almost always the case, except where everyone in the group consumes the same food in the same amounts – thus there is little variability in intake). If these two assumptions are met, along with the symmetry mentioned previously, then the EAR can be used as the cut-point for adequacy in other similar populations (as shown in Figure 4). This is called the EAR cut-point method.

Because the RDA has been so misused as a tool to assess adequacy of intakes of groups in the past by policymakers and scientists alike, it has been argued by some that it is better for scientific panels of experts not to provide, in addition to EARs, any recommended intakes because their only use is to provide guidance to the individual, and health professionals can easily develop recommended intakes from reference values that are average requirements. More recently, it is recommended to provide individual recommended intakes that easily document the percent of the population covered, such as Individual Nutrient Level_x, where x = the percent of the population

covered (usually 2SD above the mean requirement, or 97% or 98%), and provide instructions for their specific and only use: to plan diets for the individual.

AI: Used When an EAR Cannot be Determined

Although for many nutrients, enough data exist to be able to establish levels of nutrient intake at which half of the individuals in a group would be inadequate based on the criterion chosen; for some nutrients, the necessary data may be conflicting or lacking. To give some guidance to users on nutrient reference values, it is still necessary to provide quantitative numbers. To further differentiate the appropriate uses of the RDA, the DRI framework provides an additional category of a recommended intake for use with individuals to plan diets – termed the AI. Other groups have advocated that this be called ‘safe intake.’ This is a level that is considered adequate for all members of the group and thus may overestimate the needs of many, if not all. Statistically, it cannot be used as if it were an EAR to assess adequacy. It does, however, provide guidance for how much an individual should consume. In some cases, it is derived from the average intake of a population in which inadequacy appears to be nonexistent based on review of available indicators or criteria (such as is the case for vitamin K).

Reference Values: Which to Use When

As mentioned previously, there are two main uses of reference values: to assess diets for adequacy or excess and to plan diets (Figure 1). Although these may seem to be the same, in many ways the best reference values to use in these situations may differ substantially from each other on a quantitative basis. In

addition, each of these major functions is frequently applied in two different situations: to a group's intake (i.e., the intake of a population or subpopulation), or to an individual's intake.

Using DRIs to Plan Diets

If the goal is to plan a diet or menu for a specific group so that the nutrient intake of all but a small number (e.g., 2% or 3%) in the group will have their needs met, it is not necessary for each person to consume at least the RDA; this actually overstates the need of almost all individuals. It is only necessary that the nutrient be consumed such that the intake of only 2% or 3% of those in the group would be below the EAR. Thus, the goal would be to have a very low percentage of intakes below the EAR (Figure 4).

However, if one is planning a diet for the individual, and there is little knowledge about the individual other than his or her gender and age, then one would want to provide what is thought to be adequate for almost everyone in the group, which is the RDA – set at 2SD above the median or average requirement (EAR) – or the AI.

Using DRIs to Assess Diets

Frequently, such as when considering whether to fortify the food supply with a specific nutrient or when evaluating the nutritional status of a subgroup in the population, it is necessary to assess the diets of groups through surveys of food intake and from such surveys determine which nutrients may be consumed at inadequate levels. If data on intakes for the group of interest are available, and the group possesses similar characteristics to the individuals studied when deriving the EARs, it is possible to estimate the prevalence of inadequacy in the group of interest from the intake data without information on their requirements or variation in intake.

This is a key reason for establishing EARs for nutrients, and it replaces the questionable past practice of comparing group intakes to the RDA. Frequently when this was done, a group might appear to be at low risk of inadequacy as the mean intake of the group as whole for a nutrient might be at or above the RDA, despite a sizable portion of the group being below their requirements, if they had been determined (Figure 6). Whether this occurred or not would depend on whether the RDA was based on the mean intake of a population in which no one was inadequate or whether the RDA came from data in which some members of the population had inadequate intakes and thus demonstrating one or more of possible criteria of inadequacy, which is usually not possible to determine. By using the EAR as the cutoff to determine the prevalence of inadequacy (this applies to those nutrients for which requirements are symmetrically or normally distributed), it is possible to set an acceptable level of inadequacy (for example, <2–3%) in situations of scarce resources where it is not possible to implement programs that will result in all consuming a level above the EAR and thus meet their nutrient needs.

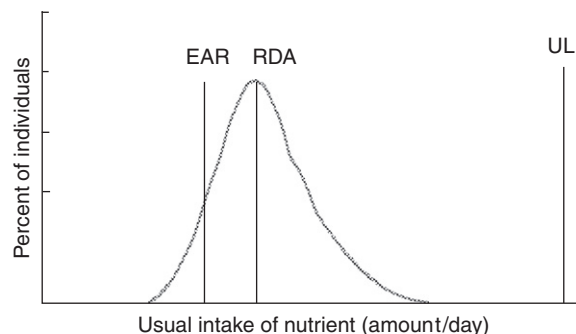


Figure 6 Use of the RDA to determine adequacy by comparing it to the average intake can allow an unacceptable prevalence of inadequacy (the percent of the population whose intakes are below the EAR will typically be greater than is considered acceptable).

DRIs for Other Nutrients and Food Constituents

As indicated previously, assumptions regarding variability and independence are involved in using EARs to estimate adequacy and to plan diets. When these cannot be followed, the Food and Nutrition Board's DRI framework included other categories of reference values to provide guidance for program planning and nutrition policy: the AI, the Estimated Energy Requirement (EER), and the Acceptable Macronutrient Distribution Range (AMDR). In the UK population averages along with minima and maxima for some energy-yielding nutrients have been established, whereas other countries have established population goals for guidance.

The EERs for use in the USA and Canada are derived from regression equations for adults and for children based on pooled data obtained from a group of international investigators. They represent the first time that energy expenditure (made by the technique of measuring doubly labeled water metabolism) was measured directly in individuals over 2 or 3 weeks for a large number of people rather than estimating the amount of time spent in various energy-requiring activities over a 24-h period and then multiplying each type of activity by indirect estimates of energy expended.

Reference values for macronutrients such as starch, fiber, other carbohydrates, various fatty acids, and other lipids such as cholesterol, are primarily related to the role each macronutrient plays in chronic disease development and risk factor reduction. As such, the data that support such reference values (AMDRs) are usually less definitive and definitely more complex than those for single nutrients that can be easily isolated and manipulated in the diet. This additional set of reference values is given as ranges to provide guidance to federal agencies and others related to nutrient intakes. The ability to identify and quantitate accepted risk factors for diseases is also important in reviewing literature to develop macronutrient ranges compatible with low risk of disease and maintenance of health.

Finally, physical activity in the form of recommended activity levels as part of energy requirements has been included in both WHO/FAO reports as well as the DRI series to highlight the very important role it plays in decreasing risk of chronic disease in terms of both maintaining sufficient energy expendi-

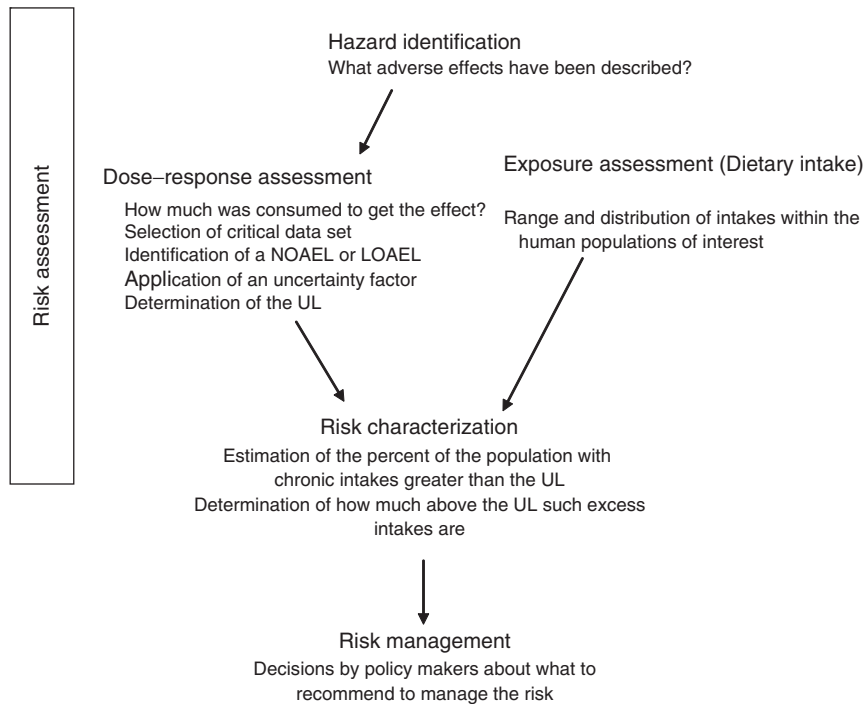


Figure 7 Steps in a model of risk assessment for nutrients.

ture to allow for maintenance of body weight and maintaining cardiovascular fitness to decrease the risk of heart disease.

Application of Risk Assessment Methodology to Nutrients

One of the many needs for reference values is to provide guidance about when intake of a nutrient may be too much: where the level of intake has the potential for an increased risk through excess consumption. In the past, this was rarely a concern, as it was difficult to consume, on a chronic basis, large enough amounts of a specific nutrient from foods to result in serious adverse effects.

Most adverse effects of overconsumption are self-limiting as they usually involve gastrointestinal disturbances (as is the case of dietary fiber) or involve objectionable and readily reversible effects (e.g., turning orange when consuming very high amounts of carotenoids from carotene-rich foods). However, instances of serious adverse effects have been reported in the last few decades due to overingestion of isolated nutrients or food constituents, typically in pill form and given in therapeutic doses, or through mistakes in fortification and enrichment of the food supply, but rarely from overconsumption of foods in their natural state.

Recent increases in demand for nutrients as a result of consumer interest in self-management of health, and provocative findings relating specific dietary constituents to possible health benefits, have provided incentives for manufacturers and food processors to increase the availability of nutrients and food components in dietary supplements and through the voluntary fortification of foods. Thus, the need for science-based reviews of data on the potential for increased risk of

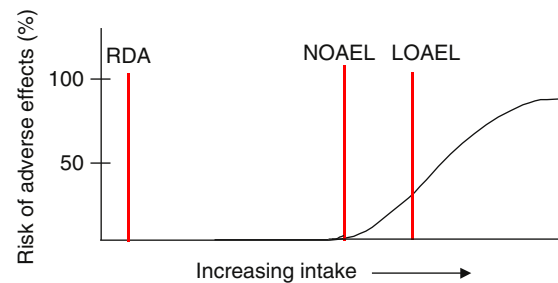


Figure 8 Identifying the hazard: dose response.

serious adverse effects that may result from chronic consumption of individual nutrients in higher amounts than typically encountered with foods has grown in importance. Such reviews have been conducted by Canadian and US scientists through the Food and Nutrition Board, by the UK's Expert Group on Vitamins and Minerals, and by the European Food Safety Authority, European Union, among others. Each has worked on developing approaches to evaluating reports of adverse effects and establishing, if possible, upper levels of intake (UL) for which little concern about risks of serious adverse effects may be expected. Although somewhat differing in the review of specific studies and in defining what might be considered serious, these efforts are all aimed at incorporating the basic components of toxicological risk assessment (Figure 7) in the review of nutrients, primarily from a qualitative perspective and on an individual nutrient-by-nutrient basis. In all cases, attempts are made to quantitate no-observed-adverse-effect levels (NOAELs), as well as lowest-observed-adverse-effect levels (LOAELs) of exposure, and then divide by an uncertainty factor, to obtain the upper reference level or limit (Figure 8).

Issues in Establishing Reference Intakes

Extrapolating Data to Other Life Stage and Gender Groups

Invariably, there is not enough information in studied populations to establish reference values directly for each subgroup. Knowledge of nutritional needs as well as response to higher levels of intake and exposure for such groups, such as during pregnancy or preadolescence, would be very useful. To provide adequate guidance when data are lacking, reference intakes are routinely obtained by extrapolating the available primary data to these important age or life stage groups from those subgroups for whom data are available. Consensus on the best methods to use for extrapolation when data are lacking, such as modeling or consideration of more sophisticated approaches than just based on body size or caloric expenditure, is needed to enhance the utility of the derived reference values.

Role of Nutrient Intake Surveys and Food Composition Databases

Surveys such as the National Health and Nutrition Examination Surveys (NHANES) and What We Eat in America Survey in the United States, the Dutch National Food Consumption Survey in the Netherlands, and the National Diet and Nutrition Surveys in UK serve as the underpinning for tracking changes in consumption and eating behavior of specific vulnerable population groups such as young children or the elderly, in order to evaluate the potential for targeted interventions, through programs aimed at changing eating behavior (e.g., the MyPlate Program, which recommends “make half your plate fruits and vegetables” in USA), through fortification of specific foods (e.g., calcium in bread in Canada), or through changes in food product formulation due to changes in labeling regulations (e.g., *trans*-fat in processed foods). The absence of surveys that link intake with health or quantifiable and validated disease indicators makes it almost impossible to determine risk of inadequacy as well as risk of excess, particularly in vulnerable groups, without very expensive laboratory tests and clinical observation.

Lack of analyzed nutrients in a variety of foodstuffs, as well as lack of valid intake data, decreases the utility of subsequent estimates of inadequacy or exposure. An issue that continues to hamper reliable estimates of intake is selective underreporting and overreporting of intakes of specific foods or portion sizes by responders in surveys, usually related to foods known to be related to causation of disease in the first case (underreporting) or considered more healthy in the second (overreporting). Although conducting large-scale surveys is costly and highly labor-intensive, poor collection of intake data and lack of replicate food composition information available to estimate intakes continue to hamper attempts to improve accuracy of the estimates. Much work is currently ongoing to increase the ability for such surveys to more accurately estimate intakes.

Approaches to Evaluating Bioactive Food Components

As new technologies, such as metabolomics, develop, which allow better understanding of cell metabolism and interaction

among nutrients within cell systems, food constituents that have previously gone unnoticed are gaining recognition of their potential roles in maintaining health and decreasing risk of chronic disease. Some food components appear to work in concert with other nutrients and chemicals and are highly active at nanogram concentration levels in cellular systems involved in decreasing inflammatory responses or cell death. These bioactive substances may be difficult to analyze in foodstuffs when they rapidly convert or oxidize into other less active compounds, making traditional methods of determining potential roles in health very difficult to apply. However, new technologies offer the opportunity to study not pathways, but integrated circuits of several systems and bioactive food components simultaneously, modeling from multiple perspectives rather than the typical linear relationships diagrammed in the metabolic pathways identified by the mid-twentieth century. Using these tools, the integrated nature and role of known and unknown chemical constituents of foods will form the basis for evaluating human nutritional requirements in the future.

Steps Toward International Consensus and Harmonization

The diversity of reference values from various countries or expert groups might mislead one to assume that there was significant variability in nutrient needs based on geographic location or genetic makeup. Many have observed that the differences are more due to the approaches taken in reviewing available data than in genetic or environmental factors. With growing consensus on how best to estimate human requirements using science-based approaches, and as new information regarding the roles genetics and environment play in disease becomes available, the variability seen in actual requirement estimates will diminish. There will continue to be a need to recognize and use information about nutrient bioavailability, which may well be different for diets based on different foods and staples and thus require different reference values for such varied situations, but human physiology is remarkably similar.

Harmonizing approaches to reviewing data and achieving consensus on terminology among scientists is an important first step to having truly borderless reference values that represent differences that are physiologically and genotypically related rather than culturally related. Following earlier efforts by a number of joint groups and countries (e.g., scientists in Austria, Switzerland, Germany; Australia and New Zealand; the USA and Canada; and Southeast Asia), a major step was taken in 2005 with the convening by the United Nations University's Food and Nutrition Program, in collaboration with the FAO, the WHO, and United Nations International Children's Emergency Fund, of a group of international experts to review harmonization of approaches for developing nutrient-based dietary standards, define key terms, and develop a framework for scientific assessment of data. More recently, the Codex Committee on Nutrition and Foods for Special Dietary Uses of the WHO/FAO Codex Alimentarius Committee has proposed using the terminology recommended by the 2005 Harmonization extragovernmental group in updating the nutrient reference values for nutrition labeling. These harmonization activities are in process. With the

enhanced level of communication brought on by computers and the Internet, such efforts are now feasible as well as critical to move forward and to be of greater use to program planners, the food industry, and others who work on a global basis.

The views expressed do not necessarily represent the views of United States Department of Agriculture or of USA.

See also: Antioxidants. Bioavailability. Dietary Guidelines, International Perspectives. Dietary Intake Measurement: Methodology. Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Energy: Adaptation; Balance. Energy Requirements. Food Composition Data. Nutrition Labeling. Nutritional Surveillance: Developed Countries; Developing Countries

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NUTRITIONAL ASPECTS OF BONE

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Introduction

The pathogenesis of poor bone health is multifactorial. Both the development of peak bone mass in the younger population and the rate of bone loss in postmenopausal women and the elderly are determined by a combination of genetic, endocrine, mechanical, and nutritional factors, with evidence of extensive interactions within and between these groups (**Figure 1**).

Endogenous factors have a critical influence on the skeleton. Monozygotic and dizygotic twin research and mother/daughter pairs show a genetic influence on bone health in the region of ~75%. In addition, the skeletal determinants of

osteoporotic fracture risk, such as areal bone mineral density (BMD), bone geometry, and bone turnover, are all subject to strong genetic influences.

Nutritional advice, which is based on sound scientific evidence, is of paramount importance to encourage the optimization of bone health throughout the life cycle. Even a small or modest effect on bone health is likely to have a significant impact on fracture prevention – for example, an increase in BMD by one standard deviation unit is likely to result in a 50% reduction in fracture rates. For effective strategies, emphasis needs to be given, in combination, at three different levels as shown in **Figure 2**: (1) Universal primary prevention, (2) selective prevention for high-risk groups and, (3) targeted prevention for individuals.

Calcium

It is well considered that calcium is critical to health. Calcium is the most abundant mineral element in the body and has two key roles in the body. The first role is structural and the second is regulatory. For its structural role, bone consists of protein matrix encased in a crystalline mineral. Approximately 1 kg of calcium is contained within the skeleton (99% of Ca is contained in bones and teeth) and it is this mineral part that contributes to the strength of bone. Bone mineral provides a huge reserve of Ca, behaving as a large ‘ion exchanger’

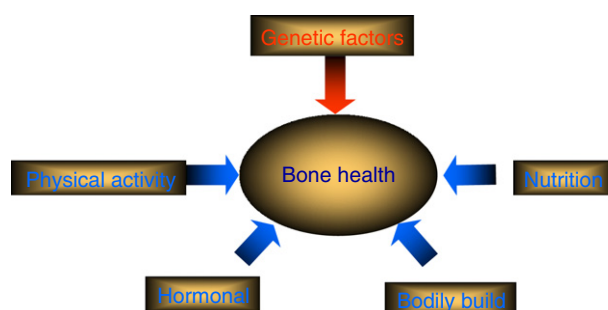


Figure 1 Modifiable (endogenous) versus nonmodifiable (exogenous) factors affecting bone health.

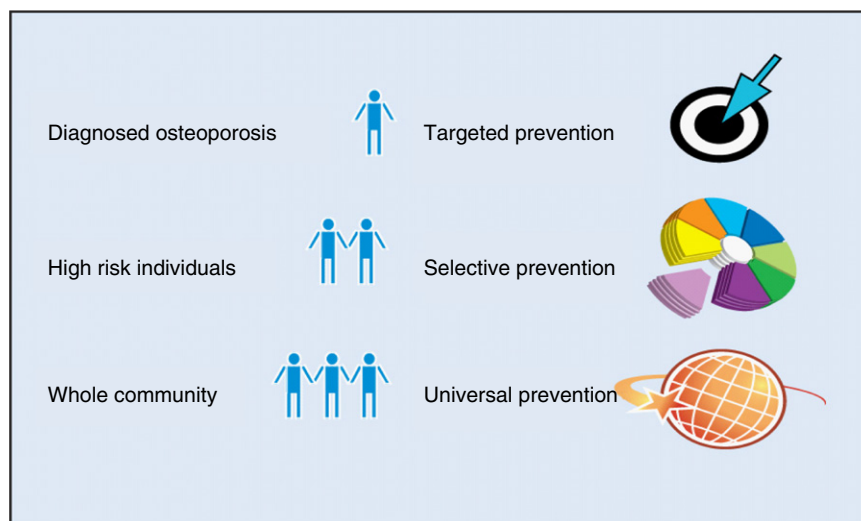


Figure 2 Nutritional approaches to osteoporosis prevention.

allowing interaction between ions in body fluids and bone. Ca is the most abundant mineral element in the body. For its regulatory role, plasma concentrations of calcium are maintained within very narrow limits ($90\text{--}110\text{ mg l}^{-1}$) and although only 1% of calcium is found in soft tissues and body fluids, this calcium is required for a number of key functions including cellular structure; inter- and intracellular metabolic function and signal transmission; muscle contractions including heart muscle, nerve function, activities of enzymes, and normal clotting of blood. The regulation of serum calcium levels is carefully maintained by calciotropic hormones (parathyroid hormone, calcitriol (hormonally active metabolite of vitamin D, $1,25\text{ (OH)}_2\text{D}$) and calcitonin.

PBM Attainment

PBM, the highest level of bone mass attained during normal growth, is one of the key factors determining bone mass and fracture risk later in life. The age at which peak mass is attained ranges from approximately 17–35 years and varies for different skeletal sites.

Calcium absorption and bone calcium deposition rates peak (approximately five times that of adulthood) in girls shortly before menarche. Thus, low calcium during growth and late menarcheal age may affect peak bone mass and consequently fracture risk later in life. There are data to show that adolescent girls are less likely than boys to meet the current recommended dietary levels for calcium and that calcium intake in girls may begin to decline around the time of puberty. The demand to provide calcium to the fetus and neonate during pregnancy and lactation is considerable, and the results of a few recent small studies on the effect of adolescent pregnancy on bone turnover and later risk for osteoporosis as well as fetal bone development highlight the need for adequate calcium intake in pregnant teens.

To date, clinical trials investigating the effect of increased calcium intake (either through foods or supplements) on peak bone mass development have been of relatively short duration, and it has been difficult to determine whether the positive effects of calcium supplementation on bone are maintained. In a very important calcium and bone study, Matkovic and colleagues looked at the effects of calcium intake over an extended period. They reported the results of a 4-year randomized clinical trial that involved 354 girls at stage 2 of puberty. The study was optionally extended for a further 3 years. The mean intake of calcium over the 7-year period was 830 mg day^{-1} , with calcium-supplemented participants receiving an additional $670\text{ mg}^{-1}\text{day}$ of calcium. The results indicated that calcium supplementation significantly influenced bone accretion in girls during the pubertal growth spurt. The effect diminished in young adulthood, but in tall girls, the significant effects remained at the metacarpals and at the forearm. Thus, calcium requirements for maximum skeletal development may be associated with bone size.

Postmenopausal Bone Loss

There are good data to suggest that calcium supplements are effective in reducing bone loss in late menopausal women

(>5 years postmenopause), particularly in those with low habitual calcium intake ($<400\text{ mg day}^{-1}$). In addition, a meta-analysis that includes 15 trials indicates that calcium supplementation at levels between 500 and 2000 mg day^{-1} reduces postmenopausal bone loss. The findings of calcium supplementation studies in the early stages of the menopause are conflicting, and this is an area for further investigation.

Vitamin D

Vitamin D is derived from both endogenous (skin) and exogenous (diet) sources. It is generally believed that the major source of vitamin D is the exposure of skin to the ultraviolet (UV) B-rays contained in sunlight. However, the relative contributions of these two sources are thought to vary widely among individuals and between different geographical areas, but as yet there are few good data available. Much of the UV in sunlight is absorbed by clouds, ozone, and atmospheric pollution. In northern latitudes, there is no UV radiation of the appropriate wavelength (280 nm–310 nm) from the end of October to the end of March; and for the remaining months of the year, 60% of the effective UV radiation occurs between 11.00 am and 3.00 pm. There are actually relatively few dietary sources of vitamin D – the major providers being fat spreads (which are fortified with vitamin D), fish, eggs, fortified cereals, and pastry products.

Although it is well documented that vitamin D synthesis from sunlight is affected by the aging process, there is a remarkable lack of awareness of this public health nutrition message. A number of recent studies indicate that children and adolescents in many different populations have poor vitamin D status, and there has been a reemergence of nutritional rickets. Lack of sufficient sunlight exposure, especially but not only in Northern countries during winter months, poor nutrition, or low milk consumption or vegetarian diet, nonwhite ethnicity, urban residence; and poverty are key factors that have been cited for the low vitamin D levels.

Vitamin D and Calcium Supplementation Studies on Bone

In younger postmenopausal women who are not vitamin D deficient, vitamin D supplementation has little effect on BMD. However, vitamin D and calcium supplementation studies have been shown to reduce fracture rates in the institutionalized elderly. There are also data to suggest an effect in free-living elderly populations. In one study, elderly American men and women (mean age 71 years) given daily treatment with 500 mg of calcium and 700 IU vitamin D₃ had a significantly reduced total number of nonvertebral fractures; however, it is important to note that the study was not specifically powered to look at fracture reduction.

Vitamin D supplementation alone seems to be marginally, if at all, effective in preventing fractures in the elderly if the dosage is not sufficient. In a Norwegian supplementation trial using cod liver oil containing $10\text{ }\mu\text{g day}^{-1}$ (400 IU) of vitamin D, fracture in 1144 nursing home residents was not prevented. This dosage of Vitamin D would be considered suboptimal on

the basis of the 2005 meta-analysis by Bischoff-Ferrari and colleagues, which shows that the dosage of oral vitamin D supplementation is critical. Their results indicate that a daily dose of 700–800 IU reduces the risk of hip and any non-vertebral fractures in ambulatory or institutionalized elderly persons, but 400 IU day⁻¹ is not sufficient for fracture prevention.

Vitamin D and Risk of Falling

Low vitamin D status has been implicated in an increased risk of falling, and a recent meta-analysis has shown that vitamin D supplementation reduces the risk of falls among institutionalized and free-living elderly. The results of a randomized trial also suggest that vitamin D supplementation for 2 years in institutionalized elderly can reduce falls, even if they are not initially vitamin D deficient. Mechanisms of action need further elucidation, but certainly muscle weakness, which can affect balance and mobility, has been implicated. Vitamin D (and calcium) supplementation may be helpful in reducing falls by improving body sway and by normalizing blood pressure.

Protein Intake and Bone

There is controversy concerning the relationship between dietary protein and bone metabolism. Although dietary protein has been shown to result in urinary calcium loss, negative calcium balance and increased bone loss, cross-sectional and longitudinal epidemiological studies examining the effect of protein intake on BMD, bone loss, and risk of fracture show mixed results: with some studies showing protein intake to be detrimental to bone health, whereas others demonstrating a beneficial effect. Conversely, protein-energy undernutrition is considered to be a risk factor for bone loss and osteoporosis. Low protein intake is related to low bone mass and increased risk of fracture, and protein supplementation has been shown to improve recovery from hip fracture.

Ecological studies have shown that worldwide *per capita* consumption of animal protein has been associated with a higher risk of hip fracture in women aged over 50 years. More recently, the correlation has been shown to be stronger with the ratio of animal protein to vegetable protein, a study which has adjusted for important cultural differences. It is important to note, however, that in these correlational studies, the unit of measurement is country and not individual and as such, these types of studies have a number of limitations, which must be considered in the interpretation of such data.

Vitamin K

Vitamin K has an important function for the skeleton; it acts as a cofactor in the posttranslational carboxylation of several bone proteins, with osteocalcin being the most abundant. Deficiency of vitamin K results in the synthesis of under-carboxylated osteocalcin (ucOC). Vitamin K₁, which is also known as phyloquinone, is a component of the Photosystem

I of plants and is present in foods of plant origin. Alfalfa and green leafy vegetables are good sources of Vitamin K₁. Vitamin K₁ is also a component of some dietary supplements. Vitamin K₂ is a bacterial form of the vitamin and is also known as menaquinone.

There are observational data to show that low serum concentrations of both vitamin K₁ and ucOC are associated with an increased risk for osteoporotic fractures. Studies examining the association between vitamin K and BMD have been inconsistent, however, suggesting that the effect of vitamin K on skeletal integrity may involve a mechanism other than one that leads just to reduced BMD.

Dietary Alkali/Potassium Consumption

Importance of Acid–Base Homeostasis to Health

Acid–base homeostasis is absolutely critical to health. It is well documented that extracellular fluid pH remains between 7.35 and 7.45 and thus it is a major requirement of our metabolic systems to ensure that hydrogen ion concentrations are maintained between 0.035 and 0.045 mEq (Figure 3). On a daily basis, humans eat substances that both generate and consume protons and as a net result, adult humans on a normal Western diet generate ~1 mEq per kg body weight of acid per day. Of course, the more acid precursors a diet contains, the greater the degree of systemic acidity. As humans become older, their overall renal function declines, which includes their ability to excrete acid. Hence, with increasing age, humans become slightly but significantly more acidic.

Skeletal Link to Acid–Base Maintenance

The theoretical considerations of the role of alkaline bone mineral may play in the defence against acidosis date back as far as the late 1880s/early nineteenth century. The fundamental concepts were established in the late 1960s/early 1970s – a number of studies published during this period provided evidence that in natural (e.g., starvation), pathological (e.g., diabetic acidosis), and experimental (e.g., ammonium chloride ingestion) states of acid loading and acidosis, an association exists with both hypercalciuria and negative calcium balance.

There are clear mechanisms for a deleterious effect of acid on bone. Novel work in the 1980s by Arnett and Dempster demonstrated a direct enhancement of osteoclastic activity following a reduction in extracellular pH. This effect was shown to be independent of the influence of parathyroid hormone. Furthermore, osteoclasts and osteoblasts appear to respond independently to small changes in pH in the culture media in which they are growing.

Observational and Intervention Studies

A variety of population-based studies published in the latter part of the twentieth century and more recently between 2001 and 2011 have demonstrated a beneficial effect of fruit and vegetable/potassium intake on indices of bone health in young boys and girls, premenopausal women, perimenopausal women, postmenopausal women, and elderly men and

Importance of acid–base regulation and health/disease outcome

When the human body is confronted with an excess of H^+ ions from the diet, it employs a number of strategies to maintain normal blood pH (7.4).

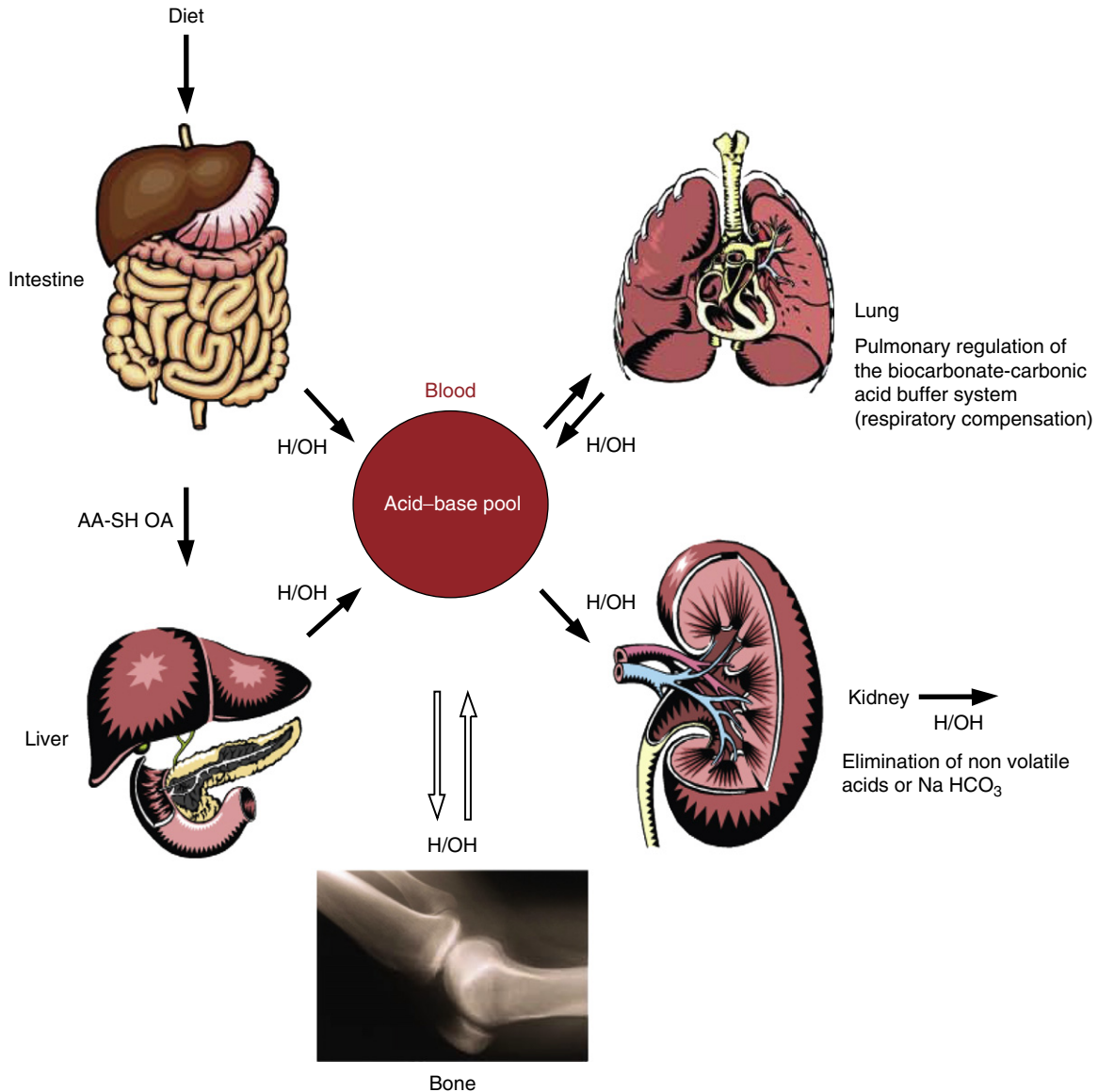


Figure 3 Importance of acid–base regulation and health/disease outcome.

women. Further support for a positive link between fruit and vegetable intake and bone health can be found in the results of the DASH (Dietary Approaches to Stopping Hypertension) and DASH-Sodium intervention trials. In DASH, diets rich in fruit and vegetables were associated with a significant fall in blood pressure compared with baseline measurements. However, of particular interest to the bone field were findings that increasing fruit and vegetable intake from 3.6 to 9.5 daily servings decreased the urinary calcium excretion from 157 mg day^{-1} to 110 mg day^{-1} . This study is the first population-based fruit and vegetable intervention trial showing a positive effect on calcium economy (albeit a secondary finding). Research is now required to determine the long-term

clinical impact of the DASH diet on bone health and fracture risk as well as clarification of the exact mechanisms involved with respect to this diet on skeletal protection.

Isoflavones and Bone Health

Soy protein consumption may also help to explain why it is so difficult to find a clear-cut answer to whether there are bone health differences between populations who follow a vegetarian-based diet and those following a nonvegetarian diet. Soy isoflavones have a chemical structure similar to that of estradiol and have been shown to possess a certain degree of weak estrogenic activity. In the animal model, comparable

favorable bone effects have been shown between 17 β -estradiol and soy protein isolate, genistein or Daidzein. Although there are studies that support a beneficial effect of soy protein isolates on bone mass in both pre- and perimenopausal women, more data are urgently required. In the most recent trials, no effect of soy isoflavones on indices of bone health have been found. Furthermore, the available epidemiological studies looking at the association between soy product consumption and hip fracture rates are conflicting.

Folate, Vitamin B₁₂, and Bone Health Link

The homocysteine–fracture risk link suggests the potential for a positive effect of vitamin B complex on the skeleton. There is increasing evidence at the experimental, clinical, and epidemiological level that raised homocysteine levels are associated with increased fracture risk. These levels can be reduced simply and cheaply through folic acid supplementation. In the absence of specific folic acid/fracture reduction trials, we cannot yet say whether this is an effective strategy for osteoporosis prevention but it is certainly an area for urgent research. There is also some evidence to show that vitamin B₁₂ deficiency *per se* adversely affects bone health through the criticality of vitamin B to the collagen crosslinks but further randomized controlled trials are urgently required.

Nutrients Adversely Affecting Bone Health

Vitamin A

Vitamin A refers to a family of essential, fat-soluble compounds called retinoids. Retinol is the principal dietary form of vitamin A. The main natural sources of vitamin A are provided by animal foods such as liver, meat, milk products, eggs, and fatty fish. In addition, some foods are fortified with vitamin A. Cod liver oil (which is commonly used in a number of population groups, particularly postmenopausal women/elderly) also contains high levels of vitamin A. There are approximately 600 or so carotenoids, with approximately 10% of these having provitamin A activity.

Vitamin A is necessary for normal bone growth. However, intakes of vitamin A > 1500 micrograms of retinol equivalents (REs) have been associated with lower BMD and higher fracture risk in populations in the US and Sweden. In both these countries dairy products and cereals are generally fortified with vitamin A. It should also be noted that high doses of pure cod liver oil can provide as much as 1200 μ g RE of vitamin A in a 10-ml dose.

Sodium

There are insufficient data to make the claim that salt is a significant risk factor for osteoporosis. However, high salt intakes have been associated with increases in urinary calcium loss. For example, it has been estimated that a 100 mmol increment in daily sodium intake is associated with an average loss of urinary calcium of approximately 1 mmol in free-living normo-calciuric healthy populations. This loss has not been specifically correlated to bone loss, however. Some studies

have shown an effect (i.e., higher sodium intake and higher bone turnover), but other studies have found no difference.

Impact of Physical Activity on Bone Health

Introduction

More than a century ago, a German scientist called Julius Wolff stated the theory, which is now called 'Wolff's Law': "Bone accommodates the forces applied to it by altering its amount and distribution of mass". More recently, this concept has been refined to a general theory of bone mass regulation, known as the mechanostat model. It is well known that in the absence of weight-bearing exercise bone loss will occur at both axial and appendicular skeletal sites. Although the exact mechanism whereby mechanical loading affects bone remains to be clarified, the scientific literature supports a positive relationship between physical activity, physical fitness, muscle strength, and bone mass at the lumbar spine and femoral neck sites.

Importance of Exercise to Bone

There is evidence that supports a positive relationship between weight-bearing exercise and bone mass in all age groups, including children, adults, and the elderly. Data also support a specific role for high-impact exercise to increase bone density in premenopausal women. Very little information is available on the relationship between exercise and fracture and is an area for further research. Although clearly exercise is of benefit to the skeleton, what remains undefined is exactly the type, intensity, and duration of weight-bearing physical activity required for optimum bone health. Furthermore, exercise may be of benefit in the prevention of osteoporosis, not necessarily via the mechanism of increasing bone mass but instead by increasing muscle strength, coordination, flexibility, and balance and thus reducing the tendency to fall.

Although bone mass has been shown to be higher in athletes involved in different sports, including tennis players, skaters, rowers, and volleyball players, there is increasing concern for the bone health of women engaged in high-intensity physical training, for whom amenorrhoea is a common characteristic. Often, these types of sports also demand extremely low body weights and there is high reported incidence of anorexia nervosa among participants. The combination of amenorrhoea (and/or anorexia) is detrimental to bone mass and there is now good evidence to show that they 'under achieve' their peak bone mass (PBM) potential and thus are at a considerably increased risk of osteoporosis and indeed, by World Health Organization criteria, are often diagnosed as having the disorder. This picture of under-nutrition, amenorrhoea, and osteoporosis is defined as the 'female athletic triad' and in 1997, the American College of Sports Medicine published a position stand to 'encourage the prevention, recognition and management of this syndrome' (American College of Sports Medicine, 1997) (Figure 4).

The exact mechanisms involved in PBM reduction remain unclarified. There are data to suggest that there is a suppression of the osteoblasts rather than an increase in osteoclastic activity, a finding that is further supported by the finding that hormone replacement therapy (HRT) is not as

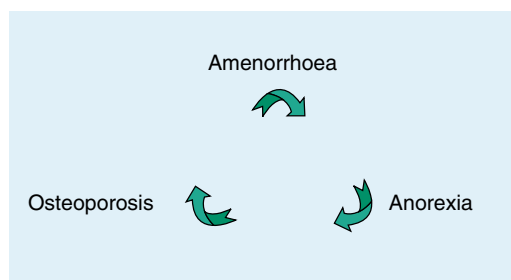


Figure 4 Detrimental effects of exercise on bone health – female athletic triad. Reproduced from American College of Sports Medicine (1997) Position stand on the female athletic triad. *Medicine and Science in Sport and Exercise* 29: i–ix, with permission from LWW.

effective in reducing bone loss in elite sportswomen as it is in young women with primary ovarian failure. Of further interest is the finding that in gymnasts, despite a high prevalence of oligo- and amenorrhoea, bone mass shows a higher than predicted value. Clearly this is an important group to study because it might provide insight into the type of mechanical loads that are most osteogenic.

Discussion

The effects of nutrition on the skeleton are key. There is evidence to suggest that such effects begin *in utero* and remain in place throughout the entire life span, providing target audiences on whom nutrition and bone health messages can be focused. On the dietary front, calcium and vitamin D are important nutrients for optimum bone health. At all costs, we must try to prevent population groups from the potential of suboptimum intakes and look to dietary strategies of fortification for particularly vulnerable groups such as the elderly, postmenopausal women, adolescent females, and amenorrheic women. Given that the decline in bone mass is seen as a natural aging phenomenon, we must encourage sufficient protein:energy nutrition in our aging population. Newer nutritional ideas, with a sound evidence-base for a positive effect are appearing, including vitamin K, phytoestrogens, and dietary alkali. Although there are plausible mechanisms for the effect of other micronutrients on bone health, such as magnesium, trace elements, vitamin C, more research is required on the specific supplementation effects of these nutrients on markers of bone health.

Concluding Remarks

It is common ground that there are genetic, environmental, lifestyle, and dietary determinants of risk of osteoporotic fracture as well as interactions between them. The key to secondary prevention is the understanding of how these components can be integrated into an effective assessment of the major risks after a previous fracture has occurred. The key to primary prevention is to understand both the pathological and physiological basis of bone fragility. It is not unreasonable to suppose that in a western lifestyle our limited and

stereotypic patterns of locomotion from middle age onwards may offer considerably less protection than, for example, the more physically demanding activity of subsistence farming.

It is absolutely critical, given the fact that by 2030, 1:4 in the adult population will be elderly, that special attention is given to nutritional strategies for the optimization of bone health throughout the life cycle. Clearly, calcium and vitamin D nutrition are of great importance. Recent data suggest that calcium works synergistically with physical activity to enhance peak bone mass development and both should be on the agenda as recommended strategies for maximizing peak bone mass attainment during growth. At the other end of the age spectrum, calcium and vitamin D have been shown to be effective strategies for fracture prevention in the elderly, particularly for those populations where vitamin D insufficiency is rife.

Data continue to accumulate showing a positive impact of dietary potassium/fruit and vegetables on skeletal integrity. Although intervention trials are urgently required, it is sensible for the clinician to promote a high potassium intake in the patient's diet, because potassium has been shown to conserve calcium. A high intake of fruit and vegetables is likely to have numerous other health-related benefits.

Further data are urgently required to enable a fuller understanding of the complex interaction between dietary factors and bone health. However, there are promising data showing a positive impact of vitamin K on reducing post-menopausal bone loss. Furthermore, caution needs to be given to the overconsumption of carbonated soft drinks in our younger population, particularly if dairy products in the diet are displaced.

In this era of functional genomics, data are now urgently required to characterize the key nutrient:gene interactions that exist and that are likely to affect bone health in both our younger and older population groups. Targeting dietary advice at those genetically susceptible to osteoporosis is likely to become a useful toolset for the practising clinician.

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NUTRITIONAL ASSESSMENT

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Anthropometry

Clinical Examination

Anthropometry

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Glossary

Kyphosis Abnormal curvature of the spine, resulting in a reduced height.

Occiput The most prominent part of the back of the cranium. Used as a reference to place the measuring tape to obtain head circumference.

Stadiometer Calibrated board used to measure height.

Stunting, stunted Chronic undernutrition, resulting in a reduced height-for-age and weight-for-age.

Uses of Anthropometric Measurements

In adults and children, anthropometric measurements can be used to estimate body fat and lean body mass, and assess their distribution and change over time. Body fat includes storage fat, found inter- and intramuscularly, around the organs and gastrointestinal tract and subcutaneously, as well as lipids in bone marrow, central nervous tissue, mammary glands, and other organs. Normal-weight men and women have approximately 10% and 20% body fat, respectively. Lean body or fat-free mass is approximately 73% water and protein with relatively small amounts of glycogen and minerals. Inadequate diets are associated with low body fat stores and reduced lean body mass in adults and growth failure of children. Consumption of food greater than requirements results in excessive body fat stores in adults and children. Body fat stores, which are too low or too high, are associated with increased risk of morbidity and mortality. The proportion and distribution of fat and fat-free mass vary with age, sex, genetics, disease, some hormones, and some drug treatments.

Different anthropometric measurements and combinations of measurements provide information on body composition and fat distribution and, therefore, nutritional status. The choice of measurements depends on the purpose of the assessment, the equipment available, the subjects being measured, and the skills of the observer making the measurements. Measurements can be made in laboratories, clinics, and hospitals using fixed, precision equipment with a high

degree of accuracy, or in the field, including people's homes or rural centers, with portable equipment.

Advantages and Limitations of Anthropometric Measurements

Anthropometric measurements are quick and relatively easy to obtain, and require inexpensive equipment. For most measurements, there are adequate measurement protocols and reference standards for comparison. Limitations include inability to detect small differences in body composition, dependency on subject's cooperation (problem with small children and handicapped adults), and require an operator with a certain amount of training and experience, particularly for skinfold measurements.

Errors of Anthropometric Measurements

All anthropometric measurements should be made as accurately as possible. Measurement errors may result in the misclassification of subjects' nutritional status or may lead to changes in nutritional status over time being over- or underestimated. Very precise and accurate measurements are needed for nutrition research and in some clinical situations. The same degree of precision may not be possible in nutritional screening and surveillance programs in field studies. Errors in

making measurements arise from the equipment, the physical state and age of the subjects, the time of day when the measurements are made, misreading of measurements by the observer, and as a result of rounding up or down to the nearest half or whole integer. These technical errors of measurement (TEM) vary with the age of the subjects, the measurements being made, and between (inter-) and within (intra-) observers. Values for a particular anthropometric measurement of a group of people by age and sex can be considered accurate if the inter- and intraobserver error is close to a reference value for TEM in a series of repeated measurements, and if there are no biases in the measurement. For measurements of subjects outside the age range, the coefficient of (*R*) can be calculated as $R = 1 - [(TEM)^2 / (SD)^2]$, where SD is the total inter-subject variance including measurement error. It has been recommended that an *R* of 0.90, i.e., a measurement 90% error-free, is an acceptable lower limit of accuracy, although an intraobserver *R* of 0.95 might be more realistic in some circumstances.

TEM can be minimized by careful training of all observers and by making measurements using appropriate equipment in triplicate and then calculating the mean. If measurements for a research study are to be made by more than one person, the interobserver measurements made must be comparable. *R* can be calculated for interobserver variability by making a series of measurements.

Anthropometric Measurements

Height

Height or stature is measured in adults and children over the age of 2 years using a stadiometer, a portable anthropometer, or a moveable headboard on a vertical measuring rod. The measuring device should be checked for accuracy using a standard 2-m steel tape. Subjects should be measured to the nearest 0.1 cm. Subjects, in minimal clothing with bare heads and feet, should stand straight, arms hanging loosely to the side, feet together and with heels, buttocks and shoulder blades in contact with the vertical surface of the stadiometer. Errors occur if subjects do not stand straight, do not keep heels on the ground, or overstretch. Diurnal variation results in people being 0.5–1 cm shorter in the evening than in the morning.

Height cannot be measured accurately in adults with severe kyphosis of the spine and in those who are bed- or chair-ridden. Because knee height is highly correlated with stature, height in such adults can be estimated from the measurement of knee height, using a sliding calliper. The regression equations, derived from a nonrandom sample of American people over the age of 60 years, are as follows: Height (cm) for men = $(2.02 \times \text{knee height, cm}) - (0.04 \times \text{age, years}) + 64.19$; height (cm) for women = $(1.83 \times \text{knee height, cm}) - (0.24 \times \text{age, years}) + 84.88$. Variations in the proportion of limb length to trunk length can lead to a standard error in the estimate (SEE) of height from knee height of ± 8 cm. Demispan, which is the distance between the sternal notch of the right collar bone and the left finger root of the middle and ring finger when the subject's arm is horizontal and

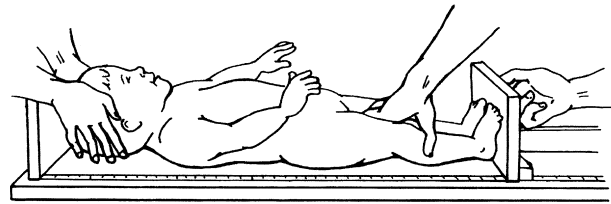


Figure 1 Measurement of recumbent length in children younger than 3 years of age. The head should be in contact with the fixed headboard, with child facing straight up. With legs fully extended, the mobile footboard should be placed firmly against the infant's heels. Reproduced with permission from Frisancho AR (1990) *Anthropometric Standards for the Assessment of Growth and Nutritional Status*. Ann Arbor: University of Michigan Press.

in line with the shoulders, can also be used to estimate height.

Length, rather than height, is measured in infants and children under the age of 3 years. Length is measured by laying a child face upwards on a measuring board with the head against the fixed headboard, and moving another board up to and resting against the child's heels with the legs straight (Figure 1). Small changes in length (± 0.5 cm) may not be significant as it is a difficult measurement to make. Children wriggle and will not stretch out their legs. Length measurements are 1–2 cm longer than height.

Height (stature) or length indicates attained size or growth of adults and children. Long periods of inadequate food intake or increased morbidity result in a slowing of skeletal growth and individuals being short for their age, or stunted. Consecutive measurements of height every 3–6 months can be used to assess growth velocity in children and to indicate the timing of the adolescent growth spurt.

Weight

Weight is measured with digital weighing scales, using a pan, basket, sling, standing platform, or chair, depending on the age and mobility of the people being measured. Weighing scales must be set on a hard, level, and even surface. Scales should be accurate, sensitive, and robust. They must be carefully maintained, calibrated, regularly checked for accuracy using known weights, and always set at zero before use. Weight is usually measured to the nearest 0.1 kg for adults and 0.01 kg for infants.

Weight measures total body mass but does not provide information on the proportions of fat, water, protein, and minerals. Adults can be heavy for height if very muscular, overfat, and big framed. With accurate scales, small changes in weight are detectable but may not necessarily reflect change in body fat or lean body mass. In healthy persons, day-to-day variation in body weight is usually small (± 0.5 kg). Consecutive measurements of weight can be used to monitor the effects of treatment such as weight loss on reduction diets or weight gain with nutritional interventions and supplementation. Weight changes are assumed to reflect changes in the amount of body fat. However, changes in body weight may also result from differences in hydration, edema, tumor growth, and trauma, as well as from factors such as the

amount of food in the gastrointestinal tract and the fullness of the bladder. Weight may remain constant if the loss of muscle mass is masked by increased fat as seen in sarcopenia, the age-related loss of muscle, or by increased fluid retention.

Weight-for-height (or length) can be used to indicate body composition in adults and is an age-independent measure of body composition in children. Growth can be measured in children by consecutive measurements of weight over time (growth velocity) or by weight-for-age if the children's ages are known.

Head Circumference

Head circumference is measured in infants and young children, to the nearest 0.1 cm, with a narrow flexible nonstretch tape laid over the supraorbital ridges and the part of the occiput, which gives the maximum circumference. The head circumference of infants increases rapidly in the first 2 years of life. Increase in head circumference in the first 2 years of life is affected by nutritional status and nonnutritional problems, including some diseases, genetic variation, and cultural practices.

Midupper Arm Circumference (MUAC)

MUAC is measured in adults and children, to the nearest 0.1 cm, using a flexible nonstretch tape laid at the midpoint between the acromion and olecranon processes on the shoulder blade and the ulna, respectively, of the arm (Figure 2). MUAC is a measure of the sum of the muscle and subcutaneous fat in the upper arm. In severe malnutrition both fat and muscle are reduced in the upper arm. Edema may increase a limb's circumference but it is not usually a problem of the upper arm.

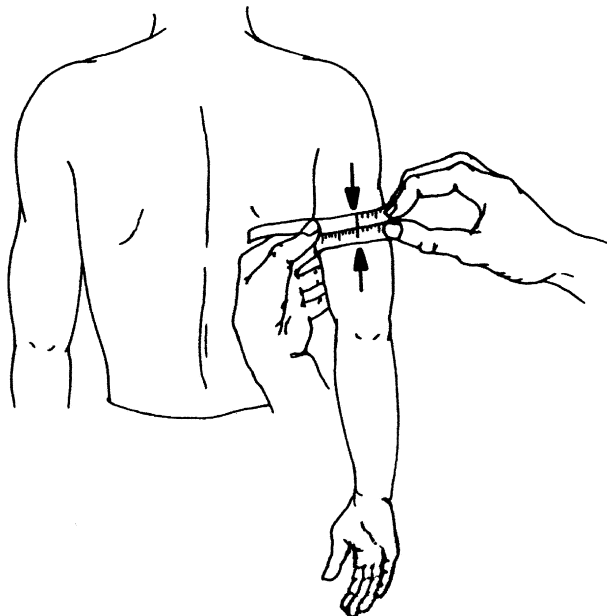


Figure 2 Measurement of upper arm circumference at the midpoint of the upper arm. Reproduced with permission from Frisancho AR (1990) *Anthropometric Standards for the Assessment of Growth and Nutritional Status*. Ann Arbor: University of Michigan Press.

MUAC can be used as a indicator of body composition in adults and children. Since MUAC increases little between the age of 6 months and 5 years, it can be used in preschool children as an age-independent screening tool for severe malnutrition. An MUAC less than 12.5 cm suggests malnutrition; an MUAC greater than 13.5 cm is normal.

Skinfold Thickness

Precision skinfold thickness callipers are used to measure the double fold of skin and subcutaneous fat to the nearest millimeter. The usual sites of measurement are at the triceps (TSFT), the midpoint of the back of the upper arm (Figure 3); the biceps (BSFT) at the same level as the TSFT but to the front of the upper left arm; the subscapular (SSFT) just below and laterally to the left shoulder blade (Figure 4), and the suprailiac (SISFT) obliquely just above the left iliac crest. Skinfold thicknesses can also be measured at the mid thigh, mid calf, and abdomen.

Skinfold thicknesses are difficult measurements to make with precision and accuracy without rigorous training. It is difficult to pick up a consistent fold of skin and subcutaneous fat; in the very obese, the skinfold may be bigger than the callipers can measure; the fold of skin and fat compresses with repeated measurements; and the careless use of the callipers causes pain, bruising, and skin damage to subjects. There is,

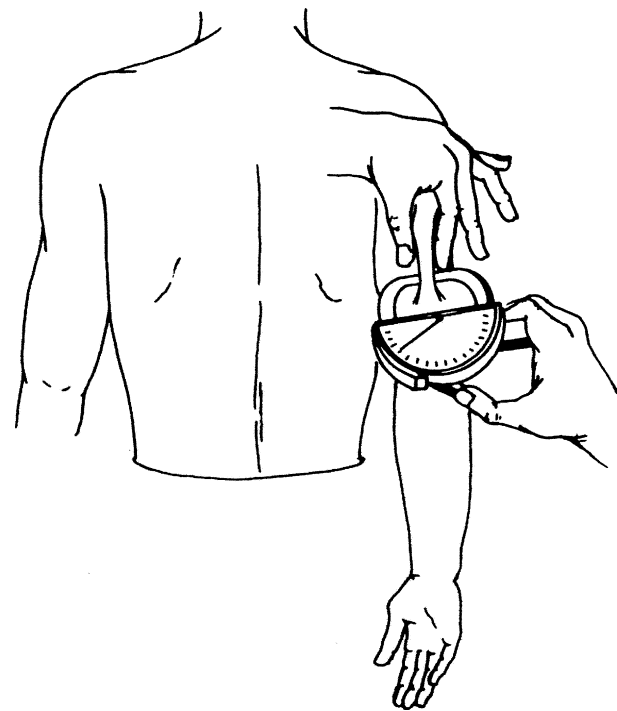


Figure 3 Measurement of triceps skinfold using a Lange caliper. With the subject's arm in a relaxed position, the skinfold is picked with thumb and index fingers at the midpoint of the arm. Reproduced with permission from Frisancho AR (1990) *Anthropometric Standards for the Assessment of Growth and Nutritional Status*. Ann Arbor: University of Michigan Press.

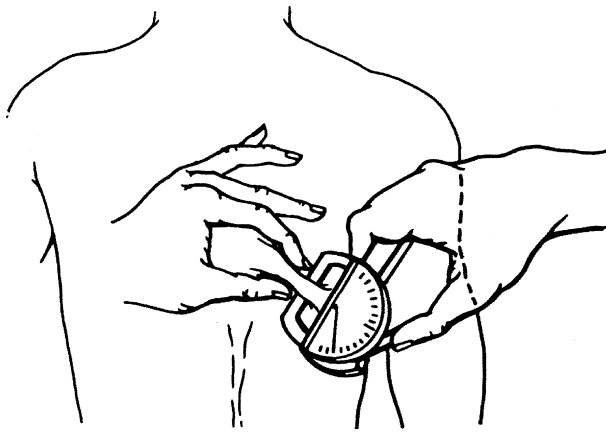


Figure 4 Measurement of subscapular skinfold using a Lange caliper. With subject's arm and shoulder relaxed, a horizontal skinfold is picked approximately 1 cm below the tip of the scapula with thumb and index fingers. The caliper is applied 1 cm from fingers. Reproduced with permission from Frisancho AR (1990) *Anthropometric Standards for the Assessment of Growth and Nutritional Status*. Ann Arbor: University of Michigan Press.

therefore, likely to be considerable inter- and intraobserver error in the measurements.

Skinfold thicknesses measure subcutaneous body fat and, therefore, indicate body composition. TSFT and SSFT indicate subcutaneous fat on the limbs and body trunk, respectively. Skinfold thickness measurements mistakenly assume that subcutaneous fat, measured at one or more selected sites, measures total body fat stores. However, subcutaneous fat at one site may not reflect fat stores at another site, and may not be positively correlated with the amount of visceral fat deposited around the internal organs of the body. Subcutaneous fat, and therefore skinfold thicknesses at the different sites, changes at varying rates with age, weight change, with diseases such as diabetes, and in women during pregnancy, postpartum, and at the menopause. Skinfold thicknesses are not useful for monitoring short-term change in fat stores. If only one skinfold thickness measurement is made, TSFT is most commonly selected. TSFT correlates with estimates of total body fat in women and children. SSFT is better than TSFT as an indicator of total body fat in men. SSFT has been shown to be a predictor of blood pressure in adults independently of age and racial group.

Waist and Hip Circumferences

Waist and hip circumferences are measured to the nearest 0.1 cm using a flexible narrow nonstretch tape in adults wearing minimal clothing, standing straight but not pulling in their stomach. Waist circumference is measured halfway between the lower ribs and the iliac crest, whereas hip circumference is measured at the largest circumference around the buttocks. Measurement error occurs if the tape is pulled too tight or loose, or if subjects wear clothes with belts or full pockets.

With increase in waist circumference there is a decrease in insulin sensitivity, and an increase risk for cardiovascular disease.

Elbow Width

Elbow width is the width of the epicondyles of the humerus with the elbow flexed at 90°. Sliding callipers are used to measure elbow width in adults to the nearest 0.1 cm. Elbow width is a measure of bone size. Frame size can be determined by comparison with reference values either by age or by height and sex.

Nutritional Indices

Most single anthropometric measurements do not in themselves assess nutritional status. Nutritional indices are derived either by combining two or more anthropometric measurements, shown in laboratory studies to be predictive of body composition, or by comparison of the anthropometric measurements with reference values of healthy, well-fed populations. A combination of these methods can also be used.

Body Mass Index (BMI)

BMI relates weight (kg) with height (m) by a simple calculation to indicate body composition ($BMI = \text{weight}/\text{height}^2$). It is the most commonly used screening measurement for both obesity and underweight, and to track growth patterns. By consensus, a healthy range of BMI for adults has been defined as the interval between 18.5 and 24.9 kg m^{-2} . Overweight or 'mild' obesity is defined from 25 to 29.9, obesity from 30 to 39.9, and morbid or severe obesity as >40 . In children, because weight and height change proportions at different stages of growth, a normalized percentile distribution is used, defining the range of desired BMI for age and gender as between the 5th and 95th percentiles. In most but not all age groups, there is a good correlation between BMI and body fat in the population, but predicting body fat from BMI at an individual level can be misleading.

Weight-for-Height

A deficit of weight relative to height indicates recent (acute) weight loss or undernutrition. Coupled with other rapid assessment methods (such as midarm circumference), a reduced weight-for-height may alert on acute, severe undernutrition requiring prompt response.

Weight-for-Age

Weight-for-age may indicate either acute or chronic weight deficit, and thus cannot be used to discriminate between these two conditions. The most common cause of a reduced weight for age is chronic undernutrition, also called stunting. It is usually accompanied by a reduced height-for-age, resulting in a child who is of small body size for his/her age, but otherwise healthy and with a BMI within the normal range.

Growth Velocity

Growth velocity, or change in weight or height over time, can be used to assess growth in children when compared with reference values by age and sex. Growth rates decline in the

first few years of life and then increase with the pubertal growth spurt. Premature and small-for-dates children and those recovering from malnutrition and severe infections tend to have higher growth velocities (catch-up growth). Growth velocities are useful to monitor growth and assess the response to therapy including nutritional supplementation.

Head Circumference-for-Age

Head circumference-for-age by sex is used by pediatricians to identify children up to 2 years of age with severe chronic malnutrition pre- and postpartum and the need for further medical investigations. It is not a good indicator of children's nutritional status.

Midupper Arm Circumference-for-Age

MUAC-for-age indicates body composition (upper arm fat and muscle) in adults and children when used with measurements of weight and height. MUAC measurements are compared with reference values by age and sex. Because the rate of change of arm circumference is slow, it cannot be used to assess growth or monitor the response to therapy.

Midupper Arm Circumference-for-Height

Midupper arm circumference-for-height (the QUAC stick) is a cheap, quick, and age-independent screening tool for children with malnutrition. It is a vertical stick on which are inscribed the 80% and 85% median reference values for MUAC and height, respectively. A child is considered malnourished if the MUAC is less than 80% of the MUAC expected for height.

Skinfold Thickness-for-Age

Measurement of BMI with skinfold thicknesses can identify people who are heavy owing to excess fat or muscle mass. A high BMI and low TSFT and SSFT indicate a large muscle mass; a high BMI and high TSFT and SSFT indicate a high subcutaneous body fat.

Midupper Arm Muscle Circumference (MUMAC) and Upper Arm Muscle Area (AMA)

MUAMC and upper AMA are estimates of upper arm muscle and, therefore, body composition. They can be used as indicators of muscle mass and protein stores. Both MUAMC and AMA are calculated from measurements of MUAC and TSFT on the mistaken assumption that the arm is cylindrical, the subcutaneous fat is equally distributed, the bone atrophies in proportion to muscle wastage in malnutrition, and the cross-sections of neurovascular tissue and bone are small. The formula, with MUAC and TSFT in millimeter, is as follows: $MUAMC = MUAC - (\pi \times TSFT)$. AMA can be calculated from revised formulae, which take account of errors resulting from the noncircular nature of muscle and the inclusion of nonskeletal muscle with MUAC and TSFT in centimeters. For men: $AMA = [MUAC - (\pi \times TSFT)/4\pi]^2 - 10.0$. For women: $AMA = [MUAC - (\pi \times TSFT)/4\pi]^2 - 6.5$.

MUAMC and AMA can be compared with reference values by age and sex. AMA cannot be used to monitor change in muscle stores because of the problems in making this measurement. The ratio of AMA to total body muscle mass changes with age and certain diseases.

Arm Fat Area (AFA)

AFA can be derived from measurements of MUAC and TSFT. AMA is a better indicator of total body fat but not percentage body fat, than TSFT alone. The formula used to calculate AFA (with MUAC and TSFT in millimeter) is $AMA = [(TSFT \times MUAC/2) - (\pi \times TSFT/4)]^2$. AMA can be compared with reference values by age and sex. Theoretically, limb fat area can be calculated for other limbs and the body trunk, but there are no reference values available.

Waist-to-Hip Ratio (WHR)

The WHR in adults discriminates between those with upper body or intraabdominal obesity (WHR greater than 1 in men and 0.8 in women) and those with lower body or peripheral obesity. Genetics, sex, and age partly determine body fat distribution. A high WHR is associated with an increased risk of premature mortality and morbidity.

Reference Values

Adults

Reference values for adults have been historically based on the relationship between body size and mortality risk. Since the early twentieth century, life insurance companies used actuarial tables to adjust premiums based on risk of premature death. This risk curve is J-shaped, and the BMI interval comprising the lowest portion of the curve was subsequently used to identify the desired or healthy BMI range. By consensus that interval was eventually defined as between BMI of 18.5 and 24.9, which is considered healthy or 'normal' BMI for adults. The segment from 25 to 29.9 is sometimes called overweight or mild obesity, from 30 to 39.9 obesity, and above, severe obesity.

There is ongoing debate on the limitations of using BMI to predict adverse health events. While the link of diseases such as type 2 diabetes and dislipidemias with obesity (i.e., high BMI) is well established, it is less clear if this indicator can be used to track risk for a number of other disorders. Furthermore, the risk level-BMI association varies with ethnicity, making difficult to adopt a universal cutoff point, as has been attempted.

Children

Traditional reference growth charts are descriptive, meaning that they reflect the body size of the population selected as 'reference'. Given the secular trends of 'healthy' populations to increase their body size, reference values derived from recent measurements would yield undesirably high BMIs. Therefore, reference charts, such as the CDC 2000, have used

older datasets, collected at a time when excess weight was less prevalent.

The World Health Organization (WHO) released in 2006 the first prescriptive reference charts for children 0–5 years. These charts reflect the growth patterns of children raised on a healthy environment and fed and stimulated according to recommended practices. The WHO subsequently released charts for 5–19 years, following the more traditional descriptive approach but introducing a number of adjustments to optimize use of the chart across countries.

See also: Dietary Intake Measurement: Methodology. Growth Monitoring

Further Reading

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Clinical Examination

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Glossary

Beriberi Clinical syndrome resulting from thiamin (vitamin B-1) deficiency.

Keratomalacia Corneal lesion characteristic of vitamin A deficiency.

Kwashiorkor Undernutrition syndrome in young children characterized by severe protein depletion (edema, skin and hair changes, etc.).

Pellagra Clinical syndrome caused by niacin deficiency.

Introduction

The two most common settings for a clinical examination are the hospital (inpatient or outpatient) and the field health care unit. In the first situation, the physician or examiner may have access to resources that are usually not available in the field. Because of this and other constraints, the assessment of nutritional status in the field is frequently more narrowly aimed at identifying a specific clinical condition or set of signs and symptoms. In either case, it is essential that information on history and physical findings be collected in a standardized manner in terms of both format and procedures. The former is usually best achieved by the use of preprinted or computerized forms. Electronic forms can be programmed to perform immediate range checking as values are entered, thus alerting the operator when values out of range are entered. Procedures for examination must be clearly defined in writing, and any health worker should be able to follow the instructions and perform an acceptable measurement. Although many components of the examination are subjective, it is important to standardize as much as possible terms such as 'minor', 'average', and 'large' within the group of examining persons, attributing a numeric value whenever possible. If data entry requires selection from a numeric scale, they should also be standardized by cross-validation with experienced personnel or by means of photographs or models.

The two components of the clinical assessment are the medical history and the physical examination (Table 1).

Medical History

The medical history for nutritional assessment is no different from a general medical history, in which familial and past and present environmental factors and their possible association with specific diseases or disease risk are considered. For the purpose of nutritional assessment, this information will be used to determine if any nutritional finding or complaint may be caused by an underlying medical condition, particularly one that remains unrecognized at the time of the examination. Additionally, specific medical conditions and their current status are important factors altering nutrient requirements and dietary prescriptions.

One specific focus of medical history in a nutritional assessment context is the exploration of gastrointestinal function. Conditions such as chronic diarrhea, gastroesophageal

reflux, and colonic disorders may be associated with reduced nutrient absorption or food avoidance that result in impaired nutritional status. Past history of gastrointestinal problems or surgery may also point to current alterations in nutrient digestion or absorption. Other important components of the medical history are history of weight loss or gain, past and present use of medications, use of special foods or formulas, changes in taste or smell, and food allergies and intolerances.

In children and adolescents, the medical history must also obtain information on neurodevelopmental stages, history of behavioral problems, and overall school performance. Food preferences must be noted, particularly in adolescence, when adoption of unconventional dietary practices is more likely to occur.

Table 1 Major components of a nutrition-oriented medical history

Medical history

- History of weight loss or gain
- Gastrointestinal symptoms (nausea, diarrhea, flatulence, pain, etc.)
- History of changes in color or texture of skin, hair, conjunctiva, buccal mucosa
- Use of medications
- Physical activity level (work-related, leisure)
- History of fatigue, shortness of breath, muscle cramps
- Other lifestyle practices
- Places of residence, travel (exposure to toxins, sunlight, food contaminants)
- In children and adolescents
 - Growth history
 - Neurodevelopmental history
 - General school performance
 - Parental and siblings' body size (body mass index)
 - Pubertal stage
 - Food preferences, fads

Dietary history

- Habitual dietary intake and preferences
- Past diet history
- Alcohol consumption
- Food allergies and intolerances
- Assessment of dietary intake
 - 24-hour recall
 - Food frequency questionnaire

A special component of the nutritional history is the assessment of habitual dietary intake. There are several approaches, all of them requiring substantial experience and standardization. These procedures, such as 24-hour dietary recall, or food frequency questionnaires, are discussed in detail elsewhere.

Physical Examination

As noted previously, anthropometric measurements are a key component of the physical examination. Measurement of weight and height is perhaps one of the most frequently performed nutritional measurements. Although its value is limited with regard to identifying specific nutrient deficiencies, it is invaluable to evaluate growth and adequacy of past and present diets in infants, children, and adolescents and to identify undernutrition and obesity in adults. Measurements should be done by trained personnel and following standard protocols. In addition to anthropometry, the physical examination focuses on signs of nutrient deficiency or excess. These signs usually appear only when the deficiency is advanced and are not to be expected in marginal deficiencies. Furthermore, the time that it takes for a deficient intake of a given nutrient to cause clinical manifestation of deficiency varies considerably, depending on whether the nutrient is stored in the body and on the initial status of the reserves. Typical signs for selected nutritional deficiencies are presented in **Table 2**. Virtually none of these signs, with the exception of Bitot's spots, are pathognomonic for one specific deficiency. However, they are useful in indicating a specific nutrient impairment and prompting further evaluation.

The physical examination should start with a general visual assessment of the patient. In children, state of alertness, willingness to engage in play, or resisting examination are important clues to energy level and physical strength. A generalized loss of fat depots, or excess adiposity as in the obese, is readily identifiable in most circumstances. A general overview can also identify pallor, loss of muscle mass, and skin changes.

Numerous signs of nutritional deficiencies can be identified in the skin and hair. Because skin exhibits a relatively rapid turnover, impairments in protein synthesis can result in fragile, flaky, and discolored skin. Vitamin A deficiency typically causes a dry, hyperkeratotic skin. The dermatitis of pellagra consists of patchy areas of hypo- or hyperpigmentations, usually in sun-exposed body regions, eventually progressing to hardened, broken surfaces. In protein-energy malnutrition, hair may become brittle, thin, and easily pluckable. Fluctuations in the rate of synthesis of hair protein may result in band discoloration, where pale and normal colors alternate, resulting in the 'banner sign', typical of kwashiorkor. Petechiae or hematomas may result from protein-energy malnutrition or vitamin K or vitamin E (in the newborn) deficiencies.

One of the most specific signs of nutritional deficiency can be identified in the eye. Vitamin A deficiency produces a series of alterations in the conjunctiva and the cornea that not only indicate a deficiency of this nutrient but also help grade its severity. The most commonly used classification of vitamin A deficiency is primarily based on eye findings, from Bitot's

Table 2 Typical clinical signs of selected nutritional deficiencies

<i>Deficiency</i>	<i>Signs</i>
Protein-energy malnutrition	Hair: depigmentation, thinning, pluckability Edema in lower extremities (generalized in severe cases) Muscle wasting Decreased subcutaneous fat Skin: diffuse depigmentation, flaky dermatosis Liver enlargement
Vitamin A	Bitot's spot Conjunctival xerosis Corneal xerosis Keratomalacia Night blindness
Riboflavin	Angular stomatitis Cheilosis Scrotal (vulvar) dermatosis Red tongue Corneal vascularization
Thiamin	Edema Hyporeflexia Muscle tenderness Cardiac enlargement Tachycardia
Niacin	Pellagroid dermatosis Scarlet, raw, fissured tongue Malar and supraorbital pigmentation
Vitamin C	Bleeding, spongy gums Petechiae Ecchymoses Epiphyseal enlargement Atrophy of lingual papillae Follicular hyperkeratosis
Vitamin D	Active rickets: rib beading, epiphyseal enlargement, persistently open fontanelle, craniotabes, hypotonia Residual rickets: frontal or parietal bossing, bowlegs, knock-knees, thorax deformities
Iron	Pale conjunctiva Atrophy of lingual papillae Koilonychia
Folic acid, B ₁₂	Usually associated with pallor of anemia Peripheral neuropathy (B ₁₂)
Iodine	Thyroid enlargement

spots to perforated keratomalacia. Conjunctival pallor has been a classic sign of anemia, but its sensitivity varies substantially depending on ethnicity, ambient lighting, and experience of the observer.

The mouth and tongue are also areas where typical manifestations of deficiency can be detected. A red tongue is a classic sign of riboflavin deficiency but has also been associated with niacin deficiency; the latter may also include fissures. Conversely, a pale tongue may indicate iron deficiency. Glossitis, with or without color changes, has been linked to pyridoxine deficiency. A similar condition, including pain and intense red color, has been associated with biotin deficiency. Angular stomatitis and ulcerations and other lip lesions are

associated with riboflavin or ascorbic acid deficiencies. In the latter, extensive involvement of the gums (swelling and bleeding) is also typical. Atrophy of the papillae occurs in vitamin B₁₂, niacin, and folate deficiencies. Excess vitamin A intake may result in discoloration of the gingival mucosa.

Rib beading (also known as rickets rosary) is a typical sign of vitamin D deficiency in children, but a similar manifestation may appear in vitamin C deficiency (scurvy). Epiphyseal enlargement and bowlegs are other classic signs of rickets. A distended abdomen is characteristic of protein–energy malnutrition in children. In the lower limbs, inspection must ascertain the presence of edema, which is also associated with protein–energy malnutrition.

Peripheral neuropathies such as those associated with beriberi or vitamin B₁₂ deficiencies may result in visible impairment of limb movements, such as the ‘foot drop’ of dry beriberi.

In preadolescents and adolescents, assessment of sexual maturation (usually following the Tanner staging) is an important component of the physical examination, although it is not always feasible due to cultural and practical reasons. Alternatively, more limited information may be obtained in girls by self-reported menarcheal status. Self-assessment of Tanner stage by comparison with photographs is another useful alternative, but use of these photographs with children may not be acceptable in some communities.

To obtain a unified rating of a person’s nutritional status, it is desirable to integrate clinical, laboratory, and functional

data into a single scoring system. Several approaches to achieve this have been proposed, and their use will depend primarily on the target population and the intended use of the score. The Subjective Global Assessment is an approach that relies primarily on data from the physical examination and thus can be readily performed after this examination has been completed. Other scoring systems, such as the Prognostic Nutritional Index or the Instant Nutritional Index, rely to variable degrees on combinations of clinical and laboratory data.

See also: Biochemical Indices. Dietary Intake Measurement: Methodology. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Nutritional Assessment: Anthropometry

Further Reading

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NUTRITIONAL CONSIDERATIONS FOR THE MANAGEMENT OF HYPERTENSION

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Glossary

DASH Dietary approaches to stop hypertension. An NIH-funded feeding trial that determined the effects of a low-fat, higher protein diet high in fruits and vegetables and dairy on blood pressure.

DASH Diet A diet characterized by eight to 10 servings of fruit and vegetables, two to three servings of non-fat to low-fat dairy, focused on whole grains, somewhat higher in protein, and high in fiber. This diet was proclaimed no. 1 in many health aspects by US News & World Report in 2012.

DASH-Sodium The second DASH trial funded by NIH that studied the effects of low, medium, and high levels of sodium in addition to the DASH diet on blood pressure.

Dietary Guidelines for Americans 2010 These guidelines are intended to be used in developing educational materials and aiding policymakers in designing and carrying out nutrition-related programs, including US Federal nutrition assistance and education programs. They also serve as the basis for nutrition messages and consumer materials developed by nutrition educators and health professionals for the general public and specific audiences, such as children.

PREMIER An NIH-funded intervention trial that focused on the ability of individuals to adhere to the DASH diet on their own, in addition to following the established recommendations for blood pressure control.

Introduction

Hypertension or high blood pressure affects approximately 30% of adult Americans and has not significantly changed over the 10-year period from 1999 to 2008, as reported in 2010 by the US Department of Health and Human Services, Centers for Disease Control and Prevention. African Americans in the US have even higher rates at approximately 40%. Increased rates of hypertension are not limited to the US. In 2009, the Canadian Community Health Survey reported that percentages of their population diagnosed with high blood pressure ranged from 14 to 18% in 45- to 54-year olds, was approximately 32% in 55- to 64-year olds, and 44–53% in those 65 and older. Hypertension is reportedly as much as 60% higher in some European countries. Hypertension is obviously more common among older persons, as evidenced by the Canadian statistics, occurring in two-thirds of those older than 65 years of age – a population that is often untreated. Estimates indicate that the lifetime risk of developing hypertension for middle aged and elderly individuals is 90%. Considering the prevalence of hypertension, both in the US and worldwide, nutritional management could have a significant impact on the overall health of humankind.

Current guidelines suggest that the values of 120/80 mmHg, previously considered normal, are now classified as prehypertensive. Classification categories are shown in **Table 1**. The recommendations for management of hypertension are built on a base of lifestyle modification for all

categories confusing parenthetical comment, with drugs not routinely promoted until the patient presents with at least stage 1 hypertension or other medically indicated reasons.

The Dietary Approaches to Stop Hypertension (DASH) and DASH-Sodium trials are still considered the most definitive studies examining diet and hypertension. The initial DASH study focused on dietary patterns to lower blood pressure while keeping sodium content of these diets constant. DASH-Sodium tested the effects of varying levels of sodium in conjunction with the DASH diet to determine whether lowering sodium intake had additional benefits. Both trials were metabolic feeding trials, each including more than 400 participants at four sites in the US. A follow-up to the DASH and DASH-Sodium was the PREMIER trial. PREMIER tested whether individuals could lower blood pressure on their own by implementing established guidelines for treating hypertension and including the DASH diet. PREMIER used a lifestyle

Table 1 Classification of blood pressure for adults 18 years of age and older

Category	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)
Normal or desirable	<120	<80
Prehypertensive	120–139	80–89
Stage 1 hypertension	140–159	90–99
Stage 2 hypertension	≥160	≥100

counseling approach and randomized more than 800 adults to one of three treatment arms: advice only, established recommendations, and established recommendations plus DASH. Compared to the advice-only group, both active interventions resulted in a significant lowering of blood pressure at 6 months with the established plus DASH arm further improving blood pressure control. At 18 months, both active interventions persisted in these lifestyle modifications, although adherence to the lifestyle changes at 18 months was not as strong as at 6 months.

Recommended Lifestyle Modifications

Whether or not one has normal blood pressure or has hypertension, adoption of a healthy lifestyle is needed. The following lifestyle modifications are recommended to prevent high blood pressure or to manage hypertension:

- Weight reduction in those overweight or obese.
- Reduce sodium.
- Increase physical activity.
- Moderation of alcohol consumption.
- Adoption of the Dietary Approaches to Stop Hypertension (DASH) eating plan, which focuses on dietary patterns to increase potassium, magnesium, and calcium.
- Stop smoking.
- Control stress.

The established recommendations for lifestyle modification used in one arm of the PREMIER clinical trial were weight loss, increasing physical activity to 180 min week⁻¹, reducing sodium intake to no more than 100 mmol day⁻¹, limiting alcohol consumption, reducing total fat intake to 30% of energy, and reducing saturated fat intake to 10% of energy. A second arm in PREMIER included essentially the same lifestyle modifications, but a lower fat diet with 25% of energy from total and 7% from saturated fat, and adherence to the DASH diet (emphasizing consumption of fruits, vegetables, and low-fat dairy products, which will be described in more detail later).

The 2010 Dietary Guidelines for Americans (DGAs) recommend that total fat intake fall within the range of 20–35% of calories for adults ages 19 years and older. The 2010 DGAs suggest that type of fat is more important than total fat in influencing cardiovascular disease (CVD) risk and both the American Heart Association and 2010 DGAs support limiting saturated fat to 10% of calories and replacing it with mono-unsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), which are associated with lower cholesterol levels and reduced risk of CVD.

Nutritional Considerations

Reduction in Sodium Intake

Current guidelines in the US suggest reducing the daily intake of sodium to approximately 100 mmol (2.3 g) of sodium or less per day. Additionally, persons 51 years of age or older, African Americans of any age, and individuals with

hypertension, diabetes, or chronic kidney disease should reduce intake of sodium even further to approximately 65 mmol (1.5 g) per day.

The DASH-Sodium trial demonstrated that reduction in sodium intake to levels from the current recommendation of 100 to 50 mmol day⁻¹ significantly reduced blood pressure in individuals following either the usual American diet or the DASH diet. Additionally phase 2 of the Trials of Hypertension Prevention (TOHP2) and Trials of Nonpharmacologic Interventions in the Elderly (TONE) documented that reducing sodium can either prevent hypertension or facilitate hypertension control. It should also be noted that salt-sensitivity increases with age, so those who demonstrate this sensitivity should maintain a reduced salt diet.

Consumers should either eliminate or limit salt added to foods in cooking and at the table as a means of reducing sodium intake. Nutrition facts labels require sodium content to be listed and wise shopping for better health means that reading labels enables consumers to be more prudent about their diets. The amount of sodium in processed food is often alarming when one looks at labels of convenience foods (e.g., boxed products one would prepare at home), soups, and processed meats (e.g., sausage, ham, and other meat products). Canned products generally contain more sodium than fresh or frozen items, unless a product is specifically labeled as 'no salt added.' The consumption of fresh, unprocessed foods should be promoted. If consumers rely on books with nutrition content of various foods, it should be noted that these are general data, often from food composition tables, and may not be an exact match for the foods actually consumed. Considering the diversity of products in current food supplies in both the US and abroad, sodium is one nutrient that varies greatly.

Knowing the variability of sodium content in foods is essential; however, it does not appear to be feasible to capture the sodium content of every food in the marketplace in nutrient databases. Because a large proportion of the population tends to eat away from home, restaurant and processed foods are a high priority. USDA food composition tables, as well as food composition tables from other countries, are commonly used in dietary assessment; therefore, it is crucial to recognize differences or changes in the sodium content of the foods.

Obviously food tables do not always reflect the values in the food as consumed. For most foods and for dietary assessment purposes, published values are reasonable.

Moderation of Alcohol Intake

The relationship between high consumption of alcohol (typically three or more drinks per day) and elevated blood pressure has been shown in numerous epidemiological studies. These effects, however, are usually transient, with blood pressure falling when consumption ceases. A drink is defined as 12 fl oz of beer, 5 fl oz of wine, or 1.5 fl oz of distilled spirits. Most evidence to date indicates that, for those who drink, alcohol should be limited to two drinks per day for men and one drink per day for women. Ideally, daily alcohol consumption should be avoided. Whenever possible, alcohol, if consumed, should be done so with meals.

Consumption of a DASH Diet (Increasing Potassium, Magnesium, Calcium, and Fiber Intakes through Increasing Intakes of Fruits, Vegetables, and Low-Fat Dairy Foods)

The contribution of minerals, in particular potassium, magnesium, and calcium, and fiber, were brought about by contributions from fruits, vegetables, low-fat dairy products, whole grains, and nuts in the DASH eating plan. The DASH diet effectively used these components through an ideal dietary pattern to result in lowered blood pressure.

Increased intakes of potassium have been associated with lower blood pressure. A meta-analysis of several trials suggested that 60–120 mmol day⁻¹ of supplemental potassium reduced systolic and diastolic blood pressure by 4.4 and 2.5 mmHg, respectively, in hypertensive individuals. In normotensive individuals, systolic and diastolic blood pressure was reduced by 1.8 and 1.0 mmHg, respectively. Dietary intake of potassium can be easily achieved through consumption of various foods.

The DASH diet, while promoting dietary patterns, was developed very carefully with particular attention paid to use of specific food choices within categories that would contribute more greatly to the intake of desired nutrients. As an example, consider the rank ordered listing of potassium content of fruits and fruit juices presented in Table 2. Where fruit is concerned, dried fruits typically have the highest potassium content, followed by raw fruits, then by frozen fruits. Canned fruit products generally do not contain as high potassium content as other forms. There is less potassium contained in fruit juices and generally the fresh forms of the juices have incrementally more than the processed forms. Fruits and juices in general contain some magnesium, another mineral of interest for the DASH investigators. Most fruits contain from 2 to 30 mg of magnesium per 100 g, but dried fruits contain

much more (between 30 and 90 mg) with amounts varying greatly. Fruit juices contain less than 20 mg of magnesium per 100 g, with most containing less than 10 mg. Fiber content of fruit ranges from approximately 7–14 g of fiber for dried fruits on a per 100 g basis, and anywhere between 1 and 5 g for other fruits per 100 g. Generally, fruit juices contribute less than 1 g of dietary fiber per 100 g, but choosing high pulp varieties of juices may provide slightly more dietary fiber.

Table 3 contains a rank ordered listing of vegetables (including beans) by content of potassium. Magnesium content of these is shown as well. The data are presented for vegetables and beans in the raw form. It is important to remember that many fresh forms are concentrated in terms of weight when cooked, especially for some types (e.g., spinach and other greens) and it is quite possible to obtain a higher mineral content from cooked vegetables because of this (especially in the case of potassium). Relatively speaking, magnesium content differs less from the fresh to the cooked state for most vegetables and beans. Most vegetables contain approximately 1–3 g dietary fiber per 100 g; beans and legumes offer approximately 5 g of dietary fiber, with some dried vegetables offering more than double this amount.

Clearly shown in Table 3 are the content differences in potassium and magnesium among vegetables and beans in this partial listing. As an example, palm hearts with high potassium contain low magnesium, as opposed to lower potassium seaweed (kelp) containing high magnesium. The referenced website for Table 3 will inform the consumer on content based on cooking or other preparations and provides amounts both on a 100 g basis as well as typical serving sizes consumed.

Nuts were included in the DASH diet, contributing potassium, magnesium, fiber, and protein. They contain fat, mostly monounsaturated, and thereby contribute energy to the diet. Table 4 includes the potassium, magnesium, and fiber content

Table 2 Fruits and fruit juices ranked by potassium content (mg per 100 g) according to range of content with listing in each section ordered from highest to lowest content within that group

Description	K range (mg per 100 g)
Dried fruit	
● Apricots, bananas, peaches, litchis, prunes	1000–1850
● Currants, raisins, Japanese persimmons, plums, Medjool dates, figs, longans, deglet noor dates	600–1000
● Pears, jujube apples	450–550
Raw fruit	
● Tamarinds, plantains, breadfruit, avocados, jackfruit, durian, guavas, bananas, passion-fruit (grandilla), European black currants, kiwifruit, persimmons	300–600
● Rhubarb, cherimoya, elderberries, soursop, currants, cantaloupe, loquats, longans, apricots, jujube, pomegranates, figs, honeydew melon, sweet cherries, prickly pears, pummelo, roselle, muscadine grapes, nectarines	200–300
● Gooseberries, quinces, oranges, mulberries, crabapples, American and European grapes, peaches, kumquats, papayas, casaba melon, oranges, sour red cherries, blackberries, plums, strawberries, raspberries, grapefruit, acerola (West Indian cherry), carambola (starfruit), rowal, pineapple, rose-apples, pears, watermelon, apples, limes	100–200
● Cranberries, java-plum (jambolan), blueberries, mammy-apple (mamey), oheloberries	30–100
Fruit juice	
● Passion-fruit juice, prune juice, pomegranate juice, orange juice, tangerine juice, grapefruit juice, orange-grapefruit juice	150–278
● Blackberry juice, pineapple juice, apple juice, apricot nectar, grape juice, lemon juice	100–135
● Lime juice, peach nectar, guava nectar, papaya nectar, tamarind nectar, guanabana nectar, mango nectar	20–75
● Pear nectar, cranberry juice cocktail	10–15

Table 3 Potassium and magnesium content of raw vegetables (including beans), rank ordered by potassium content, mg per 100 g (partial listing) (view website entry below for additional information)

<i>Vegetable</i>	<i>K (mg)</i>	<i>Mg (mg)</i>
Tomatoes, sun-dried	3427	194
Palm hearts	1806	10
Yam	816	21
Beet greens	762	70
Water chestnuts, Chinese, (matai)	584	22
Spinach	558	79
Bamboo shoots	533	3
Mushrooms, portabella	484	11
Lima beans, immature seeds	467	58
Squash, zucchini, baby	459	33
Kale	447	34
Jerusalem-artichokes	429	17
Brussels sprouts	389	23
Chard, swiss	379	81
Artichokes, (globe or French)	370	60
Fiddlehead ferns	370	34
Mustard greens	354	32
Squash, winter, butternut	352	34
Rutabagas	337	23
Sweet potato	337	25
Watercress	330	21
Beets	325	23
Broccoli	325	25
Carrots	320	12
Broccoli	316	21
Beans, navy, mature seeds	307	101
Beans, pinto, mature seeds	307	53
Cauliflower	303	15
Okra	303	57
Turnip greens	296	31
Corn, sweet, yellow or white	270	37
Tomatillos	268	20
Lettuce, cos or romaine	247	14
Peas, green	244	33
Cabbage, red	243	16
Tomatoes, red, ripe	237	11
Cabbage, savoy	230	28
Eggplant	230	14
Squash, summer	212	21
Peppers, sweet, yellow or red	212	12
Beans, snap, green or yellow	209	25
Tomatoes, green	204	10
Asparagus	202	14
Turnips	191	11
Beans, kidney, mature seeds	187	21
Lettuce, red leaf	187	12
Peppers, sweet, green	175	10
Lettuce, iceberg	152	8
Yambean (jicama)	150	12
Mung beans, mature seeds	149	21
Cucumber, with peel	147	13
Onions	144	10
Cucumber, peeled	136	12
Chayote, fruit	125	12
Onions, sweet	119	9
Squash, winter, spaghetti	108	12
Seaweed, kelp	89	121

Table 4 Potassium, magnesium, and fiber content of nuts and seeds per 100 g, ranked by potassium content (partial listing). See website below for additional information

<i>Description</i>	<i>K (mg)</i>	<i>Mg (mg)</i>	<i>Fiber (g)</i>
Nuts, pistachio nuts, dry roasted	1042	120	10.3
Nuts, ginkgo nuts, dried	998	53	9.3
Seeds, sunflower seed kernels, dry roasted	850	129	9.0
Nuts, almonds, dry roasted	746	286	11.8
Nuts, almonds	728	275	11.8
Seeds, sunflower seed kernels, dried	689	354	10.5
Nuts, almonds, blanched	687	275	10.4
Seeds, flaxseed	681	362	27.9
Nuts, hazelnuts or filberts	680	163	9.7
Nuts, cashew nuts, raw	660	292	3.3
Nuts, Brazil nuts, dried, unblanched	659	376	7.5
Nuts, hazelnuts or filberts, blanched	658	160	11.0
Nuts, cashew nuts, oil roasted	632	273	3.3
Nuts, pine nuts, pinyon, dried	628	234	10.7
Nuts, pine nuts, pignolia, dried	597	251	3.7
Nuts, cashew nuts, dry roasted	565	260	3.0
Nuts, walnuts, black, dried	523	201	6.8
Nuts, chestnuts, European, raw, unpeeled	518	32	8.1
Nuts, ginkgo nuts, raw	510	27	9.3
Seeds, sunflower seed kernels	491	129	11.5
Seeds, sunflower seed kernels, oil roasted	483	127	6.8
Seeds, sesame seeds, whole, dried	468	351	11.8
Nuts, hickorynuts, dried	436	173	6.4
Nuts, pecans, dry roasted	424	132	9.4
Nuts, walnuts, English	441	158	6.7
Nuts, pecans	410	121	9.6
Seeds, sesame seed kernels, toasted	406	346	16.9
Nuts, pecans, oil roasted	392	121	9.5
Nuts, macadamia nuts, raw	368	130	8.6
Nuts, macadamia nuts, dry roasted	363	118	8.0

of some common nuts and seeds. In the table, nuts are presented ranked order by potassium content. However, some nuts and seeds do contain magnesium and dietary fiber and were therefore encouraged in the DASH diet.

Low-fat dairy products were also an important part of the DASH diets. These were used primarily to increase the calcium content of the diets from a low content of approximately 450 mg on the control and fruit and vegetable diets to 1250 mg on the DASH diet at the 2000 kcal (8368 kJ) level. Calcium has frequently been reported to have an inverse relationship with blood pressure, but studies utilizing supplemental calcium have been inconsistent. With supplements, effects on blood pressure reduction have been negligible. Nonetheless, the blood pressure lowering effect of the DASH diet has been suggested to be in part related to the calcium content of the diet, but it should be noted that the DASH

diet also was lower in fat and higher in protein. Therefore, it is not easily attributable to one factor alone, rather a combination of several factors, as depicted in **Figure 1**.

The final point regarding composition of the DASH diet is that it included specific food choices. The diet contained whole grains, poultry, and fish (in addition to the fruits, vegetables, low-fat dairy, and nuts previously mentioned). While it was reduced in total and saturated fat, it was also reduced in meat, sweets, and sugar-containing beverages. Food was consumed as an overall pattern where it is quite possible that the interaction between food items is as important as the specific foods themselves in reducing blood pressure. Thus, the DASH diet contained dietary patterns promoted by the National Institutes of Health, National Heart, Lung, and Blood Institute. The dietary patterns of DASH are presented at three energy levels in **Table 5**.

Dietary Protein Consumption

Results of meta-analyses from several investigators indicate an inverse association between dietary protein and blood

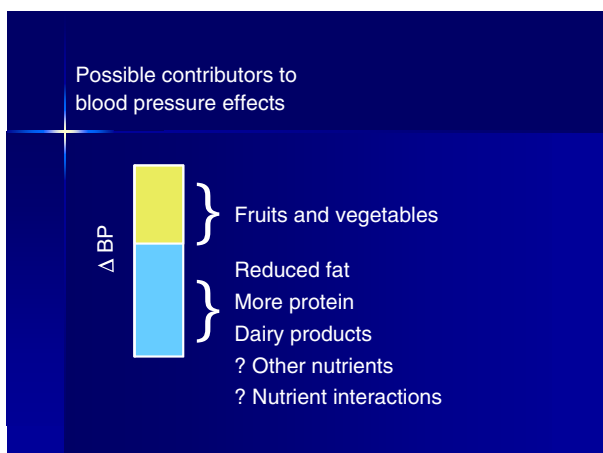


Figure 1 Assessing the effects of the DASH-Sodium diet on blood pressure. The yellow area depicts the fact that there is some certainty associated with the increases in fruits and vegetables and reduction in sodium. The light blue area represents the other components of the diet and just what might be the specific contribution of each, alone or in combination with other factors.

pressure levels. Some evidence suggests a small beneficial effect of plant protein on blood pressure, but data have not been conclusive. The DASH diet contained approximately 18% of energy from protein compared to the 15% of energy from protein in the other diets tested. Owing to the addition of low-fat dairy foods and de-emphasis on high-fat meats, it can be assumed that this elevation of protein was brought about by foods that would contribute protein from perceived beneficial sources. This is an area of dietary intake requiring further investigation.

Fish Consumption

Studies suggest that an intake of fish oil at a level of approximately 4 g per day reduces systolic blood pressure by approximately 1.7–2.1 mmHg and diastolic blood pressure by 1.5–1.6 mmHg. These effects tend to be larger in individuals older than 45 years of age and in populations with blood pressure readings greater than 140/90 mmHg. Generally there have been differences associated with fish oil capsules compared to naturally occurring sources. Differences between supplement use and consumption through food favor the food-based choice. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the main contributors of omega-3 fatty acids in fish. The DASH diet had a relatively high fish content (compared to animal meats) and this could be yet one more factor contributing to the lowering of blood pressure in the individuals in DASH.

The American Heart Association recommends eating two servings of fish per week and emphasizes that the choice should be a fatty fish (such as salmon, herring, or mackerel). Not all fish are created equal, at least in terms of contents of omega-3 fatty acids. **Table 6** provides a listing of amounts of EPA, DHA, and the combined totals in fish and other seafood sources. Descriptors include raw products that are commonly found, but realize that the intakes given are rough estimates due to potential variability in oil content within species, season, and diet. Studying the listing in **Table 6**, one can conclude that the majority of omega-3 fatty acids appear as DHA, as opposed to EPA. One interesting observation is the difference between farmed and wild versions of the same fish species, as well as the differences among species of the same fish or other seafood. Cooking methods and other preparation techniques may affect the final concentrations in raw fish. The

Table 5 Food group servings for the DASH diet at three energy levels

Food group	Daily servings, except as noted		
	1600 kcal or 6694 kJ	2000 kcal or 8368 kJ	3100 kcal or 12 970 kJ
Grains and grain products	6	7–8	12–13
Vegetables	3–4	4–5	6
Fruits	4	4–5	6
Low fat or fat free dairy foods	2–3	2–3	3–4
Meats, poultry, fish	1–2	2 or less	2–3
Nuts, seeds, dry beans	3 per week	4–5 per week	1
Fats and oils	2	2–3	4
Sweets	0	5 per week	2

Table 6 Eicosapentaenoic (EPA, 20:5 n-3) and docosahexaenoic (DHA, 22:6 n-3) acid in fish, mollusks, and crustaceans (per 100 g) for those containing at least 0.3 g EPA + DHA (partial listing). View website entry below for additional information

<i>Item</i>	<i>EPA (g)</i>	<i>DHA (g)</i>	<i>EPA + DHA (g)</i>
Fish			
Caviar, black and red, granular	2.74	3.80	6.54
Shad, American	1.09	1.32	2.41
Roe, mixed species	0.98	1.36	2.35
Mackerel, Atlantic	0.90	1.40	2.30
Salmon, Atlantic, farmed	0.86	1.10	1.97
Salmon, Chinook	1.01	0.94	1.95
Herring, Pacific	0.97	0.69	1.66
Herring, Atlantic	0.71	0.86	1.57
Anchovy, European	0.54	0.91	1.45
Salmon, Atlantic, wild	0.32	1.12	1.44
Mackerel, Spanish	0.33	1.01	1.34
Salmon, Coho, farmed	0.39	0.82	1.21
Tuna, fresh, bluefin	0.28	0.89	1.17
Salmon, Coho, wild	0.43	0.66	1.09
Bluefish	0.25	0.52	0.77
Bass, striped	0.17	0.59	0.75
Swordfish	0.11	0.65	0.75
Trout, mixed species	0.20	0.53	0.73
Salmon, sockeye	0.23	0.44	0.67
Salmon, chum	0.23	0.39	0.63
Sea bass, mixed species	0.16	0.43	0.60
Tilefish	0.09	0.35	0.43
Pollock, Atlantic	0.07	0.35	0.42
Seatrout, mixed species	0.17	0.21	0.37
Catfish, channel, wild	0.13	0.23	0.36
Mackerel, king	0.14	0.18	0.31
Pike, walleye	0.09	0.23	0.31
Snapper, mixed species	0.05	0.26	0.31
Mollusks			
Oyster, Pacific	0.44	0.25	0.69
Squid, mixed species	0.15	0.34	0.49
Mussel, blue	0.19	0.25	0.44
Oyster, Eastern, farmed	0.19	0.20	0.39
Oyster, Eastern, wild	0.18	0.14	0.31
Crustaceans			
Crab, queen	0.26	0.11	0.37
Crab, blue	0.17	0.15	0.32
Crab, Dungeness	0.22	0.09	0.31

USDA website noted as the data source for the table contains information on many additional fish/seafood varieties and provides data on cooked, smoked, and canned versions of fish and seafood where applicable. This type of information can be of use to the consumer.

Other Fatty Acid Effects

There have been some suggestions that MUFAs, particularly olive oil, may help to lower blood pressure. Olive oil has

typically been associated with the popular Mediterranean diet, which has been promoted as a treatment for CVD. It should be noted here that other oils, e.g., canola and peanut oil, have a high monounsaturated fat content. Nuts, part of the DASH diet, contain significant amounts of monounsaturated fats and fit nicely into the Mediterranean diet scheme.

Caffeine

While a link between caffeine consumption (particularly coffee) and hypertension may exist, effects of coffee drinking on blood pressure appear to be dependent on time of consumption and subsequent determination of blood pressure values. Generally, a role for caffeine intake and development of hypertension is not felt to be significant.

Weight Reduction

Obesity and overweight are now considered independent risk factors for CVD and are closely associated with hypertension. This linkage was demonstrated by the Framingham Heart Study investigators in the US in the 1960s. Obesity in the industrialized world has been increasing in epidemic proportions. The relationship between increasing body weight with increasing blood pressure has been termed obesity hypertension and treatment requires consideration of physiological changes related to this disorder. While efforts have been underway in the US to reduce overweight and obesity, current estimates are that the age-adjusted prevalence of overweight (BMI ≥ 25.0) among adults aged 20 is 34.2%, those considered obese (BMI ≥ 30.0) is 33.8%, and those extremely obese (BMI ≥ 40.0) is 5.7%. The increase in obesity is seen in all ethnic, gender, and age groups. This problem is not only confined to the average American; the US military reported that more than 50% of military personnel were overweight and more than 6% were obese in the late 1990s, despite high physical activity levels due to the rigors of basic training and regular field exercises. For the military, this reflects a trend that mirrors what is happening in the general population. Since 2003, the number of US troops diagnosed as overweight or obese has more than doubled, with the number of military service members diagnosed as overweight in 2010 reaching approximately 85 000.

In 2008, 1.5 billion adults were classified as overweight and almost 500 million as clinically obese (200 million men and nearly 300 million women); worldwide obesity has more than doubled since 1980. Developing countries such as Mexico, China, and Thailand have seen dramatic increases in obesity. Globally, prevalence in countries in intermediate development has increased more than 70% over the past decade, some approaching 100%. Alarming, this epidemic has spread to children with 43 million children under 5 years of age estimated to be overweight worldwide in 2010. Data from the US in 2007–2008 indicated that 17% of children and adolescents 2–19 years of age were overweight. Overweight children are at risk of becoming overweight adults, but more importantly are likely to experience chronic health problems

(including hypertension) associated previously with only adult obesity.

The World Health Organization (WHO) has recommended an integrated, multifaceted, population approach to be implemented to bring about effective weight management for those at risk of overweight and obesity. The key elements for individuals include the following:

- Limiting energy intake from total fats.
- Increasing consumption of fruits, vegetables, legumes, whole grains, and nuts.
- Limiting intake of sugars.
- Engaging in regular physical activity.
- Achieving energy balance and a healthy weight.

WHO suggests that the food industry can play a significant role in the promotion of healthy diets by:

- reducing fat, sugar, and sodium in processed foods,
- ensuring healthy, nutritious choices are available and affordable,
- using responsible marketing, and
- supporting regular physical activity practices in the workplace.

Obviously, it is essential to maintain a healthy body weight and thus necessary to keep a focus on energy intake in an effort to prevent overweight and obesity. With regard to hypertension, weight reduction appears to be the most promising answer in terms of potential impact on lowering blood pressure. Losing as little as 4.5 kg, or 10 lb, of body weight can reduce blood pressure. Adopting healthy eating patterns would yield additional benefits.

An additional concern related to obesity is the condition known as the 'metabolic syndrome,' which may include hypertension as one of its criteria. From a practical view, identifying dietary and lifestyle factors are important in designing a diet and exercise program to reduce detrimental health consequences. For greater success, dietary treatment needs to be individualized and encompass a variety of weight loss strategies, such as meal replacements (for initial weight loss) and a variety of macronutrient approaches that enable the patient to succeed. Follow-up evaluations are critical to monitor progress and key to weight management.

Strategies for Implementing Nutritional Changes to Control Blood Pressure

Self-Monitoring

A strategy undeniably praised for weight control is that of self-monitoring one's food intake. The self-monitoring technique has been used to help people comply with other lifestyle recommendations, e.g., to increase physical activity by recording physical activity minutes. In the PREMIER clinical trial, participants on the 'established plus DASH' arm were required to monitor intake of energy, sodium, total fat, and saturated fat, and servings of fruit, vegetables, and dairy, so as to determine their compliance to the intervention. Those participants on the 'established' arm only recorded energy, sodium, and total fat intake. Most people find these recordings

difficult but readily admit that they are successful in documenting dietary compliance if taken seriously.

Although a time-consuming and difficult task, successful diet compliers continue this behavior change strategy, in addition to others, over the long term. Those who discontinue utilizing this technique often revert back to old habits and relapse.

Working with a Dietetics Professional

Dietitians in North America and abroad typically have to meet national standards set by professional organizations, e.g., the Academy of Nutrition and Dietetics, the Canadian Dietetic Association, etc. As such, these individuals are called 'registered dietitian' or other titles used only by those who have met these standards. Although one does not necessarily need to work with a professional, for some it is often easier to implement change when individuals can clear up confusing and often conflicting information by working with a dietitian who can provide credible nutrition information. Dietitians are taught to interpret the science into meaningful terms for the consumer. Additionally, a well-trained professional will be equipped with knowledge of motivational and behavioral strategies to help effect change.

There are many important parts of behavior change that are taught to the hypertensive client. Making lifestyle changes gradually so that one adjusts to one change before making another is important. Strive for short-term, attainable goals. Getting off track is not uncommon, but figuring out what triggered the sidetrack and getting back on track are equally important. Understand that slips are inevitable; it takes time to get used to the new changes. In essence, lifestyle change is a long-term process, but worthwhile for good health.

Conclusions

Ultimately blood pressure control will mandate lifestyle changes, even if hypertensive medications are prescribed. First and foremost, body weight has to be a key focus and the goal should be to work toward an ideal body weight and avoid gaining weight during the aging process. Although it is a simple concept in theory, it is a difficult task for many to accomplish. Diet plays a key role in blood pressure control. Data suggest that increasing fruits, vegetables, and dairy products and reducing sodium should be priority when modifying dietary intake. The DASH dietary pattern approach yields an increased consumption of potassium, magnesium, and calcium, which have documented effects on lowering blood pressure as well. Increasing whole grains, nuts, and legumes will additionally improve mineral and fiber content of the diet. DASH results suggest that individuals should consume less red meat and instead choose fatty fish to increase consumption of omega-3 fatty acids. Alcohol consumption in moderation should also be encouraged. These simple, but often difficult to accomplish, strategies will help to lower blood pressure and improve risk against CVD.

See also: Hypertension: Dietary Factors

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NUTRITIONAL PROBLEMS OF PRE-SCHOOL CHILDREN

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The 'preschool period' extends from the end of infancy to approximately five years of age. It is a time when children are changing from wholly dependent infants to mobile individuals who communicate their needs verbally and eat independently. Failure to nurture children and provide them with adequate amounts of appropriate food during this period may have lifelong physical and psychological effects. Undernutrition is also directly or indirectly implicated in approximately one-third of the 10-million deaths that occur each year in children under five globally.

Young children face many challenges: their nutrient requirements are relatively high yet intake is frequently threatened. Infections, particularly diarrheal disease, respiratory infections, measles, and malaria may simultaneously induce anorexia thereby increasing energy expenditure and nutrient losses. Weaning from the breast may further reduce resistance to infection and remove a nutritional 'safety net'. The need to fuel catch-up growth after infection can further increase requirements. Effective parenting is essential to ensure that the child's needs are met and that eating habits conducive to lifelong health are established. Faltering of growth is often attributable to the inability of carers to recognize children's needs and act appropriately by feeding them responsively.

Paradoxically, the rising prevalence of obesity in industrialized countries and those in economic transition also has its origins in early life; accelerated weight gain in the preschool years is associated with an increased later risk of cardiovascular and metabolic complications later in adult life. The existence of this 'double burden' of over and undernutrition has led to the concept that these early years, particularly those before the second birthday, represent a 'window of opportunity' for investment in nutrition.

Growth in the Preschool Years

Publication of the World Health Organization growth chart in 2006 was a major achievement: it represents a 'standard' that prescribes how healthy, well-nourished children should grow. Although there is a deceleration in the rate of growth between one and five years of age, weight approximately doubles and height increases by approximately 10%. Overall healthy children become much leaner, the relative amounts of fat and fat-free mass changing substantially. There are also changes in the proportionate growth of organ systems at this age; the brain continues to grow at a greater velocity than somatic tissues, particularly during the early preschool years. Indeed it has

attained approximately 80% of its adult size by the age of two and 90% by five.

Alongside slowing of growth in late pregnancy or infancy, suboptimal growth in the early preschool years also contributes to stunting. Infections are frequent at this time of life and children must be adequately fed to support catch-up growth whilst they are well. This is not simply a matter of increasing energy intake but also of achieving adequate micronutrient supply to support the accrual of lean tissue. Low micronutrient status is common in this age group and often manifests as deficiencies of iron, zinc, vitamins A and D.

Child Development in the Preschool Years

Eating behavior causes much concern to parents during these early years. Problems such as food refusal, fussiness, and restricted dietary selection are very common and need to be handled appropriately to ensure adequate nutrient intake and to encourage the development of healthy eating and activity patterns for life. Management requires an understanding of the developmental context in which they occur. Developmental progress through the preschool years is closely linked to nutritional status because it determines how the child eats, selects food, and expends energy. Equally, disturbed nutritional status is a common consequence when developmental progress fails to follow the usual course.

As they enter the preschool years children just begin to walk; by the age of two they can go up and down stairs, by the age of three they can ride a tricycle, and by four they run with sufficient balance and skill to avoid objects. In keeping with these advances, physical activity level (PAL) rises from approximately 1.40 to 1.55 between the ages of one and five years. Children also develop their eating skills in the preschool years. At one most of them can drink from a beaker, albeit with some spillage. They need help to hold it but should be encouraged as this is more advantageous for dental health than drinking from a bottle or spouted cup. Towards the end of the first year children should be able to pick up food with their hands and transfer it accurately to the mouth. At this age they can also use a spoon; at first it may be upside down but by the end of the second year most of them will use it properly. Feeding is messy but children must pace their own eating and be allowed to handle food. Children should never be coerced or forced to eat as this will lead to aversive learning and rejection of foods. The ability to use spoon and fork together normally appears towards the end of the second year

and by four they are used accurately but not until five years or so can the child be expected to use knife and fork.

Socially, children communicate likes and dislikes increasingly effectively. In the second year of life they initially may do this by gestures such as head shaking, spitting back foods, or uttering single words but by three they will have the ability to use two or three words together to articulate preferences clearly. Between these ages imitating others becomes increasingly important and children begin to take much more interest in the appearance and texture of food, including food preparation. Capitalizing on these aspects is important in helping the child to accept a variety of foods and eat well.

The Development of Food Preferences and Eating Behavior

In the second half of infancy children show interest in, generally accept and enjoy an increasing range of tastes and textures as complementary foods are introduced. This normal liking for new foods is known as neophilia, though as the first year of life progresses into the second a more conservative approach known as neophobia gradually emerges. It has been suggested that this sequence reflects evolutionary pressures brought about by the need of toddlers to reject potentially toxic new food items as they become more mobile and explore their environment, though it is important to realize that they will also often reject the foods they once accepted. Physical properties of food (such as the presence of 'bits' or its color) may come to dominate likes and dislikes much more than the type or taste of food.

During the neophilic stage of infancy it is important to capitalize on enhanced acceptance of food items, diversifying the diet appropriately, and advancing the texture to lumpier foods. In this way the infant should at least enter the preschool years accepting a wide range of foods offering a basis on which to encourage further acceptance. If this is not done it can be more difficult to achieve change in the second year; sometimes children who did not progress to lumpier foods in infancy may still require all food to be pureed. Addressing such issues requires behavioral intervention to persuade the

child first to accept foods that dissolve in the mouth, progressing to small lumps, then larger pieces, and so on.

As they pass through the preschool years, normal children gradually learn to accept more food items. Approximately two-thirds of two year olds reject any new foods offered, whereas by three years of age this proportion has more than halved. Gaining this increased acceptance requires repeated exposure to foods and the opportunity to imitate others; eating together with peers, siblings, and other family members is thus an important learning experience. Many exposures to new food items may be needed before they become accepted, and it is important to note that food must be tasted not merely handled. Consumption may initially be confined to specific contexts, for example a food may be eaten at nursery but refused at home. Vegetables, fruits, and meat tend to be the foods most often rejected, though satiating foods seem more readily accepted. If the diet is not adequately balanced this may have the undesirable consequence of constraining micronutrient intake, for example, intakes of zinc and iron (from meat and fish), vitamin C, and vitamin A (from vegetables and fruits) may be very low.

Some evidence-based practical guidance about helping fussy preschool children to eat is given in **Box 1**.

Recognizing the extent of normal variation and the developmental context of food selection is fundamental to managing many common parental concerns about eating behavior in the young. Abnormal persistence of neophobia into the later preschool years may be a manifestation of underlying psychological disorder (e.g., autism). From a public health perspective, it is important to recognize the growing body of evidence from cohort studies that suggests dietary choices established in the preschool years strongly influence patterns of eating in later life.

Dietary Recommendations for Young Children

In the past, energy requirements of young children have been estimated from studies of dietary intake but new data on the

Box 1 Helping fussy preschool children to eat

Dos

- Encourage them to eat with the 'good' eaters among their peers and family members.
- Listen to them, talk to them about food, and engage them in preparing it.
- Observe mealtimes, avoiding distraction whilst eating.
- Allow them to eat at their own pace.
- Encourage them to try new tastes, even in tiny amounts. They can spit out if necessary.
- Repeat exposures many times at intervals if necessary. Children must taste the food, not merely handle it, if consumption is to be encouraged.
- Reward them for eating (but never offer foods or confectionery as a reward).

Don'ts

- Don't put pressure on them to finish or 'eat up'.
- Don't serve continuous snacks or meals on demand in an effort to get them to eat 'something'.
- Never force-feed.
- Don't offer liked foods as a reward (children then merely value such foods more than the food offered, thus reinforcing existing preferences).

(From "Laying the Table: Recommendations for National Food and Nutrition Guidance for Early Years Settings in England" (<http://www.schoolfoodtrust.org.uk/research/advisory-panel-on-food-and-nutrition-in-early-years>), School Food Trust.)

total energy expenditure (TEE) of preschool children have permitted a re-evaluation, which factors in the small amount of energy stored (only approximately 1% of TEE) during growth at this age. The resulting estimated average requirements (EAR) for energy are approximately 10–20% lower than those formerly set.

Although these newer estimated average requirements for energy are lower than those formerly set, they are higher than those of adults if expressed on a per unit body weight basis as the requirements for many other nutrients, particularly micronutrients. To meet micronutrient demands within the limits imposed by energy requirements for health, it is important that the diet is diversified and not overly dependent on milk. Firstly, foods have to be relatively micronutrient dense for a balanced supply of nutrients in adequate amounts; secondly meals have to be spaced across the day and snacks provided between meals so that the amount served at one meal is not more than a young child can reasonably be expected to consume. For example, three meals (breakfast, lunch, and tea) may be served to provide, respectively, 20%, 30%, and 20% of energy requirements, with the remaining 30% divided equally between a mid-morning, mid-afternoon, and a bedtime snack.

In general, children should be offered and consume items from each of the food groups every day: starchy foods (e.g., bread, cereals, pasta, rice), meat and fish (or alternatives such as pulses), vegetables and fruit, and milk and dairy foods. To achieve dietary balance these need, however, to be provided in amounts which differ proportionately from those consumed by adults. For example, although providing five portions of fruit and vegetables each day is a reasonable aim, each should be approximately 40 g rather than the 80 g recommended for an adult or the intake of other foods at mealtimes may be reduced. Similarly milk intake should be limited to approximately 400 ml per day for those under two, and then not more than approximately 350 ml per day. Providing larger amounts may lead to satiation and reduce food intake at mealtimes. Milk should not be skimmed but full fat until about two years of age and semi-skimmed thereafter until five years of age. Reduced-fat foods are unsuitable for most preschool children (see the section 'Toddler diarrhea').

Unfortunately, foods low in micronutrient density such as biscuits, crisps, and confectionery often contribute significant amounts of energy as refined carbohydrate and fat along with salt in the diets of young children. Processed meats and bread may further increase saturated fat and salt intake. Indeed it can be difficult to keep salt intakes in diet of young children within the target population average (2 g per day, 1–3 years; 3 g per day, 4–6 years), particularly where there is reliance on commercially prepared foods. Drinks may additionally offer significant amounts of sugar; preferably water should be provided between meals and milk or fruit juice (diluted to 50%) with meals.

Obesity and Overweight in Preschool Children

Over the last 30 years there has been a remarkable increase globally in the proportion of young children who are overweight or obese. In the UK, weight and length are routinely

measured in the reception school year (at age 4–5 years); during the school year 2008–09 in England some 23% of these children were overweight or obese (>85th percentile or >95th percentile BMI, respectively, on the UK1990 Reference). Across Europe the prevalence of overweight or obesity at the age of four on the International Obesity Task Force Reference seems to vary from 11% (Romania) to 33% (Spain). This recent increase in prevalence of obesity has been most evident in industrialized countries and those in economic transition globally. A UK cohort study found that risk of obesity and overweight at five years was greater among children of Asian or Afro-Caribbean ethnic origin than among those of White British or European origin. The relative contributions of diet and activity to pathogenesis in this age group are not well understood though some work suggests that there are groups of young children, particularly girls, who show strikingly low levels of activity as early as five years of age. This suggests that patterns of physical activity (as well as diet, *vide supra*) may become established even at early ages.

Definitions of overweight and obesity used clinically differ from those applied in population surveillance. In the UK the thresholds currently adopted for surveillance are BMI >85th percentile (overweight) or >95th percentile (obese) on the UK1990 reference whereas the thresholds chosen for clinical diagnosis are >91st or >98th percentile. However, where British preschool children are concerned, the matter has been further complicated since 2009 by replacement of the UK1990 reference with the World Health Organization international growth reference. The proportion of two-year-old children above the 98th percentile on the WHO reference is almost double that on the UK1990 reference, though after four years of age the discrepancy is smaller.

The contribution of preschool years to later obesity has been overlooked to an extent in the past, on the grounds that the correlation between weight in early childhood and adult life is relatively low but this view is challenged by longitudinal study of changes in weight and BMI in cohort studies. For example, a UK cohort study observed that change in weight standard deviation score between birth and five years of age predicted weight standard deviation score, BMI, and metabolic risk at nine years. Longitudinal studies in other countries have also shown that rapid increase in body mass index at this age, particularly amongst those thin in infancy, is associated with glucose intolerance and coronary heart disease in later life. Interventions to prevent excessive weight gain therefore need to begin earlier than school age.

Many preschool interventions have been tested with the intent of preventing accumulation of excess weight. They include breastfeeding promotion, parental dietary education (peer or professional), and interventions to promote physical activity. Disappointingly they seem individually to have very limited effectiveness in reducing the proportion of children who are overweight or obese. It is probable that to be effective, interventions need to be combined and supported over a longer period than has thus far been tested. For example, interventions to increase physical activity have been tested in formal settings such as nurseries but these often have not involved parents and carers sufficiently to achieve sustainability. The most effective preventive measures are likely to encompass dietary change combined with increased physical activity

through play, delivered in a family setting and closely involving parents (many of whom may be overweight or obese themselves). One example of a community approach is the Healthy Exercise Nutrition for the Really Young (HENRY) intervention, a national project in the UK aiming to educate those who work with young children about obesity prevention.

Clinical interventions for children already overweight or obese have been studied very little in children under five, the principal focus being on children of school age and adolescents. The general aim of management in such children is to maintain weight rather than promote weight loss. Parents may not appreciate their child is overweight or may even be concerned that the normal tendency to thinness at this stage of life reflects a need to eat more rather than less. Advice should focus on serving three meals, eating with family members, and restricting 'snacks', which are high in non-milk extrinsic sugars or fat and low in micronutrient value (such as confectionery and crisps). These should be replaced with foods such as vegetables, fruits, or bread and water substituted for sweetened beverages. Fruit juice should always be diluted in the ratio 1:1 and served only with food.

Weight Faltering

Growth monitoring is a fundamental aspect of both child health surveillance and pediatric clinical care. It is based on the premise that the growth trajectory of a healthy child follows the percentile channels described by a reference or 'standard' such as that described by the World Health Organization International Growth Reference of 2006. The first year of life presents some exceptions to this statement as a period in which the individual child's patterns of linear and ponderal growth may show 'physiological' catch up or catch down as a consequence of mismatch between intrauterine nutrient supply and genetic potential. Such shifts are usually complete by the end of the first year and deviations from percentile lines later thus require closer examination.

The term 'weight faltering' (previously known as 'failure to thrive') refers to a slowing of weight velocity, resulting in a downward crossing on weight percentiles on the growth chart. Absolute definition depends on the age of the child and the growth chart used; in infancy a fall through two or more percentile spaces is often cited as an objective descriptor, though this is generally unhelpful in a child over one year of age. At this time healthy children are gaining weight more slowly, which means that even a child who is gaining no weight may take up to a year to fall through two percentile spaces. Sometimes children who are underweight (weight <0.4th percentile) are also described as 'failing to thrive', though it is more helpful to consider whether children are wasted (unusually thin, representing recent or current undernutrition) or stunted (short, reflecting either genetic background or long-standing undernutrition).

The causes of weight faltering are complex and largely determined by the environment in which the child lives. In resource-poor countries curtailment of breastfeeding, recurrent acute infection, and food shortage are prevalent. In industrialized countries children who show weight faltering are

often investigated extensively with the intention of excluding underlying organic disease, though in the absence of other presenting clinical features this accounts for only a small proportion of cases, probably as few as 5–10%. Poverty, deprivation, low maternal educational status, and maternal depression have all been implicated. It is helpful to work with parents in such circumstances, helping them to recognize the child's needs to prepare appropriate food and encourage the child to eat. Working with parents in groups, for example, through a nursery or early years center, can be particularly helpful in this respect. Peer education may also be helpful.

Micronutrient Deficiency

Globally, preschool children are particularly at risk of deficiency in many micronutrients including iron, zinc, iodine, vitamin A, and vitamin D. Often these coexist. Whilst the prevalence is highest in poorer countries, particularly southern Asia and sub-Saharan Africa, iron deficiency and rickets continue to present clinically in significant numbers of children.

Iron Deficiency Anemia

Mild hypochromic anemia ($Hb < 11.0 \text{ g dl}^{-1}$) may affect as many as 10–20% of young preschool children in the UK. It is common in underprivileged urban communities, particularly those with a high proportion of children of South Asian ethnic origin. Low birth weight often predisposes to low iron stores; later, early cessation of breastfeeding with dependence on cow's milk, and foods low in iron content contribute to low intake, which is compounded by increased losses during gastrointestinal infection. Severe anemia may be a manifestation of malabsorption, for example, a consequence of celiac disease, particularly if associated with weight faltering. Both iron and folate deficiency may be present in this condition.

Rickets and Vitamin D Deficiency

The prevalence of rickets in industrialized countries has markedly declined over the last century but over the last decade a resurgence has been reported in the UK and other countries. Softening of the bone matrix in rickets predisposes to fractures and to bony deformities such as *genu varum* and delayed closure of the cranial sutures. Deficiency of vitamin D may result in other clinical presentations in young children including hypocalcaemic seizures and laryngospasm (stridor). Rickets in Northern industrialized countries is commonest among (but not unique to) black and ethnic minority population groups, particularly those of African and south Asian ethnic background. Many mothers in these groups have low vitamin D status throughout pregnancy, particularly during the winter months, thus predisposing the infant to low vitamin D status at birth. Low intake of vitamin D through infancy, coupled with increasing tendency to protect children from ambient sunlight through use of clothing and sunscreens, further increase risk.

Conventionally vitamin D deficiency is defined by the presence of a plasma 25-hydroxycholecalciferol concentration

$<25 \text{ nmol l}^{-1}$. Levels above this threshold are unlikely to be associated with bone disease but those below 50 nmol l^{-1} are termed 'insufficient' as levels may fall during winter to those associated with deficiency. Prevention of vitamin D deficiency and rickets entails the consumption of dietary supplements by groups at greatest risk, coupled with encouragement to expose skin safely to sunlight during the summer months. Pregnant and breastfeeding women should consume 10 mcg (400 IU vitamin D daily (600 IU is recommended in the USA). Policy on the use of supplements in infants varies between countries; in the UK it is recommended that all breastfed infants should be given a supplement of 7 mcg per day by seven months of age, starting earlier than this if the mother's vitamin D status is uncertain, and continuing until at least four years of age. In high-risk groups, for example, those with dark skin or those with reduced sunlight exposure, supplementation should continue through childhood.

In some other European countries and the USA, supplementation from birth is recommended. Infants who are not breastfed do not require supplementation until consumption of infant formula (which is fortified with vitamin D) falls below 500 ml per day.

Food Allergy and Intolerance

Carers may attribute a number of eating-related symptoms in young children to 'allergy', leading to avoidance of certain foods. The prevalence of such perceived allergy is much higher than true prevalence but belief in 'allergy' may sometimes lead to worrying and uninformed dietary restrictions that place the child at risk of nutrient deficiency.

'Food allergy' and 'food intolerance' may nevertheless both occur in young children and cause serious clinical manifestations. In both, the child exhibits a reproducible adverse response to a food or food component; in the case of allergy this is immunologically mediated, whereas a variety of mechanisms may explain intolerance. The latter include pharmacological responses to food components or inability to metabolize a food component (e.g., hereditary fructose intolerance or lactose intolerance). Both allergy and intolerance need to be distinguished from psychological aversion to foods, which is quite common in this age group (*vide supra*), particularly during the earlier preschool years.

Food allergy is usually mediated through release of immunoglobulin E in response to antigen exposure and thus manifests with immediate symptoms when the offending food is ingested: these may include swelling and erythema of the face, lips, and tongue, periorbital or generalized edema, and rarely anaphylactic shock. Acute gastrointestinal symptoms such as vomiting, pain, or diarrhea may also occur. Sometimes symptomatology is more chronic, for example, worsening of wheezing or eczema. Symptoms resolve when the food is omitted from the diet and recur when it is reintroduced. It is important, however, to stress that neither manoeuvre should be attempted without professional supervision. Firstly, exclusion of potential antigens without expert dietetic advice is often unsuccessful because common antigens (e.g., cow's milk protein, nut proteins, and soya proteins) are present in a large number of commercially available foods. Secondly,

unsupervised dietary manipulation may lead to restriction of nutrient intake, particularly of calcium and micronutrients. Thirdly, challenge with suspected antigens should always be conducted where facilities for resuscitation are available because the severity of symptoms may vary from one exposure to another; sometimes a second exposure gives rise to severe and life-threatening symptoms even though these may previously have been mild.

Dietary challenge is not always necessary to establish the diagnosis, as skin prick testing or specific IgE measurement can be helpful if the clinical history is strongly suggestive. Most young children grow out of egg or milk allergies by school age, but nut, fish, soya, and wheat allergies are generally more persistent and may remain a problem into adult life. Serial measurement of specific IgE can be helpful in determining whether resolution is likely. Parents may need to be provided with an adrenaline injection device and instructed in its use. Desensitisation through subcutaneous administration of specific antigens may in future prove an effective therapy where allergic symptoms persist.

Toddler Diarrhea ('Nonspecific Childhood Diarrhea')

Disturbances of bowel habit are common in preschool children. 'Toddler diarrhea' is the episodic passage of frequent, loose watery stools containing undigested vegetable material in children who are otherwise well. Affected children do not become dehydrated, nor do the stools contain mucus or blood; diarrhea often alternates with periods of constipation. They thrive normally and do not show features of nutrient malabsorption. Children usually outgrow this condition by the age of five or six, suggesting that a developmental dysregulation of bowel motility is responsible. Commonly identified dietary factors include over consumption of sugar sweetened beverages (e.g., squashes), low-fat diet, or the increasing use of artificial fat replacers, and low digestibility carbohydrates in commercially available foods. Increasing dietary fat intake may be helpful through promoting ileal braking and thus delaying intestinal transit. It can also be helpful to advise on the consumption of fruit and vegetables, which may either be too high or too low.

See also: Appetite: Psychobiological and Behavioral Aspects. Body Composition. Breast Feeding. Celiac Disease. Early Origins of Disease: Non-Fetal. Energy Requirements. Food Allergies: Diagnosis and Management. Food Choice: Behavioral Aspects. Food Intolerance. Growth and Development: Physiological Aspects. Growth Monitoring. Malnutrition: Secondary, Diagnosis and Management. Nutritional Aspects of Bone. Obesity: Childhood Obesity. Pediatric Feeding Disorders: Feeding Children Who Can't or Won't Eat. Vitamin A: Deficiency and Interventions; Physiology, Dietary Sources, and Requirements. Vitamin D: Physiology, Dietary Sources, and Requirements. Zinc: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements

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NUTRITIONAL REQUIREMENTS OF INFANTS

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Abbreviations

AI Adequate intake
DRI Dietary Reference Intakes

EAR Estimated average requirement
RDA Recommended Dietary Allowance
UL Upper level

Glossary

Adequate Intake (AI) The recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate; used when an recommended dietary allowance cannot be determined.

Dietary Reference Intakes (DRI) The nutrient reference values developed by the Institute of Medicine in Washington, DC, USA that comprise four nutrient-based reference values that can be used to assess or plan the diets of healthy people. The reference values include the four other Glossary terms defined here.

Estimated Average Requirement (EAR) The average daily nutrient intake level that is sufficient to meet the nutrient requirements of half of the healthy individuals in a particular life stage and gender group.

Recommended Dietary Allowance (RDA) The average daily nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97–98%) healthy individuals in a particular life stage and gender group.

Tolerable Upper Level (UL) The highest average daily nutrient intake level that is likely to pose no risk of adverse health effects to almost all individuals in the general population. As intake increases above the UL, the potential risk of adverse effects may increase.

Introduction

Optimal nutritional support of infants in the first year of life is essential to attain normal trajectories of growth and development. Additionally, emerging evidence from epidemiological and animal research supports the hypothesis of an interaction effect or programming of genes by nutrition (as an environmental exposure) even during neonatal and early childhood that impacts on the growth and body composition of the child. Detrimental exposures in early life may also enhance the susceptibility to various chronic diseases developing in later life. For example, greater growth velocity in infancy, which may be the result of excess nutrition, has been tracked to risk for later obesity and cardiovascular disease. Various infant feeding practices have also been associated with risk of later obesity. For example, a randomized trial in infants fed on formula with differing protein content compared with breast-fed infants demonstrated that early rapid growth was greater in infants fed on formula with higher protein intakes compared with breast-fed groups. Based on this observation, it was predicted that the observed higher protein intake from formula compared with the breast milk would produce about a 13% higher risk for later obesity. Although suggestive of an early nutrition programming effect, there is not yet sufficient information that can be applied as a basis to set the dietary standards for infants.

Recommended nutrient intakes or dietary standards are produced by many countries as well as key international agencies such as the Food and Agriculture Organization/World Health Organization (FAO/WHO). The importance of adopting the quantity and quality of nutrients in human milk as the reference standard in determination of the nutrient recommendations for infants younger than 1 year of age has been reinforced by agencies who set dietary recommendations such as the Institute of Medicine (IOM) in Washington, USA. For infants, the recommended intakes are usually intended for term-born, healthy, and normally growing infants who have a birth weight of more than 2500 g (and thus not small for gestational age). In this article, the nutrient requirements outlined reflect recent reports of the Dietary Reference Intakes (DRIs) for USA and Canada as published by the IOM. The key changes in the derivation of the DRIs for infants compared with the previous dietary standards from USA and Canada include adoption of human milk as the reference model for setting recommended nutrient intakes for infants, simplification of age groupings within the first year, no specific provision of dietary recommendations for formula-fed infants, and gender-specific DRI values for fewer nutrients and only where data were available to support such gender specificity. Another major change from previous dietary standards is that upper levels (ULs) for nutrient intake were defined for the first time. Unfortunately, for infants, few ULs were established due

to a paucity of relevant age-specific data; even for those aged 1–18 years, the UL values were often extrapolated from adult values.

This article provides an overview of key concepts and examples of the DRIs specific for infants, future needs for additional research, and practical aspects of meeting the dietary recommendations for infants.

DRIs for Infants

For infants, evaluation of evidence to establish the DRIs consistently revealed a paucity of appropriate studies on which to base an Estimated Average Requirement (EAR) or UL. A Recommended Dietary Allowance (RDA) could not be calculated if a value for the EAR was not established, in which case the recommended intake was based on an Adequate Intake (AI). The nutrient recommendations for infants from birth through 6 months of age for all nutrients except for energy were set as an AI, a value based on “an observed or experimentally determined estimate of nutrient intake by a group of infants who are apparently healthy and assumed to be maintaining normal growth” (IOM, 2010). An AI value does not reflect an average requirement but rather an intake based on approximations or estimates of nutrient intakes that are assumed to be adequate. The mean intake of a nutrient was calculated based on the average concentration of the nutrient in human milk from 2 to 6 months of lactation using consensus values from several reported studies, multiplied by an average volume (0.780 l day^{-1}) of human milk. The predicted daily volume of breast milk ingested by an infant was based on observational studies that used test weighing of full-term infants. For infants aged 7–12 months, the AI for many nutrients was based on mean observed nutrient intake from human milk in the second 6 months (0.6 l day^{-1}), in addition to the published values for intake of nutrients from complementary or weaning foods if such data were available.

Assuming an adequate intake of milk for all infants was considered a valid approach because there is evidence that the volume of milk produced during the early months of lactation is very consistent among women irrespective of their racial, cultural, or nutritional diversity or variations in their body size. The volume of milk produced increases with the size of the infant, when twins are nursing, and in response to increased frequency of nursing. Using consensus values for the nutrient content of human milk was deemed appropriate because for many nutrients – energy, macronutrients, and macrominerals – maternal diet does not influence the nutrient content of the milk. The exceptions to this include the fatty acid profile, selenium, iodine, and the water-soluble vitamins. Although human milk is known to contain many non-nutrient bioactive factors, such as immune and growth factors and live enzymes, these were not considered to have an impact on nutrient needs *per se*.

For nutrients for which intake data were not available for those aged older than 6 months, the EAR or AI was derived by extrapolation from intake estimates of older children or adults using the formula with adjustments for metabolic body size,

growth, and variability:

$$\text{EAR}_{\text{infant or child}} = \text{EAR}_{\text{child or adult}} \times F,$$

where $F = (\text{weight}_{\text{infant or child}} / \text{weight}_{\text{child or adult}})^{0.75} (1 + \text{growth factor})$ or occasionally by extrapolating up from intake of breast-fed infants with similar adjustments using the formula

$$\text{AI}_{6-11 \text{ months}} = \text{AI}_{0-5 \text{ months}} \times F,$$

where $F = (\text{weight}_{6-11 \text{ months}} / \text{weight}_{0-5 \text{ months}})^{0.75}$.

For a few nutrients, such as iron and zinc, sufficient metabolic data were available to derive an EAR using modeling or factorial methods.

Because no specific AIs were derived for formula-fed infants, it is incumbent on the industry to design formulas with a quantity and quality of nutrients, which when fed will provide the amount of nutrients that meets the RDA or AI. An approach to establish the amount of nutrient needed by formula-fed infants is addressed under the section titled ‘Special Considerations’ in each DRI report. When possible, a DRI called the tolerable UL was defined as “the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population” (IOM, 2002). Chronic consumption of nutrients above the UL increases the potential risk of adverse effects, the latter varying by nutrient. For infants, data were only available to reliably estimate ULs for vitamins A and D and the minerals fluoride, selenium, zinc, iron, and now calcium in the recently revised report (IOM, 2010). Although adequate data were not available to define an UL for infants for other nutrients, it is important to note that intake for nutrients for which an UL does not exist should only be consumed from food or formula and not from supplements. Also notable is that the UL for iron for infants is only relevant to intake from supplements and not foods.

Summary of DRIs for Infants: Macronutrients – Energy, Carbohydrate, Fat, Protein, and Amino Acids

Energy

The estimated energy requirement (EER) for infants was derived by summing the predicted total energy expenditure (TEE) and energy deposition for growth. Because the energy needs for growth decelerate with advancing age, an equation for EER was established for three age intervals during the first year of life (Table 1). The TEE is calculated using an equation (Table 1) based on energy expenditure measured by doubly labeled water and adjusted for weight of the child. The EER is then the sum of TEE for an individual child plus the predicted energy deposition for age (Table 1). No adjustment for physical activity was included in the EER for infants. Examples of the EER for males and females using the reference weights are shown in Table 1 for infants at five age intervals during the first year of life. At most ages beyond the first 2 months of life, the values for EER exceed the average energy provided (500 kcal) by human milk assuming a volume of intake (0.780 l day^{-1}) from human milk.

Table 1 DRI estimated energy requirement (EER) for infants

<i>Equations</i>		
0–3 months	$(89 \times \text{weight of infant (kg)} - 100) + 175$ (kcal for energy deposition)	
4–6 months	$(89 \times \text{weight of infant (kg)} - 100) + 56$ (kcal for energy deposition)	
7–12 months	$(89 \times \text{weight of infant (kg)} - 100) + 22$ (kcal for energy deposition)	
<i>Calculated EER for age using reference weights for age</i>		
<i>Age (months)</i>	<i>Males (kcal day⁻¹)</i>	<i>Females (kcal day⁻¹)</i>
1	472	438
3	572	521
6	645	593
9	746	678
12	844	768

DRI, Dietary Reference Intake.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

By comparison, the energy requirements of infants during the first year of life recommended in the FAO/WHO/UNU (United Nations University) report (2004) are remarkably similar to that of the DRI report (IOM, 2005) as the approaches included the same parameters. In both the reports, estimates of TEE were based on the analysis of experiments using doubly labeled water and calculated by monthly age intervals, to which an assessment of energy deposition for growth was added. Any small variation in estimates of daily energy requirement by age between the FAO/WHO/UNU and DRI values is attributed to variations in estimates of mean weight, energy expenditure, or energy deposition for age by gender as can be appreciated by comparing the detailed tables outlining the energy requirements by monthly age intervals in the respective reports (IOM, 2005; FAO/WHO/UNU, 2004).

Carbohydrate

The AI for carbohydrate for infants through 1 year of age is based on the average carbohydrate intake from human milk and complementary foods for the 7–12-month age group (Table 2). Although the carbohydrate from human milk is almost exclusively lactose and that from infant's formula may be lactose, sucrose, or glucose polymers alone or in combination, there is no evidence that nonlactose-containing formulas vary from lactose present in human milk with regard to available energy.

Fat

As for other nutrients, the AI for fat intake is based on the average intake of fat from human milk alone or in addition to complementary foods after 7 months of age (Table 2). Although infant formulas are designed to contain a percentage of energy as fat similar to human milk (approximately 50%), the type of fat in formulas varies widely, including sources such as safflower, sunflower, soybean oil, and coconut and palm oils, usually in some combination.

Table 2 DRI for macronutrients for infants – carbohydrate, protein, fat, and essential fatty acids

<i>Nutrient^a</i>	<i>0–6 months</i>	<i>7–12 months</i>
Carbohydrate, AI (g day ⁻¹)	60	95
Protein		
AI (g day ⁻¹)	9.1	–
RDA (g day ⁻¹)	–	13.5
Total fat, AI (g day ⁻¹)	31	30
Linoleic acid (<i>n</i> -6), AI (g day ⁻¹)	4.4	4.6
α -Linolenic acid (<i>n</i> -3), AI (g day ⁻¹)	0.5	0.5

^aNo upper levels of nutrients were set for any macronutrients.

DRI, Dietary Reference Intake; AI, Adequate Intake; RDA, Recommended Dietary Allowance.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

Linoleic Acid (*n*-6) and α -Linolenic Acid (*n*-3)

The *n*-6 fatty acids are essential for infants, and in extreme long-term deficiency, skin lesions and delayed growth may develop. Linoleic acid serves as a precursor of arachidonic acid (AA), which is required for the synthesis of prostaglandins and other eicosanoids. The *n*-3 fatty acids are also essential as a precursor of docosahexaenoic acid (DHA), which comprises a large percentage of the fatty acids incorporated into developing brain and retina, and of eicosapentaenoic acid, which is the substrate for eicosanoid synthesis. Human milk is a natural source of both fatty acid families, including the long-chain polyenoic derivatives DHA and AA. The pattern of all fatty acids in human milk, including the polyenoic fatty acids, is dependent on the maternal diet. The AI established for infants for *n*-6 and *n*-3 fatty acids is based on the average content in human milk reported for North American women with the addition of that from complementary foods during months 7–12 (Table 2).

Feeding of mother's milk compared with cow milk-based infant formula has been associated with a positive benefit to developmental outcomes (cognitive, motor, and vision) in both retrospective and prospective studies (but not randomized trials for obvious ethical reasons). To date, investigations of the nutrient(s) possibly responsible for the observed benefits of mother's milk on neurodevelopment have focused on the long-chain polyenoic fatty acids DHA and AA. These fatty acids represent the greatest proportion of polyenoic fatty acids contained in phospholipids of neural and retinal tissues, and they are present naturally in human milk. Globally, infant formulas are now available for infant feeding that are enriched in DHA and AA.

A positive benefit of breast feeding compared with formula feeding on short-term visual and developmental outcomes in term and premature infants has been observed in several studies. However, the evidence of a benefit is more consistently observed in premature than in term infants, perhaps due to a greater immaturity of their enzymatic pathway to convert α -linolenic and linoleic acids to the long-chain polyenoic derivatives. Owing to the conflicting evidence, specific requirements for DHA and AA for term infants were not included in the recent DRI report.

Protein

For infants aged 0–6 months, the AI for protein is based on the intake from human milk (Table 2). For infants aged 7–12 months, sufficient information was available from nitrogen balance studies and protein deposition to derive an EAR based on the factorial method. For both males and females, this averaged to 1.1 g protein per kg body weight per day. The RDA was set as the EAR + 2 standard deviations (based on coefficients of variation observed in adults), which yielded a value for protein intake of 1.5 g kg⁻¹ day⁻¹. Because the absorption and digestibility of protein contained in the infant formula may be less efficient than that from the human milk, the quantity of protein contained in infant formulas may have to be adjusted depending on the protein source used.

The FAO/WHO recommendations for safe level of protein intakes for infants at mean age of 0.5 year is 1.31 g kg⁻¹ day⁻¹ and for those aged 1 year, it is 1.14 g kg⁻¹ day⁻¹ (Joint WHO/FAO/UNU, 2002). However, in calculating total safe level of intakes, the average infant weight for age used was different from the DRIs and different weights were applied for males and females. Thus, the total recommended intakes from WHO/FAO/UNU (2002) compared with the DRIs (IOM, 2005) (Table 2) are higher for males (10.2 g day⁻¹ at 0.5 year and 11.6 g day⁻¹ at 1 year) and lower for females at 1 year (10.8 g day⁻¹).

Amino Acids

The DRI for the essential (indispensable) amino acids for infants was derived from the content of human milk for ages 0–6 months. For older infants, an EAR was derived for these amino acids using a factorial estimate that was based on the amino acid needs for growth or protein deposition, with adjustments for efficiency of protein deposition and maintenance requirement. The RDA was determined by adding the coefficient of variation derived for maintenance and protein deposition to the value for the EAR. No values were set for UL for any of the amino acids. A summary of the AI and RDA for the indispensable amino acids of infants is provided in Table 3.

Other Macronutrients

For infants, no DRI was set for saturated fat, monounsaturated fat, *trans*-fatty acids, and cholesterol or dietary fiber. Although some dietary fiber is present in the diet after solid foods are introduced, there are no data on fiber intakes in such young age groups and no theoretical basis exists, which establishes a need for fiber at less than 1 year of age.

Macrominerals: Calcium, Phosphorus, Magnesium, and Fluoride

The AI for infants for the 'bone' minerals are summarized in Table 4. The content of human milk was used as the basis to derive the AI for calcium, phosphorus, and magnesium for infants aged 0–6 months and with the addition of intake from complementary foods for those aged 7–12 months. For calcium, the values reflect those provided in the revised DRI report for Calcium and Vitamin D (IOM, 2010). The recommended AIs do

Table 4 DRI for minerals for infants – calcium, phosphorus, magnesium, and fluoride

Nutrient	0–6 months	7–12 months
Calcium		
AI (mg day ⁻¹)	200	260
UL	ND	ND
Phosphorus		
AI (mg day ⁻¹)	100	275
UL	ND	ND
Magnesium		
AI (mg day ⁻¹)	30	75
UL	ND	ND
Fluoride		
AI (mg day ⁻¹)	0.01	0.5
UL (mg day ⁻¹)	0.7	0.9

DRI, Dietary Reference Intake; AI, Adequate Intake; UL, upper limit; ND, not determinable due to lack of data of adverse effects in infants.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

Table 3 DRI for indispensable (essential) amino acids

Amino acid ^a	0–6 months AI (mg kg ⁻¹ day ⁻¹) ^b	7–12 months RDA (mg kg ⁻¹ day ⁻¹)
Histidine	23	32
Isoleucine	88	43
Leucine	156	93
Lysine	107	89
Methionine + cysteine	59	43
Phenylalanine + tyrosine	135	84
Threonine	73	49
Tryptophan	28	13
Valine	87	58

^aNo upper levels were set for any of the indispensable amino acids.

^bAI values shown as amino acid in mg kg⁻¹ day⁻¹ can be converted to milligram amino acid per day by multiplying with the reference weight of 6 kg for infants 0–6 months of age.

DRI, Dietary Reference Intake; AI, Adequate Intake; RDA, Recommended Dietary Allowance.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

not differ substantially from those in the 1997 DRI report, the slight difference is due to the variation in values applied for the calcium content of breast milk. The AI value of 200 mg day⁻¹ for breast-fed infants from 0 to 6 months was substantiated by considering that average calcium absorption in infants is around 60% thus yielding retention of calcium of 120 mg day⁻¹, a value approximately 20% higher than the estimated accretion of calcium for an infant of about 100 mg day⁻¹. For infants aged 6–12 months, recent data on calcium intakes from solid foods for formula-fed infants were used to add to the intake from breast milk to yield an AI of 260 mg day⁻¹.

For fluoride, intake from human milk was the reference for the first 6 months only. After 6 months, the AI for fluoride was set at 0.05 mg kg⁻¹ day⁻¹ and adjusted to a reference weight for age, based on the well-documented evidence of the benefit of fluoride intake for the prevention of dental caries (Table 4).

Microminerals/Trace Elements

The AI for iron for ages 0–6 months is based on the concentration of iron in human milk albeit low (approximately 0.35 mg l⁻¹) but assumes that the infant is born with maximal iron stores due to transplacental transfer of iron from an iron-replete mother. If the latter conditions do not apply, then an exogenous source of iron such as iron drops may be required. For infants aged 7–12 months, an EAR and RDA were developed based on a factorial modeling method that summed basal loss of iron with needs for growth, increasing hemoglobin mass, and iron stores. This value was then adjusted for iron bioavailability using a factor of 10% for infants due to a medium bioavailability of iron from infant cereals, which are generally the major dietary source of iron in weaning foods before meats are introduced (Table 5). An UL was established (Table 5) for iron based on the risk of adverse gastrointestinal side effects from supplemental (not food) iron.

For zinc, an AI was based on the human milk model only for the 0–6 months age group (Table 5). The zinc content of human milk declines rapidly during the first 6 months (from 4 to 1.2 mg l⁻¹), so the AI was based on a milk zinc concentration of 2.5 mg l⁻¹. This value cannot be directly applied to infants being fed with cow milk- or soy-based infant formulas because zinc absorption is significantly lower from these feeds compared with human milk. The EAR for the 7–12 months age group was set using a factorial method that summed obligatory losses with requirements for growth and adjusted for fractional absorption of dietary zinc from human milk and complementary foods. The RDA was derived by adding twice the coefficient of variation of 10% to the EAR (2.5 mg day⁻¹ of zinc) for infants aged 7–12 months (Table 5). An UL was set for zinc on the basis of the possibility of an adverse effect of high-zinc intakes on copper status.

For the trace elements, such as chromium, copper, iodine, manganese, molybdenum, and selenium, an AI was set for infants of age 0–6 months based on the human milk model (Table 5). For the age group 7–12 months, data on intake from complementary foods were only available to set an AI for chromium, copper, and selenium (Table 5). For iodine and molybdenum, the AI represents an extrapolation up from the AI values for the age group 0–6 months based on differences in metabolic body weight (kg^{0.75}). For manganese, the AI

Table 5 DRI for micronutrient/trace minerals for infants – chromium, copper, fluoride, iodine, iron, manganese, molybdenum, selenium, and zinc

Nutrient	0–6 months	7–12 months
Chromium		
AI (mg day ⁻¹)	0.2	5.5
UL	ND	ND
Copper		
AI (mg day ⁻¹)	200	220
UL	ND	ND
Iodine		
AI (mg day ⁻¹)	110	130
UL	ND	ND
Iron		
AI (mg day ⁻¹)	0.27	–
RDA (mg day ⁻¹)	–	11
UL (mg day ⁻¹)	40	40
Manganese		
AI (mg day ⁻¹)	30	75
UL	ND	ND
Molybdenum		
AI (mg day ⁻¹)	2	3
UL	ND	ND
Selenium		
AI (mg day ⁻¹)	15	20
UL (mg day ⁻¹)	45	60
Zinc		
AI (mg day ⁻¹)	2	–
RDA (mg day ⁻¹)	–	3
UL (mg day ⁻¹)	4	5

DRI, Dietary Reference Intake; AI, Adequate Intake; ND, not determinable due to lack of data of adverse effects in infants; RDA, Recommended Dietary Allowance; UL, upper limit.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

represents an extrapolation down from the AI for adults as described previously (Table 5). Owing to the lack of relevant information, no UL values for infants younger than 1 year were established for chromium, copper, iodine, manganese, or molybdenum, but intakes of these nutrients should be limited to foods and not supplements. An UL was established for selenium due to the known chronic toxicity of excessive selenium ingestion, which is manifested clinically as brittleness and loss of nails and hair. The UL was set for infants based on the highest known intake of selenium from human milk and adjusting for a reference infant weight (Table 5). The UL value pertains to intake from both foods and supplements.

The trace elements such as arsenic, boron, nickel, silicon, and vanadium are known to have a role in human metabolism, but due to lack of information, DRI values, including UL, could not be established for infants.

Globally, deficiencies of iron, iodine, and zinc in infants are still widespread despite the international efforts to develop sustainable food fortification and supplementation programs. In North America, the prevalence of iron deficiency anemia is relatively low at 4–5% owing to the promotion of breast feeding and the widespread fortification of infant formulas and cereals with iron. In developing countries, prevalence of anemia can be 50% or more by the age of 1. Premature

low-birth weight (<2.5 kg) infants represent a particular risk group for iron deficiency owing to a major reduction in transplacental transfer of iron when birth occurs during the third trimester of pregnancy, the period when most iron is transferred to the fetus as long as the mother is not iron deficient. Infants of low birth weight require iron supplementation in a liquid form until complementary foods containing iron can be introduced. Use of weaning foods that are not iron fortified and that often contain phytic acid, a strong inhibitor of iron absorption, is a key causative factor for high rates of anemia in many developing countries.

Fat-Soluble Vitamins: A, K, E, and D

For vitamins A, K, and E, the AI for infants aged 0–6 months was based on the human milk model as previously described (Table 6). For vitamins A, K, and E, the AI for infants aged 7–12 months was extrapolated up from the values for infants aged 0–6 months using a reference weight for infants at this age. There are two important points with respect to the AI established for vitamin K. First, the AI was set assuming that the infants had received a prophylactic injection of vitamin K just after birth. Because vitamin K is not readily transferred to the fetus while *in utero*, and human milk is relatively low in vitamin K, newborn infants, at least in North America, routinely receive an injection of vitamin K within a few hours after birth. Second, the AI set for ages 7–12 months may be lower than the actual intake of vitamin K once a child's diet of complementary food becomes varied. Any evaluation of dietary intake of vitamin K should use the recently updated vitamin K values for raw and cooked foods available on the website of the US Department of Agriculture National Nutrient Database for Standard Reference, Release 17. Although carotenoids are present in human milk, a factor to calculate their bioconversion to vitamin A is not known and so their contribution to vitamin A was not included.

For vitamin D, setting an AI could not be based on the content of human milk as it contains only marginal amounts of

vitamin D. An AI for vitamin D of 400 IU day⁻¹ was set for infants from 0 to 12 months, based on this intake being associated with maintenance of serum 25-hydroxyvitamin D level higher than 30 nmol l⁻¹ and likely closer to 50 nmol l⁻¹, which represent a vitamin D status that is above that usually associated with clinical rickets in infants. The revised AI for vitamin D of 400 IU day⁻¹ for infants now set by the IOM was previously established by the Canadian Pediatric Society (2003) and the American Academy of Pediatrics (2008). For breast-fed infants to meet the AI of 400 IU of vitamin D per day, they must be provided with a vitamin D supplement. For formula-fed infants, intake of nearly 1000 ml day⁻¹ is required to achieve the AI for vitamin D because infant formulas in North America are regulated to contain 400 IU l⁻¹ of liquid formula.

Water-Soluble B Vitamins, Folate, Choline, and Vitamin C

The AIs for infants aged 0–6 months for most water-soluble vitamins were based on the content of human milk (Table 7). This approach may be problematic for water-soluble B vitamins, in which the milk content is dependent on maternal intake of vitamins. An example of clinical relevance is a vegan mother who may have subclinical vitamin B₁₂ deficiency and produce vitamin B₁₂-deficient milk. For vitamin C, the effect of maternal supplementation on milk content remains uncertain, but available reports do not indicate that excessive amounts of vitamin C are secreted in milk, even in mothers taking supplements of 1000 mg or more. For those aged 7–12 months, the AI for thiamin, riboflavin, niacin, folate, pantothenic acid, and choline was derived by extrapolating down from values for older children or adults due to a lack of information of dietary intake of these nutrients from solid foods. Tolerable ULs for infants were not established for any of the water-soluble vitamins.

Water and Electrolytes

Optimal water intake in infants is more critical than at any other period of life. Not only do infants have a higher total body water content per body mass than children or adults but also they have a higher water turnover rate, a less well-developed sweating mechanism, and a little ability to indicate when they are thirsty. The AI for water intake of infants aged 0–6 months is 0.7 l day⁻¹ and is based on the water content of human milk. Assuming that the infants are breast fed on demand, infants will drink to meet their thirst needs; thus, even in hot and humid climates, supplemental water may not be required. The AI for water intake of 0.8 l day⁻¹ set for infants aged 7–12 months is based on the sum of the water content of human milk, complementary foods, and beverages, the latter obtained from reported food intakes from surveys in the USA.

For sodium and potassium, the AI of 0.12 and 0.4 g day⁻¹, respectively, is based on the human milk model. For 7–12 months, the AI for sodium is 0.37 g day⁻¹ and for potassium, it is 0.7 g day⁻¹ based on the sum of observed intakes from human milk and complementary foods. No ULs were established for infants due to the lack of data on adverse effects of these nutrients on infant health. However, particularly because the renal excretory capacity of young infants may not be able

Table 6 DRI for fat-soluble vitamins

Nutrient	0–6 months	7–12 months
Vitamin A		
AI (mg day ⁻¹)	400	500
UL (mg day ⁻¹)	600	600
Vitamin D		
AI (IU day ⁻¹)	400	400
UL (IU day ⁻¹)	1000	1500
Vitamin E		
AI (mg day ⁻¹)	4	5
UL	ND	ND
Vitamin K		
AI (mg day ⁻¹)	2.0	2.5
UL	ND	ND

DRI, Dietary Reference Intake; AI, Adequate Intake; ND, not determinable due to lack of data of adverse effects in infants; UL, upper limit.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

Table 7 DRI for water-soluble vitamins

Nutrient	0–6 months	7–12 months
Vitamin C		
AI (mg day ⁻¹)	40	50
UL	ND	ND
Thiamin		
AI (mg day ⁻¹)	0.2	0.3
UL	ND	ND
Riboflavin		
AI (mg day ⁻¹)	0.3	0.4
UL	ND	ND
Niacin		
AI (mg day ⁻¹)	2	4
UL	ND	ND
Vitamin B ₆		
AI (mg day ⁻¹)	0.1	0.3
UL	ND	ND
Folate		
AI (mg day ⁻¹)	65	80
UL	ND	ND
Vitamin B ₁₂		
AI (mg day ⁻¹)	0.4	0.5
UL	ND	ND
Pantothenic acid		
AI (mg day ⁻¹)	1.7	1.8
UL	ND	ND
Biotin		
AI (mg day ⁻¹)	5	6
UL	ND	ND
Choline		
AI (mg day ⁻¹)	125	150
UL	ND	ND

DRI, Dietary Reference Intake; AI, Adequate Intake; ND, not determinable due to lack of data of adverse effects in infants; UL, upper limit.

Source: Data from Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrates, Fiber, Fat, Protein, and Amino Acids (Macronutrients)*. Washington, DC: National Academy Press.

to handle excessive amounts of ingested electrolytes, the DRI report notes that the intake of sodium, chloride, and potassium should be limited to human milk (or infant formula) and solid foods appropriate for age.

Research Needs

The paucity of sound evidence on which to provide a substantial basis for estimating the nutrient requirements for infants is highlighted at the end of each article in the DRI reports. Because infants and children are not just 'little adults,' the DRI values must be carefully defined for the specific stages of growth and development and with consideration for nutritional programming that occurs in early life in response to dietary exposures as our knowledge of this area becomes more complete.

Assessment of Growth as an Indication of Adequate Nutrition

Assessment of growth in weight, length, and head circumference is an internationally accepted measure of health and

nutritional status of infants, albeit not an indicator that it is nutrient specific. The interpretation of growth measures requires comparison with reference data from normal populations of infants that have been compiled into growth charts with percentiles indicated. The growth charts from the WHO are recommended as they are based on an internationally represented population of infants breast fed during the first year of life and followed up till 5 years of age. These WHO growth standards can be downloaded and copied from their website. The WHO growth standard has been adopted by health professional societies in many countries, including Canada.

Practical Aspects of Meeting the Nutrient Needs of Infants

Adequate amounts of breast milk meet the nutrient needs of most infants for the first 6 months of life. However, there is no universal agreement on the optimal duration of exclusive breast feeding and the precise timing or the order of introduction of complementary foods. Internationally, recommendations from most health agencies state that the ideal feeding of infants is exclusive breast feeding for the first 6 months of life with appropriate introduction of foods from 6 months onward, including partial breast feeding through 2 years of age or beyond. When assessing the intakes of infants fed with marketed formulas, it must be kept in mind that the intakes of most nutrients will exceed the new DRI values for the AI given that these are based on the composition of human milk. In many cases, the greater concentration of nutrients in infant formula is appropriate due to lower digestibility or bioavailability of nutrients from cow milk- or soy-based protein in formulas compared with human milk.

The introduction of complementary foods, especially solids and eventually finger foods, is important for infants to develop normal oral and motor skills related to eating and to attain AIs of nutrients that may be low in breast milk (e.g., protein or iron). In a report by the March of Dimes, three common inappropriate complementary feeding practices were delineated: (1) introducing foods too early or too late, (2) introducing foods of low nutrient density, and (3) feeding contaminated foods. It is noted in the report that early introduction of foods may reduce the intake of breast milk due to limited gastric capacity of very young infants or precipitate an allergic reaction in infants with a family history of food allergy or atopy. By delaying the introduction of foods beyond 6 months, there is increasing risk of deficiencies of nutrients known to be relatively low in breast milk and yet essential to support rapid growth of infants, such as iron and zinc. The choice of first foods is important so that they contain adequate energy and micronutrients to meet the needs of infants. For example, reduced-fat cow's milk (2% skim and 1% fat) should not be fed to infants before 2 years of age. Excessive amounts of fruit juices or 'empty calorie' fast foods should not be fed to infants. To achieve AIs of micronutrients, such as iron, choice of nutrient-fortified foods (e.g., iron-fortified infant cereal or other weaning food) may be required in areas where natural sources of micronutrients are not available. Finally, both solid and liquid foods offered to infants need to be free of contamination because the transmission of infections

through food is thought to be a primary cause of diarrhea in young infants, particularly in developing countries.

The March of Dimes report (2002) outlined three key recommendations for ensuring optimal nutrition of term-born infants through breast feeding and complementary feeding practices. The rationale for each recommendation and suggestions for implementation strategies on a global basis are provided in the report. The three key recommendations are as follows:

Recommendation 1: Promote and support exclusive breast feeding for 6 months, with the introduction of complementary foods and continued breast feeding thereafter – up to 2 years of age or longer as mutually desired by the mother and infant.

Recommendation 2: Promote and support programs to ensure that pregnant women and breast-feeding mothers receive adequate nutrient intakes.

Recommendation 3: Promote the appropriate introduction of safe, nutritionally adequate, and developmentally appropriate complementary foods.

The recommendations from guides to infant feeding from the Canadian Pediatric Society (2005); the European Society of Gastroenterology, Hepatology and Nutrition (ESPGHAN) (2008, 2009); or from agencies such as the March of Dimes (2002) are generally universally applicable, with recognition of variations in foods available on the market. Following such guidelines will ensure that infants attain nutrient intakes that match the nutrient requirements as set out in the dietary standards such as the DRI reports.

See also: Amino Acids: Chemistry and Classification; Metabolism. Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Breast Feeding. Calcium. Carbohydrates: Requirements and Dietary Importance. Fats and Oils. Folic Acid. Iron: Physiology, Dietary Sources, and Requirements. Lactation: Dietary Requirements. Magnesium. Phosphorus: Physiology, Dietary Sources, and Requirements. Protein: Requirements and Role in Diet. Vitamin B₆: Physiology. Vitamin E: Metabolism and Requirements. Vitamin K

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Further Reading

ESPGHAN Committee on Nutrition (2008) Complementary Feeding: A Commentary by the ESPGHAN Committee on Nutrition. *Journal of Pediatric Gastroenterology and Nutrition* 46: 99–110.

NUTRITIONAL SUPPORT

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Adults, Enteral

Infants and Children, Parenteral

In the Home Setting

Adults, Enteral

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Adults, Enteral

Definition

Enteral feeding is a method of providing nutrients directly into the gastrointestinal (GI) tract when a person cannot receive food orally. It is used in patients who have an adequate functional GI tract and can digest and absorb food but in whom oral intake is inadequate to maintain or restore optimal nutritional status. Also known as tube feeding, enteral nutrition (EN) delivers nutrients directly to the stomach or intestines through a thin flexible tube. It is administered through a nasogastric tube placed via the nose, or a percutaneous tube placed into the stomach (gastrostomy) or the small intestine (jejunostomy). EN is generally considered safer and the preferred method of delivering nutritional support over parenteral nutrition. In this article, the use of enteral feeding is reviewed.

Types

EN requires liquid formulas to be administered through a tube in the upper GI tract. Once it has been decided that an individual is a candidate for EN, the tube type and appropriate route of access for tube placement can be selected. The type of feeding tube can be divided into two categories: nasogastric or nasoenteral tubes, which enter the GI tract through the nose or gastrostomies and jejunostomies, which enter through the abdominal wall. In general, feedings administered through nasal tubes is indicated for periods lasting less than 2 weeks whereas feedings that require a longer duration of time use more permanent tubes (gastrostomies and jejunostomies).

The access selection for enteral feeding depends on multiple factors:

1. Length of time that enteral feeding will be administered.

2. Degree of risks for aspirations or device displacement.

3. Patency of upper GI tract.

4. Planned or prior GI surgery.

5. Administration issues that could include volume and formula viscosity (**Figure 1**).

Access

Nasogastric Route

Short-term feeding tubes should be used for patients who are expected to receive EN for less than 2 weeks or for whom a long-term feeding tube is not an option. Nasogastric tube placement is one of the most common types of enteral access and can be used to safely administer short-term EN and or in patients who are at risk of aspiration. A nasogastric tube passes through the nose into the stomach and is used when there is adequate GI function. It does not require surgery and can be performed bedside. Nasogastric tubes are made from silicone or polyvinyl, and vary in length from 30 to 43 in with diameters of 8–14 French. Bolus or continuous infusions can be used to administer feedings.

Nasoduodenal or Nasojejunal Route

Similar to nasogastric tubes, although longer, these tubes are also used for short-term feeding (2 weeks), in patients with gastric dysmotility, nausea or vomiting, gastroparesis, and high-risk patients. A nasojejunal tube is more difficult to insert and place. It is inserted through the nose and passed through the esophagus into the stomach. Feedings require an infusion pump for administration. Tubes can be advanced with endoscopic or fluoroscopic guidance.

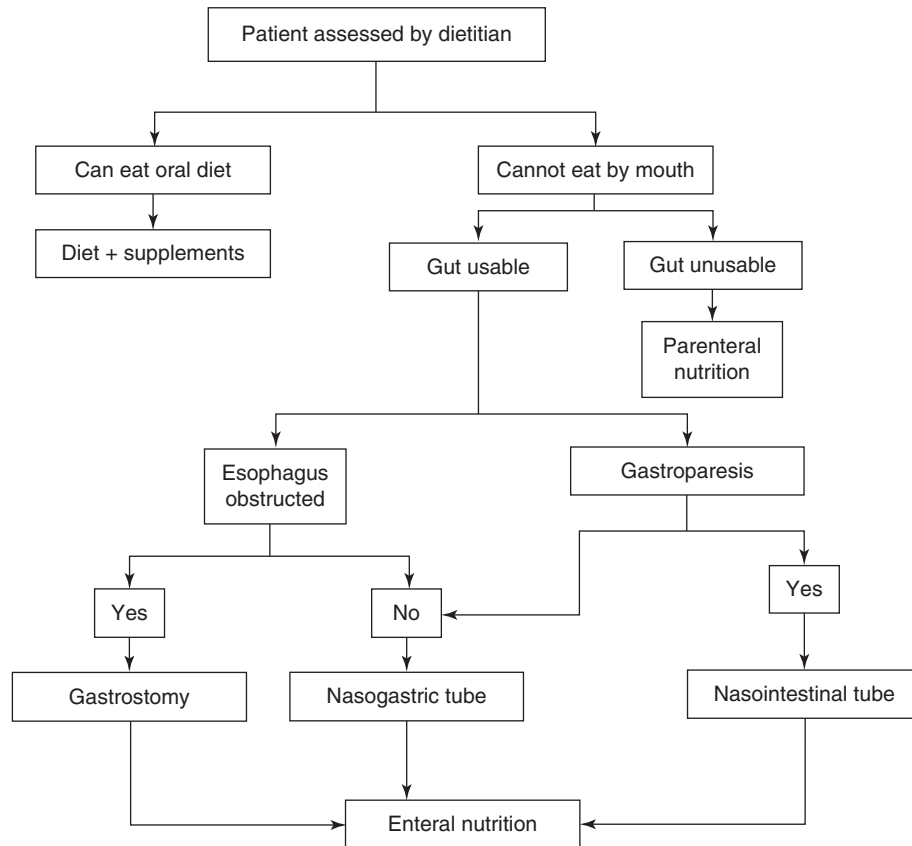


Figure 1 Algorithm for nutritional support. Reproduced from Encyclopedia of Human Nutrition, Enteral Nutrition, Adults 2005.

Percutaneous Endoscopic Gastrostomy (PEG) or Percutaneous Jejunostomy (PEJ)

PEG is used in patients who will need long term, full, or supplemental feedings lasting more than 4 weeks. Requiring moderate to deep sedation, the PEG tube can be placed directly into the stomach through the abdominal wall using an endoscope. Tubes can then be advanced by endoscopic guidance into the duodenum and then brought out through the abdominal wall to provide the access route for enteral feedings. PEG tube feeding has many advantages in terms of long-term feeding options including easier to place than a jejunostomy, less expensive, and easier to maintain. Disadvantages are that it cannot be used in patients with obstructions and there may be a moderate risk of aspiration in high-risk patients. The tube diameters commonly used range from 6 to 8 mm French units. In general, small-diameter tubes should be avoided in patients with poor gastric emptying who require intragastric administration of medication.

Percutaneous jejunostomy (PEJ) is also used in long-term feeding, in patients who require full or supplemental feedings for more than 4 weeks. PEJ can safely feed patients with gastric resection, gastric dysmotility, and patients with a higher risk for aspiration. The PEJ tube is the most difficult to insert, requiring a higher skill level, and can be placed percutaneously. Additionally, because the tubes are typically smaller in size, they may clog more easily. That said, overall, PEG and

PEJ tubes, if maintained properly, can function well with few complications.

Feeding Formulas

A wide selection of enteral feeding formulas is commercially available. Although the composition of formulas varies widely, almost all formulas are nutritionally complete when administered in the appropriate quantities. Since 1989 the US Food and Drug Administration has used the term, 'Medical Foods' to define EN products, meaning they must be used under the supervision of a doctor. Formulas are selected based on an assessment of the patient's age, medical concern, nutritional status, and ability to absorb and digest nutrients. Nutrition related factors that can be used in the formula selection process include (1) a patient's energy, protein, and fluid requirements, (2) patient's need for fiber modifications, and (3) food allergies and intolerances such as gluten sensitivity. The types of formulas are classified as follows.

Feeding Formula Classification

1. Polymeric formulas: The most commonly used of the enteral feeding formulas, polymeric formulas can be used orally or through a tube. These formulas contain whole

proteins and oligosaccharides with fat partly as long-chain triglycerides (LCT) and partly as medium-chain triglycerides (MCT). Lactose and gluten-free, polymeric formulas are also low in osmolality.

2. **Elemental formulas:** These formulas are used for patients that have malabsorptive conditions, which may include short gut, Crohn's or pancreatitis among other conditions. These formulas require less digestion than polymeric formulas so they are often used in patients with impaired digestion or absorption. Elemental formulas are amino acid based and are hyperosmolar. Semielemental diets have a mix of peptides and free amino acids, with a lower osmolality content than the elemental diets. They are considerably more expensive than standard formulas.
3. **Disease-specific formulas:** These formulas are designed to provide specific nutrient needs based on specific illnesses. They are intended for patients who have specific metabolic requirements: liver, renal and pulmonary diseases, diabetes, and metabolic disorders. They may be enriched with branched-chain amino acids (BCAA), glutamine, omega-3 fatty acids, and arginine whereas others might have a high-fat content. Effectiveness of disease-specific formulas is controversial with mixed research results.
4. **Modular formulas:** These formulas are designed for supplemental use. They may add calorie or protein density and can be used to tailor tube feedings to individual nutritional needs as a supplement to commercial enteral formulas. Specific nutrient composition of the patient can be customized. Disadvantages include the risk of bacterial contamination from excessive handling of formulas.

Compositions

Polymeric Formulas

These formulas are the standard formulas that are most commonly used for enteral feedings. They contain whole proteins, complex carbohydrates, and mostly LCTs for fat with varying percentage of free water.

Standard formulas provide 1.0 and 1.2–2.0 cal ml⁻¹ of formula. This affects the free water provided per milliliter, as a 1.0 formula provides ~85% free water and a 2.0 formula provides ~70% free water.

On average, carbohydrate percentage ranges from 51% to 57% and formulas may include fiber or not include fiber. Polymeric formulas contain a moderate amount of fat with percentages ranging from 29% to 33% to meet essential fatty acid needs and contain a mix of LCT and MCT, with the majority coming from LCT. Sources of fat are of vegetable origin and include corn, canola, soybean, sunflower, and safflower oils.

Standard formulas contain intact protein including casein, egg albumin, and whey protein among others sources. Protein may have the most variability in standard formulas and is one of the deciding factors of which formula will be chosen. Protein percentages often range from 14% to 19%.

Elemental or SemiElemental Formulas

These formulas require less digestion than polymeric formulas because they contain protein and carbohydrates that have been partially or fully broken down and require minimal digestion. They do not contain lactose or fiber and are used for patients that have impaired digestion.

The protein in these formulas are in the form of peptides, fat as LCT or MCT or a combination of both, and carbohydrates as partially hydrolyzed starch maltodextrins to facilitate digestion and absorption.

Elemental formulas provide 1–1.5 kcal ml⁻¹. Fat from plant sources such as corn oil, soybean oil and canola oil provide 1–5% of calories and protein from hydrolyzed casein, whey, or soy protein, among other sources provides, 12–20% of total calories.

Disease-Specific Formulas

Diabetic

Diabetic formulas often contain a mix of soluble and insoluble fibers, with a composition of 31–40% carbohydrate, 42–49% fat, and 17–20% protein content. Lower in carbohydrates and higher in fat, fiber is added to help in maintaining glycemic control by slowing gut transit and the absorption of glucose. Consequently the high fiber and high-fat content of diabetic EN formulations, may cause slow gastric emptying which could be problematic for diabetic patients with gastroparesis. It is important to avoid overfeeding diabetic patients to prevent excess hyperglycemia. In difficult to manage patients, the initial use of standard formulas with insulin, as needed for glycemic control, is appropriate with the use of diabetic formulas.

Renal

Renal formulas are designed to provide optimal nutrition whilst minimizing blood urea nitrogen, maintaining water and electrolyte balance and reducing accumulation of the level of toxic products. These formulas also contain a lower electrolyte content as well as lower potassium, phosphorus, and magnesium and are concentrated to reduce volume for fluid restriction.

They provide 1.8–2 cal ml⁻¹ and are lower in protein, 7–18% with 34–58% carbohydrates, and 35–48% fat.

Renal patients receiving dialysis require an increased amount of protein due to protein loss during dialysis. If a patient has not begun dialysis treatment, and a protein restriction is indicated, renal formulas may be too high in protein. There are specialized renal formulas available with a very low protein profile, providing approximately 10% of calories from protein, which can be used.

The need for a decreased electrolyte formula will depend on dialysis and subsequent serum levels and urinary output. If a patient is receiving continuous renal replacement therapy, there may be no need for a renal formula at all. Volume status and electrolyte labs will dictate if this formula is required. These formulas will meet the RDIs for renal patients

if the patient receives on average one liter of enteral formula daily.

Hepatic

The disease-specific formulas for liver disease contain higher proportions, 40–50%, of the BCAAs valine, leucine, and isoleucine and lower levels of aromatic amino acids (AAAs) tryptophan, tyrosine, and phenylalanine, to counteract a BCAA:AAA imbalance. Patients with hepatic encephalopathy tend to have decreased levels of BCAAs and increased levels of AAAs. An altered ratio may increase AAA transport through the blood–brain-barrier, causing a ‘false neurotransmitter effect’ that could precipitate encephalopathy.

Energy density is slightly increased in these enteral formulas containing 1.5–2 cal per cc of fluid, allowing for full caloric provision in smaller volumes which could help with delayed gastric emptying, complaints of fullness, and to meet fluid restrictions. Recent clinical trials looking at the benefit of BCAAs in these patients have been inconclusive.

Pulmonary

The metabolism of macronutrients produces carbon dioxide, with the breakdown of carbohydrate producing the greatest amount. Based on these concepts, an enteral formula was developed to provide a higher fat content, and reduced carbohydrate content. The fat content in these formulas is 50% and is derived from soy or corn oil versus approximately 30% in standard formulas, protein is 7–18%, carbohydrates 34–58%, and 1.8–2 kcal ml⁻¹.

Immune Enhancing

Specialized formulas have been designed to improve immune function in the immunocompromised critically ill patient. Specifically patients admitted with traumatic injuries, burns or requiring major abdominal surgeries and at high risk for development of infections and other ICU and postsurgical complications. These formulas may include arginine, glutamine, omega-3 fatty acids, probiotics, and fiber and often are peptide based to help maximize absorption.

Arginine

Arginine is not a traditional ‘essential’ amino acid, but becomes a conditionally essential amino acid during periods of stress including burns and trauma. It is important for T-cell functioning, collagen synthesis and production of growth hormone, prolactin, somatostatin, insulin, and glucagon. Following trauma and surgery there is a drop in arginine synthesis. Multiple studies have demonstrated a decrease in postoperative infections and hospital length of stay in patients when using an arginine containing formula in conjunction with other immune-enhancing ingredients, particularly, omega-3 fatty acids.

Glutamine

Glutamine is the most abundant nonessential amino acid in the body and like arginine it becomes a conditionally essential

amino acid in states of stress. It is the preferred fuel source for the small bowel enterocyte, which is thought to help maintain its structure and function during times of stress. In septic and malnourished patients, muscle glutamine is depleted, and it is hypothesized that in these patients the availability of glutamine lymphocytes and the gut is reduced, resulting in increased risk of sepsis. Although enteral formulas designed to improve immunity have given mixed results, glutamine supplementation has not been shown to be harmful and has reduced complications in patients with bone marrow transplantation, after surgery, and in those with critical illness and burns. Studies using parenteral glutamine have generally been more positive than those employing enteral glutamine.

Omega-3 Fatty Acids

The fatty acids found in fish oil, called eicosapentaenoic (EPA) and docosahexaenoic (DHA) are precursors of prostaglandins and thromboxanes that upset the prothrombotic effects of similar compounds derived from linoleic acid. They promote anti-inflammatory cascade under stress conditions and may be beneficial in patients with acute respiratory distress syndrome and severe acute lung injury.

Probiotics

The administration of a probiotic with EN may have a role in reducing septic complications in patients with transplantation, major abdominal surgery and severe trauma. Although, differences in species of probiotics may have different effects of variable impact on patient outcomes. One report of adverse outcomes in a randomized trial in patients with severe pancreatitis has generated some concern about the broad usage of probiotics in critically ill patients.

Modular

These formulas can provide macronutrients and micronutrients separately and can be used to provide additional calories or protein. Typically modular formulas contain only one or two macronutrients and are used to enhance other formulas. Protein modular products are powders and must be mixed with water before administration. Carbohydrates are in the form of glucose polymers and fat as triglycerides of long-chain polyunsaturated or medium-chain fatty acids. Some modular formulas can be combined with liquid vitamins and minerals for individualization for patients with unique needs. Normally, modulars are added to meet nutritional needs in patients who have disproportionate requirements.

Indications and Contradictions

EN is the preferred method of feeding patients who cannot eat, absorb, or use a normal diet in the presence of a usable GI tract.

The following are indications for EN:

1. Critical care patients, including those with trauma and burns, and also after major surgery.

2. Anorexia in patients with malignant disease, sepsis, liver and renal failure, and inflammatory bowel disease (IBD).
3. Upper GI obstruction or ulceration of the pharynx, esophagus, stomach, and duodenum may prevent the ingestion of normal food. Examples of these conditions are cancer, central nervous system disorders, and stenosis following ulceration.
4. Pancreatic disease: In patients with pancreatitis it may be possible to feed a low-fat enteral formula through a NJ route beyond duodenum without causing increased disease activity or pain.
5. Short bowel and severe malabsorption: In controlled trials enteral diets are not better absorbed than normal food. Therefore, the presence of a short bowel per se is not an indication for enteral feeding. However, some patients with severe malabsorption may benefit from the use of elemental diets.
6. IBD: In IBD, enteral feeding is used in the following situations:
 - i. Profound anorexia preventing the ingestion of a normal diet.
 - ii. Abdominal discomfort due to partial bowel obstruction or intestinal inflammation.
 - iii. In Crohn's disease some controlled trials have suggested that enteral feeding induces a remission comparable to that seen with steroids.
7. Dementia: Patients unable to feed themselves because of mental changes.
8. Fistulas of the distal small bowel or colon.
9. Nausea or vomiting. Patients who suffer from nausea or vomiting due to a gastric disorder can be fed into the jejunum.
10. Recurrent aspiration. Formula should be delivered through a jejunostomy.
11. High nutritional requirements that are not being met by oral intake (mostly applying to patients with burn injury who require increased nutrient intake).
12. Cancers of the head and neck.
13. Dysphagia associated with disabling neurologic conditions including amyotrophic lateral sclerosis (ALS) and multiple sclerosis (MS).
14. Neoplasms: Advanced primary and secondary intracranial tumors.
15. Coma.
16. Severe head injuries.
17. Cerebrovascular accidents.
18. Transition from parenteral nutrition.

The following are contradictions for EN:

1. Mechanical obstruction that cannot be bypassed.
2. Intractable vomiting or diarrhea refractory to medical management.
3. Extreme malabsorption.
4. Short bowel syndrome (100 cm small bowel remaining).
5. Paralytic ileus.
6. High output fistula.
7. Peritonitis.
8. Mild GI bleeding.
9. Hemodynamic instability.

10. Inability to access GI tract.
11. Imminently terminal disease.
12. Aggressive interventions not desired.

Methods of Infusion

Enteral feedings can be administered by continuous drip, bolus feedings or intermittent infusions. The method selected depends on the stability of the patient, gastric emptying rate, caloric and protein needs, patient mobility and central access route.

Continuous drip – tube feedings are administered for 24 h with minimal interruptions. An advantage of this type of feeding is that it may be tolerated well in critically ill patients. A disadvantage is that an infusion pump is usually required, to ensure accuracy of volume is delivered. Initiation begins at 25–50 ml h⁻¹ and is increased by 50 ml h⁻¹ every 4–8 h.

Intermittent infusions – Larger volumes (240–480 ml) are administered 3–6 times per day infused over longer periods (30–60 min). This type of feeding may result in more adequate volumes being administered. Another advantage is that it allows some time off and the feedings are usually given by a gravity drip over period of 30 min to 1 h. The gravity feeding system is less expensive than a pump. Although a disadvantage is that the higher rates may not be tolerated well. Initiation of feeding begins with 1/2–1 can per feeding increased by 1/2 can per feeding per day until the goal is met.

Bolus feed – bolus feeding is the preferred method because it allows the patient more freedom, is easier to use, takes less time, and is cheaper. The bolus method does not require a pump and can be given in larger volumes (240–480 ml) administered 3–6 times per day over short periods of time (10–15 min). Rapid installation of feeding into GI tract can be given by syringe or funnel. The majority of patients tolerate this method. To avoid aspiration the bolus feeding must be given when the patient is sitting or reclining at 45°. Initiation of feeding begins with 1/2–1 can per feeding and can be increased by 1/2 can per feeding per day until the goal is met.

EN is viewed as the safest and most efficacious method to support nutritional status in patients who are unable to eat orally. If managed properly, enteral feeding is associated with improved clinical outcomes and reduced infectious complications and is the preferred form of nutritional support.

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Infants and Children, Parenteral

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Glossary

Central venous lines or catheters (CVL) Indwelling intravenous catheters, either silastic or polyurethane, placed with the tip in the superior vena cava to allow long-term provision of hypertonic dextrose solutions.

Enteral nutrition (EN) Nutrition through the gastrointestinal tract, either orally or through tube feedings.

Essential fatty acids Fatty acids which cannot be synthesized by humans and which are required in the diet, including linoleic, arachadonic, linolenic acids, docosahexanoic acid (DHA), and eicosapentanoic acid (EPA).

Macronutrients Carbohydrates, fats, or proteins required in large quantities in the diet, mainly for energy.

Micronutrients Vitamins or minerals essential in small quantities for normal physiologic functions but unable to be synthesized by humans and required in the diet.

Multivitamin infusion (MVI) A solution containing recommended requirements of essential vitamins and minerals.

Parenteral nutrition (PN) Nutrition delivered intravenously, not enterally.

History and Introduction

Parenteral nutrition (PN) allows provision of nutrients intravenously, containing adequate amounts of energy, carbohydrate, protein, fat, minerals, and vitamins, while bypassing the gastrointestinal tract. It has improved survival of many thousands of patients who cannot or will not eat or absorb enough to maintain their weight or nutritional balance because of a wide variety of acquired or congenital diseases. From its beginnings in the late 1960s, it has expanded rapidly to become available for many patients in the USA and in Europe, but its expense and complications preclude wide availability in many countries. Before the availability of parenteral nutrition, as many as 30–50% of hospitalized patients had unrecognized malnutrition from chronic diseases and would remain for weeks without adequate nutrition to maintain weight or lean body mass, making them susceptible to infections, poor wound healing, and other complications. Surprisingly, it has been difficult to demonstrate substantial reductions in morbidity or mortality with parenteral nutrition except in moderately or severely malnourished patients or those with long-term intestinal failure. It is difficult to estimate the exact impact, but an American registry, the Oley Foundation, enumerated 10 035 Medicare beneficiaries on home parenteral nutrition in 1992, giving a rough estimate of 40 000 patients on home parenteral nutrition in the USA. Approximately 15–20% of these patients were children.

One of the first attempts at parenteral nutrition was carried out by Sir Christopher Wren in 1656. He infused ale, opium, and beer intravenously into animals. Parenteral nutrition that we are most familiar with for patient support has been available for approximately 40 years. The research carried out by Dr Stanley Dudrick and others allowed the support of the first pediatric patient on intravenous nutrition. The provision of intravenous nutrition was challenged by the development of several factors, before its completed use in patient support, including catheter access,

sterility of solutions, and the optimal form of each macro- and micronutrient.

Indications for PN

There are many circumstances where PN is necessary and life sustaining. The indications for use have not changed dramatically over the years since the development of PN. Congenital malformation of the intestine, specifically small bowel atresia, was the diagnosis in the first cases PN was used in this age group. Congenital malformations of the gastrointestinal tract continue to be one of the leading reasons for its use as well as acquired diseases including necrotizing enterocolitis. Other indications include severe malabsorption, intestinal dysmotility, other congenital defects, and those with hematology–oncology diseases (Table 1).

Dextrose

The primary source of energy during intravenous therapy is usually provided by dextrose (D-glucose). This is especially true in infants and children when higher energy requirements often necessitate glucose infusion rates of up to $15 \text{ mg kg}^{-1} \text{ min}^{-1}$ or more. Not until 1945 did Zimmerman report the first attempt at infusing intravenous solutions through a catheter placed in the superior vena cava. Experiments performed by Dudrick in Beagle puppies advanced the glucose infusion solutions closer to what is utilized currently with hypertonic dextrose solutions. In current practice hypertonic solutions are infused through a catheter with its tip centrally located in the superior or inferior vena cava. It continues to be the major energy component of intravenous support.

Initial doses of glucose should be approximately $5\text{--}7 \text{ mg carbohydrate kg}^{-1} \text{ min}^{-1}$ with incremental increases of $2\text{--}5 \text{ mg kg}^{-1} \text{ min}^{-1}$. Recommended maximum glucose infusion rate is $12\text{--}14 \text{ mg carbohydrate kg}^{-1} \text{ min}^{-1}$ for infants.

Table 1 Conditions commonly requiring parenteral nutrition

Conditions	Examples/Comments
Surgical gastrointestinal disorders	Gastroschisis, omphalocele, tracheoesophageal fistula, intestinal atresias, meconium ileus, peritonitis, malrotation and volvulus, diaphragmatic hernia, prolonged postoperative ileus, Hirschsprung's disease, and intestinal dysmotility
Short bowel syndrome	
Prematurity	
Congenital heart disease	
Pancreatitis	
Gastrointestinal fistulas	
Bone marrow transplantation	
Acute intestinal disease	Antibiotic colitis, necrotizing enterocolitis, inflammatory bowel disease, chronic or secretory diarrhea
Hypermetabolic states	Burns, multiple trauma
Chronic idiopathic intestinal pseudoobstruction	

Source: Adapted with permission from Hendricks KM, Duggan C, and Walker WA (eds.) (2000) *Manual of Pediatric Nutrition*, 3rd edn. London: BC Decker.

Frequent monitoring of blood glucose and urine for glucosuria are important parameters to follow to assess tolerance to increasing glucose infusion rates. It is important to avoid excessive carbohydrate intake to minimize complications from potential hyperglycemia with subsequent osmotic diuresis. In addition, hepatic steatosis can occur with overfeeding. Hyperglycemia may ensue even without excess carbohydrate infusion in certain clinical situations, such as sepsis, renal failure, and certain medication including corticosteroids. Glucose infusion rates should be decreased if hyperglycemia ensues; however, it may still be necessary to add insulin to control blood glucose to provide adequate support.

Protein

Another vital macronutrient that needs to be provided is protein. Initial experiments from the 1930s were done with plasma as the protein source, where investigators achieved positive nitrogen balance. In the early 1900s research began into the development of protein hydrolysates and crystalline amino acids.

Vitrum, a company in Sweden, produced the first commercially available casein hydrolysate solution. It was developed by Arvid Wretling who hydrolyzed casein enzymatically then dialyzed the mixture to remove large polypeptides. Wretling went on to modify it further and eventually replaced the hydrolysates in the 1970s.

The development of amino acid solutions specifically for infants took place in the early 1980s. These solutions provided conditionally required amino acids for the immature organ systems of premature infants and newborns. They were formulated based on the postprandial plasma amino acid levels of breast fed infants. Special amino acid solutions for renal or liver failure are also available, which have increased amounts

of branched chain amino acids. Studies with the solutions for liver failure have shown they may be beneficial in adult patients with encephalopathy. Glutamine is a much-researched amino acid that could not initially be added to PN solutions due to shelf instability in liquid form. If it is added as a dipeptide it has been found to be more stable. Not all studies have shown clear benefit to its addition in patients for gut adaptation or prevention of bacterial translocation.

Recommendations for initiation and advancement of protein is $1\text{--}2\text{ g kg}^{-1}\text{ day}^{-1}$ and advance by $1\text{ g kg}^{-1}\text{ day}^{-1}$ to goal (see Table 1 for protein requirements). Initiation at the higher dose is more common practice for premature infants based on more recent studies that have demonstrated good tolerance and optimal metabolic profiles with earlier and higher protein within the first 24–48 h of life. Blood–urea nitrogen is monitored for tolerance to amino acid infusion. Prealbumin levels are helpful to monitor adequacy of protein intake.

Lipid Emulsions

Glucose was the only nonprotein source of energy until intravenous lipids were developed between 1920 and the 1960s. The first emulsion available for clinical use was Lipomul, a cottonseed oil based formulation. Because there were many adverse effects from its use it was withdrawn from clinical use in the mid 1960s. Wretling, after extensive testing, developed Intralipid, a soybean-based emulsion, in 1961. It was well tolerated and is the most familiar intravenous fat emulsion currently available. It is available in 10%, 20%, and 30% solutions. The advantage of 20% and 30% over the 10% is the lower ratio of phospholipids to triglyceride, which minimizes the increase in plasma lipoprotein X levels. Lipid emulsions provide essential fatty acids, in addition to providing a concentrated energy source, particularly advantageous for patients requiring fluid restriction. Trials are underway for the use of emulsions that contain a blend of long chain fats with medium chain fats and those with fish oil blends. Also structured fat emulsions are being studied for clinical use. These specialized emulsions may have advantages in patients with liver disease and those with sepsis.

Lipid emulsions are usually initiated at $1\text{ g kg}^{-1}\text{ day}^{-1}$ and advanced to $2\text{--}3\text{ g kg}^{-1}\text{ day}^{-1}$ or 30–50% of total energy. More recent practice has been to limit to $1\text{ g fat kg}^{-1}\text{ day}^{-1}$ for patients who are anticipated to need PN for greater than two weeks as a hepatoprotective measure. In addition, recent investigational use of a fish oil based emulsion has been found to be efficacious in reducing serum bilirubin levels in patients who have required long-term PN support. The mechanism is unclear, however a couple of proposed theories is less productions of inflammatory thromboxanes, prostaglandins, and leukotrienes and the absence of phytosterols. Serum TG levels are monitored for tolerance. Hypertriglyceridemia may occur in situations of stress, sepsis, and renal and liver insufficiency/failure. In addition, a number of medications can cause hypertriglyceridemia. In these situations a reduction in fat infusion is warranted, usually by infusing over 18–20 h instead of 24 h.

A minimum of 3–5% of total energy requirements is necessary to meet essential fatty acid requirements.

Micronutrients

To provide complete nutritional support, micronutrients, electrolytes, and minerals also need to be included in the parenteral solution. Addition of adequate amounts of calcium and phosphate together in one solution may be particularly problematic without precipitation occurring. Solubility guidelines are available accounting for the brand and percentage of amino acids and other salts, which impact the pH of the solution. Compounding guidelines for the order of addition of calcium and phosphorus, amounts of other additives and the temperature of the solution are other factors to optimize the solubility. Filters in the delivery system also help in minimizing the risk of occlusion of the catheter if a solution should precipitate, especially with so-called '3-in-1' solutions, where lipids are mixed with the glucose/amino acid solution. Further studies have evaluated the stability of the variety of nutrient components in these solutions.

Before the availability of vitamins and minerals, plasma levels of micronutrients fell rapidly while infusing only macronutrients. The only commercial preparation initially available was a trace element solution that had iron and iodide. The first commercial preparation of multivitamins for intravenous use was in the 1960s and lacked folic acid, vitamin B₁₂ and K, and biotin. It also had very high concentrations of vitamins A and D and thiamin. Because of the variability in practice, there was increased risk of toxicity to vitamins A and D and deficiencies of other vitamins. Recommendations were made for intravenous pediatric and adult intravenous preparations in 1975. By 1978 there was a commercial multivitamin preparation that met these recommendations. Current preparations available contain all vitamins for which there are Dietary Reference Intake values, with the exception of choline. A Food and Drug Administration mandate now requires the addition of vitamin K to all preparations. Differences between the pediatric and adult forms of multivitamins (MVI) include amounts of B vitamins and vitamin D (see Table 4). There is currently no multivitamin preparation designed specifically for the premature infant. Dosing recommendations of Pediatric MVI for this group are based on weight (1/3 vial for <500 g; 2/3 vial for 500–1000 g and full vial for over 1000 g).

Trace element deficiencies have been noted in patients receiving long-term parenteral-nutrition support. The first case of chromium depletion was reported in 1977; selenium deficiency in 1979 and molybdenum in 1981. There are now many trace element solutions available with a variety of combinations of minerals and range from solutions appropriate to meet the needs of premature infants through the adult population. They are also available as single elements to tailor a solution as necessary. Contamination of trace elements can occur in parenteral solutions. Aluminum is one element that has been under recent scrutiny with the Food and Drug Administration mandate to minimize the amount patients receive. It was initially found to be in high concentrations in the casein hydrolysates and continues to be found in high concentrations in a variety of intravenous preparations. Over time aluminum can deposit in the bone, interfering with bone calcium uptake and deposition in the brain may impair neurological development.

Table 2 Pediatric parenteral nutritional requirements

	< 2000 g	0–4 years	5–18 years
Energy (kcal kg ⁻¹ d ⁻¹)	100	80–90	40–70
Protein (g kg ⁻¹ d ⁻¹)	3–4	2.0–3.0	1–1.5
Fat (g kg ⁻¹ d ⁻¹)	<3	<3	<2
Sodium (mEq kg ⁻¹ d ⁻¹)	2–3	2–4	2–4
Potassium (mEq kg ⁻¹ d ⁻¹)	2–3	2–4	2–4
Chloride (mEq kg ⁻¹ d ⁻¹)	2–3	2–4	2–4
Calcium (mEq kg ⁻¹ d ⁻¹)	3–4.5	2–3	.5–2.5
(mg kg ⁻¹ d ⁻¹)	60–90	40–60	10–50
Magnesium (mEq kg ⁻¹ d ⁻¹)	.35–.6	.25–.5	.25–.5
Phosphate (mM kg ⁻¹ d ⁻¹)	1.5–2.5	1–2	1–2
Zinc (mcg kg ⁻¹ d ⁻¹)	400	300	100
Selenium (mcg kg ⁻¹ d ⁻¹)	1–3	1–3	1–2
Trace elements (ml l ⁻¹)	2	2	2
Multivitamins (ml d ⁻¹)	5	5	5–10

Parenteral iron supplementation is controversial due to its potential risk of anaphylaxis and possible effect of providing a nutrient source for bacteria during sepsis episodes. However, if the enteral route is contraindicated for prolonged course or malabsorption and blood transfusions are not being given, consideration for iron dextran supplementation is warranted (Table 2).

Metabolic Complications

Liver Disease

Although PN may be life-sustaining, long-term use may be detrimental to the liver. The severity of injury ranges from reversible transaminase elevations to severe cholestasis and cirrhosis, especially in infants with short bowel syndrome. It is still not clear whether this is due mainly to a nutrient deficiency, toxicity, or some physiological process missing because of the lack of enteral feeding. Prevention and treatment strategies continue to include minimizing or preventing episodes of sepsis, providing enteral feedings, moderating energy intake to provide adequate for growth, but not to overfeed, cycling parenteral nutrition infusion, reduction of copper and manganese, use of an amino acid solution developed for infants, treatment/prophylaxis for bacterial overgrowth and the use of ursodeoxycholic acid. Our common practice has been to reduce lipid exposure to 1 g kg⁻¹ day⁻¹ among infants likely to require long-term PN. Intravenous lipid emulsions are a rich source of linoleic acid, an omega-6 polyunsaturated fatty acid, and may enhance production of the proinflammatory cytokines. Increased leukotriene B₄ synthesis by the hepatic macrophages will draw additional polymorphonuclear leukocytes that intensify the inflammatory response to endotoxin by release of reactive oxygen species.

Bone Disease

The development of osteopenia is another complication that is common with long-term parenteral-nutrition support. The reasons are multifactorial and include relative immobility,

inability to provide adequate calcium and phosphorus with solubility limitations, and hypercalciuria. It has also been suggested that the dose of vitamin D in the multivitamin preparation may contribute to bone disease. Excessive vitamin D may suppress parathyroid hormone secretion and directly cause bone resorption. Although aluminum is still present in some intravenous solutions, including calcium gluconate, vitamins, trace elements, the amounts are much less than those seen with the casein hydrolysates and are not believed to be a significant contributor toward the development of metabolic bone disease. Prevention and treatment strategies include maximizing calcium and phosphorus in parenteral-nutrition solutions, especially in growing children, providing enteral supplementation of these minerals as feasible and provide weight bearing physical therapy as possible.

Micronutrient Deficiency and Excess

If a patient is entirely parenteral-nutrition dependent, it is known that certain micronutrients need to be provided. Some parenteral-nutrition solutions require the addition of carnitine and selenium (if not provided in multi trace element solutions) and iron dextran (if the patient is not receiving transfusions or tolerating enteral iron). All serum levels should be monitored on a monthly basis or every 6–12 months if in the long-term phase of support. There may be other micronutrients not yet identified that may be deficient in the purified PN solution, hence another reason to begin enteral feedings as soon as feasible. Monitoring for excess losses is also important. For example, with increased stool/ostomy losses, the patient may require increased zinc in the parenteral-nutrition solution (Table 3).

Excess micronutrients can be caused by contamination, such as the case with aluminum, discussed earlier, or because of clearance. Copper and manganese can accumulate and become directly hepatotoxic because both elements depend on the biliary pathway for excretion. Therefore, in the presence of cholestasis, there will be increased intrahepatic accumulation. Manganese has also been reported to deposit in brain tissue, so copper and manganese levels should be monitored routinely.

Catheter Complications

Complications with central venous catheters most frequently include obstructions, infections, and occasional leakage and perforation. Although parenteral nutrition can be temporarily provided through peripheral intravenous catheters, the high osmolarity of intravenous glucose-electrolyte solutions often cause phlebitis and loss of access. Therefore, long-term access has required placement of a central venous catheter, placed via the internal or external jugular vein or a subclavian vein. There is also increased placement of peripherally inserted catheters by a team of specially trained staff and by an interventional radiologist. Tip position in the superior vena cava or SVC-right atrial junction should be verified radiographically to reduce complications from venous thrombosis or rare perforations. Central placement allows rapid dilution of hypertonic solutions in a large-diameter vein to minimize obstruction or

Table 3 Suggested monitoring schedule for inpatients receiving parenteral nutrition

Parameter	Daily	Weekly ^a	Periodically ^a
Weight	x		
Fluid balance	x		
Vital signs	x		
Urine sugar	x		
Catheter site/function	x		
<i>Laboratory (serum)</i>			
Sodium		x	
Potassium		x	
Chloride		x	
Bicarbonate		x	
Glucose		x	
Urea Nitrogen		x	
Creatinine		x	
Triglycerides		x	
Calcium		x	
Magnesium		x	
Phosphorus		x	
Albumin or prealbumin		x	
Transaminases		x	
Bilirubin		x	
Selenium			x
Copper			x
Zinc			x
Iron			x

^aWeekly or more often as necessitated by clinical course.

thrombosis. Catheters for central venous access have been made of polyvinyl chloride, polyurethane, and silastic, often with a Teflon cuff to anchor the catheter subcutaneously. However, formation of a fibrin sheath is common, often with a biofilm, which may harbor infectious organisms and prevent penetration of antibiotics. Central catheter obstructions can often be visualized by ultrasound or inserting radioopaque dye in the catheter. A thrombus can often be lysed with installation of a small bolus of tissue plasminogen activator. Long-term anticoagulation with coumadin, low-dose coumadin, or low molecular weight heparin has been advocated by some to avoid repeated catheter obstruction, venous thrombosis, superior vena cava obstruction, and potential pulmonary emboli.

Obstructions caused by precipitation of calcium-phosphate salts or medications may be susceptible to installation of a small amount of dilute acid, and those due to fatty material might be dissolved with dilute ethanol. For long-term home parenteral use, some patients have preferred use of implantable ports, which can be accessed through the skin daily with a special needle. Recently, peripherally inserted central catheters (PICC) have been used for longer periods up to a month or more without requiring a surgical procedure.

Infections

Patients who require parenteral nutrition are often predisposed to infectious complications. The catheter hub is often the entry site with skin flora such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, or *Candida* being the most common

Table 4 Comparison of parenteral multivitamin preparations for pediatric and adult

Vitamin	MVI Pediatric (Hospira)	Infuvite (Baxter) and MVI adult with vitamin K (Hospira)
A (IU)	2300	3300
D (IU)	400	200
E (IU)	7	10
K (mCg)	200	150
Ascorbic acid (mg)	80	200
Thiamine (mg)	1.2	6
Riboflavin (mg)	1.4	3.6
Niacin (mg)	17	40
Pantothenate (mg)	5	15
Pyridoxine (mg)	1	6
B ₁₂ (mCg)	1	5
Biotin (mCg)	20	60
Folate (mCg)	140	600

Source: Adapted with permission from Hendricks KM, Duggan C, and Walker WA (eds.) (2000) *Manual of Pediatric Nutrition*, 3rd edn. London: BC Decker.

organisms, along with Gram negative enteric bacteria possibly from bacterial translocation. Antibiotic treatment through the central line is often successful without replacement of the catheter, using antibiotic combinations such as vancomycin and gentamicin, or with periodic indwelling infusions of a 70% ethanol lock (Table 4).

Summary

Advancements in the technology, production and manufacturing of intravenous solutions have progressed over the past 40 years. Along with improvement in the solutions available for dextrose, amino acids and lipid emulsions, there has also been progress with the delivery systems, catheters, and improved sterile techniques for line and skin care to reduce overall complications.

Ongoing research and product development in areas associated with long-term PN support is vital for future patient

management to be able to continue to provide optimal support with minimal risk for those patients for whom PN is life sustaining.

See also: Nutritional Support: Adults, Enteral; In the Home Setting. Parenteral Nutrition

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In the Home Setting

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The prevalence of nutritional problems in developed societies is a cause of growing concern. At one end of the nutritional spectrum, the obesity 'epidemic' is spreading at an alarming rate. At the other end of the spectrum, protein-energy malnutrition and nutrient deficiencies are also common, especially in the elderly and in those with disease. **Table 1** shows the frequency of specific vitamin deficiencies and underweight (body mass index $<20 \text{ kg m}^{-2}$) in people aged 65 years or older, residing in the United Kingdom. Complimentary information on protein-energy status can be obtained by considering simple criteria, such as those used by the 'Malnutrition Universal Screening Tool' (MUST) (**Figure 1**). This tool, which depends on weight loss and body mass index (and an acute disease effect, which does not normally apply to community patients), has been used to help estimate the distribution of malnutrition in the United Kingdom. Approximately 2% of individuals with medium and high risk of malnutrition are in hospital, 5% in care homes, and the remaining 93% in the community, of which 2–3% live in sheltered housing. Similar distributions are thought to exist in other countries although in low-income countries, the proportion of malnourished people in institutions is expected to be even less than in more developed countries. National surveys in various countries have demonstrated that malnutrition is common on admission to institutions, for example, approximately 28% among those admitted to hospitals in the United Kingdom (MUST criteria). This means that national strategies to combat malnutrition must consider its origins and causes in the community and attempt to prevent them at an early stage. However, because there are so many people who are discharged from the hospital in a malnourished state, often more malnourished than on admission, there should be an opportunity to identify them before discharge from the hospital and initiate treatment there, which can continue in the community. The same principles apply to individuals attending hospital outpatient clinics. The use of a consistent framework for identifying and treating malnutrition within and between care settings is important in facilitating continuity of care during the patient journey from one setting to

another. Unlike other screening tools, MUST was developed for this specific purpose and adopted nationally in a number of countries with this in mind.

This article focuses on the treatment of malnutrition (rather than obesity) in the home setting. This treatment includes dietary counseling and fortification, oral nutritional supplementation (mixed macro- and micronutrient supplements), and artificial nutritional support (enteral tube feeding (ETF) and parenteral nutrition (PN)). The simplest and most commonly used treatment involves oral nutritional support, which is considered before home enteral tube feeding (HETF) and home parenteral nutrition (HPN).

Oral Nutritional Support

Dietary Counseling and Fortification

Dietary counseling, usually provided by a dietitian, is an integral part of oral nutritional support. It includes advice on dietary fortification, which is often the first-line treatment of malnutrition in the home and other care settings. Counseling may involve advice on eating patterns (e.g., eating certain types of snacks at particular times of day) or addition of energy- and protein-rich food ingredients (e.g., cream, milk, oil, butter, sugar, and skimmed milk powder) to meals. Commercial energy- and protein-containing supplements can also be used to improve intake without substantially altering the intake from normal food and drink. The use of nutritionally fortified food snacks as part of the diet may improve both the intake and the status of micronutrients. However, the success of these dietary strategies is limited in patients with severe anorexia, those living in poverty and due to other social factors, and in those with inadequate motivation. Thus, patients may find it difficult to purchase, manipulate, or prepare their meals. Financial or other forms of social support, such as help with shopping, cooking (or provision of 'meals on wheels'), and help with eating, may do much to improve intake in some individuals. Although dietary counseling, with or without

Table 1 Proportion of subjects aged 65 years or older with selected vitamin deficiencies and body mass index $<20 \text{ kg m}^{-2}$

Free living (%)		Institutions (%) ^a		Criteria	
Vitamin deficiencies					
Folate deficiency	29	35	Red blood cell concentration	<345 mmol l ⁻¹	
Severe deficiency	8	16		<230 mmol l ⁻¹	
Thiamine deficiency	9	14	Erythrocyte transketolase activation coefficient (ratio)	> 1.25	
Vitamin B ₁₂ deficiency	6	9	Plasma concentration	< 118 pmol l ⁻¹	
Vitamin D deficiency	1-2	1-5		< 12 mmol l ⁻¹	
Vitamin C deficiency	14	40	Plasma concentration	< 11 mmol l ⁻¹	
Severe deficiency	5	16		< 5 mmol l ⁻¹	
Underweight	3	16	Body mass index	< 20 kg m ⁻²	

^aRegistered residential homes (57%), nursing homes (30%), dual-registration homes (9%), and other facilities (4%).

Source: Based on the National Dietary and Nutrition Survey (1998) in the United Kingdom.

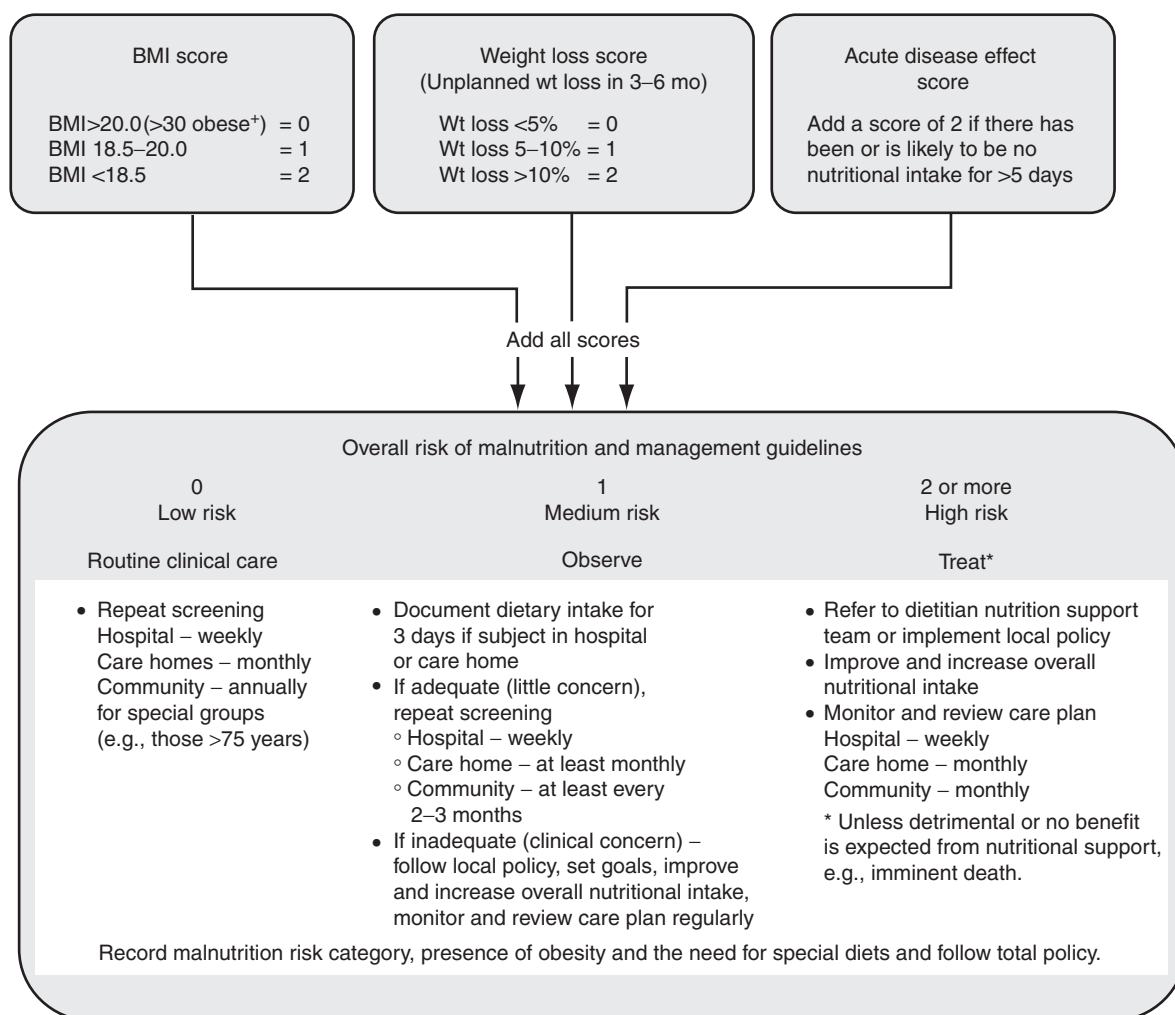


Figure 1 Malnutrition Universal Screening Tool (MUST). A copy of MUST and further details on taking alternative measurements, special circumstances, and subjective criteria can be downloaded at www.bapen.org.uk

dietary fortification, is widely used in clinical practice, there is little research supporting its clinical efficacy in patients at risk of malnutrition in developed countries.

Oral Nutritional Supplements

Mixed macro- and micronutrient liquid sip feeds and other oral nutritional supplements (bars, powders, and puddings) are widely used in the treatment of malnutrition in the community setting. A systematic review of 78 randomized controlled trials (RCTs) (including 44 RCTs from the community setting) suggests that oral nutritional supplements can improve energy and nutrient intakes, improve body weight (or attenuate weight loss), and improve a number of functional and clinical outcomes in various patient groups (Table 2). Meta-analyses of RCTs involving hospital and community settings suggest significantly lower mortality and complications in favor of oral nutritional supplements (typically 1.05–2.5 MJ day⁻¹ (250–600 kcal) daily). The evidence base in care homes is more limited, but a recent systematic review suggests that oral nutritional supplements can produce clinical

or functional benefits, such as improved healing of pressure ulcers and fewer infections.

For some patients, nutrition via the oral route is either unable to meet the nutritional requirements (e.g., patients with a poor appetite) or contraindicated (e.g., a cerebrovascular accident patient with aspiration and intestinal failure). For such patients, HETF and HPN may be required, although the treatment is usually initiated in hospital.

Artificial Nutritional Support: Home Parenteral Nutrition and Home Enteral Tube Feeding

Patients suffering from chronic conditions often prefer to be treated in the familiar surroundings of their home rather than in hospital. When the treatment involves sophisticated techniques, it is essential that either the patient or the caregiver is adequately trained to distinguish between problems that can be easily remedied at home and those that need expert advice and treatment in hospital. With the increasing pressure for hospital beds and the increasing cost of hospital care, many

Table 2 Summary of significant functional and clinical outcome improvements following oral nutritional supplementation in community patients from randomized controlled trials

Patient group	Functional/clinical outcome
Chronic obstructive pulmonary disease	Respiratory muscle function Hand grip strength Walking distances
Elderly	Reduced number of falls Increased activities of daily living Muscle power
HIV/AIDS	Cognitive function
Liver disease	Lower incidence of severe infections Lower frequency of hospitalization
Malignancy	Immunological benefits
Osteoarthritis	Increased activities of daily living ^a Improved osteoarthritis index ^a

^aNutritional supplement also containing immunoglobulin G (90 mg).

Source: Reproduced from Stratton RJ, Green CJ, and Elia M (2003) *Disease-Related Malnutrition: An Evidence-Based Approach to Treatment*. Oxford: CABI Publishing.

forms of treatment that were previously restricted to the hospital environment have extended to the community, including renal dialysis, cytotoxic drug therapy, HETF, and HPN. HETF has grown rapidly so that its prevalence in several developed countries is now several times greater than in hospital. In contrast, PN is still practiced less commonly outside hospital than in hospital and is likely to remain so in the foreseeable future. Both forms of treatment have led to the development of professional teams specializing in nutritional support in both the hospital and the community. These teams deal with problems ranging from simple day-to-day management issues to difficult ethical problems, such as concerning withholding or withdrawing nutritional support.

Origins and Development

The first report of HPN appeared in 1970 in North America and in the late 1970s in Europe. The number of people receiving HPN has increased considerably since then but remains substantially lower than that for HETF (Figure 2).

HETF is a much older technique than HPN, with the first reports appearing centuries ago. Accurate information on the number of people receiving HETF is difficult to obtain, because HETF tends to be initiated from many centers, and centralized reporting and record keeping in most countries are not fully established. There has been rapid growth in HETF attributable to developments in tube technology (flexible fine-bore tubes) and endoscopic procedures for placement of gastrostomy tubes (facilitating easier initiation and management of long-term feeding), as well as the development of home care services provided by commercial enteral feeding companies. In many developed countries, there is considerably more ETF taking place in the community than in the hospital. In the United Kingdom, there has been a steady growth in the number of people receiving HETF, and in 2007, it was estimated that there were approximately 30 000 people receiving this treatment at a given point in time (point prevalence). As with HPN, HETF is less common in Europe

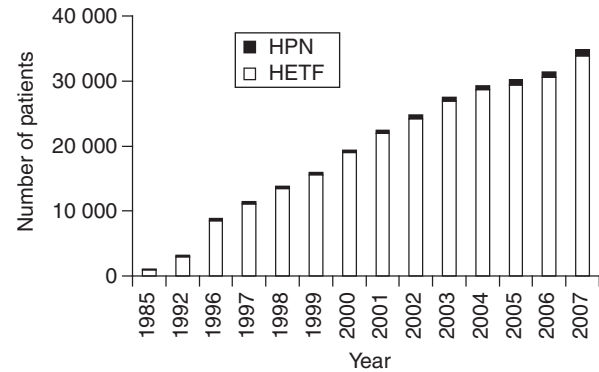


Figure 2 Estimated growth in point prevalence (amount of feeding taking place at a given point in time) in home enteral tube feeding (HETF) and home parenteral nutrition (HPN) in the United Kingdom.

than in North America and is practiced substantially less in Eastern Europe, India, and China than in high-income industrialized countries in the West.

In addition to differences in the prevalence of HETF and HPN between countries, there are also differences in practice of HETF and HPN within countries, which are unlikely to be due to chance. For example, the point prevalence of HPN in the United Kingdom varied from 3.7 to 22.5 patients per million of the population in 2006. This is likely to be due to availability of expertise and support staff, resources to fund treatment, reimbursement policies, and attitudes/policies toward the use of artificial nutrition.

The wide variations in the prevalence of home artificial feeding throughout the world are related to health-care economies. There is a relationship between expenditure on health care, as a percentage of gross domestic product (GDP), and the incidence of HPN and HETF. In India, Pakistan, and Africa, where spending on health is low, home artificial nutrition is less common. In Western Europe, where health care accounts for a greater proportion of GDP, home artificial nutritional support is more common. In the United States, with an even greater expenditure on health care, the prevalence of HPN and HETF is higher than anywhere else in the world.

Indications

Home Enteral Tube Feeding

The indications for HETF are different for adults and children. In adults, the most common indications are neurological disorders of swallowing resulting from cerebrovascular accidents, Parkinson disease, and obstructive lesions of the upper gastrointestinal tract. These mainly affect older individuals so that in various countries approximately half of HETF is administered to individuals aged 65 years or older. In children, HETF is usually used in conditions that lead to failure to thrive, such as cerebral palsy, cystic fibrosis, congenital malformation, and metabolic disorders. As with HPN, one of the main differences in the indications for HETF between countries concerns malignant disease. In North America, ~40% of people receiving HETF have been reported to have malignant disease and more than 50% in Italy. In the United Kingdom, as in many other countries, the proportion of patients

receiving HETF with cancer has steadily grown over time. The main malignancies in the United Kingdom are head and neck tumors and malignancies of the upper gastrointestinal tract (mainly obstructive oropharyngeal and esophageal cancers). The age distribution of people receiving HETF is influenced by the indications. Because disorders of swallowing (due to strokes, motor neurone disease, and other neurological conditions) and cancer of the upper gastrointestinal tract tend to occur in older age groups, adults receiving HETF tend to be elderly (with more than 65% of those in the United Kingdom being older than 60 years, 43% older than 70 years, and 19% older than 80 years in 2007). In recent years, there has been a trend to provide HETF to an older and more disabled population. Recent surveys in the United Kingdom suggest that approximately one-third of patients are house or bed bound and nearly half require total help to manage their tube feeding. Because the majority of these patients with high levels of disability are at home (spending <1% of their time in hospital), there are resource implications associated with the provision of health care by the underrecognized and underappreciated voluntary caregivers. Approximately 20% of those receiving HETF are children, and many children who started HETF because of cerebral palsy or congenital handicap continue tube feeding into adulthood.

Home Parenteral Nutrition

The main indications for HPN are Crohn disease, mesenteric vascular ischemia, motility disorders, surgical complications (e.g., enterocutaneous fistulae), and malignant disease. Patients receiving HPN are usually younger than those who receive HETF, although there is an overlap. There are also differences between the practices of HPN in different countries. One of the main differences concerns malignant disease. In the United States, 40–50% of patients receiving HPN have been reported to have cancer, and similar, if not higher, percentages have been reported in some European countries, such as Italy. Early reports from the United Kingdom and Denmark suggested that only a small proportion of HPN (~5%) involved patients with cancer, although this has increased with time. For example, in the United Kingdom, it has steadily increased so that by 2007 more than 15% of patients starting HPN had cancer. There has also been an increase in age (due at least partly to the increasing use of HPN in patients with cancer and mesenteric vascular ischemia) so that by 2007 nearly one-third of all patients receiving HPN in the United Kingdom were older than 60 years.

Organization

The organization and management of HETF and HPN have evolved over time. For example, delivery of feeds and equipment to the first patients who received HPN or HETF was undertaken by the hospitals that initiated the treatment. As the number of patients receiving such treatment increased, commercial organizations have established an organizational infrastructure for delivering feed and ancillary equipment through a national and international network. Some companies employ

doctors, nurses, and other staff so that they can provide most of the care, although this practice varies from country to country. In many countries, there is joint care between commercial companies and the national health-care systems.

HETF is initiated by many centers or hospitals, and some patients are followed up as outpatients. However, it is impractical to follow up many severely disabled patients in hospital, because they are house bound. Patients receiving HPN are often managed by centers with expertise in nutritional problems (e.g., in France, Denmark, and the United Kingdom). It has been suggested that all patients on HPN should be managed at such centers, but traveling to distant centers may require considerable time, effort, and expense. It is possible for patients to be managed more locally, especially if they are uncomplicated. It remains to be demonstrated if locally managed patients have better satisfaction and similar outcomes as those managed by larger centers. Of course, it is possible to have a system that combines local care and more distant specialist care when required.

Funding arrangements also vary. In several countries, home nutritional support is either totally or partially funded by the National Health Service, but payment may also be provided by private insurance and individual patients. Sometimes, confusion exists about the funding arrangements even in the same country, and this may limit and delay the use of HETF or HPN.

Patient organizations have developed in some countries, such as Patients on Intravenous and Nasogastric Nutrition Therapy (PINNT) in the United Kingdom. This organization provides support and information to people on home feeding, and it contributes to all levels of the operation of the British Association for Parenteral and Enteral Nutrition (BAPEN), through which it influences policy and decision making. Furthermore, as the feeding equipment for use at home was found to be impractical because it was originally designed for hospital use, PINNT has redesigned the equipment specifically for home use.

Standards of Care

Several surveys have identified inadequacies in training, support, and follow-up of patients receiving HETF and HPN. Specific problems include lack of written instructions about how to manage simple problems that may arise during feeding, lack of telephone contacts for use in emergency, lack of confidence, and inadequacy of equipment for home use. Such surveys have also highlighted the importance of a multidisciplinary approach and the need to undertake home visits to assess the status of severely disabled patients who cannot easily attend a hospital. Pressure on hospital beds has meant that some patients are discharged home before they have been adequately trained, and the care of such patients is sometimes passed on to other health-care workers who have little experience of home nutritional support. Because HPN is relatively uncommon in the population, general practitioners may have never encountered patients on this form of therapy and are therefore poorly equipped to manage them. The needs of patients may change during the course of their treatment; therefore, there is a need to establish an organizational infrastructure for continuity of care for HETF and HPN over time.

and from one health-care setting to another. Many hospitals do not have a nutrition team or policies that embrace the needs of people receiving artificial nutrition at home.

A series of guidelines for the management of artificial nutrition in the community have been developed by BAPEN (Tables 3 and 4). The guidelines cover aspects of training before discharge from hospital (although training can take place at home) and the support required from trained

specialist staff once the patient is at home. A national and local organizational structure for delivering the support would aid the process.

Monitoring

The basic elements of monitoring are similar for both HETF and HPN. They include an assessment of the activity of the

Table 3 Standards of practice for home enteral tube feeding (HETF)

<i>Structure</i>	<i>Process</i>	<i>Outcome</i>
There will be a training program for the health-care professionals involved in the care of patients receiving HETF.	Discharge planning will be performed only by professionals who have the necessary experience or who have undertaken a course of training in the topic.	The patient has confidence in the hospital team planning his or her discharge.
There will be a model of care for patients needing HETF.	The members of the multidisciplinary team will be involved in writing the 'mission statement' on which the model is based.	The patient will know the benefits, aims, and objectives of the HETF team.
There will be a relaxed, quiet area suitable for private discussion.	There will be a caring and compassionate atmosphere with adequate time for discussion.	The patient will feel able to express his or her fears and expectations.
The discharge planning documentation will include sections on domestic, family, and social circumstances.	The nutrition team will evaluate, with the patient and family, how HETF will alter his or her way of life.	The patient will believe that the feeding system can be integrated into an acceptable way of life.
There will be written patient/carer learning goals of HETF.	A designated nurse or dietitian will be responsible for teaching the patient according to his or her individual capacity for learning.	The patient will be able to demonstrate the necessary skills and achieve all the learning goals.
There will be an instruction manual for HETF.	Information and procedures will be regularly updated in order to reflect developments and innovations in tube feeding, access, nutrients, and delivery systems.	The patient will perform therapy based on current practice.
A relative, friend, or appropriately trained health-care professional will be available to deliver therapy if the patient is unable to do so.	The nurse/dietitian will help the patient identify the most appropriate carer. A community nurse will be given the opportunity to visit the patient in hospital and observe therapy before the patient is discharged.	The patient has confidence that safe care will be available at home.
Access to the gastrointestinal tract will be achieved by a tube suitable for long-term use.	The patient, nurse, and doctor will choose the most appropriate tube and access site.	The patient will use a feeding tube that is acceptable and accessible.
There will be a policy for sharing care with the patient's general practitioner (GP).	The GP will be contacted and a shared care protocol agreed.	The patient will know the responsibility of each health-care professional.
Written information describing HETF will be available for the GP.	The hospital team will provide the GP with the information before the patient is discharged, together with the discharge date and on-call telephone numbers.	The patient will have confidence in his or her GP's knowledge of HETF.
There will be written procedures for the management of feeding tubes.	The nurse/dietitian will adapt the procedures according to the patient's physical skills and domestic circumstances.	The patient's daily life will not be restricted by prolonged inappropriate procedures.
There will be a written prescription for the enteral feed (and other prescribable items).	The patient's GP will be contacted and advised on how to prescribe the feed.	The patient will have the enteral feed available at home on the day of discharge.
There will be an on-call system for providing expert advice to the patient by telephone day and night.	The nurse/dietitian/doctor will explain the system to the patient and identify the professions involved.	The patient will know the names and telephone numbers of health-care professionals to contact in case of emergency by day or night.
Information will be available describing how the nutrient solutions and supplies will be provided following discharge.	The nurse/dietitian will explain the ordering system and discuss storage, depending on the patient's home circumstances.	The patient will know how to obtain supplies and store them, and dispose of unwanted material.
There will be a postdischarge monitoring protocol, established by the nutrition team.	Monitoring will be performed by a designated health professional as defined by the protocol.	The patient will know what the follow-up arrangements are.

Table 4 Standards of practice for home parenteral nutrition (HPN)

<i>Structure</i>	<i>Process</i>	<i>Outcome</i>
There will be a training program for health-care professionals involved in the care of patients receiving HPN.	Discharge planning will be performed only by professionals who have the necessary experience or who have undertaken a course of training in the topic.	The patient has confidence in the hospital team planning his or her discharge.
There will be a mode of care for patients needing home intravenous nutrition.	All members of the multidisciplinary team will be involved in writing the 'mission statement' on which the model is based.	The patient will know the beliefs, aims, and objectives of the HPN Care Team.
There will be relaxed, quiet area suitable for private discussion.	There will be a caring and compassionate atmosphere with adequate time for discussion.	The patient will feel able to express his or her fears and expectations.
The discharge planning documentation will include sections on domestic, family, and social circumstances.	The nutrition team will evaluate with the patient and family how the HPN will alter his or her way of life.	The patient will believe that the feeding system can be integrated into an acceptable way of life.
There will be written patient/carer learning goals for HPN.	A designated nurse will be responsible for teaching the patient according to his or her capacity for learning.	The patient/carer will be able to demonstrate the necessary skills and achieve all the individual learning goals.
There will be an instruction manual for HPN.	Information and procedures will be regularly updated to reflect developments and innovations in venous access, nutrient solutions, and delivery systems.	The patient will perform therapy based on current practice.
A relative, friend, or appropriate health-care professional will be available to deliver therapy if the patient is unable to do so (e.g., parent or guardian of a child).	The health-care professional will help the patient to identify the most appropriate carer. The district nurse will be given the opportunity to visit the patient in hospital and observe therapy before the patient is discharged.	The patient has confidence that safe care will be available at home.
Venous access will be achieved by a central venous catheter suitable for long-term use.	The patient, nurse, and doctor will choose the most appropriate catheter and access site.	The patient will use a central venous catheter that is acceptable and accessible.
There will be written procedures for the management of central venous catheters.	The nurse will adapt the procedures according to the patient's physical skills and domestic circumstances.	The patients's daily life will not be restricted by prolonged inappropriate procedures.
There will be a policy for sharing care with the patient's general practitioner (GP).	The GP will be contacted and a shared care protocol agreed.	The patient will know the responsibility of each health-care professional.
Written information describing HPN will be available for the GP.	The hospital teams will provide the GP with the information before the patient is discharged, together with the discharge date, and on-call telephone numbers.	The patient will have confidence in his or her GP's knowledge of HPN.
There will be a written prescription for the nutrition solutions (and other prescribable items).	The patient's GP will be contacted and advised on how to prescribe the feed.	The patient will have the feeding solution available at home on the day of discharge.
There will be a list of the required equipment, e.g., refrigerator, infusion pump, syringes, sterile gloves, and telephone.	Before discharge, the patient's home health authority will be provided with the list and asked to arrange supply by making local arrangements or establishing a contract with a commercial supplier.	The patient will have all the necessary supplies at home on the day of discharge.
There will be an on-call system for providing expert advice to the patient by telephone day and night.	The nurse will explain the system to the patient and identify the professions involved.	The patient/carer will know the names and telephone numbers to contact in case of emergency by day or night.
Information will be available describing how the nutrient solutions and supplies will be provided following discharge.	The nurse will explain the chosen supply system and discuss storage depending on the patient's home circumstances.	The patient will know how to obtain supplies, store them, and dispose of unwanted material.
There will be a postdischarge monitoring protocol, established by the nutrition team.	Monitoring will be supervised by the nutrition team.	The patient will know the date of the first outpatient visit and what monitoring will be performed.

underlying disease, the nutritional and metabolic state of the patient, and complications associated with nutritional support (Table 5). The clinical history alerts the attending health professional to the general well-being, as well as the likelihood

of specific problems, such as dehydration, electrolyte imbalance (e.g., diarrhea), local infection (e.g., local redness and swelling near the catheter exit site or peristomal area), blocked tubes and catheters, and so on. Catheter-related sepsis is an

Table 5 Some complications associated with parenteral nutrition and enteral tube feeding

	<i>Parenteral nutrition</i>	<i>Enteral tube feeding</i>
Mechanical	Catheter malposition Insertion trauma (e.g., pneumothorax, brachial plexus injury, cardiac arrhythmia) Catheter blockage, kinking, or occlusion Catheter embolus Air embolus Clot embolus (from catheter tip) Lack of access site	Tube malposition (e.g., into lung) Insertion trauma; perforation of esophagus, stomach, and small bowel; peritonitis and peristomal leakage and inflammation Tube blockage, e.g., kinking or occlusion
Feed/flow	Nutrient overload (e.g., hyperglycemia, infusional hyperlipidemia)	Diarrhea or constipation
Infections	Catheter-related sepsis Infected feed/administration set	Bloated abdomen/cramps Regurgitation/aspiration of feed
Metabolic	Fluid and electrolyte disturbances Hyperglycemia Deficiency syndromes, e.g., trace elements and vitamins Nutrient overload (see above) and toxicity (e.g., some trace elements)	Infected feed administration set Infection around gastrostomy Fluid and electrolyte disturbances Deficiency syndromes (rate with standard feeds given to typical patients) Hyper/hypoglycemia
Organ tissue dysfunction	Abnormal liver function, intestinal atrophy, metabolic bone disease	Mainly disease-related, abnormal liver function Aspiration pneumonia
Psychological	Anxiety, depression, disturbance in self-image, social isolation	Anxiety, depression, disturbance in self-image, social isolation
Financial	Economic issues vary from center to center and country to country	Economic issues vary from center to center and country to country

important complication of PN, and aspiration pneumonia is an important complication of ETF. The patient/caregiver should have written instructions about basic procedures, which aim to reduce complication rates, and how to deal with simple problems and to recognize those that they cannot readily deal with. Specialist advice should be available 24 h a day. The frequency of complications depends at least partly on the support provided by health professionals.

Dietary intake should be monitored, especially in patients whose clinical status is changing. Appropriate dietary advice may facilitate return to normal oral feeding in some patients. In those with a swallowing difficulty, it may be necessary to assess whether swallowing has improved, with input from speech and language therapists, so that unnecessary HETF is not continued when full oral feeding becomes possible. Studies in the United Kingdom suggest that 15% of patients receiving HETF can revert to full oral feeding after 1 year. Blood tests should be carried out at intervals to check for metabolic stability and specific nutrient deficiencies (e.g., vitamins, minerals, and trace elements) and toxicities. The frequency with which tests are carried out depends on the patient (e.g., whether the patient is receiving HETF or HPN), the duration of feeding, the extent of oral intake, and disease activity.

Outcome

The most important predictor of the outcome in patients receiving home artificial nutritional support (enteral or

parenteral) is the underlying disease. Therefore, mortality statistics strongly depend on the initial indications. Nevertheless, a few conclusions can be made. First, the complications associated with artificial nutritional support vary but are reported to be responsible for less than 3–5% of deaths. Second, the outcome is dependent not only on the type of disease but also on the stage of the disease (e.g., patients with advanced HIV who start HPN are only expected to survive a few months, whereas patients with less-advanced disease are expected to survive longer). Third, the outcome of patients receiving HPN and HETF for a variety of conditions is available from the British Artificial Nutrition Survey (Table 6). For patients on HPN, overall mortality at 1 year is 13%, with 19% returning to oral feeding and the majority continuing with HPN. Patients with Crohn disease often have a good prognosis (with 2.5% mortality and 38% returning to oral feeding within 1 year). For patients on HETF, typically an older patient group, mortality is higher overall (36% at 1 year) and the outcome varies according to age and condition. The outcome data for two common conditions in adults and children receiving HETF are shown in Table 6.

Assessments of quality of life, using EuroQol, suggest that the majority of patients receiving HETF and HPN have some problems (moderate or extreme) with mobility, self-care, usual activities, pain/discomfort, and anxiety/depression (five EuroQol dimensions). Mean quality-of-life scores (0, 'worst imaginable health state'; 100, 'best imaginable health state') in adults receiving HPN (53 ± 18) are higher than those for adults receiving HETF (42 ± 27), but both are considerably lower than the scores obtained from the general population,

Table 6 Twelve-month outcomes for patients receiving home enteral tube feeding (HETF) and home parenteral nutrition (HPN)

<i>Continuing</i>		<i>Discontinuing</i>			
<i>Continues (%)</i>		<i>In hospital (%)</i>	<i>Transferred to oral (%)</i>	<i>Withdrawn/refused (%)</i>	<i>Died (%)</i>
HETF					
All adults (<i>n</i> = 33 955)	47.1	0.6	15.3	0.8	36.3
Cerebrovascular accident (<i>n</i> = 11 621)	51.1	0.4	9.6	0.5	38.3
Esophageal cancer (<i>n</i> = 2406)	23.8	1.0	24.9	1.3	49
All children (<i>n</i> = 6946)	70.6	0.5	20.8	0.7	7.3
Cerebral palsy (<i>n</i> = 977)	87.9	0.3	5.6	0.2	5.9
Congenital handicap (<i>n</i> = 607)	85.5	1	7.1	0	6.4
HPN					
All adults (<i>n</i> = 538)	63.6	1.1	19.3	3.2	12.8
All children (<i>n</i> = 79)	64.6	1.3	19.0	0	15.2

even when adjusting for age. For HETF patients, quality-of-life scores have been found to be similar for those living at home and those in nursing care.

Intestinal Transplantation

In some patients with irreversible intestinal failure, intestinal transplantation can be considered as an alternative to long-term PN. The first intestinal transplantation in humans was undertaken in the early 1960s. Limitations in technical expertise and immunosuppressive therapy meant that none of the original patients survived beyond 76 days. From 1985 to 1990, a series of 20 patients were given cyclosporine, but only two patients were able to resume normal nutrition and most of the grafts failed. The development of new immunosuppressive agents, particularly tacrolimus, resulted in renewed interest in intestinal transplantation. Furthermore, since 1990, there has been greater standardization of patient selection, operative procedures, and postoperative care mainly in centers specializing in intestinal transplantation. The total international experience is still limited with less than 2000 patients reported in 2009 (1031 children and 733 adults). Some of the transplants were isolated intestinal grafts, others were intestinal–liver transplants, and the remaining few were multivisceral transplants that included the small intestine. Better graft and patient survival rates have been reported more recently, especially in the more experienced centers. For example, in North America, the 1-year graft survival rate for intestinal and multiorgan transplants increased from 52% in 1997 to 75% in 2005. The associated patient survival increased from 57% to 87%, but in more recent years centers with greater experience have reported a 1-year survival rate more than 90%. Early referral appears to be associated with better outcome. One study that assessed the quality of life reported an improvement after transplantation and less dependency on narcotics.

It appears that intestinal transplantation has become a realistic lifesaving option for some people who cannot be maintained on HPN. However, it is not yet the treatment of choice in patients who can be successfully maintained on HPN without noteworthy complications. Nor is it the treatment of choice in patients who are likely to deteriorate rapidly

from other causes, such as aggressive multisystem disease, or likely to improve so that they can resume oral nutrition (e.g., patients with healing intestinal fistula or those with short bowel syndrome, in which benefits from intestinal adaptation may continue for up to 1–3 years). A better understanding of the immune response to the transplanted intestine and better immunosuppressive therapy, surgical techniques, and post-operative management are required. Appropriate selection and referral of patients to specialist centers are also important criteria that affect clinical outcomes.

Ethical Issues

The provision of nutritional support to people who are chronically sick, who have rapidly progressive disabling diseases, or who are terminally ill raises many ethical questions. Opinions about withholding or withdrawing artificial nutritional support vary from country to country because of different clinical, religious, and social beliefs and differences in national economies, some of which cannot support large-scale expensive long-term treatments. Thus, there is little home artificial nutrition in countries with poor economies. In more developed economies, the types of patients being fed may also vary considerably. For example, parenteral and enteral nutrition in patients with cancer are used more frequently in Italy than in the United Kingdom, suggesting that clinical attitudes to this type of nutritional support vary. The sanctity of human life is a belief that is strongly held by many religions, but when these conflict with medical judgment, public policies normally override personal religious beliefs. A common ethical controversy concerns the need to provide food and fluid to prolong life in severely disabled patients, such as those with severe neurological problems (e.g., cerebrovascular accident) or those approaching the ends of their lives. Although health professionals have a duty to prolong life, it seems inappropriate to prolong suffering. There has been controversy as to whether the provision of food and fluid by a feeding tube placed in the stomach or small intestine should be regarded as an essential part of care or medical treatment. The highest legal authorities in countries such as the United States and England have ruled that this is medical treatment. From an ethical perspective, there is no difference between withholding

and withdrawing treatment, but in practice it is often more difficult to withdraw treatment once it has begun than to not initiate it. Joint discussions at the outset between mentally capable patients, family members, and health-care workers can do much to prevent future ethical dilemmas.

Conclusions

Home nutritional support, including both oral and artificial (enteral and parenteral) methods of feeding, is an important modality of treatment that is being used for an increasing number of people with disease and disability who are managed in the community. The identification of individuals who are at increased risk of malnutrition and who may benefit from additional nutritional support is a vital first step, which can be undertaken using a validated screening tool (such as MUST; [Figure 1](#)). Oral nutritional support, including liquid multivitamin supplements, is of value in improving the nutritional intake and functional well-being of patients with malnutrition in the community. Without ETF, many patients with persistent swallowing difficulties would die; similarly, without PN, many patients with persistent intestinal failure would not survive. Although these forms of home therapy can be lifesaving, they may restrict normal lifestyle and lead to life-threatening complications. These complications can be prevented or treated by establishing an adequate organizational infrastructure. This should include education and training of both health workers and patients/caregivers as well as a management structure that allows all patients to be followed up and, when necessary, admitting patients to the

hospital for more intensive investigations and therapy. Ethical difficulties about withholding or withdrawing artificial nutritional support are likely to continue and to vary with time and from country to country. Intestinal transplantation is becoming a potentially realistic option for a few patients with irreversible intestinal failure who cannot be adequately maintained on long-term PN, but it has not yet become part of routine clinical care in the same way as renal transplantation has become routine in patients with renal failure, who would otherwise receive a lifelong treatment with dialysis.

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NUTRITIONAL SURVEILLANCE

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Glossary

AMPM Automated Multiple Pass Method, a computerized method for collecting interview-administered 24-h dietary recalls in person and by telephone. Developed by the USDA Food Surveys Research Group, the research-based approach employs five steps that are designed to enhance data collection and reduce respondent burden.

DPAS The Global Strategy on Diet, Physical Activity was adopted by WHO member states in 2004. It is a prevention-based strategy aimed at significantly reducing the prevalence of NCDs and their common risk factors, mainly unhealthy diet and physical activity.

EuroFIR The European Food Information Resource Consortium is a partnership between several universities

and 25 countries to develop and integrate a comprehensive cohort and validated network of food composition databanks in Europe.

INFOODS Established in 1984 to stimulate and coordinate efforts to improve the quality and availability of analytical food data worldwide.

NCDs Noncommunicable diseases such as diabetes or cardiovascular disease.

Nutritional surveillance A system established to continuously monitor the dietary intake and nutritional status of a population or selected population groups using a variety of data collection methods whose ultimate goal is to lead to policy formulation and action planning.

Nutritional surveillance is defined as a system established to continuously monitor the dietary intake and nutritional status of a population or selected population groups using a variety of data-collection methods whose ultimate goal is to lead to policy formulation and action planning. Continuous monitoring varies among countries such that annual, bi-annual, or other data-collection timeframes are determined by the funding and commitment of national governments. Increasingly, countries have come to recognize the need to collect dietary information in a systematic manner to make science-based policy decisions related to diet and health.

This article describes nutritional surveillance systems in developed countries. Emerging nutritional and health issues informed by these systems are reviewed. Examples of surveillance systems in Australia, Canada, Europe, Japan, New Zealand, South Korea, and the United States are described.

Uses of Nutritional Surveillance Data

Most notably data generated from these systems are categorized broadly as (1) nutritional monitoring and surveillance,

(2) research relating diet to health, and (3) informing the development of or enhancing existing nutritional policies and programs. **Figure 1** illustrates the interrelationships between data in a nutritional surveillance system. These data are used by government agencies, the private sector, the academic community, and the public. Government agencies use the data for nutritional policy, research, and programs. The private sector uses the data for marketing, product development, food labeling, and food safety compliance. Academics use the data to conduct research, develop programs, and train students.

Data uses for public policy enable informed decision-making regarding cost-effective programs, policy, and regulations. For example, data can be used to:

- assess the status of national populations and identify high risk or vulnerable groups;
- identify problems that inform the development of new policies and programs;
- formulate food enrichment, fortification, and food labeling policies and regulations;
- determine risk and inform regulatory decisions;

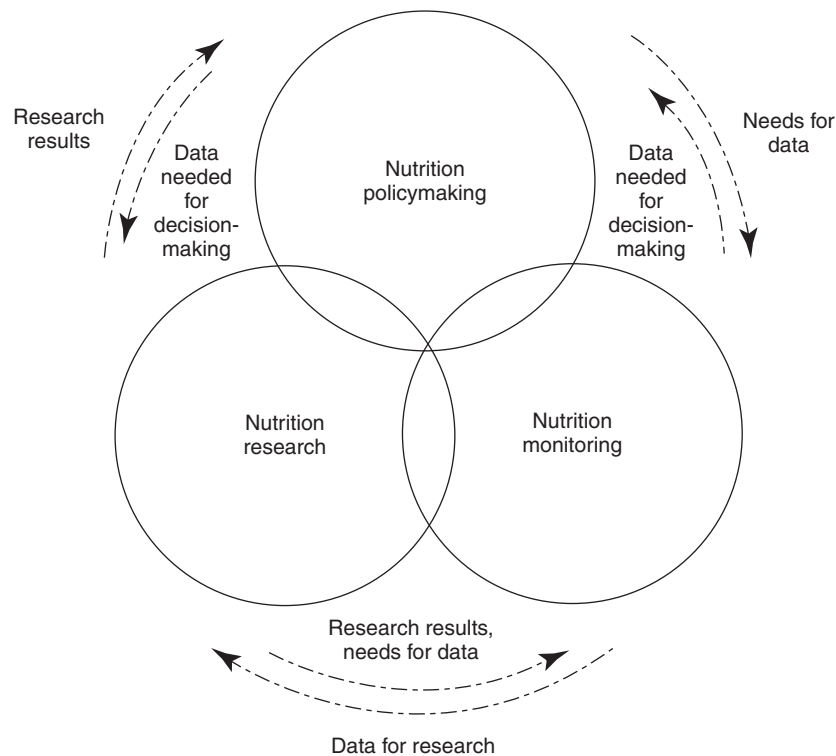


Figure 1 Relationships among nutrition policymaking, nutrition research, and nutritional monitoring. Reproduced from US Department of Health and Human Services/US Department of Agriculture (1993) Ten-year comprehensive plan for the National Nutrition Monitoring and Related Research Program. *Federal Register* 58: 32752–32806.

- develop and update socio–culturally appropriate foods and food guidance;
- evaluate the effectiveness of existing policies and programs and;
- conduct epidemiological, socioeconomic, behavioral, and marketing research.

Nutritional Surveillance Systems

The purpose of a nutritional surveillance system is to depict the relationship between food and health. Key information collected through the system includes data on nutritional and health status, food consumption patterns (household and individual levels), nutrient intakes, food supply, and food composition. There are many other influential factors on these data such as socio–demographic; culture; food knowledge, attitudes, and behavior; and food security. Although **Figure 2** highlights the traditionally accepted relationship between food and health, **Figure 3** begins to depict a new paradigm where globalization is included and may eventually include the effects of climate change.

Dietary Data Collection Methods

Food Supply Data

Food supply data reflect the type and amount of food available for consumption in a country. Food balance sheets are the

primary tool used for assessing available food at the country level. This indirect measurement method estimates the amount of food consumed by a country's population at a certain point in time. Food balance sheets do not measure actual food consumption, but rather food disappearance. Calculations are based on using beginning and ending inventories, with the difference as the amount of food consumed. The beginning inventory includes measurements of food production, imports and exports, adjustments for nonhuman food consumption and estimates of waste. Mean per capita annual food consumption is determined by dividing total disappearance of food by a country's population. Each food commodity is then multiplied by the appropriate nutrient values and the results expressed as either kilograms per year or grams per day of individual food commodities and nutrient availability per person. Major limitations to this method include (1) the accuracy of data may be questionable, (2) data only represent food available for consumption, (3) does not account for waste, and (4) does not indicate how food was distributed among individuals. However, food balance sheets are useful for comparing available food supply within and between countries, monitoring trends, and forecasting food consumption patterns over time. They are also useful in formulating agricultural policies related to food production and consumption. For example, **Table 1** highlights the use of food balance sheets data and shows per person food consumption in kilocalories per day worldwide and by region of the world from 1990–1995 to 2003–2005.

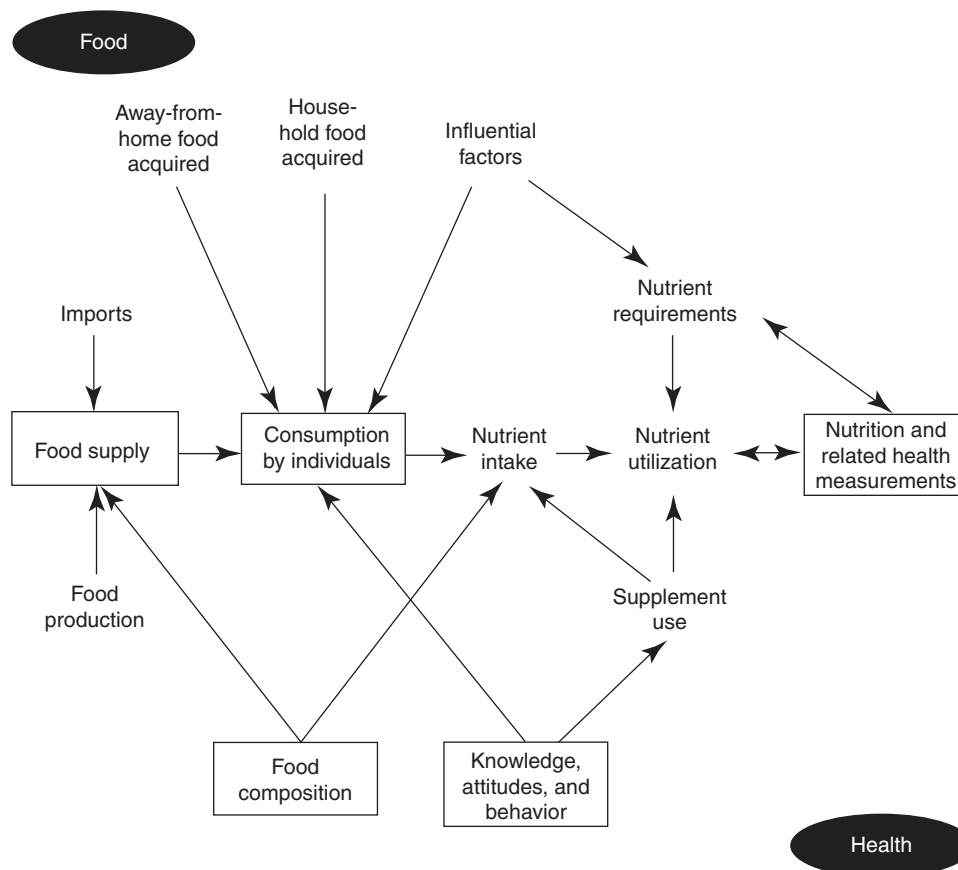


Figure 2 A conceptual model of the relationships of food to health. Reproduced from US Department of Health and Human Services/US Department of Agriculture (1993) Ten-year comprehensive plan for the National Nutrition Monitoring and Related Research Program. *Federal Register* 58: 32752–32806.

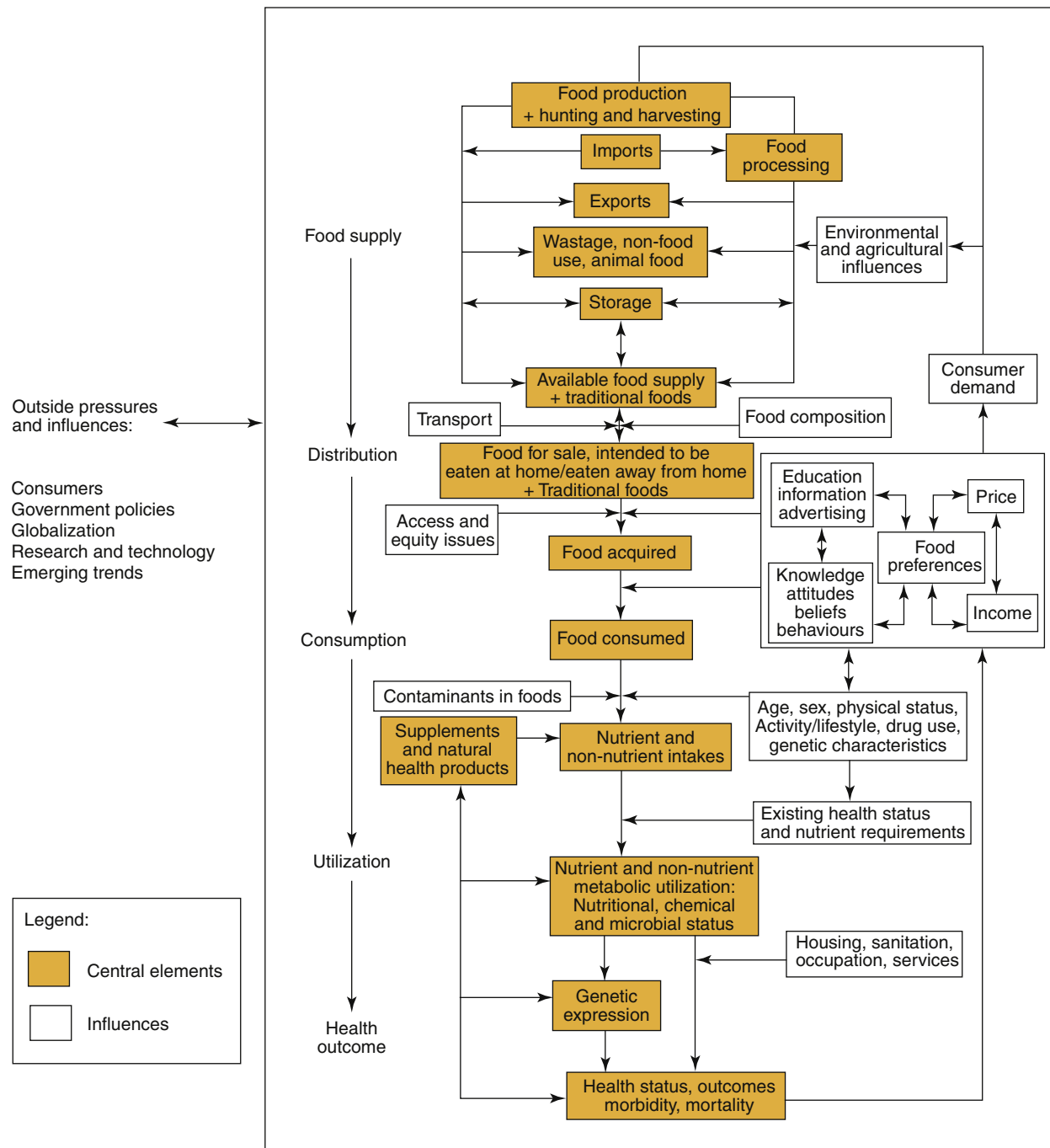
Food Consumption by Household

Several methods of assessing food consumption by households have been devised, which consider the per capita food consumption of the household. These methods account for all food and beverages on hand in the home at the beginning of the survey period, all items purchased or grown during the survey period, and all that remains at the end of the survey. They vary by the level of respondent burden and extent of recall or recordation expected. Four methods are primarily used: the food account, list-recall, inventory, and food record methods. For the food account, the head of household records daily the type and amount of food entering the household. The list-recall method includes an interview where the head of household recalls the foods used by the household on an 'as purchased' basis. The inventory and food record methods are probably the most burdensome to the respondent because they require a daily record of food acquired and changes to the food inventory, with detailed weighing and measuring of food. None of these methods provide data on actual food intake by individuals within the household or food consumed away from home. However, data from household food consumption surveys indicate the kinds, quantities, money value, and nutrient content of food used, which in turn, provides valuable data for determining the effects of income, household

size, and other socioeconomic factors on total food and food group consumption. These data demonstrate how diets at the household level conform to nutritional and household budgeting constraints.

Food Consumption by Individuals

A variety of methods are available to assess the food intake by individuals: diet history, 24-hour dietary recall, food record, and food frequency questionnaire. All of these methods provide advantages and limitations, and their applicability depends on the purpose and design of the survey. Diet histories can provide detailed information, but they require respondents to make judgments about their usual food habits and can be very burdensome. Dietary recalls are most appropriate for assessing the intakes of groups of people, and the current gold standard requires at least the collection of two days of intake to assess an individual's usual intake. Food records are thought to be the best method for assessing individual dietary intake, but they are time consuming and may impart bias if respondents modify their eating behavior due to the data collection method. Although food frequency questionnaires provide less detailed information, they are designed to provide usual intake data and are particularly useful for epidemiological studies of large population groups. For all these methods, advanced



Adapted from the conceptual framework of the Australian food and nutrition system

Ref: Ian H. Lester. AUSTRALIA'S Food and Nutrition.

Australia Government Publishing Service, Canberra. 1994

Figure 3 Conceptual model of the Canadian food and nutritional system adapted from the Australian food and nutritional system conceptual framework, www.hc-sc.gc.ca/fn-an/surveill/conceptual_model-eng.php. Reproduced from US Department of Health and Human Services/US Department of Agriculture (1993) Ten-year comprehensive plan for the National Nutrition Monitoring and Related Research Program. *Federal Register* 58: 32752–32806.

computerized systems provide standardized probes and multiple inquiry passes of intake have improved data collection, coding, and the estimation of nutrient intake and dietary patterns.

Examples of Nutritional Surveillance Activities

Most developed countries support a nutritional surveillance system and many conduct combined nutritional and health

Table 1 Per person food consumption by region

<i>World/Region</i>	<i>1990–1995 (kcal per capita per day)</i>	<i>1995–2000 (kcal per capita per day)</i>	<i>2003–2005 (kcal per capita per day)</i>
World	2669	2710	2778
Africa	2343	2335	2389
Asia	2539	2628	2646
Central and South America	2790	2825	2960
Caribbean	2269	2346	2565
Europe	3237	3206	3335
North America	3498	3616	3799
Oceania	3095	3048	3063
Developed Countries	3256	3269	3394
Developing Countries	2507	2569	2633

Source: Adapted with permission from Food and Agriculture Organization of the United Nations (2009) *Summary of World Food and Agricultural Statistics*, FAO Statistics Division.

surveys. **Table 2** presents some of these surveys by region of the world and the method of dietary data collection selected by each country. For Asia, surveys conducted by Japan in 2007 and South Korea in 2005 represent their most recently reported data collection. The 2007 National Nutrition Survey in Japan collected dietary histories and behavior information, in addition to other assessments, from almost 9000 respondents aged one year and older. Whereas, the 2005 National Health and Nutrition Survey in South Korea included a 24-hour dietary recall, dietary behavior, food frequency questionnaire, anthropometric, biochemical, and clinical examinations.

With 24 European Union (EU) member countries plus Norway, a considerable amount of data is available through the European Nutrition and Health Report 2009. This report provides (1) an overview of available data on food and nutrient intake, (2) identifies major national and regional health and nutritional issues, (3) describes trends in food supply, (4) compares average daily individual food availability at the household level, (5) evaluates individual food consumption in adults and energy and nutrient intake in all ages, (6) describes data on diet-related health indicators and status, and (7) analyzes food and nutritional policies in EU countries. Individual national reports provide more detailed information on nutritional and health status in different participating countries, with much of that information obtained from national surveys. As evidenced by **Table 2** various dietary intake methodologies are used and anthropometrics appears to be collected by a majority of the surveys, with a few adding the collection of physical activity assessments. To date the 2009 report is the most current, comprehensive study of nutritional surveillance in a region of the world. It highlights both the considerable improvements in the quality of data collection and assessment methods employed and the continuing need for harmonization of databases and survey methods, which would allow more robust comparisons of data across countries.

The United States has one of the most comprehensive nutritional surveillance systems in the world. Since 1999, the periodic National Health and Nutrition Examination Survey (NHANES) became a continuous national nutritional surveillance system. A key component of the survey is the

Automated Multiple Pass Method (AMPM), a computerized method for collecting interview-administered 24-hour dietary recalls in person and by telephone. The research-based, multiple pass approach employs five steps that are designed to enhance data collection and reduce respondent burden. Dietary data is collected from a nationally representative sample of 5000 individuals yearly and is released in 2-year cycles. The NHANES 2010–2011 is currently in the field. In Canada, the Canadian Community Health Survey, Cycle 2.2, Nutrition also used the AMPM to collect 24-hour recalls. Dietary data for greater than 35 000 individuals six months and older was released in Wave 1 (2005), Wave 2 (2006), and Wave 3 (2008).

In 2007, Australia conducted the National Children's Nutrition and Physical Activity Survey, which combined the collection of two days of dietary intake on children, aged 2–16 years old with anthropometric and physical activity assessments to strengthen the monitoring of childhood obesity in the country. A new National Nutrition and Physical Activity Survey Program, which will collect dietary data on 35 000 individuals plus an additional 15 000 individuals from indigenous population groups in the country is planned for 2011–2012. New Zealand's most recent survey, the Adult Nutrition Survey, was conducted in 2008–2009. Twenty-four-hour recalls, anthropometrics, biochemical, clinical, and blood pressure assessments were completed on respondents 15 years and older. Results from this survey are due out in 2011.

Food Composition Databases

Reliable food composition data is essential in calculating the food intake by individuals and ultimately assessing the nutritional status of population groups. Factors such as sampling, variability, and analytical methods used to determine the nutrient content of food are important in the development of these databases. To better expand the benefits of nutritional surveillance considerable work has been done to try and harmonize food composition tables regionally. Harmonization requires comparable criteria at the food level (number

Table 2 Nationwide food consumption surveys with individual-based dietary intake data

Country	Year	Survey	Population (ages in years)	Sample Size	Dietary method ^a	Other information ^b
Asia						
Japan	2007	National Nutrition Survey	1 +	8 885	DH, DB	A, BC, CE, BP, PA Rcl
South Korea	1998	National Health and Nutrition Survey	1 +	39 060 (10 876 dietary)	24-h recall, DB, FFQ	A, BC, CE
	2001	National Health and Nutrition Survey			24-h recall, DB, FFQ	A, BC, CE
	2005	National Health and Nutrition Survey	3 +	6 436	24-h recall, DB, FFQ	A, BC, CE
Europe						
Austria	1991–1994	Austrian Study on Nutritional Status (ASNS)	6–18	2 173	7d FR	
	1993–1997	ASNS	19–65	2 065	24-h recall, DH	A
	1998–2002	ASNS	19–60	2 580	24-h recall	A
	2007–2008	ASNS (compilation of 9 studies)	55 + 6 +	645 7 416	24-h recall, 3d FR (subsample)	A
Belgium	1980–1985	Belgium Interuniversity Research on Nutrition and Health	25–74	10 971	1d FR (DH in subsample)	A, BC, MH
	2004	National Food Consumption Survey	15 +	3 249	24-h recall	A
Denmark	1985	Dietary Habits in Denmark	15–80	2 442	DH	A
	1995	Danskernes Kosvaner	1–80	3 098	7d FR	
	2000–2006	National Survey of Dietary Habits and Physical Activity	4–75	4 120 (Yr 2000–2002) 3 247 (Yr 2003–2006)	7d FR 7d FR	A A
Finland	1992	Dietary Survey of Finnish Adults (FINDIET 1992)	25–64	1 861	3d FR	
	1997	FINDIET 1997	25–64 65–74	2 862 290	24-h recall	
	2003–2005	Type I Diabetes Prediction and Prevention (DIPP) Nutrition Study	1–6	2 535	DH	
	2005	Nationwide Survey of Breastfeeding and Complementary Feeding	<1	10 500	DH	
	2007	FINDIET	25–64, 65–74	2 039	48-h recall, 3d FR (subsample)	
France	1993–1994	Etudes Nationale des Consommations Alimentaires	2–85	1 500	7d FR	A
	1998–1999	Individual National Food Consumption Survey	3–14 15 +	1 018 1 985	7d FR	
	2006–2007	French Nutrition and Health Survey (ENNS)	3 +	1 675 (3–17) 3 115 (18–74)	3–24-h recall	A, BC, PA recall

(Continued)

Table 2 Continued

Country	Year	Survey	Population (ages in years)	Sample Size	Dietary method ^a	Other information ^b
Germany	1985–1989	National Nutrition Survey in Former West Germany	4–65 +	24 632	7 d FR KN, ATT, BH	A, BC
	1991–1992	National Nutrition Survey in East Germany	18–79	1 897	DH	
	1998	German Nutrition Survey	18–79	4 030	DH (4-week recall and FFQ) 3 d FR, FFQ	A, BC, CE, PA
	2003–2006	German Health Interview and Examination Survey for Children and Adolescents (KIGGS)	0–17	17 641		
Ireland	2005–2007	German National Nutrition Survey II	14–80	19 329	24-h recall, DH, 4 d FR	A
	1990	Irish National Nutrition Survey	10–65 +	1 214	DH	A (self-reported)
	1997–1999	North–South Food Consumption Survey	18–64	1 379	7 d FR, ATT	
	2003–2005	National Children's Food Survey	5–12	800		
	2005–2006	National Teens' Food Survey	13–17	441	7 d FR	
	2007	National Survey of Lifestyle, Attitudes and Nutrition (SLAN)	18 +	10 364	FFQ	CE (subsample)
Italy	1994–1996	INN-CA 1994–96	0–94	2 734	7 d FR	
The Netherlands	1987–1988	The Dutch National Food Consumption Survey (DNFCS-1)	1–85	5 898	2 d FR	A (self-reported)
	1992	DNFCS-2	1–92	6 218	2 d FR	A (self-reported)
	1997–1988	DNFCS-3	1–97	6 250	2 d FR	A (self-reported)
	2003	DNFCS-young adults	19–30	750	2–1 d FR	A
	2005–2006	DNFCS-young children	2–6	1 279	2–24-h recall	A
	1993	National Dietary Survey	13	1 705	FFQ	A (self-reported)
Norway	1993–1994	National Dietary Survey among Adults NORKOST	18	1 564	ATT, BH	
	1997	National Dietary Survey among Adults NORKOST	16–79	3 144	FFQ, ATT, BH	A (self-reported)
	1999	National Dietary Survey	16–79	2 672	FFQ, ATT	A (self-reported)
	2000–2001	National Dietary Survey	6 and 12 months, 2 years	2 400 2 010	FFQ	
Portugal			4	391	4 d FR	A, PA
			9	810	4 d FR	A, PA
			13	1 005	4 d FR	A, PA
	1980	Portuguese Food Consumption Survey	1–65 +	13 080	1 d FR, 24-h recall, FFQ	A, BC, CE, MH
Sweden	1989	Household Food Survey, HULK	1–74	2 036	7 d FR	A (self-reported)

United Kingdom	1997–1998 2003	Riksmaten Children's National Food Survey	18–74 4 8 11 16–64	1 215 590 889 1 016 2 197	7d FR 4d FR 4d FR 4d FR 7d FR	A (self-reported) A A A A, BC, CE, BP
	1986–1987	The Dietary and Nutritional Survey of British Adults	16–64	2 197	7d FR	A, BC, CE, BP
	1992–1993	Natl. Diet and Nutritional Survey (NDNS)	1.5–4.5	1 675	4d FR	
	1994–1995	NDNS	65+	1 687	4d FR	A, BC, CE, BP
	1997	NDNS	4–18	1 701	7d	
	2000–2001	NDNS	19–64	2 000	7d FR, BH	A, BC, CE, BP
	2003–2005	Low Income Diet and Nutrition Survey		3 728	4–24-h recall, BH, ATT	A, BC, BP, CE
North America Canada	1970–1972 2004–2005 2004	Nutrition Canada National Population Health Survey Canadian Community Health Survey, Cycle 2.2, Nutrition Wave 1 (2005) Wave 2 (2006) Wave 3 (2008)	0–65+ 12+ 6 months +	12 795 35 107	24-h recall, FFQ FFQ 24-h recall (2 nd Rci subsample)	A, BC, MH A, PA recall
	1977–1978	Nationwide Food Consumption Survey (NFCS)	(Households)	30 467	24-h recall, 2d FR	
	1987–1988	NFCS	(Households)	25 100		
	1985–1986	Continuing Survey of Food Intakes by Individuals (CSFII)	1–5 19–50 F 19–50 M All	3 200 6 400 1 100 15 192	24-h recall, 2d FR	
	1989–1991	CSFII	All	15 192	24-h recall, 2d FR (subsample), KN, ATT, BH (subsample)	A, BC, MH
	1994–1996	CSFII	All	16 103	24-h recall, 2d FR (subsample), KN, ATT, BH (subsample)	A, BC, MH
	1998	CSFII	0–9	5 559	2–24-h recall, KN, ATT, BH (subsample)	A, BC, CE, MH
	1970–1974	National Health and Nutrition Examination Survey (NHANES I)	1–74	20 749	24-h recall, FFQ	A, BC, CE, MH
	1976–1980	NHANES II	1–74	20 322	24-h recall, FFQ	A, BC, CE, MH
	1982–1984	Hispanic HANES	6 months–74	11 653	24-h recall, FFQ BH	A, BC, CE, MH
United States ^c	1988–1994 1999 +	NHANES III NHANES 1999–2000	2 months + Birth +	33 994 9 965	24-h recall, FFQ 24-h recall (2 nd day by phone), FFQ (by mail for 2 years +)	A, BC, CE, MH A, BC, CE, MH
		NHANES 2001–2002	Birth +	11 039 (5000 dietary)		

(Continued)

Table 2 Continued

Country	Year	Survey	Population (ages in years)	Sample Size	Dietary method ^a	Other information ^b
Oceania Australia		NHANES 2003–2004	Birth +	10 122 (5 000 dietary)		
		NHANES 2005–2006	Birth +	10 348 (5 000 dietary)		
		NHANES 2007–2008	Birth +	10 149 (5 000 dietary)		
		NHANES 2009–2010	Birth +			In the Field
	1983 1985 1995	National Dietary Survey of Adults National Dietary Survey of School children National Nutrition Survey	25–64 10–15 2 +	6 295 5 224 13 858	24-h recall 1d FR 24-hour recall (2 nd 24-h recall from subsample) FFQ	A, BC, CE, MH A, BC, BP A, BP
New Zealand	2007	National Children's Nutrition & Physical Activity Survey	2–16	4 400	24-h recall (2 nd by phone)	A, 2 PA Rci, PR (on 5 + subsample)
	2011–2012	National Nutrition and Physical Activity Survey Program (planned)		35 000 15 000 (Indigenous)		
	1977 1989 1997	National Diet Survey Life in New Zealand Survey National Nutrition Survey (adults)	20–74 15 + 15 +	1 938 1 702 4 636	24-h recall 24-h recall, FFQ, ATT, BH 24-h recall, FFQ, KN, ATT, BH	A A, BC, CE A, BC, CE
	2002 2008–2009	Children's Nutrition Survey Adult Nutrition Survey	5–14 15 +	3 275 Results due in 2011	24-h recall, FFQ, BH 24-h recall	A, BC A, BC, CE, BP

^aDietary method. 24-h recall; 1d FR; 1-day food record; FFQ, food frequency questionnaire; DH, dietary history; KN, dietary knowledge; ATT, dietary attitude; BH, dietary behavior.

^bOther information. A, anthropometry; BC, biochemical tests; BP, blood pressure; CE, clinical exam; MH, medical history; F, Female; M, Male; PA Rci, physical activity recall; PR, pedometer record.

^cStarting in 1999, the CSFII and NHANES merged and became one integrated national survey.

of foods covered, food classification and descriptions systems, and representativeness of nationally consumed foods), component level (coverage, identification, definition, analytical methods), and value level (missing nutrient data, documentation). Issues related to data management, including the compilation, software capacity, evaluation, data interchange, and ownership also must be considered. There are currently greater than 150 food composition tables or nutrient databases in use worldwide. Many are based on the USDA National Nutrient Database for Standard Reference available on the Nutrient Data Laboratory website, www.ars.usda.gov/nutrientdata. For several years there has been a concerted effort to improve harmonization of analytical methods, definition, and mode of expression of foods and nutrients. INFOODS was established in 1984 to stimulate and coordinate efforts to improve the quality and availability of analytical food data worldwide. The European Food Information Resource Consortium, EuroFIR, is a partnership between several universities and 25 countries to develop and integrate a comprehensive cohort and validated network of food composition databanks in Europe. FAO also maintains a comprehensive list of food composition tables, which are grouped by region of the world at www.fao.org/infoods/directory_en.stm.

Emerging Nutritional and Health Issues

Obesity continues to be a major health issue for the developed world. Obesity has been linked to multiple health disorders, such as Type 2 diabetes, cardiovascular disease, hypertension and stroke, some cancers, and disability. Globally, greater than one billion adults are overweight and at least 300 million are obese. Moreover, childhood obesity is one of the most critical public health challenges of the 21st century. The problem is global and the prevalence has increased exponentially. Worldwide, in 2010 the number of overweight children under the age of five is estimated to be greater than 22 million. Overweight and obese children are more likely to become obese adults and more likely to develop noncommunicable diseases (NCDs) such as diabetes or cardiovascular disease at a younger age. In 2004, a Global Strategy on Diet, Physical Activity, and Health (DPAS) was adopted by WHO member states. The primary purpose of DPAS, a prevention-based strategy, is to significantly reduce the prevalence of NCDs and their common risk factors, mainly unhealthy diet and physical activity. The role of WHO in implementing the DPAS is to provide leadership, evidence-based recommendations and advocacy for international action to improve dietary practices and increase physical activity. Thus in 2009, the WHO Forum and Technical Meeting in Population-based Prevention Strategies for Childhood Obesity was held in order to identify priorities for population-based strategies to prevent childhood obesity and to define roles and responsibilities for various stakeholders.

An aging population also remains an emerging global public health issue. On October 1, 2010 at the 20th Anniversary of the International Day of Older Persons, it was announced that the world's population is now as old as it has ever been. Women comprise the majority of the older population in

most countries, because globally they tend to outlive men. In 2010, one in every 10 persons was aged 60 or more and by 2050, that figure will be one in 5. Because both lean body mass and basal metabolic rate decline with age, maintaining adequate nutritional status among the elderly is especially important. In some instances nutrient requirements may be reduced, where requirements for other essential nutrients may rise in later life. Although WHO has developed a policy framework that promotes active aging and focuses on the prevention and reduction of the burden of disabilities and associated NCD risk factors, there continues to be an increasing demand worldwide for WHO guidelines that address the nutritional needs of the growing elderly population.

Despite access to nutritious foods and nutrition marketing strategies, poor nutrition is growing in affluent developed countries. Studies reveal that adult intakes for certain vitamins and minerals, including vitamin A, E, D, and folate, are below the recommended intakes in some countries. Notably the 2010 US Dietary Guidelines concluded that the under-consumption of vitamin D, calcium, potassium, and dietary fiber for both adults and children was a substantial public health concern. Economic globalization, with increasingly powerful transnational companies shaping global consumer behaviors, in many ways has created an environment of unhealthy food choices, such as fast food and high-sugar beverages. Environmental and climate changes may also place the nutritional status and dietary behavior of populations even in developed countries at risk.

The ability to monitor these emerging nutritional and health issues becomes increasingly important for enabling countries to better make decisions regarding cost-effective programs, policy, and regulations. As evidenced by the number of surveys conducted since 2005, countries appear committed to strengthening and maintaining national nutritional surveillance systems.

See also: Biochemical Indices. Dietary Intake Measurement: Methodology. Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Food Composition Data. Nutritional Assessment: Anthropometry; Clinical Examination. Nutritional Surveillance: Developing Countries

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Developing Countries

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Glossary

Demographic and Health Surveys Large scale national health survey.

Gross National Income Gross domestic product less net taxes on production.

Low birth weight A baby born weighing less than 2500 g.

Introduction

Nutritional surveillance has been defined in the previous article as a system to continuously monitor the dietary intake and nutritional status of a population or select population groups, using a variety of data collection methods, with the ultimate goal of improving policies and programs to address the situation. The challenges to meet this goal in developing countries are many, and differ from those of more industrialized countries for a number of reasons. First, in most developing countries the prevalence of problems related to nutritional deficiency is higher than in industrialized countries and varies greatly within and among regions and countries. Second, continuous national monitoring (e.g., through the health care system) is not well established in many countries and resources for national surveys are often scarce. Third, during the past decade there has been a dramatic increase in the prevalence of overweight and obesity and their related morbidities in developing countries. Together, the problems related to under- and over-nutrition present unique challenges to national and international policy makers and heighten the need for nutritional surveillance systems that are able to provide useful information in a timely fashion for policy and program decision making related to multiple nutritional problems within the same country. Finally, the recent food and financial crises have led to further vulnerability of the poor, particularly those living in some regions such as Sub-Saharan Africa. Effective surveillance systems that identify those at risk in a timely fashion, and provide information on the determinants of nutritional problems and on coping strategies adopted by the population could do much to improve programs and the welfare of those likely to be affected most.

Information to Inform Policy-Making and Program Planning

In developing countries, the need for nutritional interventions is great. Accurate and timely information on the prevalence and distribution of nutritional problems is vital to inform policy-making and programs-planning. Health and nutritional policies and programs should use information from nutritional surveillance to identify populations or sub-groups within regions and countries at greatest need and to guide the design of appropriate

interventions that address the relevant causes of health and nutritional problems. At all stages of planning, those responsible for data collection should interact directly with policy makers to ensure that the information is collected, analyzed, and presented in a way that is meaningful to them. Unfortunately, to date the number of successful examples of the use of nutritional surveillance to inform policy-making and program design, particularly in developing countries, is still limited.

The responsibility for making nutritional surveillance action-oriented lies with all those involved – donors, agencies, researchers involved in data collection and analysis, and policy makers. In many developing countries the lack of existing information systems and limited local resources implies that external funds, often from donor agencies, will be required for surveillance activities. Once data on the nutritional situation become available, policy makers may need to seek technical assistance from experts in the field to assist with the design of interventions with high potential for impact on nutritional outcomes. Evaluation of policies and programs is essential to complete the cycle and determine whether modifications to policies and programs are needed. Although information collected as part of nutritional surveillance does not permit causal attribution of any changes in nutrition and health indicators over time to specific interventions, it can be used to evaluate trends over time in the presence/absence of such interventions. This again, may imply the need for external funds. Researchers or national or international agencies may need to become advocates to promote dialog with policy makers and to convince donors of the importance of investment in this process.

Types and Sources of Nutritional Surveillance Data in Developing Countries

Information collected as part of a nutritional surveillance system should include not only the prevalence and distribution of nutritional problems, but also information that will permit an analysis of their direct and indirect causes. A conceptual framework, such as that of The United Nations Children's Fund (UNICEF), for understanding the causes of malnutrition should be used to determine the types of information that are needed. The exact information needed

therefore may be context specific, depending on what pre-existing information is available. For example, if recent national data show an adequate national food supply, but a high prevalence of malnutrition in children less than five years of age, information related to household food security, individual food consumption, breast and complementary feeding practices, and other causes of childhood malnutrition such as infections may be needed. On the other hand, if an increasing prevalence of overweight and obesity is the primary concern in the country, information on dietary intake, physical activity patterns and environmental factors that favor excess weight gain will be needed to understand the causes of this increase and design appropriate interventions.

Details of different methods to collect data at the national, household, and individual level are described in the previous article and will not be reviewed here. Rather, this article focuses on some of the specific strengths, limitations, and applications of each type of data as they apply to nutritional surveillance in developing countries and describes some additional methods that have been adapted for use in developing countries.

National Food Supply

The Food and Agriculture Organization of the United Nations (FAO) compiles and monitors food supply data for many developing countries. The estimates are typically based on food balance sheets supplied from each country's national records. The information is usually converted to per capita food availability and is presented for developing countries as a whole, by region, sub-region, and for over 100 individual countries. Similar information on food supply is also available for many countries (e.g., in Latin America–Mexico, Brazil, Argentina, and Chile) from national statistical institutes, as well as regional organizations (e.g., the Council for Statistics in Latin America). Much of this information can be accessed free of charge through local Internet sites.

The quality of data used to generate food balance sheets can vary greatly among countries. In general, the methods are thought to underestimate total per capita energy availability in developing countries. In some countries, particularly those where small-holder agriculture is still common, this may be related to underestimates of true production due to a less centralized economy.

Food supply data is essential to make comparisons between and across regions and to monitor trends. For example, according to the FAO food supply data, approximately 925 million people had inadequate dietary energy intake (<1800 kcal person⁻¹ day⁻¹) in 2010, representing 16% of the population of developing countries. Although lower than the past few years (e.g., estimated at 1.023 billion in 2009) this rate is still higher than before the 2008–09 economic crisis. Furthermore, the gain has been mainly in Asia with little improvement in other regions, particularly Sub-Saharan Africa. Ideally this type of information should be used to promote agricultural and trade policies that will enhance the food supply.

National food supply data should not be used to identify specific populations at risk nor does an adequate national

food supply imply that there is no hunger in the country. Because data are available at the national level only, they cannot be used to analyze diversity within the population or identify vulnerable groups. Thus, although food supply data is useful for trend analysis, they should not be used to assess changes in food consumption or food security within specific population groups.

Trends toward a decline or increase in the food supply can also be identified using food balance information. Declines may reflect an unstable political environment or conditions that influence food production. Ideally, this information should be used to influence agricultural policy to stimulate higher levels of production. However, war or other political strife may impede this process thus information that explores the causes of insufficient supply might be useful to consider appropriate interventions. The data should not be used to predict food shortages or famine, first because it is not useful to identify vulnerable groups within a population and because vulnerable groups may already be experiencing shortages by the time this information is available and processed.

Household Food Consumption and Food Security

Household food consumption provides an estimate of the food available to be consumed on a *per capita* basis (e.g., kcal person⁻¹ day⁻¹), whereas food security commonly refers to the adequacy (real or perceived) of the food available to meet the needs of the household members, taking into consideration cultural and personal preferences. Perceived food security differs from the methods described below because instruments collect information on the degree to which household members worry about running out of food, and modify habits due to a lack of food, without making any quantitative estimation of food availability.

The documentation of household food consumption and security in developing countries continue to be of great interest because of their relationship to specific health and nutritional indicators and as a means of monitoring the impact of political and environmental change on these outcomes. There have also been a number of efforts to document the impact of poverty alleviation programs on food consumption and food security. Whether qualitative assessments of food insecurity or quantitative estimates of food availability are best for identifying those at highest risk and evaluating impact of programs is not yet clear and may depend on the population and circumstances, for example, whether the risk is long term or short term due to natural disaster or conflict.

Traditional methods to assess household food consumption include those that collect data over a period of time, often 7 days, by asking the respondent to keep a record of food entering the home (food account method) or by quantifying the foods consumed at each meal (household food record method). Other methods may include an inventory of foods available in the home over a period of time or the list-recall method, whereby the respondent is asked to recall all foods purchased, produced, or received by other means over a given time, the quantity, and purchase or trade price. These methods have many limitations in a developing country. For example, respondents may have limited literacy and numeracy skills. In

this case, field workers would be responsible for data collection, resulting in increased survey time and costs. Many poor households have little or no food stores in the home and inventory methods may not provide an accurate estimate of household consumption if foods are purchased in small quantities for daily consumption. Furthermore, as is always a limitation with recall methods, misreporting either purposeful or due to erroneous recall can be an issue and must be addressed as part of training and quality control. Finally, these methods often rely on telephone or costly house-to-house surveys, the resources for which may not be available. Household consumption surveys are implemented in many countries for purposes other than nutritional surveillance. For example, household income and/or expenditure type surveys are used for estimating levels of poverty, for setting consumer price index among other purposes. Although they are subject to the limitations listed above, such surveys may be useful for example, to explore patterns of use of fortified foods or the identification of foods that might be appropriate for fortification.

In the past decade or so, there has been considerable interest in dietary diversity as an indicator of household food security. The dietary diversity score is also used to assess intake of individuals. The principle is the same, but the respondent is asked to list all foods or food groups consumed by the individual. This method provides qualitative information on all foods or food groups, including meals and snacks that were consumed over a given period of time (often 1, 3, or 7 days) by all members of the household. Each food or food group is assigned a value based on its nutrient density, bioavailability, and typical portion size. Portion size is included because some foods, nuts for example, may have high nutrient density but are typically consumed in small quantities. Points are then summed and the adequacy of dietary diversity assessed based on this score. Reasonable correlations have been found between dietary diversity, household socio-economic status, and household consumption as assessed by more traditional methods. The major advantage of this type of instrument is that it is simple and less time-consuming than other household consumption methods, with important implications for its use in large surveys. Although the use of this type of instrument in nutritional surveillance systems is still limited, its potential as a simple method to assess and monitor household food security appears promising.

Individual Nutritional Status and Dietary Intake

Information on the dietary intake and nutritional status of individuals in a population is essential for monitoring trends in these indicators over time and in response to political and environmental changes, as a means of identifying groups for intervention, and to assess the impact of interventions on nutritional status of the population. Although dietary intake and simple anthropometric measurements such as weight and height have often been the focus of health and nutritional surveys, it is essential that other indicators of nutritional status, such as micronutrient deficiencies also be documented as they continue to be important public health problems in most developing countries.

As discussed in the introduction, information regarding factors that are direct (e.g., the prevalence of infections) and indirect (e.g., maternal education and family socio-economic status) causes of nutritional problems will increase the usefulness of nutritional surveillance information for policy makers. Many nutritional surveillance systems have dealt with this daunting list of indicators by focusing efforts on specific high-risk groups – a logical decision in light of limited resources. Thus, more information is currently available for children younger than five years of age and women of reproductive age than for older children, adolescents, adult men, and older adults. With the increasing prevalence of overweight and obesity, particularly in school-age children and adults, this focus should be modified in countries undergoing nutritional transition. Although not evident in all developing countries, this transition is particularly striking in some middle-income Latin American countries such as Mexico and Chile, but is also documented in India and other parts of Asia.

The coexistence of malnutrition and 'over-' nutrition represents an important challenge to all those involved in nutritional surveillance. For funders, the population groups being monitored may need to be expanded with important cost implications; malnutrition in children and pregnant and lactating women has not disappeared in developing countries with the increase in overweight and obesity. For those involved in data collection, these additional nutritional problems imply the development and validation of new instruments to measure causes of overweight and obesity, for example, physical activity. For policy makers, the burden lies in the need for policies and programs that respond to two extremes of nutrition problems, often occurring in the same communities and even households or individuals. For example, programs designed to improve dietary intake in household members at risk for nutritional deficiencies (e.g., children less than two years of age) should not result in excess energy intake among those members of the household at risk for overweight and obesity (e.g., school-aged children). Thus program evaluations must be designed to detect both desirable and unexpected or undesirable outcomes.

The choice of which indicators are most appropriate for monitoring the nutritional status and dietary intake of individuals within populations depends on the country context and the specific objective of the surveillance system. For example, in countries where food shortages are common, indicators that are particularly sensitive to change, such as the prevalence and severity of low mid-upper arm circumference in children younger than five years of age should be used. If the objective is to document trends in the nutritional status of a population where micronutrient deficiency and overweight and obesity are the principal problems, then the prevalence of these should obviously be monitored.

Information on the intake and nutritional status of individuals is available from a variety of sources in developing countries. We present a description of the types of information available for children younger than five years of age (Table 1) and adults (Table 2) from a variety of information sources. Much of this information is obtained from large-scale multinational health and nutritional surveys and from databases maintained by international organizations such as FAO

Table 1 Surveys in low and lower middle-income countries with individual-level data on indicators of nutritional status in children under five years of age^a

Region/country	Survey year ^b	Age (years)	Sample size ^c	Indicators of nutritional status in children under five included in surveys ^d	Source ^e
<i>East Asia and Pacific</i>					
Cambodia	2005	0–4.99	3 587	Anthropometry, use of nutritional supplements (Vit A, Fe), anemia, use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality, micronutrient intake (Fe, Vit A).	DHS
China	2000	0–4.99	16 491	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe).	FAO, WHO
Indonesia	2007	0–4.99	5 539	Use of nutritional supplements (Vit A), complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Kiribati	2000/ 07	0–4.99		Prevalence LBW, feeding practices, underweight.	UNICEF
Korea, Democratic Republic	N/A				
Lao People's Democratic Republic	2000	0–4.99	1 347	Anthropometry, use of nutritional supplements, micronutrient deficiencies (I, Vit A), breast and complementary feeding practices.	WHO, NS
Marshall Islands	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A).	UNICEF
Micronesia, Federated States	2000/07	0–4.99		Prevalence LBW, feeding practices, underweight.	UNICEF
Mongolia	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Myanmar	2000/ 07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Papua New Guinea	1982–83	0–4.99	27 464	Anthropometry, Complementary feeding practices, Micronutrient deficiencies (I, Vit A).	WHO, NS (Rural)
Philippines	2008	0–4.99	24 308	Breastfeeding, complementary feeding practices, use of nutritional supplements (Vit A, Fe), immunization coverage, morbidity, mortality, micronutrient intake (Fe, Vit A).	DHS
Samoa	2009	0–4.99	1 408	Breastfeeding, complementary feeding practices, use of nutritional supplements (Vit A, Fe), immunization coverage, morbidity, mortality, micronutrient intake (Fe, Vit A).	DHS
Solomon Islands	2000/07	0–4.99		Prevalence LBW, feeding practices.	UNICEF
Thailand	1987	0.3–3.0	1 857	Anthropometry, mortality, morbidity, immunization coverage.	DHS
Timor-Leste	2000/07	0–4.99		Prevalence LBW, breastfeeding duration, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Tuvalu	2000/07	0–4.99		Prevalence LBW.	UNICEF
Tonga	2000/07	0–4.99		Prevalence LBW.	UNICEF
Vanuatu	1996	0–4.99	1 194	Anthropometry, dietary intake, micronutrient deficiencies (Fe, I, Vit A).	FAO, NS
Vietnam	2002	0–2.99	1 304	Mortality, immunization coverage, breastfeeding, use of nutritional supplements (Vit A).	DHS
<i>Europe and Central Asia</i>					
Armenia	2005	0–4.99	1 293	Anthropometry, use of nutritional supplements (Vit A), anemia, use of iodized salt, complementary feeding practices, micronutrient intake (Fe, Vit A), immunization coverage, morbidity, mortality.	DHS
Georgia	2000/07	0–4.99		Prevalence LBW, feeding practices, use of iodized salt.	UNICEF
Kosovo	N/A				
Kyrgyz Republic	1997	0–2.99	1 015	Anthropometry, complementary feeding practices, anemia (hemoglobin), immunization coverage, morbidity, mortality.	DHS

Moldova	2005	0–4.99	1 498	Anthropometry, complementary feeding practices, micronutrient intake (Fe, Vit A), anemia, immunization coverage, morbidity, mortality.	DHS
Tajikistan	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Turkmenistan	2000	0–4.99	2 928	Anthropometry, complementary feeding practices, micronutrient intake (Fe, Vit A), anemia, use of iodized salt, immunization coverage, morbidity, mortality.	DHS
Ukraine	2007	0–2.99	643	Complementary feeding practices, mortality.	DHS
Uzbekistan	1996	0–2.99	989	Anthropometry, complementary feeding practices, anemia, micronutrient deficiency (Vit A), immunization coverage, morbidity, mortality.	DHS
<i>Latin America and the Caribbean</i>					
Belize	2000/07	0–4.99		Prevalence LBW, feeding practices, use of iodized salt.	UNICEF
Bolivia	2008	0–4.99	8 422	Anthropometry, mortality, morbidity, complementary feeding practices, use of nutritional supplements (Vit A, Fe), anemia, micronutrient intake (Fe, Vit A), use of iodized salt, immunization coverage	DHS
Ecuador	1998	0–4.99	2 998	Anthropometry, complementary feeding practices.	PAHO
El Salvador	2002–03	0.25–4.99	-	Anthropometry, complementary feeding practices.	WHO
Guatemala	2002	0.25–4.99	6 308	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe, I, Vit A), complementary feeding practices.	DHS
Guyana	1997	0–4.99	289	Anthropometry, complementary feeding practices.	PAHO
Haiti	2005–06	0–4.99	2 841	Anthropometry, mortality, morbidity, complementary, feeding practices, use of nutritional supplements (Fe), anemia, use of iodized salt, immunization coverage.	DHS
Honduras	2005–06	0–4.99	9 595	Anthropometry, mortality, morbidity, complementary feeding practices, use of nutritional supplements (Vit A, Fe), anemia, use of iodized salt, immunization coverage.	DHS
Nicaragua	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A).	UNICEF
Paraguay	1990	0–4.99	3 389	Anthropometry, use of nutritional supplements (Vit A), micronutrient deficiencies (Fe, I, Vit A), complementary feeding practices.	DHS
<i>Middle East and North Africa</i>					
Djibouti	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A)	UNICEF
Egypt, Arab Republic	2008	0–4.99	9 103	Anthropometry, mortality, morbidity, immunization coverage, feeding practices, use of nutritional supplements (Vit A), foods and liquids consumed, micronutrient intake (Vit A), use of iodized salt.	DHS
Iraq	2000/07	0–4.99	N/A	Prevalence LBW, feeding practices, use of iodized salt.	UNICEF
Jordan	2007	0–4.99	8 607	Anthropometry, anemia, micronutrient intake (Vit A), mortality, morbidity, immunization coverage.	DHS
Morocco	2003–04	0–4.99	5 311	Anthropometry, immunization coverage, morbidity, mortality.	DHS
Syrian Arab Republic	2000/07	0–4.99		Prevalence LBW, feeding practices, use of iodized salt.	UNICEF
Tunisia	1988	0.3–3.0	2 023	Anthropometry, immunization coverage, morbidity, mortality.	DHS
West Bank and Gaza	N/A				
Yemen, Republic	1997	0–4.99	7 501	Anthropometry, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
<i>South Asia</i>					
Afghanistan	2000/07	0–4.99		Feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Bangladesh	2007	0–4.99	5 312	Anthropometry, use of nutritional supplements (Vit A), complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Bhutan	1999	4.5–4.99	2 981	Anthropometry, micronutrient deficiencies (Fe, I, Vit A).	FAO, WHO, NS

(Continued)

Table 1 Continued

Region/country	Survey year ^b	Age (years)	Sample size ^c	Indicators of nutritional status in children under five included in surveys ^d	Source ^e
Maldives	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Nepal	2006	0–4.99	5 262	Anthropometry, use of nutritional supplements, anemia (hemoglobin), use of nutritional supplements (Vit A, Fe), micronutrient intake, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Pakistan	2006–07	0–4.99	8 367	Immunization coverage, morbidity, mortality, breastfeeding supplementation, micronutrient intake.	DHS
Sri Lanka	1995	0.25–4.99	2 782	Anthropometry, micronutrient deficiencies (Fe, I, Vit A).	FAO, NS
<i>Sub-Saharan Africa</i>					
Angola	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Benin	2006	0–4.99	13 099	Anthropometry, dietary intake, use of nutritional supplements (Fe), anemia, use of iodized salt, micronutrient intake, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Burkina Faso	2003	0–4.99	8 628	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe, I, Vit A), immunization coverage, complementary feeding practices, morbidity, mortality.	DHS
Burundi	1987	0.3–36	1 929	Anthropometry, immunization coverage, morbidity, mortality.	DHS
Cameroon	2004	0–4.99	3 705	Anthropometry, use of nutritional supplements, anemia, immunization coverage, complementary feeding practices, use of iodized salt, morbidity, mortality.	DHS
Cape Verde	2005	0–2.99	1 223	Use of nutritional supplements, micronutrient deficiencies (Fe), immunization coverage, complementary feeding practices, morbidity, mortality.	DHS
Central African Republic	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Chad	2004	0–4.99	4 635	Anthropometry, use of nutritional supplements, immunization coverage, complementary feeding practices, use of iodized salt, morbidity, mortality.	DHS
Comoros	1996	0–2.99	921	Anthropometry, immunization coverage, complementary feeding practices, morbidity, mortality.	DHS
Congo, Democratic Republic	2007	0–4.99	3 631	Anthropometry, anemia, use of iodized salt, use of nutritional supplements (Vit A), immunization coverage, complementary feeding practices, morbidity, mortality.	DHS
Congo, Republic	2005	0–4.99	4 472	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe, I, Vit A), immunization coverage, complementary feeding practices, morbidity, mortality.	DHS
Côte d'Ivoire	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Eritrea	2002	0–4.99	5 466	Anthropometry, use of nutritional supplements, use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Ethiopia	2005	0–4.99	4 586	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Gambia, The	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF

Ghana	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Guinea	2005	0–4.99	2 786	Anthropometry, complementary feeding practices, immunization coverage, anemia (hemoglobin), use of iodized salt, morbidity, mortality.	DHS
Guinea-Bissau	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Kenya	2008–09	0–4.99	5 470	Anthropometry, use of nutritional supplements (Vit A, Fe), use of iodized salt, complementary feeding practices, immunization coverage, micronutrient intake (Vit A, Fe), morbidity, mortality.	DHS
Lesotho	2004	0–4.99	1 620	Anthropometry, use of nutritional supplements (Vit A), use of iodized salt, complementary feeding practices, anemia, immunization coverage, micronutrient intake (Vit A, Fe), morbidity, mortality.	DHS
Liberia	2007	0–4.99	5 166	Anthropometry, use of nutritional supplements (Vit A, Fe), complementary feeding practices, micronutrient intake (Vit A, Fe), immunization coverage, morbidity, mortality.	DHS
Madagascar	2008–09	0–4.99	5 436	Anthropometry, use of nutritional supplements, use of iodized salt, complementary feeding practices, micronutrient deficiencies (Fe), immunization coverage, micronutrient intake (Vit A, Fe), morbidity, mortality.	DHS
Malawi	2004	0–4.99	8 520	Anthropometry, anemia, use of nutritional supplements (Vit A), use of iodized salt, micronutrient intake (Vit A, Fe), complementary feeding practices, immunization coverage morbidity, mortality.	DHS
Mali	2006	0–4.99	11 877	Anthropometry, use of nutritional supplements, anemia, use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Mauritania	2000–01	0–4.99	3 554	Anthropometry, use and nutritional supplements, use of iodized salt, complementary feeding practices, morbidity.	DHS
Mozambique	2003	0–4.99	8 697	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe, I, Vit A), use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Niger	2006	0–4.99	4 185	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe, I, Vit A), use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Nigeria	2008	0–4.99	19 896	Anthropometry, use of nutritional supplements (Vit A, Fe), anemia, use of iodized salt, complementary feeding practices, micronutrient intake (Vit A, Fe), immunization coverage, morbidity, mortality.	DHS
Rwanda	2005	0–4.99	3 859	Anthropometry, anemia, use of nutritional supplements (Vit A), use of iodized salt, micronutrient intake (Vit A, Fe), complementary feeding practices, immunization coverage, mortality.	DHS
São Tomé and Príncipe	2008–09	0–4.99	1 544	Anthropometry, use of nutritional supplements, micronutrient deficiencies (Fe), use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Senegal	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF
Sierra Leone	2008	0–4.99	2 764	Anthropometry, use of nutritional supplements (Vit A, Fe), anemia, use of iodized salt, complementary feeding practices, micronutrient intake (Vit A, Fe), immunization coverage, morbidity, mortality.	DHS
Somalia	2000/07	0–4.99		Feeding practices, use of nutritional supplements (Vit A), use of iodized salt.	UNICEF

(Continued)

Table 1 Continued

Region/country	Survey year ^b	Age (years)	Sample size ^c	Indicators of nutritional status in children under five included in surveys ^d	Source ^e
Sudan	2000/07	0–4.99		Prevalence LBW, feeding practices, use of nutritional supplements (Vit A), Use of iodized salt.	UNICEF
Swaziland	2006–07	0–4.99	2 940	Anthropometry, Use of nutritional supplements (Vit A, Fe), Anemia, use of iodized salt, complementary feeding practices, micronutrient intake (Vit A, Fe), immunization coverage, morbidity, mortality.	DHS
Tanzania	2004	0–4.99	7 989	Anthropometry, use of nutritional supplements (Vit A, Fe), anemia, use of iodized salt, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Togo	1998	0–2.99	3 260	Anthropometry, immunization coverage, morbidity, complementary feeding practices.	DHS
Uganda	2006	0–4.99	2 687	Anthropometry, use of nutritional supplements (Vit A, Fe), anemia, use of iodized salt, micronutrient intake, complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Zambia	2007	0–4.99	5 602	Anthropometry, use of nutritional supplements (Vit A), anemia (hemoglobin), micronutrient intake (Vit A, Fe), complementary feeding practices, immunization coverage, morbidity, mortality.	DHS
Zimbabwe	2005–06	0–4.99	4 860	Anthropometry, use of nutritional supplements (Vit A), anemia, micronutrient intake (Vit A, Fe), complementary feeding practices, immunization coverage, morbidity, mortality.	DHS

^aAll samples include both sexes. Income according to World Bank classification: Low income (per capita Gross National Income (GNI) < US\$995); Lower middle-income GNI US\$996–3945.

^bWhen two surveys available since 2000, both survey years are listed, e.g., 2000/07 denotes survey in 2000 and in 2007. Indicators listed are for most recent survey. N/A = Not available.

^cTotal sample size reported. Actual sample sizes differ by variable.

^dFe = Iron, deficiency may denote Anemia (below hemoglobin cut-off) or low serum ferritin; I = Iodine, deficiency defined as low urinary iodine excretion; Vit A = Vitamin A, deficiency based on low serum retinol; Prevalence LBW = Percentage of infants born with birth weight < 2500 g; Micronutrient intake = Percentage of youngest children living with their mother who consumed fruits and vegetables rich in vitamin A and fruits and vegetables rich in iron in the 24 hours preceding the survey.

^eDHS = Demographic and Health Surveys; FAO = Food and Agriculture Organization of the United Nations; WHO = World Health Organization; NS = nationally representative health or nutrition survey not included in FAO or WHO database; PAHO = Pan American Health Organization; UNICEF = The United Nations Children's Fund.

Table 2 Surveys in low and lower middle-income countries with household consumption and individual-level data on indicators of nutritional status in adults^a

Region/country	Survey year ^b	Age (years)	Sample size ^c	Information available at household or individual-level (adults) ^d	Source ^e
<i>East Asia and Pacific</i>					
Cambodia	2005	15–49	23 554	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
China	2006	> 18	19 000	Anthropometry, dietary intake, dietary energy intake (kcal person ⁻¹ day ⁻¹).	NS, FAO
Indonesia	2007	15–54	41 653	Use of nutritional supplements (Vit A), food consumption, nutrient intake.	DHS, FAO
Kiribati	2006	25–64	1 351	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Korea, Democratic Republic	2007	19–100	243	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Lao People's Democratic Republic	2001	> 15	5 942	Anthropometry, dietary intake, use of nutritional supplements, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Marshall Islands	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Micronesia, Federal States	2002	25–64	1 474	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Mongolia	2005	15–65	3 404	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Myanmar	N/A			Food consumption nutrients.	FAO
Papua New Guinea	2005	15–59	11 128	Anthropometry, anemia, use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Philippines*	2008	20–39	3 123	Use of nutritional supplements (Vit A, Fe), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Samoa	2009	15–54	3 964	Use of nutritional supplements (Vit A, Fe), consumption of fruits and vegetables, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Solomon Islands	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Thailand	2004	15–100	38 984	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Timor-Leste	2009	15–49	17 213	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Tuvalu	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Tonga	2000	15–70	608	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Vanuatu	2000	20–60	800	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Vietnam	1997	15–49	4 212	Anthropometry, vitamin A deficiency, dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO, NS
<i>Europe and Central Asia</i>					
Armenia	2005	15–49	8 013	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Georgia	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Kosovo	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Kyrgyz Republic*	1997	15–49	3 848	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Moldova	2005	15–59	9 948	Anthropometry, anemia, use of nutritional supplements (Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Tajikistan*	2003	25–49	1 295	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Turkmenistan*	2000	15–49	7 919	Anthropometry, use of nutritional supplements (Vit A, Fe), use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Ukraine*	2002	15–49	707	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Uzbekistan	2002	15–59	7 796	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO

(Continued)

Table 2 Continued

Region/country	Survey year ^b	Age (years)	Sample size ^c	Information available at household or individual-level (adults) ^d	Source ^e
<i>Latin America and the Caribbean</i>					
Belize	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Bolivia	2008	15–64	22 993	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Ecuador	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
El Salvador*	2003	15–49	11 723	Micronutrient deficiencies (Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	NS, FAO
Guatemala	2008–09	15–49	24 706	Anthropometry, micronutrient deficiencies (Fe, I) dietary energy intake (kcal person ⁻¹ day ⁻¹).	NS, FAO
Guyana	2008–09	15–49	8 518	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Haiti	2005–06	15–59	15 715	Anthropometry, anemia, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹), micronutrient intake (Fe, Vit A).	DHS, FAO
Honduras*	2005–06	15–49	17 855	Anthropometry, dietary intake, anemia, use of nutritional supplements (Vit A, Fe), micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Nicaragua*	2005–06	15–49	5 485	Anthropometry, use of nutritional supplements (Fe, folic acid), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, NS, FAO
Paraguay	1992	20–74	1 606	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
<i>Middle East and North Africa</i>					
Djibouti	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Egypt, Arab Republic*	2008	15–49	16 527	Anthropometry, use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), foods and liquids consumed, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Iraq	2006	25–65	4 483	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Jordan*	2007	15–49	10 876	Anthropometry, anemia (hemoglobin), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Morocco*	2003–04	15–49	16 798	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Syrian Arab Republic	2004	18–65	1 936	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Tunisia	1988	15–49	4 184	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
West Bank and Gaza	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Yemen, Republic*	1997	15–49	5 479	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
<i>South Asia</i>					
Afghanistan*	2002	15–49	555	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Bangladesh	2007	15–54	3 921	Anthropometry, use of nutritional supplements (Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Bhutan	2007	25–74	2 431	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Maldives	2009	15–64	8 858	Anthropometry, micronutrient intake (Fe, Vit A), use of nutritional supplements (Fe), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Nepal	2006	15–59	15 190	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Pakistan	1994	15–100	8 568	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	WHO, FAO
Sri Lanka*	2006–07	15–49	14 692	Anthropometry, anemia, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO

Sub-Saharan Africa

Angola	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Benin	2006	15–64	23 115	Anthropometry, anemia, use of iodized salt, micronutrient intake, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Burkina Faso	2003	15–59	16 082	Anthropometry, anemia, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Burundi	2010	15–59	13 359	Anthropometry, anemia (hemoglobin), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Cameroon	2004	15–59	15 936	Anthropometry, anemia (hemoglobin), use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Cape Verde	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Central African Republic	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Chad	2004–05	15–59	7 972	Anthropometry, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Comoros	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Congo, Democratic Republic	2005	15–59	10 197	Anthropometry, anemia, use of iodized salt, use of nutritional supplements (Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Côte d'Ivoire	2005	15–49	9 686	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Eritrea*	2002	15–49	8 754	Anthropometry, use of iodized salt, food consumption.	DHS, FAO
Ethiopia	2005	15–59	20 103	Anthropometry, anemia, use of iodized salt, use of nutritional supplements (Vit A, Fe), micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Gambia, The	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Ghana	2008	15–59	9 484	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Guinea	2005	15–59	11 128	Anthropometry, anemia, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Kenya	2008–09	15–54	11 909	Anthropometry, use of iodized salt, use of nutritional supplements (Vit A, Fe), food consumption.	DHS, FAO
Lesotho	2009–10	15–59	10 941	Anthropometry, anemia, use of iodized salt, use of nutritional supplements (Vit A), Dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Liberia	2007	15–49	13 101	Anthropometry, use of iodized salt, use of nutritional supplements (Vit A), Micronutrient intake (Fe, Vit A), Dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Madagascar	2008	15–59	25 961	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Malawi	2010	15–54	31 000	Anthropometry, anemia, use of nutritional supplements (Vit A), micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Mali	2006	15–59	18 790	Anthropometry, anemia, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Mauritania	2000–01	15–59	9 919	Anthropometry, use of iodized salt, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Mozambique	2003	15–59	15 318	Anthropometry, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Niger	2006	15–59	12 772	Anthropometry, micronutrient deficiencies (Fe), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Nigeria	2008	15–59	48 871	Anthropometry, use of nutritional supplements (Vit A), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Rwanda	2005	15–59	16 141	Anthropometry, anemia, use of nutritional supplements (Vit A), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
São Tomé and Príncipe	2008–09	15–59	4 911	Anthropometry, micronutrient deficiencies (Fe), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Senegal	2005	15–59	18 363	Anthropometry, anemia, dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Sierra Leone	2008	15–59	10 654	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO

(Continued)

Table 2 Continued

Region/country	Survey year ^b	Age (years)	Sample size ^c	Information available at household or individual-level (adults) ^d	Source ^e
Somalia	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Sudan	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Swaziland	2006–07	15–49	9 143	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Tanzania	2009–10	15–49	13 000	Anthropometry, anemia, use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Togo	N/A			Dietary energy intake (kcal person ⁻¹ day ⁻¹).	FAO
Uganda	2006	15–54	11 034	Anthropometry, anemia, deficiency of vitamin A (serum retinol), use of nutritional supplements (Vit A, Fe), use of iodized salt, micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Zambia	2007	15–59	13 646	Anthropometry use of nutritional supplements (Vit A), micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO
Zimbabwe	2005–06	15–54	16 082	Anthropometry, anemia, use of nutritional supplements (Vit A), micronutrient intake (Fe, Vit A), dietary energy intake (kcal person ⁻¹ day ⁻¹).	DHS, FAO

^aAll samples include both sexes with the exception of countries with (*) in which individual-level data were found for women only. Income according to World Bank classification: Low income (Gross National Income (GNI) < US\$995); Lower middle-income GNI US\$996–3945.

^bMost recent survey year. N/A = Survey year not available.

^cTotal sample size reported. Actual sample sizes differ by variable.

^dFe = Iron, deficiency may denote Anemia (below hemoglobin cut-off) or low serum ferritin; I = Iodine, deficiency defined as low urinary iodine excretion; Vit A = Vitamin A, deficiency based on low serum retinol.

^eDHS = Demographic and Health Surveys; FAO = Food and Agriculture Organization of the United Nations; NS = nationally representative health or nutrition survey not included in FAO database. WHO = World Health Organization.

and the World Health Organization (WHO). A number of countries conduct periodic nationally representative health and nutritional surveys, and information may also be available from smaller scale health and nutritional studies and from routine growth monitoring and promotion programs. The following sections provide a brief description of each of these types of information and their application in the developing country context. We have included all countries classified as low and lower middle-income. For this classification, we use the categories developed by the World Bank, which establishes cut-off points based on per capita Gross National Income (GNI). Information from 2009 was used for inclusion in the table including countries classified as low income (per capita GNI <US\$995) and lower middle-income (per capita GNI US\$996–3945).

Multination Health and Nutritional Surveys

Over the past few decades, the Demographic and Health Surveys (DHS) have been conducted in many countries in all regions of the world. The DHS are nationally representative surveys that include household and individual health and nutritional indicators. The surveys are large, typically 5000 to 30 000 households and are conducted periodically, often at five-year intervals. The data included in the survey vary by country (Tables 1 and 2) but typically include as a minimum anthropometric measurements and hemoglobin concentration (prevalence of anemia) of children and women of reproductive age, and breast and complementary feeding practices. One of the major strengths of the DHS is that they use standardized questionnaires, which allow for comparisons across survey years and countries. Information from DHS is readily available on the Internet.

The WHO Global Database on Child Growth and Malnutrition provides a compilation of information from nationally representative and smaller-scale surveys conducted in a number of countries. To be included in the database, a number of criteria must be met for data collection, analysis and presentation. This facilitates the comparison of information that has been collected in different countries and regions. Nutrition Country Profiles are also compiled by FAO and include national-, household-, and individual-level data. The national data are obtained from the United Nations global data banks and is supplemented for many countries by data from local institutions and independent experts. Considering this broad range of sources, many differences in methodology of data collection, analysis and presentation may exist, and should be taken into consideration when comparing data from different countries.

The World Health Organization, through the Micronutrients Unit, Department of Nutrition for Health and Development also maintains the Vitamin and Mineral Nutrition Information System (VMNIS). The objectives of VMNIS are to systematically retrieve and summarize information on the vitamin and mineral status of populations, facilitate access to data and summary variables at the global, regional, and national level and track progress toward elimination of vitamin and mineral deficiencies. As of 2010, the VMNIS includes a section with tools and resources to help countries assess and monitor vitamin and mineral status of their populations. Data on the following biochemical indicators are included in the

VMNIS Micronutrients Database: hemoglobin, serum ferritin, serum transferrin receptor protein, serum/plasma retinol, serum/plasma retinol binding protein, urinary iodine excretion, serum/plasma zinc, serum/plasma folate, red blood cell folate. The VMNIS Micronutrients Database also includes clinical indicators of vitamin A and iodine deficiencies (i.e., night blindness, goiter) and reports prevalence of anemia, vitamin A, and iodine deficiency. Obviously, whether these indicators are reported for each country depends on the information available from surveys. VMNIS collects data from multinational surveys such as DHS, but great effort is also made to compile and obtain information from national health and nutritional surveys. The inclusion of these data in global surveillance is extremely useful as generally access to such information outside of the country where it is generated is limited. Thus, the VMNIS performs a unique and useful function in compiling and making accessible data from all regions around the globe. The updated VMNIS (to be completed in 2011) should strengthen surveillance of micronutrient status globally and facilitate its utilization for policy-making.

National Health and Nutritional Surveys and Small-Scale Surveys

Nationally representative health and nutritional surveys have the major advantage that data may be representative of the population in the country, at national level and by regions. Small-scale surveys do not usually provide nationally representative data, but have the strength that the survey may be targeted to specific high-risk groups, thus providing data for those to whom policy makers may need to target interventions.

Information on nutritional status of individuals, particularly children, may also be collected at the local community level through national growth monitoring and promotion activities as part of government or nongovernmental agency programs. Many such activities stress a high level of local involvement in data collection and can be very useful to provide feedback for decisions on resource allocation that need to be made at a local level. Data can then be aggregated to higher administrative levels and can be used for regional and national resource allocation. Although this type of surveillance may not have the same level of data quality control as the larger, more heavily supervised surveys, they have the advantage of being readily available and may promote a higher level of community involvement.

See also: Biochemical Indices. Dietary Surveys: Surveys of Food Intake in Groups and Individuals. Food Security. Growth Monitoring. Hunger. Nutritional Assessment: Anthropometry. Nutritional Surveillance: Developed Countries

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NUTRITION AND HIV/AIDS

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Glossary

AIDS Acquired immunodeficiency syndrome – a severe disease of the human immune system caused by the human immunodeficiency virus (HIV).

Antiretroviral drugs (ARVs) Drugs used to treat HIV.

Antiretroviral therapy (ART) Treatment with combinations of several drugs against HIV.

Body mass index (BMI) Calculated as weight in kg per height in meters squared; often used as an indicator of overall nutritional status.

CD4 cells A subgroup of lymphocytes which are targets of HIV infection. Destruction of CD4 cells is both a cause and a marker of HIV/AIDS disease progression; therefore

blood CD4 count is often used to measure the degree of immunodeficiency.

HAART Highly active antiretroviral therapy; treatment for HIV/AIDS combining at least three antiretroviral drugs.

HIV Human immunodeficiency virus – a family of closely related viruses which infect and destroy the human immune system.

Lipodystrophy Altered proportion and distribution of body fat, often as a consequence of antiretroviral therapy and associated with blood lipid abnormalities.

NFκB A gene transcription factor with a central role in inflammatory responses.

Introduction

HIV infection and nutrition are intimately linked at many levels: biological, clinical, social, and economic. Nutritional problems have been a defining characteristic of human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) since the early years of the epidemic. In Africa, AIDS was first recognized as 'slim disease' because sufferers experienced severe weight loss. HIV infection can lead to malnutrition, although poor diet can in turn speed disease progression. In contrast, in high income countries, HIV/AIDS and antiretroviral therapy (ART) for the disease have been associated with abnormal fat metabolism and distribution

and with chronic diseases associated with overnutrition. Thus HIV/AIDS is a challenge to nutritionists working at both the individual patient level and the public health level globally (**Figure 1**).

HIV/AIDS

AIDS is a disease of the human immune system caused by HIV. HIV is transmitted through direct contact of a mucous membrane or the bloodstream with a body fluid, such as blood, genital secretions, or breast milk of someone who is infected with the virus. The transmission most commonly occurs

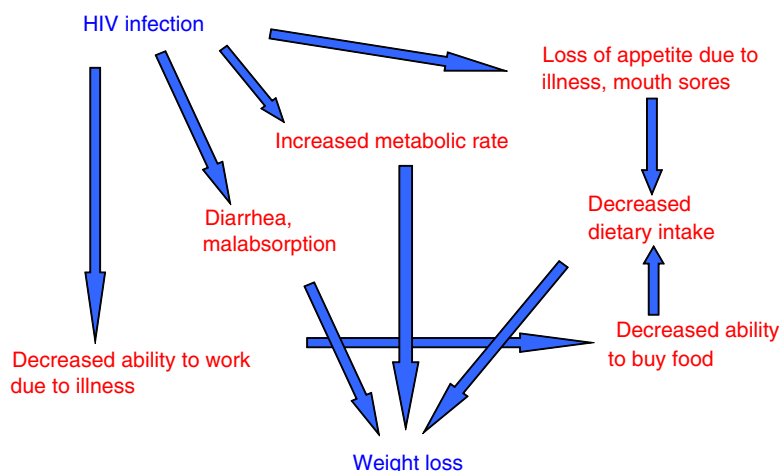


Figure 1 Clinical and socioeconomic pathways whereby HIV/AIDS can cause weight loss.

during sexual contacts and from mothers to infants during pregnancy, at delivery or through breastfeeding. HIV can also be transmitted through transfusion of blood products or injection with contaminated needles.

HIV infects and destroys cells of the immune system, resulting in immunodeficiency. Key cells involved in this process are the CD4 subclass of lymphocytes. The number of CD4 cells in the blood is often used as an indicator of the degree of HIV-induced immunodeficiency, that is, disease progression. A low number of CD4 cells is associated with an increased risk of opportunistic infections which are infections which do not normally cause disease in otherwise healthy people but which do in people who are immunosuppressed. Common opportunistic infections include *Pneumocystis jirovecii* pneumonia, disseminated cryptococcosis, and cerebral toxoplasmosis. In addition, certain infections such as tuberculosis can occur more frequently and cause more serious disease among HIV-infected people than among HIV-uninfected people.

HIV-immunosuppressed individuals are also at higher risk of developing cancers such as Kaposi sarcoma, invasive cervical carcinoma, Burkitt, immunoblastic, or primary central nervous system lymphomas.

There is currently no cure for HIV infection or AIDS. Vaccines have been investigated but are not publicly available at the moment. Current treatment for HIV infection is based on highly active antiretroviral therapy (HAART). This consists in a combination of at least three drugs belonging to at least two types or 'classes' of antiretroviral agents (ARVs). HAART has significantly improved prognosis and quality of life for people living with HIV. However, access to treatment remains a challenge throughout the world, especially, but not only, in resources-poor countries.

Effects of HIV/AIDS on Nutritional Status

Calories and Macronutrients

HIV, like many infections, raises metabolic rate. Among asymptomatic patients at an early stage of HIV infection, resting metabolic rate is increased by about 10%. Acutely ill patients may have higher resting metabolic rates but this is offset by their lower level of activity so that total energy expenditure may not be increased. They may also be anorexic so that acute illness is not the best time to try to improve nutritional status. There is evidence that weight loss during HIV infection is more a result of decreased food intake owing to anorexia than it is to raised metabolic rate owing to infection. When patients receive therapy for HIV or opportunistic infections and begin to feel better, their energy needs to support regain of weight and increased activity can become very high, up to 50% greater than normal healthy requirements. Provision of adequate food to cover the extra energy needs can be difficult for many in low income countries.

Although energy needs change during HIV infection, there is no evidence that protein needs, as a percent of food energy, are changed. Therefore, it is usually best to meet energy needs through increased intake of a balanced diet which will then provide adequate protein.

Tissue lost during illness and that regained during recovery may differ in proportions of fat and lean. There is a tendency for increased central fat deposition and a loss of lean tissue as a result of this weight cycling. This can have important implications for health since loss of lean tissue is associated with decreased ability to work or manage other aspects of daily living as well as with increased risk of mortality. On the other hand, given the role of central fat in regulation of the immune system and in increasing systemic inflammation, it is possible that increased central fat has short-term health benefits for HIV-infected people at high risk of infectious disease, even though it may increase their long-term risk of chronic diseases. This is an area requiring considerable further research.

Micronutrients

Anorexia and increased wastage of some micronutrients during illness, for example, fecal zinc losses during diarrhea, may result in deficient micronutrient status. However, infections change plasma levels of many markers of micronutrient status as part of the normal acute phase response and this does not necessarily imply changed micronutrient status itself. Therefore, it is not always easy to determine the effects of HIV/AIDS on micronutrient status. The best indication of inadequate micronutrient status has come from intervention trials which in some cases have shown benefits of improving micronutrient status in HIV/AIDS (see Micronutrient Intervention Studies).

Antioxidant micronutrients may be especially important because infection is an oxidative stress: the immune response to combat infection involves generation of activated oxygen metabolites and increased energy expenditure. High cellular oxidant levels can lead to increased inflammation through regulation of the transcription factor, NFκB. Although inflammation is an essential part of the response to infection, both inflammation and activated oxygen metabolites can cause tissue damage. Therefore inflammation and production of oxygen metabolites must be controlled and limited to the site of the immune response. Adequate status of antioxidant micronutrients – vitamin E, vitamin C, and selenium – is needed for this.

Gastrointestinal Function

Nutrition and gastrointestinal function are obviously intimately linked. HIV infection can disrupt these links. HIV and opportunistic infections can lead to diarrhea, malabsorption, and nutrient wastage with consequent macro- and micro-nutrient deficiencies. Nutritional deficiencies can further impair immune functions and the ability to repair damaged tissues.

Nutritional Intervention Studies

Macronutrient Intervention Studies

Many governments, nongovernmental organizations, religious, and other community groups have responded to the

crisis of malnutrition in HIV/AIDS patients by various types of food and nutrition interventions. Many projects are small scale and few have been formally evaluated. Where evaluations have been conducted, the effectiveness of the program for improving nutritional status, health, and survival has generally been disappointing.

A Cochrane systematic review of macronutrient interventions for people with HIV/AIDS found evidence that interventions increased protein and energy intake but no evidence that this had any benefits for weight, body composition, or health. However, trials were few, fairly small, and conducted mainly in high income countries where under-nutrition is fairly uncommon.

Since that review there have been a few macronutrient intervention studies among malnourished or food insecure HIV-infected people in low income countries. Provision of macronutrient supplements, as extra food rations or specific composite foods such as fortified blends or lipid-based supplements, can in some cases and in the short term increase body mass index (BMI) or improve compliance with ART. However, there has been little or no documented benefit for health or survival. Reasons for this are currently unclear. It may be that macronutrient deficiencies, even among malnourished HIV-infected people, are not limiting factors for health. It may be that diarrhea and anorexia limit intake and thus effectiveness of the foods provided. It is also possible that the foods provided are not consumed in sufficient amounts, because of sharing or selling, to have detectable benefits for health.

One area where macronutrient intervention trials have been conducted and programmes evaluated is management of severe malnutrition among HIV-infected young children. In HIV-endemic countries, a high proportion of children referred to malnutrition wards are HIV-infected. A recent meta-analysis has shown that mortality rates are higher for HIV-infected than uninfected malnourished children and are above target levels even in facilities with high quality care as evidenced by low mortality rates among HIV-uninfected children. For those HIV-infected children who do survive, it seems that weight gains are similar to gains of uninfected children. It is important to test HIV status of children admitted for severe malnutrition in high HIV prevalence areas in order to permit simultaneous management of HIV infection with ARVs and opportunistic infections (prophylaxis and treatment), and, hopefully, improve survival. It is notable that nutritional care of HIV-infected malnourished children has usually been initiated on malnutrition wards, not in clinics managing HIV. Fortunately some clinics managing HIV services for prevention of mother-to-child HIV transmission are beginning to incorporate into their services interventions to prevent severe malnutrition rather than to worry later about treating it.

Micronutrient Intervention Studies

Micronutrient interventions appear to have somewhat greater impact on health of HIV-infected people than do macronutrient interventions. This may be because, compared with macronutrients, micronutrient deficiencies are more important limiting factors for health of people with HIV or simply

because micronutrient supplements are easier to deliver in the face of anorexia and are less likely to be shared or sold. There is evidence that vitamin A supplements have similar benefits for reducing mortality and diarrhea among HIV-infected children as among other children. Multiple micronutrient supplements have decreased mortality of HIV-infected adults. Multiple micronutrients given to HIV-infected pregnant Tanzanian women improved many aspects of maternal and child health. However, these studies were conducted before the widespread availability of ARVs in the communities and it is unclear whether micronutrient interventions would have added benefits when given with ARVs. Furthermore, there is very limited information regarding the optimal micronutrient supplements for efficacy and safety among different patient groups.

The micronutrient intervention data is from research trials and, although micronutrient supplements are often taken by people with HIV/AIDS in high income countries and micronutrient interventions have been implemented in some parts of Africa, there is little programmatic evidence for the effectiveness of these interventions, mainly because of lack of evaluations. It seems to be generally assumed that providing nutrients to people with nutritional deficiencies must be beneficial but, in fact, the experience with macronutrient interventions for people with HIV/AIDS indicates that this is not necessarily true. Demonstration of program effectiveness is essential for program sustainability.

Interactions Between Antiretroviral Drugs and Nutrition

ARVs to decrease viral load and improve health, although they do not cure the infection, have been available in high income countries for many years and in Africa during the past decade. Treatments are constantly improving but continue to have side effects, such as nausea or taste abnormalities, which can affect dietary intake. Many ARVs need to be taken with food and, as mentioned above (*see Effects of HIV/AIDS on Nutritional Status*), treating the infection can lead to regain of weight which requires additional food. Therefore, increasing availability of ART may actually be increasing rather than decreasing the need for nutritional support of people with HIV/AIDS (**Figure 2**).

There is evidence that provision of ART can improve growth in children. It may not be possible to completely restore adequate growth in HIV-infected children since stunting, that is, poor length growth, is generally irreversible after age about 2 years. There is some danger that additional weight will then be deposited as fat. Children and their carers should be provided information about healthy eating and exercise, similar to that provided for HIV-uninfected children but possibly even more important.

One side effect of some ARVs is altered proportion and distribution of body fat referred to as lipodystrophy. Partly not only in association with cycles of weight loss and gain (*see Effects of HIV/AIDS on Nutritional Status*) but also as a result of some ARVs themselves, there is a tendency toward decreased peripheral fat and increased central fat. This has cosmetic and social implications which in the past may have

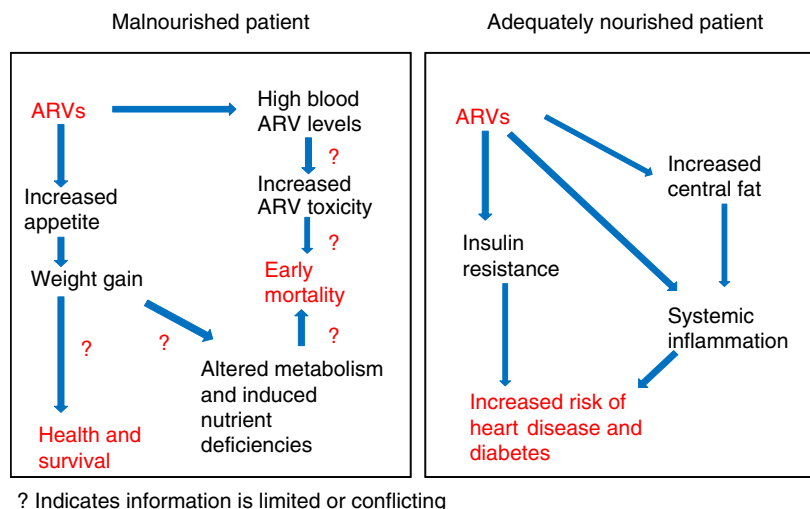


Figure 2 Interactions between antiretroviral drugs (ARVs) and nutrition.

decreased compliance with ART. There are more important metabolic effects as well such as increased plasma cholesterol and triglycerides and increased insulin resistance. As for HIV-uninfected people, these abnormalities are associated with increased risk of chronic diseases such as cardiovascular disease and diabetes. Newer drugs do not have such strong lipodystrophic effects so changing regimen may ameliorate the problems. Other interventions which could potentially promote deposition of lean rather than fat tissue are provision of androgenic drugs, supplementation with micronutrients, or exercise. Although historically it may have been odd to worry about excess fat deposition in Africa, even poor African countries are now undergoing the nutrition transition as evidenced by rising prevalence of overweight and obesity. It will require sensitive and intelligent nutritional counseling to promote good nutrition and exercise for preventing overweight and chronic diseases among HIV-infected Africans who have been more usually worried about weight loss and malnutrition.

There is some evidence that ARVs are metabolized differently, that is, more slowly, among malnourished people and this may lead to increased drug toxicity. Differences in metabolism and toxicity, although supported by only limited evidence, are not surprising given that drug metabolism and excretion involve enzymes which often have micronutrients as cofactors and may use oxidative metabolism. In Africa with the ART roll-out, it has now become apparent that, unlike in high income countries, starting ART is associated with a high mortality in the first few months of taking the drugs. Mechanisms for the high early mortality are still under review but one risk factor which consistently remains significant even in multivariable analyses controlling for CD4 count and co-infections such as tuberculosis, is malnutrition, as represented by low BMI. Low BMI, in addition to being associated with loss of lean tissue, may be a marker for metabolic derangements such as low plasma phosphate. There is concern that when ARVs are first provided and recovery begins, increased metabolic rate could further lower the phosphate problem causing a refeeding syndrome with increased risk of death.

Infant Feeding and HIV/AIDS

One topic in the area of nutrition and HIV which has attracted huge amounts of research, commentary, and concern is HIV and infant feeding. Early in the epidemic it became apparent that HIV can be found in breast milk and that breastfed infants can acquire the infection from their mothers (about 15% of infants of HIV-infected mothers). Mother-to-child transmission can also occur *in utero* (to about 7% of infants of infected mothers) and at delivery (to about 15% of infants of infected mothers) (Figure 3). With the advent in Africa around 2000 of single dose nevirapine, an ARV, to mothers and infants at delivery, the proportion of infants infected at delivery decreased so that breastfeeding became the major mode of mother-to-child HIV transmission.

In high income countries HIV-infected mothers have access to ART during pregnancy and at delivery and are advised not to breastfeed. Therefore few infants in these countries become HIV-infected. However, in low income countries, notably in Africa where HIV prevalence is highest, not breastfeeding is associated with a high risk of morbidity and mortality from other infectious diseases. Furthermore, safe and nutritious alternatives to breast milk are not available or affordable for many African women. HIV-infected African women and health services supporting them have been faced with very difficult decisions regarding the best infant feeding mode. Not only do women need to make a feeding decision in the absence of specific information about their own child's risk of infection, but also they need to consider the social implications of not breastfeeding in societies where breastfeeding is almost universal and not breastfeeding advertises a positive HIV status.

World health organization (WHO) has regularly updated its recommendations based on research on HIV and infant feeding. Whereas this was done for the best of reasons – to save children's lives – the rapidly changing recommendations have resulted in some confusion among women and health care workers. Current recommendations are to provide ART to women during late pregnancy and at delivery and to the

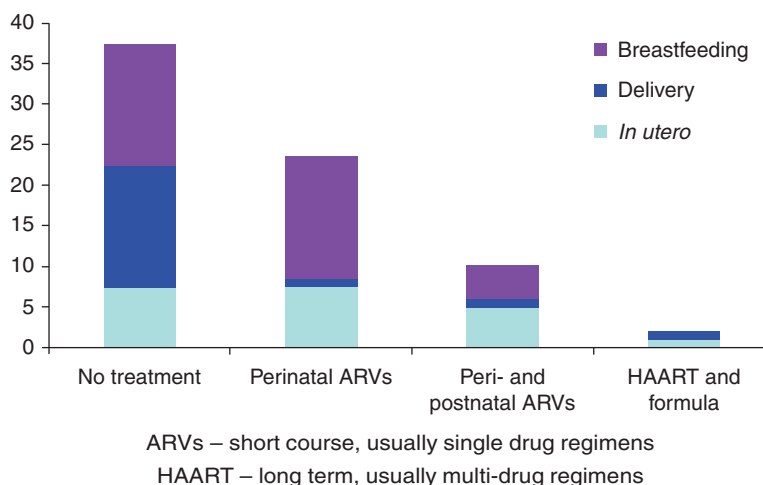


Figure 3 Estimated rate and timing of mother-to-child HIV transmission under different treatment conditions.

mother or infant throughout the period of breastfeeding. Although this has been shown to reduce mother-to-child transmission to less than 10% in research studies, this is an expensive recommendation to implement – not primarily in terms of drug costs but more because of costs of health workers' time needed – and so is not yet widely implemented in Africa.

It has recently become apparent that even those children of HIV-infected mothers who escape HIV infection themselves are at increased risk of nutritional and health problems compared to HIV-unexposed children. These HIV-exposed, uninfected children tend to be smaller than HIV-unexposed children and to suffer more episodes of serious morbidity and higher mortality. It is as yet unclear whether nutritional or other interventions can improve these children's growth and health.

Social and Economic Interactions with HIV/AIDS

In HIV-endemic Africa, HIV/AIDS is not only a disease of individuals but also of the whole society. HIV/AIDS strikes mainly young and middle-aged adults who are at their most economically productive ages. These adults are normally also the main carers of young children. Loss of caring capacity for children can cause them long term health and emotional problems. Additional financial losses occur because other family members are needed as carers, because of overt medical costs or less obvious costs such as for transport to clinic visits, or because of funeral expenses. Therefore HIV infection of one member of a household can affect the entire household and can be devastating to families, many of whom are near the poverty line already in Africa.

The best approaches for improving nutrition of HIV-infected individuals and HIV-affected families are not clear. Should individuals be targeted on the basis of low BMI or on the basis of food insecurity? Which types of food support and which type of targeting are most cost-effective for outcomes in the patient? Should we also be considering family outcomes such as family members in employment and children in schools? What are the ethics of providing food support to HIV-

affected food insecure families in areas where large proportions of the whole population are food-insecure? There is an urgent need for research addressing these questions.

Conclusions

Since early in the HIV/AIDS epidemic it has been clear that nutrition plays an important role. Paradoxically, nutrition may be of even greater importance with the increased availability of ART. The problems are complex and multifactorial and there remain many questions as to how best these can be solved, particularly in Africa where both the HIV epidemic and nutritional problems are most widespread and acute. Evaluation of nutrition intervention programmes in HIV care is essential since nutrition interventions may be expensive but can be justified if they improve patient and family outcomes.

See also: Dietary Modulation of Inflammation. Infection: Nutritional Management in Adults. Nutrition and Susceptibility to Tuberculosis. Supplementation: Developing Countries. Tuberculosis: Nutritional Management

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NUTRITION AND SUSCEPTIBILITY TO TUBERCULOSIS

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Glossary

Bacillus of Calmette and Guérin vaccine A tuberculosis vaccine used in many parts of the world containing an attenuated strain of tubercle bacillus developed by repeated culture on medium containing bile.

Cell-mediated immunity The principle host defense against tuberculosis (TB) mediated primarily by T lymphocytes rather than antibodies.

Droplet nuclei Microscopic particles (1–5 mm in diameter) that can be expelled when a person coughs,

sneezes, shouts, or sings. The droplets produced by an infectious TB patient can carry tubercle bacilli and can remain suspended in the air for prolonged periods of time.

Hematogenous reseeding The process by which bacilli make their way back to the lung through the bloodstream where they infect all parts of the lung.

Undernutrition A type of malnutrition caused by the lack of food or failure of the body to absorb or assimilate nutrients properly.

Undernutrition is an important risk factor for the development of tuberculosis (TB) both at the individual level and at the population level. Undernutrition profoundly affects cell-mediated immunity (CMI), and CMI is the principal host defense against TB. Although these concepts may be widely accepted, the relative and attributable risks of TB due to undernutrition are not well known. Moreover, the effects of specific nutrients have not been established. Recent evidence suggests that overweight and obesity may actually decrease the risk of developing TB. This article will summarize available evidence on the relationship between nutritional status and the risk of TB.

Understanding the link between undernutrition and susceptibility to TB is based on a conceptual model for the transmission and pathogenesis of TB. *Mycobacterium tuberculosis* is transmitted by the aerosol route when an individual who has pulmonary or tracheobronchial TB produces droplets containing viable *M. tuberculosis*. The moisture content of smaller droplets evaporates quickly and such 'droplet nuclei' are the main vehicle for airborne transmission of TB. Of those who become infected, ~90% will remain free of TB, whereas ~10% will develop active TB disease at some time during their life. The risk of an infection progressing to active TB disease depends on the host's immune system and the microbe's virulence. The influence of nutritional status on host defenses is a central theme of this article.

Once the inhaled mycobacteria reach the alveoli, alveolar macrophages bind and internalize the bacilli, leading to the activation of these macrophages and to the production of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and other chemokines. These chemical messengers recruit macrophages and other immune response cells to the site of infection. As the inflammatory response progresses, the mycobacteria and infected macrophages are taken

in the draining lymph to the hilar and mediastinal lymph nodes, and then via the thoracic duct into the blood stream, disseminating to potentially every organ or tissue in the body. Bacilli make their way back to the lung through the bloodstream where they infect all parts of the lung in a process known as hematogenous reseeding, which apparently occurs in all infected individuals. Blood-borne organisms establish 'secondary' granulomas, in which the organisms may persist despite an active immune response, and which are often the sites of reactivation of TB are described below.

Mycobacterial antigens are presented to CD4+ (helper) and CD8+ (cytotoxic) T lymphocytes in the lymph nodes initiating the CMI. The population of TB-specific T lymphocytes expands and circulates to sites of active infection where they produce macrophage-activating cytokines such as interferon- γ (IFN- γ). The combination of IFN- γ and TNF- α from phagocytes further activates macrophages to inhibit mycobacterial growth. During this period, the host begins to express evidence of a TB-specific T-lymphocyte-mediated immune response as manifested classically by a positive tuberculin (PPD) skin test. The process by which macrophages inhibit or destroy mycobacteria may also cause local tissue destruction and necrosis. Immunological feedback mechanisms modulate the intense inflammatory response to limit tissue damage while controlling the infection. Nevertheless, local tissue destruction in the lungs can lead to the formation of cavities, characteristic of contagious, and reactivation TB.

In the large majority of people, the adaptive immune response controls the infection without overt symptoms or other clinical manifestations of TB disease. In a small minority of individuals, estimated to be <5%, the immune response will not control the infection, and the infection progresses to active TB disease known as 'progressive primary TB'. However, the risk of progression is much greater in persons with immune

systems weakened by undernutrition, human immunodeficiency virus infection, immunosuppressive medications, and extremes of age. In persons whose immune systems successfully control the primary infection, mycobacteria may remain viable within granulomas for years or decades. Among individuals with a PPD skin test and no history of active TB disease, the risk of developing reactivation TB disease over the individual's lifetime is also estimated to be ~5%, but increases greatly in persons with weakened immune systems.

Although there is little evidence of increased risk of primary infection, the risk of progression from infection to disease increases substantially in undernourished individuals. This is likely due to the adverse effects of undernutrition on CMI, which is essential for control of mycobacterial growth. Protein undernutrition clearly compromises CMI. Experimental animal studies have demonstrated that even modest protein deprivation impairs host responses to *Bacillus of Calmette and Guérin* (BCG) vaccination and tuberculin skin test responsiveness after aerosol exposure to *M. tuberculosis*. Protein repletion rapidly restores these responses. Protein from the diet and from somatic stores is crucial for many aspects of host defense against TB as described above. Essential amino acids play important physiological roles apart from serving as building blocks for protein synthesis. Other macronutrients may also influence the immune system, especially fatty acids, including n-6 and n-3 polyunsaturated fatty acids (PUFA). n-3 PUFA have a direct influence on immune cell membrane composition and function, as well as on the production of eicosanoids and other inflammatory mediators. High intakes of long chain n-3 PUFA dampen inflammatory responses and ameliorate chronic inflammatory conditions such as rheumatoid arthritis, potentially at the cost of increasing susceptibility to infection including TB (see below). The balance of n-3 and n-6 PUFA may be a key determinant of their effects on the immune system and disease resistance.

Recent findings suggest that chronic excess caloric intake may actually decrease the risk of TB. A 2009 study from China demonstrated that persons who were overweight and obese had ~1/4th to ~1/5th the risk of developing TB, respectively, compared with persons having normal body mass index (BMI). These findings are consistent with other studies, which are described below. The mechanisms by which excessive body mass decreases TB incidence are ripe for investigation. Recent research demonstrates that adipose tissue may be a reservoir for nonreplicating *M. tuberculosis*, and that such bacilli accumulate triacylglycerol in intracytoplasmic lipid bodies. A triacylglycerol synthase-encoding gene (*tgs1*) in *M. tuberculosis* is a member of the DosR regulon that may control the development of a nonreplicating 'persister' phenotype in the mycobacteria. Induction of the isocitrate lyase gene, *icl1*, in these mycobacteria allows a shift to utilization of lipids as a source of carbon and energy. Indeed, Roth speculated that obesity may have provided an evolutionary advantage during TB epidemics in the past, predisposing to obesity the modern day descendants of the survivors. Taken together, these recent data suggest the presence of a nonreplicating bacterial population, which persists especially in adipose tissue. Such mycobacteria may stop replicating or replicate more slowly, decreasing the probability of active TB disease.

Micronutrient deficiencies, for example, vitamin A, may affect resistance to TB by altering the function of the respiratory mucosa and the integrity of pulmonary epithelial tissues. Vitamins of the B complex and vitamin C play important roles in B-cell mediated humoral immune responses, but at present there is less evidence for their role in CMI. Vitamin B₁₂ may be an exception to this generalization; B₁₂ repletion in patients with pernicious anemia has been shown to reverse skin test anergy. The antioxidant functions of vitamin C, vitamin E, selenium, and glutathione have critical roles in protection against oxidative stresses, including reactive oxygen intermediates that play effector roles in cellular immunity as described below. Vitamin E may have other effects on both cellular and humoral limbs of the immune system as well, especially in relation to n-3 PUFA. At least one trial of vitamin E supplementation showed a benefit on clinically relevant measures of T-cell function. However, high doses of vitamin E may have adverse effects. Vitamin D may have a role in anti-TB immunity through activation of macrophages. Induction of a cell-surface receptor for the vitamin D metabolite, 25-hydroxycholecalciferol, as well as the hydroxylase, which forms 1,25-dihydroxycholecalciferol, appear to be part of the mechanism that helps these cells destroy the pathogen. These vitamin D effects are linked with production of the endogenous antibiotic peptide, cathelicidin. Reactive oxygen intermediates (e.g., hydroxyl radical, singlet oxygen, hydrogen peroxide, etc.) are also involved. Nitric oxide plays an important protective role in murine models of TB, but its role in human TB is not well established.

Dietary mineral deficiencies also affect CMI. Zinc deficiency interferes with T-cell replication and maturation leading to lymphopenia. Zinc deficiency is also associated with elevated glucocorticoid levels that suppress CMI. Iron plays a critical role in support of CMI, however, iron is also critical to the replication of mycobacteria and other pathogens. Mycobacteria have evolved intense iron-scavenging mechanisms, and iron deficiency may have worse consequences for the microbe than the host. Thus, the impact of iron on susceptibility to TB is difficult to predict.

Despite the seemingly clear pathways through which nutrition affects CMI and resistance to TB, the evidence in humans linking undernutrition to TB risk is indirect and surprisingly weak from the perspective of scientific rigor. The bulk of evidence in humans comes from a large body of uncontrolled observations during famines, wars, and natural disasters, as well as ecological studies comparing low income with affluent countries. In these complex circumstances, the effects of undernutrition cannot be disentangled from the effects of poor housing, overcrowding, lack of medical care, poor hygiene, social disruption, and poverty. Although much of this observational evidence is scientifically weak, it constitutes a large body of repeated observations supporting a strong relationship between undernutrition and TB. In addition, the decline in TB in the past century in developed countries often is ascribed to improvements in living conditions, especially nutrition, a concept championed in seminal work by Thomas McKeown. The remainder of this article summarizes the evidence from observations in human populations and from experimental animal models with relevance to human TB.

Early research on the interaction of nutrition and TB was exhaustively reviewed in the classic text by Scrimshaw, Taylor, and Gordon. The studies in humans were either ecological or uncontrolled observational studies. For example, studies of the sharp increases in TB morbidity and mortality in France and Germany during the two World Wars, or in the Warsaw ghetto during World War II, do not isolate the effects of undernutrition from the impact of extreme crowding, social and environmental degradation, lack of medical services, and catastrophic social circumstances. Although highly suggestive, the impact of starvation on TB morbidity and mortality independent of other circumstances cannot be isolated in these studies.

Three ecological studies present compelling evidence that undernutrition, isolated to some extent from other confounding circumstances, plays a direct role in TB morbidity and mortality. During World War I, neutral Denmark exported the bulk of its meat, fish, poultry, and dairy products to support the war effort elsewhere so the local diet lacked these protein-, vitamin-, and mineral-rich foods. During that period, TB rates increased similarly to those in the warring countries. However, in 1918 Germany blockaded Denmark making exports impossible, creating a local surplus of these foods. TB rates in Denmark plummeted whereas rates in the neighboring countries continued to increase unabated. The second study involves the Trondheim, Norway Naval Training School, where an extremely high rate of TB among recruits in the early twentieth century was ascribed to crowding, poor housing, and unhygienic conditions. TB rates did not decrease after improved housing and hygiene were provided. However, when the diet was fortified with milk, margarine, cod liver oil, whole wheat bread, and fresh fruits and vegetables, TB morbidity promptly declined to the prevailing level for young adults of that area. After World War II, Leyton reported on British and Russian prisoners of war (POW) held in the same German POW camps, sharing the same living conditions and diet, except the British received Red Cross food supplements amounting to 30 g protein and 1000 kcal day⁻¹. In a subsequent radiographic survey, the TB rate among the British POW was only 1.2% whereas the rate in Russian POW was 15%–19%. In the malnourished prisoners, TB was more severe, the onset was more rapid, and patients died rapidly with large pulmonary cavities and massive tissue breakdown. Granuloma formation was poor in the malnourished prisoners, supporting the idea there was a deficit of CMI in this group.

Protein-energy undernutrition compromises CMI and may exacerbate TB, however, TB itself can adversely affect nutritional status. Understanding the temporal relationship between the onset of undernutrition and the development of TB is crucial to correctly assess any possible cause–effect relationship. Cross-sectional and case–control studies generally suffer from the same flaw: Patients with and without TB disease are compared in terms of their concurrent nutritional status. Although these studies demonstrate substantial macro- and micronutrient deficits in TB patients, they are not useful in determining the effect of undernutrition on susceptibility to TB because TB causes undernutrition. The intrinsic uncertainty over the chronological sequence of cause and effect in case–control and cross-sectional studies becomes intractable.

After intestinal bypass surgery for morbid obesity, patients experience rapid weight loss and malabsorption due to their short-circuited bowels. In several case series, the postoperative incidence of TB was 10- to 100-fold higher than historical or population comparison groups. Similarly, partial gastrectomy for ulcer disease was shown to predispose men to TB, especially among men whose weight was <85% of ideal.

Two cross-sectional studies on vitamin D metabolism in relation to TB have focused on the molecular and cellular mechanisms of the interaction rather than on the direction of causality. Cells recovered by bronchoalveolar lavage (BAL) and peripheral blood mononuclear cells from TB patients both produced 1,25(OH)₂D₃, the amount correlating with the number of CD8+ T lymphocytes but not with other cell types. CD4+ T lymphocytes in BAL fluid from TB patients expressed specific receptors for 1,25(OH)₂ vitamin D₃ but not 25(OH)D₃. Because 1,25(OH)₂D₃ can improve the capacity of macrophages to kill mycobacteria, these results support the conclusion that cellular interactions mediated partly by 1,25(OH)₂D₃ may be important in resistance to TB.

The unique strength of cohort studies is that nutritional status is measured before the onset of TB. Only two cohort studies have examined the relationship between micronutrients and TB incidence. In the 1940s, Getz and coworkers enrolled a cohort of 1100 men who were free of TB at baseline, and followed them for up to 5 years with serial clinical, radiographic, and laboratory examinations. Plasma vitamin A levels were low in 13 of 16 men (81%) who developed TB compared to 318 of 1058 (30%) of those who did not. Similarly, plasma vitamin C levels were low in 100% of the subjects who developed active TB compared to 117 of 1013 (11%) of those who did not. Exposure to TB did not differ between the groups. In a Finnish study on cancer prevention, investigators randomized 26 975 healthy male smokers aged 50–69 years to supplementation with tocopherol, β-carotene, both, or neither. The subjects were followed for a mean of 6.7 years. In >173 000 person-years of follow-up, 167 cases of TB were detected. Higher intakes of fruits and vegetables were associated with lower risk of TB (the adjusted relative risk of TB was 0.4; 95% confidence interval, 0.24–0.69). This study is noteworthy for its size and data quality, however, detecting TB through hospital discharges selects TB patients who were sick enough to require hospital admission. Lower dietary intakes of key nutrients may have been associated with higher rates of hospitalization rather than (or in addition to) higher rates of TB.

As part of the long-term follow-up of participants in large-scale BCG vaccine trials in Georgia and Alabama, Comstock, and Palmer reported the incidence of TB was 2.2 times higher in children with 0–4 mm subcutaneous fat than in children with 10 mm subcutaneous fat. Cegielski examined the relationship between undernutrition, as determined in the first National Health and Nutrition Examination Survey (NHANES-1), and TB incidence as ascertained in the NHANES-1 Epidemiological Follow-up Study. NHANES-1 was a cross-sectional survey based on a representative sample of the US population from 1971 to 1975. In the Follow-up Study, the adult subjects of NHANES-1, aged 25–74 years at baseline, were followed up until 1992. Having BMI, average skin-fold thickness, or mid-upper arm cross-sectional muscle area in the

lowest decile of the population increased the adjusted hazard of TB from 6- to 10-fold, controlling for other known risk factors for TB.

In a related vein, three massive studies focused on 'body build' as a risk factor for TB incidence. Palmer *et al.* reported on the relationship between TB incidence and naturally acquired delayed-type tuberculin sensitivity among nearly all US Navy recruits from 1949 to 1951. Of 68 754 subjects with follow-up data, 8704 (12.7%) had tuberculin sensitivity recorded as >0 mm induration. During 4 years of follow up, 109 developed TB: 28 among those with 0 mm skin test reactions, 29 among those with 1–9 mm reactions, and 57 among those with 10 mm or greater reactions. Later, these investigators related the risk of TB to the recruits' height and weight data on a stratified random sample of 1138 subjects. TB incidence was 75/105 for those 15% or more below the median weight for height, decreasing to 19/105 for those at least 5% overweight for height. Edwards *et al.* extended this study to more than 823 000 Navy recruits and found that TB developed threefold more often in young men 10% or more below their ideal body weight compared with those 10% or more above their ideal body weight. Rather than attribute these results to nutritional status, the authors concluded that there was an association between 'body build' and risk of TB disease. The relationship between body build and TB was reviewed by Snider in 1987. One study stands out. Norway screened 42%–85% of the population older than age 14 years for TB with radiography from 1963 to 1975. Height and weight were measured accurately for approximately 80% of those screened. As reported by Tverdal *et al.*, more than 1.7 million Norwegians were followed up via the national notification system through 1982 (i.e., 8–19 years of follow up; mean, 12.1 years). A total of 2531 incident cases of TB were identified. The incidence of pulmonary TB declined logarithmically with increasing BMI for both sexes, all age groups, and at all durations of follow up. The age-adjusted incidence of pulmonary TB was five times higher in the lowest BMI category than in the highest. Even though the study was based on BMI, Tverdal argued that the observed relationship was a function of body build. Comstock suggested body build may influence susceptibility to TB because of differences in pulmonary mechanics, but no data supporting this hypothesis exist. Interpreting the findings in terms of unknown factors associated with body build rather than the most obvious explanation, i.e., nutritional status, ignores the fundamental relationship between body weight, caloric intake, and energy expenditure. A more nuanced view may be that body habitus is a function of genetic endowment and of nutrient intake/physical activity, each of which affects the incidence of TB in complex ways.

A unique study of the effect of micronutrient supplementation on TB incidence was reported by Downes in 1949. In a controlled trial among the families of black TB patients in the Harlem ghetto of New York City, 194 of 218 families under public health supervision were examined and divided into two groups matched for family size. The families were allocated alternately to receive vitamin and mineral supplements versus no supplements. The two groups were similar in terms of prior attack rates and mortality from TB, prevalence of TB at the start of the study, sputum smear positivity among the index

cases, and relation of the index case to the rest of the family. In addition, the groups were similar in terms of their economic status, crowding, and eating habits. After 5 years of follow-up, the risk of TB in the control group was 2.8-fold higher than the supplemented group. However, there was substantial non-compliance with the supplements. Compared with those who actually took the vitamin supplements throughout the observation period, the risk of TB in the control group was 5.9-fold higher. Therefore, vitamin supplementation substantially reduced the risk of TB among family contacts of active TB cases.

The effects of undernutrition on the immune response to mycobacterial proteins closely related to the CMI required for resistance to infection with *M. tuberculosis*, namely delayed-type hypersensitivity (DTH) responses, have been studied following BCG vaccination. Satyanarayana *et al.* showed that milder grades of undernutrition did not affect the tuberculin skin test response 6 months after immunization with BCG, but children with kwashiorkor were skin test negative. Chandra and Newberne demonstrated that the DTH skin test response to tuberculin was reduced in protein-energy undernourished children and adults. Among TB patients, PPD skin test reactivity was directly proportional to serum transferrin level, a sensitive indicator of protein undernutrition. Similarly, malnourished individuals did not develop skin test responses to tuberculin as often or as large after BCG vaccination as did well-nourished children. Importantly, this defect has been demonstrated even in modest protein-energy undernutrition.

Experimental animal models allow investigators to elucidate the causal links between nutritional deficiencies, immune system function, and TB in ways not possible in human studies. The link between diet, antimycobacterial immunity, and resistance to TB has been investigated in a highly relevant guinea pig model of low-dose pulmonary TB that mimics the pathogenesis of TB in humans. Moderate, chronic deficiencies of protein and other nutrients (e.g., zinc, vitamin D) induced in guinea pigs had many of the metabolic hallmarks of human dietary deficiencies. Groups of BCG-vaccinated and non-vaccinated guinea pigs were given different diet treatments and then challenged with an aerosol containing a low dose of virulent *M. tuberculosis*. Antigen-specific immune responses *in vitro* and *in vivo* were assessed several weeks later and the ability of the guinea pigs to control the infection was determined quantitatively by culture of viable mycobacteria from the lungs and spleens.

Moderate, chronic protein deficiency (modeled by a 10% ovalbumin-based diet) over several weeks resulted in a dramatic loss of CMI in infected guinea pigs. Protein deprived animals had much smaller DTH reactions and their T lymphocytes proliferated poorly to PPD *in vitro* and produced significantly less interleukin (IL)-2 and IFN- γ . Macrophages from protein malnourished guinea pigs produced less TNF- α in response to infection with virulent *M. tuberculosis*. Protein-deficient guinea pigs were unable to form mature, well-circumscribed granulomas in the lung. BCG-induced protection was diminished by protein deficiency. Protein undernutrition altered the numbers of CD4+ and CD8+ T cells in the spleen and bronchotracheal lymph nodes draining the infected lung. Thus, protein deficiency was accompanied by impairment of the normal trafficking of T lymphocytes that would be required for the formation of protective granulomas

perhaps, due to diet-induced changes in the production or function of chemokines, or by perturbations in the expression of adhesion molecules on T cells or endothelial cells.

Macrophages from TB patients are known to produce suppressive factors for T cells, including transforming growth factor-beta (TGF- β). Alveolar macrophages effectively down-regulate T-cell activation in an attempt to mitigate potentially damaging pulmonary inflammation in response to inhaled antigens. Alveolar macrophages from protein-deficient guinea pigs exerted a 10-fold greater suppression of T cells compared to cells from normally nourished animals, perhaps due to the greater levels of TGF- β produced by these cells. Recombinant human TGF- β injected daily into guinea pigs infected with virulent *M. tuberculosis* suppressed T-cell functions and impaired bacillary control in a manner similar to dietary protein deficiency. Thus, macrophages from protein-deprived guinea pigs appear to be more suppressive for T lymphocyte functions, and this suppression may be mediated, in part, by overproduction of TGF- β .

One of the most important findings from this model is that the profound loss of T-cell-mediated resistance that accompanies chronic dietary protein deprivation was substantially and rapidly reversible. Protein-deficient, BCG-vaccinated guinea pigs given a normal diet beginning on the day of pulmonary challenge with *M. tuberculosis* displayed DTH reactivity and control of bacillary growth within a few weeks that were indistinguishable from those in BCG-vaccinated animals that had never been protein deficient. Similar results were observed in protein-malnourished mice. Using a high-dose, intravenous challenge model, Chan and colleagues observed many of the same T-cell defects that have been reported in low protein guinea pigs, including inability to control the virulent infection, impaired granuloma formation, and recovery of resistance following refeeding with an adequate diet. These studies confirm the fundamental nature of the effects of protein deprivation on susceptibility to TB even when host species, and infection dose and route are altered.

Recently, the guinea pig and mouse models of low-dose, pulmonary TB have been used to demonstrate the significant effects of dietary n-3 PUFA on TB resistance. Guinea pigs fed a diet enriched in fish oil or transgenic *fat-1* mice producing n-3 PUFA endogenously were more susceptible to infection with virulent *M. tuberculosis*. Immune cells from *fat-1* mice or cells from wild-type mice cultured in medium containing n-3 PUFA produced less TNF- α , IL-6, and IL-1 β , and exhibited reduced oxidative burst and impaired phagosome-lysosome fusion. These n-3 PUFA-enriched macrophages were significantly impaired in their ability to control *M. tuberculosis* over several days of culture.

This article has reviewed and critiqued published studies in human populations and in relevant animal models covering the *in vivo* evidence relating the risk of TB due to nutritional status and nutritional factors. Although TB is clearly related to undernutrition, the risk relative to specific levels and types of protein-energy deficiency and micronutrient deficiencies remain to be defined. Analysis of the NHANES-1 Epidemiological Follow-up Study provides plausible estimates of a 6- to 10-fold increase in relative risk among undernourished adults as well as a substantially decreased risk in overweight and obese individuals. Severe protein-energy deficiencies may

increase the relative risk more than mild or moderate undernutrition, but severe undernutrition affects fewer people, even in low-income countries, except during famines, war, natural disasters, etc. Mild to moderate protein-energy or micronutrient deficiencies affect more people at risk for TB, so prevention efforts should target those groups as well. The population attributable risk of TB due to undernutrition may be substantial, especially in populations where both TB infection and undernutrition are prevalent. Undernourished individuals have an increased likelihood of primary or latent infection progressing to active disease. In populations with substantial latent TB infection, the occurrence of undernutrition may be an important determinant of the incidence of reactivation TB. In many developing countries, the risk of becoming infected with TB is as high as 1%–2% year⁻¹ of life. The United Nations Food and Agriculture Organization estimates that one billion people are undernourished. When combined with an estimate of two billion people latently infected with *M. tuberculosis*, even modest decreases in resistance affecting such large numbers of people may result in substantial increases in TB incidence. The potential public health impact of undernutrition on the global incidence of TB was summarized in a US Surgeon General's Report on Nutrition and Health, which emphasized that undernutrition was the leading cause of acquired, correctable immune system dysfunction throughout the world. Population groups at highest risk for poor nutrition are also at high risk for TB; poverty is the common denominator.

Disclaimer: The views and opinions expressed in this article are those of the authors and do not necessarily represent an official position of the US Centers for Disease Control and Prevention.

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NUTRITION LABELING

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Glossary

BOP Back of food package.

FOP Front of food package.

GDA Guideline daily amount, the amount of energy and maximum amount of certain nutrients a person should be eating in a day.

GDA labeling Labeling format where information on key nutrients is provided together with % of GDAs.

Traffic light labeling Labeling format where traffic light colors (green, amber, red) are used to signal levels of certain key nutrients.

Nutrition labeling refers to the provision of information on a food product's nutritional content on the package label. It can serve both public health and commercial purposes. From a public health perspective, the aim of nutrition labeling is to provide information that can enable consumers to make healthier choices when choosing food products. Nutrition labeling is thus closely linked to the notion of the informed consumer, that chooses products according to their aims, on the basis of the information at their disposal. Because many consumers are assumed to be interested in making healthy choices, but the nutritional content of food products may not always be clear to consumers, nutrition labeling can contribute to making the nutritional content more transparent, thus reducing the frequency of unhealthy choices. Nutrition labeling is sometimes also motivated by consumers' right to know, implying that the availability of information on the nutritional content on food products is of value in itself, no matter how this impacts consumer choices. Another argument for nutrition labeling is that making information about nutritional content transparent will lead to healthier products, partly because consumers will avoid products that the label shows to be nutritionally deficient, but also because food producers will try to avoid marketing products that appear, according to the label, as nutritionally problematic, for example, because of a high content of saturated fat or salt. Nutrition labeling could thus encourage the reformulation of products toward a healthier nutritional profile. From a commercial perspective, nutrition labeling is a means to convey the message that a certain product has a healthy nutritional profile. Healthiness is increasingly being used by food manufacturers as a positioning parameter when launching new products, and nutrition labeling can be a credible way of supporting this positioning.

Forms of Nutrition Labeling

Today, it is common to distinguish between nutrition labels that are on the back of the food package (BOP) and those that are on the front of the package (FOP). BOP nutrition labels are

mostly in the form of tables, sometimes also lists, that list the nutritional content of the product with regard to a number of key nutrients. Most BOP labels list information on energy, fat, saturated fat, salt, and sugar, but lists can be considerably longer. The information is provided with regard to a standardized unit, which may be 100 g of the product, the whole product, one unit of the product, or a specified portion size. In addition, the information may be provided as a percentage of the recommended daily values or guideline daily amounts (GDAs), to aid consumers to get a grasp of whether a certain amount in grams is high or low (**Figure 1**). An example is shown in **Figure 1(a)**.

BOP nutrition labels were for many years the only nutrition information generally available. They have been criticized for small print, lack of legibility, and technical language. Also, consumer research indicates that only a small fraction of consumers bother to look at the back of a package before making a choice. FOP nutrition labels have therefore been developed as a complement to BOP nutrition labels. The basic idea of FOP nutrition labels is to provide simplified information on nutritional content that is easily legible and easily understood.

Different forms of FOP nutrition labels have been developed and promoted. A basic distinction is between schemes that are key-nutrient based and schemes that are logo-based. The basic idea of logo-based schemes is that food products within a certain food category are divided into two groups, more and less healthy. Products in the healthier group are then allowed to carry a logo that signals this information. Criteria for the assignment of products into the healthier or less healthy group can be category-specific or a combination of category-specific and general criteria. Logo schemes are run by public bodies, by independent foundations (sometimes industry-sponsored), and by health NGOs. The Swedish Keyhole is an example of a government-supported logo scheme; products entitled to carry the Keyhole are superior to other products in the same category in terms of fat, sugar, salt, and fiber content. This logo is now being spread to other Scandinavian countries. The Choices International Foundation awards its Choices logo to products based on nutritional

profile criteria defined for each product category, mostly with regard to saturated fat, trans fat, sodium, added sugar, and fiber content. Various heart associations have developed health logos as well, like the American Heart Association Heart-Check Mark for foods that meet certain criteria with regard to, among others, fat and sodium. A few cases of graduated logos (dividing food products into more than two categories, depending on their nutritional profile, and communicating this by points or stars) have also been seen. Examples of health logos are in **Figure 1(b)**.

Key nutrient based FOP nutrition labels provide information on key nutrients, usually energy, fat, saturated fat, salt, and sugar, in clearly legible font and a fixed format, either per 100 g or per portion. This is usually supplemented by some interpretational aid to consumers. One such aid, promoted mainly by the food industry, is to supplement the gram-based information with percentages of GDAs. GDAs are defined as the amount of energy and maximum amount of some nutrients (e.g., fat, saturated fat, salt, and sugars) a person should be eating in a day and usually reference values for an adult woman are used. This scheme has been criticized on various grounds, especially by consumer associations, the main arguments being that consumers find it difficult to interpret percentages and that the percentages are usually computed per portion. Portion sizes may not always be realistic, and actual portions consumed may vary considerably over consumers. Another interpretational aid, promoted by the British Food Standards Agency and a number of consumer organizations,

supplements the gram-based information with so-called traffic light colors, i.e., red, yellow, and green, depending on whether the content of the particular nutrient is high, medium, or low. This scheme has been criticized as well, mostly by industry, for being too directive, and because the most common way of assigning the colors is based on a per-100 g scheme that may sometimes lead to misleading results. Hybrid schemes, combining GDAs with traffic lights, have also been proposed. Examples of such schemes are in **Figure 1(c)**.

History of Nutrition Labeling

Nutrition labeling developed in the 1970s in the wake of increased concern about health and nutrition. It is today governed by a combination of legislation and voluntary arrangements.

In the US, nutrition labeling was mentioned as a means to support the goal of public health in the White House Conference on Food, Nutrition, and Health in 1969, and a voluntary nutrition labeling scheme went into effect in 1975. Only products making nutritional claims or with added nutrients were required to have a nutrition label. This changed with the United States Nutrition Labeling and Education Act (NLEA) from 1990. The NLEA requires that packaged foods have a Nutrition Facts Panel, listing selected nutrients per serving in grams as well as per Recommended Daily Values. Later, similar rules were adopted for meat, poultry, and eggs.

Nutrition information				Guideline daily amounts	
Typical values (Cooked as per instructions)	Per 100g	Per pack	% Based on GDA for women	Women	Men
Energy	610 kJ 146 kcal	2580 kJ 618 kcal	30.9%	2000 kcal	2500 kcal
Protein	4.8 g	20.3 g	45.1%	45 g	55 g
Carbohydrates of which sugars of which starch	12.8 g 2.7 g 10.1 g	54.1 g 11.4 g 42.7 g	23.5% 12.7%	230 g 90 g	300 g 120 g
Fat of which saturates mono-unsaturates polyunsaturates	8.4 g 3.8 g 3.5 g 1.1 g	35.5 g 16.1 g 14.8 g 4.7 g	50.7% 80.5%	70 g 20 g	95 g 30 g
Fibre	2.0 g	8.5 g	35.4%	24 g	24 g
Salt of which sodium	0.5 g 0.2 g	1.9 g 0.8 g	31.6% 33.3%	6 g 2.4 g	6 g 2.4 g

(a)

Figure 1 Examples of nutrition labels. (a) Example of BOP nutrition table, (b) examples of health logos, and (c) examples of key nutrient based FOP schemes. These examples are taken from work in the FLABEL project (see www.flabel.org), courtesy of C. Hodgkins, University of Surrey.



Keyhole: Government system for identifying products that are healthiest within a product category

Each serving contains ...

MED	LOW	HIGH	HIGH	MED
Calories 618	Sugar 11.4 g	Fat 35.5 g	Saturates 16.1 g	Salt 1.9 g
30.9%	12.7%	50.7%	80.5%	31.6%

of your guideline daily amount

System containing energy and key nutrient information, % guideline daily amounts and traffic light colors



BRITISH HEART FOUNDATION

Heart Foundation system for identifying products that are heart healthy within a product category

Each serving contains ...

Calories	Sugar	Fat	Saturates	Salt
618	11.4 g	35.5 g	16.1 g	1.9 g

System containing energy and key nutrient information and traffic light colors



Food industry system for identifying products that are healthiest within a product category

Each serving contains ...

Calories	Sugar	Fat	Saturates	Salt
618	11.4 g	35.5 g	16.1 g	1.9 g
30.9%	12.7%	50.7%	80.5%	31.6%

of your guideline daily amount

System containing energy and key nutrient information and % guideline daily amounts

(b)

(c)

Figure 1 Continued.

This panel was and is usually BOP. This compulsory information is often supplemented today by voluntary FOP information, often in the form of logos. The proliferation of logos has given rise to concerns about consumer understanding and about consumers possibly being misled. There are also initiatives in the US to provide nutrition labeling not only on prepacked food, but also on restaurant menus.

In the European Union (EU), nutrition labeling was about to be made compulsory at the time of writing. It used to be voluntary unless the product carries a health or nutrition claim, although in fact by far the majority of products carried the BOP nutrition table, and many products these days also carry some FOP nutrition information. The most widespread FOP nutrition information is the provision of key nutrient information per portion supplemented with GDA percentages, i.e., the industry-sponsored system. However, many major retail chains have adopted their own FOP nutrition labeling scheme for their private label products, and with rapidly

growing market shares of private label products in Europe these labeling schemes have high visibility on the market. Retailer-adopted FOP schemes include GDA-based systems, traffic-light based systems and various hybrids. The new EU legislation will most likely include a FOP scheme in addition to the BOP nutrition table.

Mandatory nutrition labeling legislation can also be found in many other countries, including Argentina, Australia, Canada, Israel, Malaysia, and New Zealand.

Effects of Nutrition Labeling on Consumers

Research trying to establish to which extent nutrition labeling is successful in improving people's diet has been going on for several decades, but clear-cut conclusions have not been obtained. Usually, a sequence of effects is distinguished, all of which have to occur if nutrition labels are to have the effect of

making consumer choices healthier. First, if consumers are exposed to nutrition labels, they need to notice and actually read them. Second, they need to understand the label and make correct inferences about the healthiness of the product. Third, this understanding actually has to make a difference in the choice that consumers makes, i.e., they choose something different than they would have without the label. Fourth, a healthier choice in one product category may be offset by other changes in behavior, like making healthier choices in other product categories, increasing the quantity of consumption, or even changes in meal patterns and eating habits. The incidence of these effects will, among other factors, be influenced by people's motivation to eat healthily and their nutritional knowledge.

Studies consistently show that most people are aware of the link between health and eating, and many people have an aim of making healthy choices. When asked, many people therefore usually show an interest in getting nutrition information, especially on calories and fat, but also about sugar, salt, carbohydrates, vitamins, and calcium. However, healthiness is often only one among several other criteria when choosing food, like taste, family liking, and price. The question of whether consumers actually do pay attention to and read nutrition labels when shopping has been investigated in different ways. Most studies are based on self-reports, i.e., respondents are asked to indicate how often they read nutrition labels when shopping. These studies show that around 50% of the population (most studies done in the US and the UK, but also a range of other countries especially in Europe) claim to read nutrition labels always or often. These numbers are most likely inflated by a social desirability bias. Studies based on observation of shoppers arrive at much lower numbers, mostly less than 20% and sometimes considerably lower. As for understanding and making the right inferences about healthiness, it seems that the introduction of FOP labeling schemes has made a difference compared to the difficult-to-read tables on the back. A range of studies have compared the major FOP labeling schemes in terms of understanding and correct inferences. The major result so far is that, for the purpose of making intra-category comparisons of products with regard to their healthiness, providing key nutrients FOP in a legible and comparable format is key, and the addition of other elements – GDA percentages, traffic light colours – only leads to minor improvements in understanding and correct health inferences. Results are less clear-cut for other types of uses of nutrition labeling information, especially when making comparisons across product categories and combining different products into meals or shopping baskets. Making the right inferences from health logos depends, naturally, on whether shoppers have been familiarized with the logo and have built up an understanding of what the logo stands for. Studies also show that the healthiness of a product is inferred from other pieces of information as well, even when a FOP nutrition label is present. Examples of this are the degree of processing (the less the healthier), the use of additives, the origin, the vegetable content (in products like ready meals).

Laboratory studies have demonstrated that nutrition labels can influence consumer choices, but evidence on real life choices is scarce. It is even more difficult to establish a link between nutrition labeling and not only brand choice, but

also overall dietary intake. Correlational evidence has been provided on links between self-reported use of nutrition labels and self-reported food intake, but such studies do not allow a causal interpretation. Studies comparing purchase patterns before and after the introduction of the NLEA in the US provide mixed evidence. Unintended consequences have been demonstrated as well – for example, that information on low fat content can increase subsequent calorie intake by decreasing feelings of consumption guilt.

Not surprisingly, people differ in their interest in, understanding and use of nutrition labeling information. Gender, age, and social class have been shown to make a difference, with women, older people, and people in higher social classes having more interest, and younger people and higher social classes having more relevant knowledge.

Different stakeholders draw different conclusions from these results. Some believe that the documented interest of consumers in nutritional information suggests a huge potential for healthier choices if products were labeled in the best possible way, i.e., with a nutrition label format that maximizes attention and is easy to understand and apply. Others point out that the bulk of food purchases are habitual, low-involvement purchases, made within a few seconds, with very limited or no information uptake taking place. Effects of nutrition labeling on consumer food choice will therefore by necessity be limited, except for certain segments (people with an above average interest in healthy eating) and certain situations (new products, products with a health positioning).

Effects of Nutrition Labeling on Producers

If nutrition labels indeed have an impact on consumer choice and make them demand the healthier varieties, the supply of food products should, in the long run, become healthier. However, effects of nutrition labels on food supply need not be contingent on consumers using them. Two mechanisms have been discussed. Key-nutrient based nutrition labeling results in increased transparency with regard to the nutritional content of food products, and food manufacturers may not want to be associated with products that have a very high content of nutrients where intake is recommended to be limited, like salt or sugar. This creates incentives for reformulation of products so that the nutritional profile on the label will look better. A lot of anecdotal evidence exists that this is actually taking place when especially FOP nutrition labels are introduced, for example, when retailers require them. Health-logo labeling schemes, when run well and well-established on the market, can achieve brand-like status, and manufacturers may find it attractive to be allowed to have this logo on their products. This encourages product reformulation or new product development in such a way that the product qualifies for the logo. This effect of health logo schemes has been demonstrated in a study on the Choices logo.

Outlook

With legislation already enacted or on the way in many parts of the world, nutrition labeling is about to become standard

on food packages. The introduction of FOP nutrition labels has been a major step in improving accessibility, readability, and understandability of nutrition information on food packages. Although this development has a clear potential for enabling consumers to make healthier choices, it is important to see nutrition labeling in two contexts. One is the food label of which the nutrition label is a part. Food labels are becoming loaded with more and more information – food products are positioned by manufacturers on factors like being healthy, traditional, sustainable, local, natural, etc., all of which need to be communicated, and the nutrition label is accompanied by eco-labels, labels on protected origin, and a wealth of other information. In an overloaded information environment, consumers will in the future be still more selective with regard to the information that they will process. The other context is the totality of nutrition-related communication directed at consumers. Nutrition labels provide product-specific information, but they cannot convey general nutritional information and, most important, they will never be a main factor in motivating consumers to make healthy choices. Only when nutrition labeling is embedded in an overall scheme aimed at stimulating health motivation and nutrition knowledge will it have an effect on the healthiness of consumer food choices.

See also: Food Choice: Behavioral Aspects

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NUTRITION TRANSITION, DIET CHANGE, AND ITS IMPLICATIONS

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Glossary

Dietary change Foods consumed and overall food patterns.

Edible oils Vegetable oils extracted from oil seeds.

Globalization Commonly refers to increasing global norms in economic order, trade in goods and services or set of changes associated with the rapid exchange of goods, services, ways of thinking.

Mass media All modern forms of communication: radio to TV to computer, cell phones, movies, magazines, etc.

Nutrition transition Stages of patterns of eating, drinking, and moving.

Obesity BMI ≥ 25 .

Role of income Measure of amount of money or money in kind we obtain over a period of time.

Sugar-sweetened beverages Calorically sweetened waters -carbonated or noncarbonated, flavored in any manner from colas to energy drinks to vitamin waters.

Urbanization Residence in urban area or process of modernization of a community to bring set of services and infrastructural changes linked with urban residency.

The world is experiencing rapid shifts in structures of diet and body composition, with resultant important changes in health profiles. In many ways, these shifts are a continuation of the large-scale changes that have occurred repeatedly over time; however, the changes facing low- and moderate-income countries appear to be very rapid. Broad shifts continue to occur throughout the world in population size and age composition, disease patterns, and dietary and physical activity patterns. The former two sets of dynamic shifts are termed the demographic and epidemiological transitions. The latter, whose changes are reflected in nutritional outcomes, such as changes in average stature and body composition, is termed the nutrition transition. These three relationships are presented in **Figure 1**.

Human diet and activity patterns, and nutritional status, have undergone a sequence of major shifts, defined as broad patterns of food use and their corresponding nutrition-related diseases. During the past three centuries, the pace of dietary and activity changes appears to have accelerated to varying degrees in different regions of the world. Furthermore, dietary and activity changes are paralleled by major changes in health status as well as by major demographic and socioeconomic changes. Obesity emerges early under these shifting conditions, as does the level and age composition of morbidity and mortality. Although there are five broad nutrition patterns dating back to the origins of modern man, the focus of this article is on the three most recent periods (**Figure 2**). For convenience, the patterns are outlined as historical developments; however, earlier patterns are not restricted to the periods in which they first arose but, rather, they continue to characterize certain geographic and socioeconomic sub-populations. The first two patterns relate to earlier periods in

the evolution of humans – the first pattern of collecting food and the second pattern of famine. The following are the three later periods:

Pattern 3: Receding famine: The consumption of starchy staples was predominant and continues to be so, but these items become less important in this low-fat diet as limited amounts of fruits, vegetables, and animal protein are increasingly added to the low-fat and high-fiber diet. Many earlier civilizations made great progress in reducing chronic hunger and famines, but only in the last third of the past millennium have these changes become widespread, leading to marked shifts in diets. However, famines continued well into the eighteenth century in some parts of Europe and remain common in some regions of the world. Activity patterns are shifting and inactivity and leisure have become a part of the lives of increasingly more individuals.

Pattern 4: Nutrition-related noncommunicable disease (NR-NCD): A diet high in total fat, cholesterol, sugar, and other refined carbohydrates, low in polyunsaturated fatty acids and fiber, and often accompanied by an increasingly sedentary life is characteristic of most high-income societies (and increasing proportions of the population in low-income societies), resulting in increased prevalence of obesity and contributing to the degenerative diseases that characterize the final epidemiologic transition stage.

Pattern 5: Behavioral change: A new dietary pattern appears to be emerging, evidently associated with the desire to prevent or delay degenerative diseases and prolong health. Whether these dietary changes, instituted in some countries by consumers and in others also prodded by government policy, will create a large-scale transition in dietary structure and body composition remains to be seen.

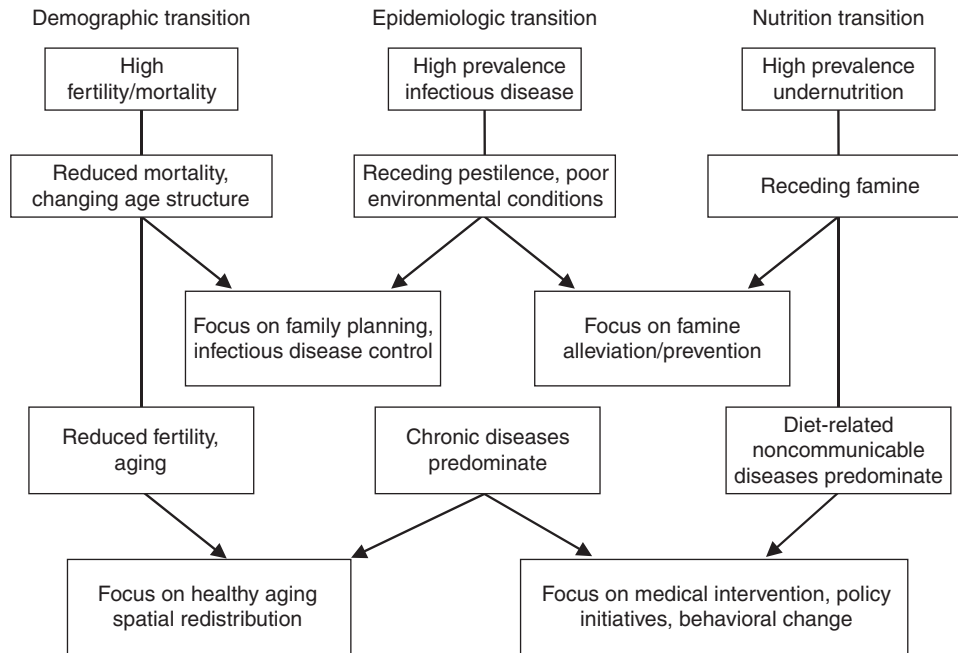


Figure 1 Stages of health, nutritional, and demographic change. Reproduced from Popkin BM (2002) The shift in stages of the nutrition transition in the developing world differs from past experiences! *Public Health Nutrition* 5(1A): 205–214, with permission from Cambridge University Press.

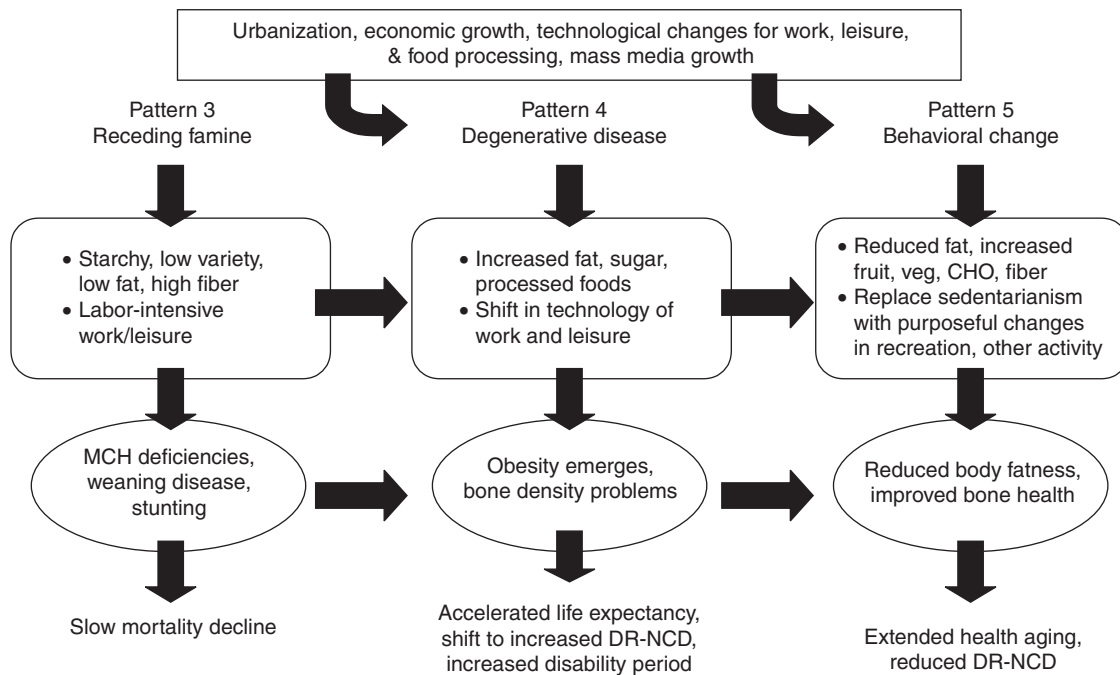


Figure 2 Stages of nutrition transition. Reproduced from Popkin BM (2002) The shift in stages of the nutrition transition in the developing world differs from past experiences! *Public Health Nutrition* 5(1A): 205–214, with permission from Cambridge University Press.

Our focus is increasingly on patterns 3–5, particularly the rapid shift in much of the world's low- and moderate-income countries from the stage of receding famine to NR-NCD. **Figure 2** presents this focus. The concern about this period is so great for many that the term 'nutrition transition' is synonymous with this shift from pattern 3 to 4.

Shifts in Dietary and Activity Patterns and Body Composition Seem to be Occurring More Rapidly

The pace of the rapid nutrition transition shifts in diet and activity patterns from the period termed the receding famine pattern to one dominated by NR-NCDs seems to be

accelerating in the lower- and middle-income transitional countries. We use the word 'nutrition' rather than 'diet' so that the term NR-NCDs incorporates the effects of diet, physical activity, and body composition rather than solely focusing on dietary patterns and their effects. This is based partially on incomplete information that seems to indicate that the prevalence of obesity and a number of NR-NCDs is increasing more rapidly in the lower- and middle-income world than it has in the West. Another element is that the rapid changes in urban populations are much greater than those experienced a century ago or less in the West; yet another is the shift in occupation structure and the rapid introduction of the modern mass media. Underlying such changes is a general concern for rapid globalization as the root cause.

Clearly, there are quantitative and qualitative dimensions to these changes. On the one hand, changes toward a high-density diet, reduced complex carbohydrates, increased added sugar and other caloric sweeteners, and inactivity may be proceeding faster than in the past. The shift from labor-intensive occupations and leisure activities toward more capital-intensive, less strenuous work, and leisure is also occurring faster. On the other hand, qualitative dimensions related to multidimensional aspects of the diet, activity, body composition, and disease shifts may exist. The social and economic stresses that people face and feel as these changes occur may also be included.

Scholars often note that the pace and complexity of life, reflected in all aspects of work and play, are increasing exponentially. There are also unanticipated developments, new technologies, and the impact of a very modern, high-powered communications system. It is this sense of rapid change that makes it so important to understand what is happening and anticipate the way in which changes in the patterns of diet, activity, and body composition are occurring. Although the penetration and influence of modern communications, technology, and economic systems related to 'globalization' have been a dominant theme of the past few decades, there seem to be some unique issues that have led to a rapid increase in globalization and its impact.

Stating that globalization is the cause results in a focus on broad and vaguely measured sets of forces; this ignores the need to be focused and specific, which would allow us to develop potentially viable policy options. It is difficult to measure each element of this globalization equation and its impact. These processes certainly have been expanded, as indicated by enhanced free trade, a push toward reduction of trade barriers in the developing world, and the increasing penetration of international corporations into the commerce in each country (measured by share of gross national product (GNP) or manufacturing). Similarly, other economic issues related to enhanced value conferred to market forces and international capital markets are important. Equally, the increasing access to Western media, the removal of communication barriers enhanced by the World Wide Web, cable television (TV), mobile telephone systems, etc., are important. The accelerated introduction of Western technology into manufacturing and the basic sectors of agriculture, mining, and services is also a key element.

Another way to understand the types of changes that the developing world is facing is to consider an urban squatter's life and a rural villager's life in China approximately 20 years

ago and today. During the 1970s, food supply concerns still existed; there was no TV, limited bus and mass transportation, little food trade, minimal processed food, and most rural and urban occupations were very labor intensive. Today, work and life activities have changed: small gas-powered tractors are available, modern industrial techniques are multiplying, offices are automated, soft drinks and many processed foods are found everywhere, TVs are in approximately 89% of households (at least one-fifth of which are linked to Hong Kong Star and Western advertising and programming), younger children do not ride bicycles, and mass transit is being heavily used. Considering that such changes are also occurring in much of Asia, North Africa, the Middle East, Latin America, and many areas (particularly cities) in sub-Saharan Africa, it is evident that the shift from a subsistence economy to a modern, industrialized one occurred in a span of 10–20 years, whereas in Europe and other industrialized high-income societies, this occurred over many decades or centuries.

To truly measure and examine these issues, we would need to compare the changes in the 1980–2000 period for countries that are low and middle income to changes that occurred a half-century earlier for the developing world. However, data on diet and activity patterns are not available, and there are only minimal data on NR-NCDs and obesity.

The elements of the nutrition transition known to be negatively linked with NR-NCDs are obesity, adverse dietary changes (e.g., shifts in the structure of diet toward a greater role for higher fat and added caloric sweeteners in food, reduced fruit and vegetable intake, reduced fiber intake, greater energy density, and greater saturated fat intake), and reduced physical activity in work and leisure. The causes of these elements of the nutrition transition are not as well understood as the trends in each of them. In fact, few studies have attempted to research the causes of such changes, and only a few data sets allow such crucial policy analyses to be undertaken.

Obesity Trends

The most commonly measured health outcome of the shifts in the structure of diet is obesity. The shifts in adult overweight and obesity in the developing world in the past 10–30 years are far faster than in the higher income countries. The author examined the shifts in body composition among Chinese adults aged 20–45 years during an 8-year period. Not only did the mean body mass index (BMI) increase but also the shape of the BMI distribution curve changed during the 8-year period. From 1989 to 1997, the proportion of underweight men and women declined considerably and the prevalence of both overweight and obesity increased greatly. In fact, the proportion of overweight and obesity in men more than doubled from a prevalence of 6.4 to 14.5%, and that for women increased much more slowly from 11.5 to 16.2%.

China is not unique; here, data from a few low- and middle-income countries are presented to compare their increase in the annual prevalence of overweight and obese adults with that of the US. **Figure 3** presents the annualized increase in the percentage points of prevalence for data from high-income countries with comparable data. **Figure 4** shows how rapidly overweight and obesity have emerged in Mexico as a

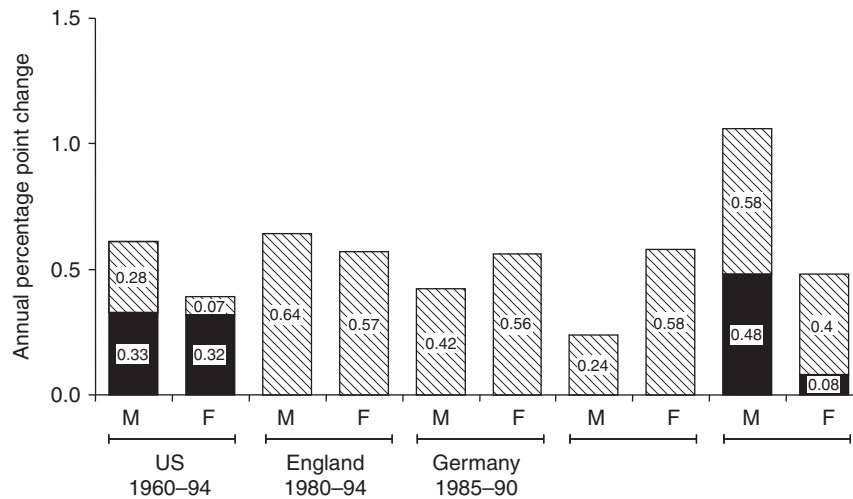


Figure 3 Obesity trends among adults in the US and Europe (the annual percentage point increase in prevalence). Reproduced from Popkin BM (2002) The shift in stages of the nutrition transition in the developing world differs from past experiences! *Public Health Nutrition* 5(1A): 205-214, with permission from Cambridge University Press.

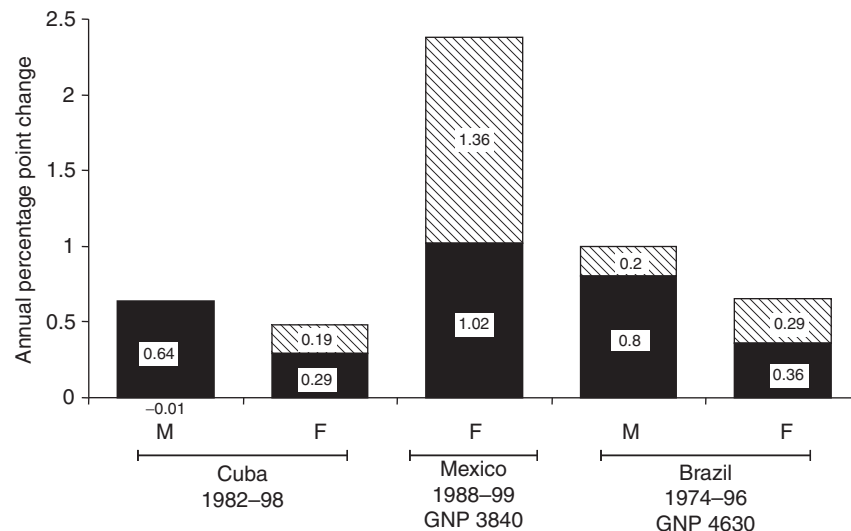


Figure 4 Obesity trends among adults in Latin America (the annual percentage point increase in prevalence). Data from Rodríguez-Ojea A, Jiménez BA, and Esquivel M (2002) The nutrition transition in Cuba in the nineties: an overview. *Public Health Nutrition* 5(1A): 129-33, and Rivera JA, Barquera S, Campirano F, Campos I, Safdie M, and Tovar V (2002) Epidemiological and nutritional transition in Mexico: rapid increase of non-communicable chronic diseases and obesity. *Public Health Nutrition* 5(1A): 113-22, with permission from Cambridge University Press.

major public health problem. Compared with the US and European countries, where the annual prevalence increase in overweight and obesity is approximately 0.25 each, the rates of change are very high in Latin America. Cuba's data only represent Havana. Similar shifts in the prevalence of obesity are presented for North Africa and the Middle East and Asia in Figures 5 and 6, respectively.

What is important to note is that the increase in the proportion of the adult population that is overweight is far greater in all the lower income countries than in the USA or most European countries. Only Spain, with its large shift in

overweight in the past decade, is close to the speed of change in these countries.

Dietary Changes: Shift in the Overall Structure over Time

The diets of the developing world are shifting equally rapidly. There are no good-quality data for most countries on total energy intake, but there are reasonable data to examine shifts in the structure of the diet. Food balance data were used to examine the shift over time in the proportion of energy from fat.

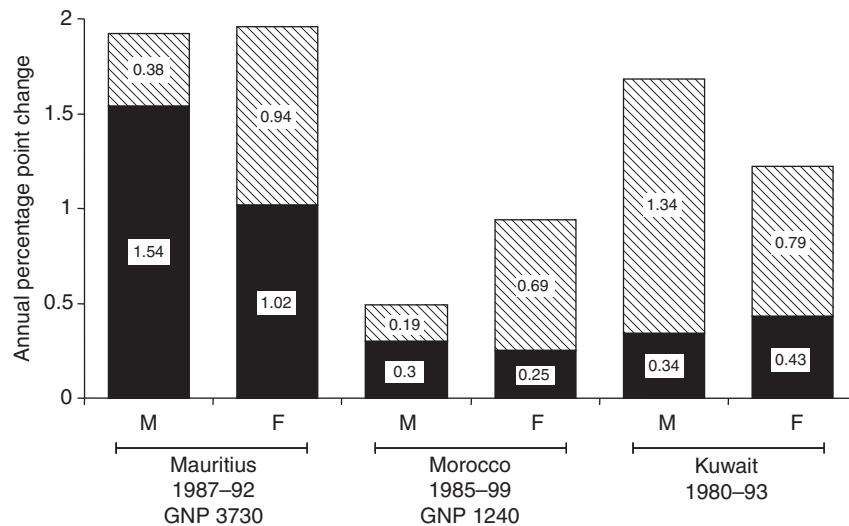


Figure 5 Obesity trends among adults in North Africa/Middle East (the annual percentage point increase in prevalence). Data from Benjelloun S (2002) Nutrition transition in Morocco. *Public Health Nutrition* 5(1A): 135–40; Hodge AM, Dowse GK, Gareeboo H, Tuomilehto J, Alberti KG, and Zimmet PZ (1996) Incidence, increasing prevalence, and predictors of change in obesity and fat distribution over 5 years in the rapidly developing population of Mauritius. *International Journal of Obesity* 20: 137–46; Al-Isa AN (1995) Prevalence of obesity among adult Kuwaitis: a cross-sectional study. *International Journal of Obesity and Related Metabolic Disorders* 19(6):431–3, and Al-Isa AN (1997) Changes in body mass index (BMI) and prevalence of obesity among Kuwaitis 1980–1994. *International Journal of Obesity* 21: 1093–9, with permission from Nature.

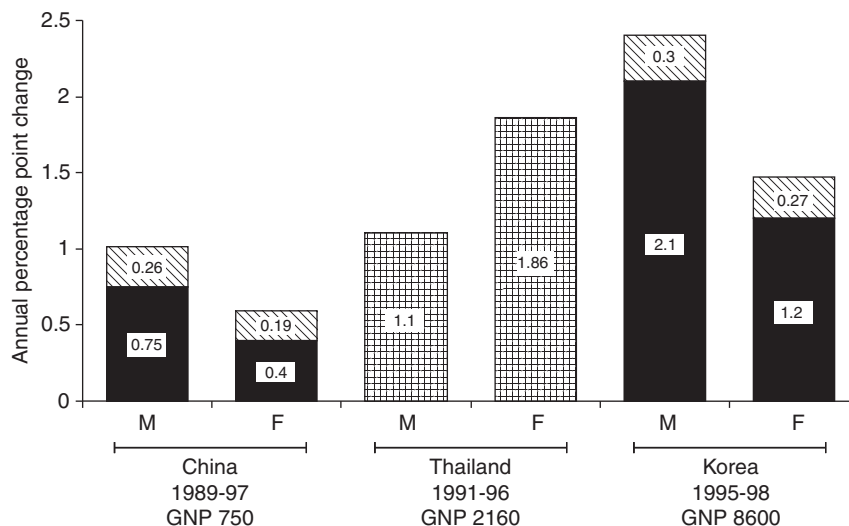


Figure 6 Obesity trends among adults in Asia (the annual percentage point increase in prevalence). Data from Kosulwat V (2002) The nutrition and health transition in Thailand. *Public Health Nutrition* 5(1A): 183–89; Du S, Lu B, Zhai F, and Popkin BM (2002) A new stage of the nutrition in China. *Public Health Nutrition* 5(1A): 169–74, and Lee M-J, Popkin BM, and Kim S (2002) The unique aspects of the nutrition transition in South Korea: the retention of healthful elements in their traditional diet. *Public Health Nutrition* 5(1A): 197–203, with permission from Cambridge University Press.

The drastic changes in the aggregate income–fat relationship from 1962 to 1990 are displayed in **Figure 7** by the estimated regression lines based on cubic polynomial regressions. Most significantly, even the poor nations had access to a relatively high-fat diet by 1990, when a diet deriving 20% of energy (kcal) from fat was associated with countries having a GNP of only \$750 per capita, whereas in 1962 the same energy diet (20% from fat) was associated with countries

having a GNP of \$1475 (both GNP values in 1993 dollars). This drastic change arose from a major increase (10–13%) in the consumption of vegetable fats by poor and rich nations; similar increases (3–6%) also occurred in middle- and high-income nations.

At the same time, there were decreases in the consumption of fat from animal sources for all except the low-income countries. The availability of animal fats continued to be

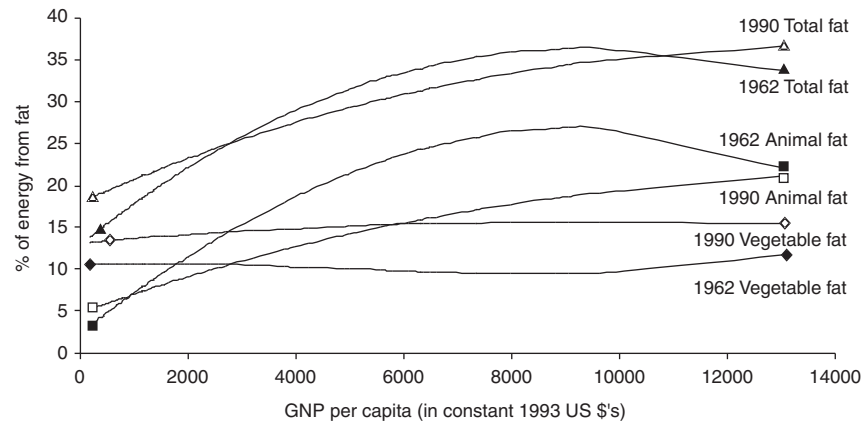


Figure 7 Relationship between the percentage of energy from fat and GNP per capita, 1962 and 1990. Reproduced from Guo X, Mroz TA, Popkin BM, and Zhai F (2000) Structural changes in the impact of income on food consumption in China, 1989–93. *Economic Development and Cultural Change* 48: 737–60.

linked to income, although less strongly in 1990 than in 1962. These decreases, combined with the increase in vegetable fat intake for all income countries, resulted in an overall decrease in fat intake for moderate-income countries of approximately 3% but an increase in approximately 4% or 5% for low- and high-income countries. **Figure 7** shows these substantial shifts in the relationships between GNP and the composition of diets over time.

Vegetable fats in 1990 accounted for a greater proportion of dietary energy than animal fats in the lowest 75% of countries (all of which have incomes less than \$5800 per capita) of the per capita income distribution. The absolute level of vegetable fat consumption increased, but there remained at most a weak association between GNP and vegetable fat intake in these aggregate data. The change in edible vegetable fat prices, supply, and consumption is unique because it equally affected rich and poor countries, but the net impact is relatively much greater on low-income countries. Recent analysis in China shows that the pace of change for increased energy density and animal source foods in the diet has accelerated.

There is also an equally large and important shift in the proportion of energy from added caloric sweeteners in the diets of lower income countries. In fact, an additional 100–200 kcal per day was available for daily consumption from added caloric sweeteners in the diet in 2000 compared with 1962 in the developing world. In the US, this added caloric sweetener increase derives mainly from soft drinks and fruit drinks, but in many other countries the source of this increase is other foods, even basic processed foods that have sweeteners added to them. High-fructose corn syrup (HFCS) is used as the sweetener of choice in some cases by food companies. Fructose, be it from HFCS, sugar, or other caloric sweeteners, appears to have unique adverse cardiometabolic attributes. When we specifically examine the combined effect of these various shifts in the structure of rural and urban Chinese diets, we find an upward shift in the energy density of the foods consumed. In this study, the kilocalories of energy intake from foods and alcohol per 100 g of food in both urban and rural Chinese adult diets increased by more than 10% (to 2.42) between 1989 and 1997. These present very rapid shifts in energy density. It is important to note that the value of 2.42

is not comparable with the normal measure of energy density of the diet. The normal method includes full measures of all beverages, whereas the Chinese Food Composition Table, from which these data were extracted, measures only a few beverages (milk, coconut juice, sugarcane juice, spirits, beer, wine, champagne, and brandy) and excludes many beverages, particularly tea and coffee. A number of clinical investigations have varied the energy density of the diet in *ad libitum* studies. Each study shows that increases in energy density, often as small as 1–1.3 kcal g⁻¹, can increase the total energy intake. For these reasons, energy density changes in China, and most likely in other developing countries, are critical components of dietary change to be monitored.

Rapid Social Change is Important: Urbanization, Rapid Demographic Change, and Other Behavioral Changes are Occurring Simultaneously

Diets have shifted in urban areas in a far more dramatic fashion than in rural areas. We do not focus on many of the complex issues related to the type of urban change that has occurred. Nevertheless, critical sociodemographic issues include the following:

- Rapid reductions in fertility have enhanced the shift in the age distribution.
- Urbanization continues unabated in Asia and Africa. More poor will reside in urban than rural areas in future decades.
- Economic changes, particularly increased income and income inequality, appear to define changes in many regions of the developing world.
- Globalization of mass media is occurring at an earlier stage of economic development than occurred in higher income countries in the past.

Urbanization

In other published works, we have shown how the structure of diet has shifted markedly as populations have urbanized. This

relationship will, by itself, shift the structure of diet significantly at the national level as urbanization continues and as the proportion of the population in urban areas grows.

Structural Shifts in Income–Diet Relationships are Occurring

Changes in dietary behavior can be caused either by shifts in the composition of society regarding the plurality of the educated, rich, or urban residents or by changes in the actual behavior of those with specific characteristics. The latter type can include a change in consumption behavior such that for the same level of education or income, a person would buy different amounts or types of commodities at different points in time. Research conducted in China shows that there have been profound behavioral shifts of this type during the past decade (i.e., for each extra dollar of income, additional high-fat foods are purchased versus what would have been purchased in previous years for the equivalent extra dollar). Economists speak of this effect as one that shows how the decision-making demand pattern for food has changed; thus, for the same income level, the patterns of demand have changed significantly from earlier periods. The explosion in access and exposure to mass media may very well have created this situation.

Mass Media

There is no doubt that access to modern mass media has increased very rapidly, particularly in the past decade. Elsewhere, we have shown worldwide trends. It is most useful to examine the proportion of households in a country that have TV sets to gain an insight into this topic. Again, we use China Health and Nutrition Survey (CHNS) data to demonstrate the types of changes in one setting. Overall, 88.5% of Chinese households in the CHNS sample had TVs in 1997. It is important to note that not only was the proportion of individuals with access to TV shifting, but also the types of programs and access to Western influences. In the 1980s, cable systems in China did not provide outside programming, but by 1997 approximately one-fourth of Chinese provinces provided access to Phoenix Star TV, a Hong Kong TV system that relies heavily on US and British programming and provides modern TV advertising.

Again, although there are no extensive data on the proportions of Chinese households with access to mass media 30–70 years ago, it is certain that the penetration into Chinese households in 1997 was far greater than it was into US households 50 years ago, when TV was in its infancy.

Health Effects: Is the Biology Different? Rather, Do We Have Different Social Structures and Body Composition Patterns That Affect BMI–Disease Relationships? Are There Genetic Variants That Are Important?

There are a number of different ways these questions could be answered in the affirmative. One is whether the body composition and other unmeasured race/ethnic factors affect susceptibility to NR-NCDs. Another might be whether previous

disease patterns (e.g., the presence of malaria or other tropical diseases) led to disease patterns that predisposed the population to certain problems. One component of this may be the fetal insult syndrome developed and popularized by Barker.

There is a growing body of research that shows that the international standards, used to delineate who is overweight and obese, are not appropriate for many large subpopulations in the world. For instance, a BMI of 25 in an Asian adult appears to have a far greater adverse metabolic effect than in a Caucasian adult. In fact, the World Health Organization and the International Obesity Task Force formed a group of scientists and agencies in Asia to review this topic. This group held international meetings and has proposed lower BMI cutoffs for Asians of 23 for overweight and 25 for obesity. In one article comparing China, the Philippines, and US Hispanics, blacks, and whites, the odds of being hypertensive were higher for Chinese men and women compared with other subpopulation groups at lower BMIs in the 23–25 range. Ethnic differences in the strength of the association between BMI and disease outcomes warrant further consideration.

Zimmet and others who have focused on this issue as it relates to lower-income countries believe that the highest genetic susceptibility for adult-onset diabetes is for Pacific Islanders, American-Indians, Mexican-Americans and other Hispanics, and Asian Indians. Those with modest genetic susceptibility include Africans, Japanese, and Chinese. The age of onset (usually after 50 years of age) of noninsulin-dependent diabetes mellitus is much lower for these susceptible populations, and it appears that the prevalence is higher for a given level of obesity and waist-to-hip ratio. Zimmet summarizes a large selection of literature that has explored these issues relating to diabetes among susceptible populations.

It is not clear how much of this difference between subpopulations regarding BMI–diabetes or other BMI–morbidity relationships is a function of differences of body composition, metabolic or genetic factors, or social causes. We have shown that part of the apparent race–hypertension relationship may also be explained by socioeconomic status (SES).

Another dimension relates to the issue of inflammatory burden. Evidence that inflammation plays a central role in cardiovascular disease (CVD), particularly at all stages of atherosclerosis, is persuasive. This position is supported by basic science and epidemiology. As reviewed in a meta-analysis, the magnitude of the associations between CVD outcomes and levels of inflammatory factors, such as C-reactive protein, albumin, white blood cell count, and fibrinogen, is surprisingly consistent across studies, despite differing designs, populations, duration of follow-up, and case definitions.

There is another pathway related to the role of previous health problems that is less understood and no real documentation of its impact (e.g., malnutrition that caused a virus to mutate, parasitic infections that affected long-term absorption patterns, or a parasite that is linked to an unknown genotype – comparable to sickle cell anemia and its evolutionary linkage with malaria). We have no basis for speculation about this potential pathway.

One pathway – the effect of fetal and infant insults on subsequent metabolic function – has the potential to be very critical to South Asia and some other regions with very rapid shifts in obesity. If the rapid shifts toward a positive energy

imbalance are occurring concurrently with higher levels of low birth weight in a population, then this becomes a much more salient aspect of this argument. For the developing world, where intrauterine malnutrition rates are high and there is a high prevalence of nutrition insults during infancy, the work of Barker and many others portends important potential effects on the prevalence of NR-NCDs in the coming decades. Not only is there an emerging consensus that fetal insults, particularly with regard to thin, low-birth-weight infants who subsequently face a shift in the stage of the transition and become overweight, are linked with an increased risk of NR-NCDs, but also infancy may equally be a period of high vulnerability. Three studies by Hoffman suggest that fat metabolism of stunted infants is impaired to the extent that this may lead to increased obesity and other metabolic shifts. Other work on the role of stunting on obesity suggested such an effect, but Hoffman's work suggests the mechanism and fits with the correlational work.

The CVD Epidemic is Beginning

Evidence from many developing countries shows that nutrition-related chronic diseases prematurely disable and kill a large proportion of economically productive people, a preventable loss of precious human capital. This includes countries in which HIV/AIDS is a dominant problem. Four out of five deaths from nutrition-related chronic diseases occur in middle- and low-income countries. Reddy reviewed these data and noted that the current high burden of NCDs is highlighted by the estimates for 1998, which indicate that these disorders contributed to 58.8% of global mortality and 43% of the global burden of disease, measured as disability-adjusted life-years lost. The contribution of low- and middle-income countries to this burden is large; approximately 77% of the total mortality and 85% of the total burden of disease attributable to NCDs arise from these countries.

The burden of cardiovascular disease alone is now far greater in India, and also in China, than in all economically developed countries in the world combined. Low-income communities are especially vulnerable to nutrition-related chronic diseases, which are not just diseases of affluence. CVD, cancer, diabetes, neuropsychiatric ailments, and other chronic diseases are becoming major contributors to the burden of disease, even as infections and nutritional deficiencies are receding as leading contributors to death and disability.

Furthermore, CVD in the developing world emerges at an earlier age. As Reddy notes, in 1990, 46.7% of CVD-related deaths in developing countries occurred below the age of 70 years, in contrast to only 22.8% in the high-income industrial countries. The Global Burden of Disease Study projected that 6.4 million deaths would occur due to CVD in the developing countries in 2020, in the age group of 30–69 years.

A World Health Report has updated this analysis and focuses on the important role of obesity and CVD and cancer deaths in the developing world. There are major differences in the profiles of the CVD epidemic across the developing world. For instance, hypertension and stroke are more likely to emerge in east Asia, whereas diabetes occurs earlier in south

Asia. As would be expected from the dietary and obesity data noted previously, CVD levels are far greater in urban areas of the developing world, but often the opposite is true in the developed higher income countries.

Social Burden of Changes in Diet, Body Composition, and Health

In higher-income countries, increasingly higher-income groups are following a more healthful lifestyle, whereas the poor are not. Thus, higher-income Americans consume a more healthful diet pattern, exercise more, and smoke less, and similar patterns can be found in other high-income countries. In contrast, the prevailing opinion has been that the opposite is found in the developing world, namely that the poor are less likely to have a heavy burden of NR-NCDs compared with the rich. This is changing rapidly. It has been shown that obesity has declined among the better educated and increased among the lower educated in southeastern Brazil. It has also been shown that not only are less healthful dietary patterns consumed by higher income Chinese, but also other dimensions of lifestyle (inactivity, smoking, and drinking) are poorer among the higher SES Chinese. In other research, scholars of China have shown a rapid shift in food consumption patterns among different income groups in China that seems to indicate a shift in the burden of poor diets toward the poor in China. It has been shown that for countries with a GNP per capita of more than \$2500, the likelihood is very high that there will be more obesity among the lower SES groups compared with higher SES groups.

The Future

Consuming a more tasteful and richer diet is a goal of most of the world's population. As shown here, dietary change is universal. In particular, a rapid change is being seen in the poorest areas of the world. The challenge is to learn how to continue to improve the palatability and quality of our diet while doing so in a more healthful manner.

See also: Dental Disease: Etiology and Epidemiology. Dietary Intake Measurement: Methodology. Famine: Causes, Consequences, and Responses. Fats and Oils. Obesity: Definition, Etiology, and Assessment

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NUTS AND SEEDS

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In botanical terms, the word ‘nut’ is used to describe a wide range of seeds, mostly from trees, with a tough, often lignified, seed coat, or shell. True nuts include the chestnut, brazil nut, and hazelnut. In practice, these are usually classified together with certain other so-called nuts, for example the almond, cashew, and peanut, and other seeds, which are all used in similar ways in the diet. Nuts and seeds come from a diverse range of different plants, so their nutritional composition is quite varied, but like most plant seeds they contain a food reserve designed to meet the needs of the developing plant embryo. In many nuts and seeds this is fat, but in others it is starch or other polysaccharides. Therefore, these foods are concentrated sources of dietary energy, as well as sources of protein, unsaturated fatty acids, various micronutrients, and fiber (nonstarch polysaccharides (NSP)).

Nuts and seeds have a wide range of uses. In the typical Western omnivorous diet they tend to be used either as snack items or added as minor ingredient to savory and sweet dishes, but they have wider applications in vegetarian diets as important sources of protein and other nutrients. Certain nuts and seeds are also made into spreads, for example peanut butter and tahini (sesame seed spread).

Types

The major types of nuts and seeds grown for human consumption are shown in Table 1.

Nuts

Almond

The almond (*Prunus amygdalis* var. *dulcis*), sometimes called the sweet almond, is one of the oldest nut crops. It is believed

to have originated in Southeast Asia but is now grown more widely, including in southern Europe, Africa, southern Australia, and California. It is closely related to peaches and plums, but in the almond, in contrast to these other fruits, the ‘flesh’ or mesocarp becomes hard and dry as it matures, and splits open to leave the thin shell or endocarp, which contains the edible almond seed or ‘nut.’ The nuts are eaten fresh, often in the ground form in prepared dishes, as well as roasted and salted.

Another species, *Prunus amara* or the bitter almond, is inedible but is cultivated for its oil, which is also present in the sweet almond and in the kernels of apricots and peaches. This oil contains benzaldehyde, the essential oil, and hydrocyanic acid, from which the benzaldehyde is separated to be used in flavorings and perfumes.

Brazil Nut

The triangular-shaped Brazil nut (*Bertholletia excelsa*) grows in large forests in the Amazon river basin in South America. The nuts are actually hard-shelled seeds, which are produced in groups of between 12 and 30 within a large, hard, thick-walled woody fruit or pod. The sweet-tasting nut meat is consumed in the fresh state and Brazil nut oil may be extracted for use as a lubricant.

Cashew Nut

The cashew (*Anacardium occidentale*) originated in Brazil but is now cultivated extensively in all tropical areas, notably in India and East Africa. The cashew fruit, which contains the seed or ‘nut,’ hangs at the end of what is referred to as the cashew ‘apple’ – the edible swollen fruit stem or pedicel. The fruit itself is kidney-shaped, about the size of a large bean, and has a two-layered shell. The outer layer of this shell contains a caustic oil that must be burned off before the nut is touched. The nuts are then roasted again or boiled to remove other toxic substances and the second shell is removed. The nuts may also be used as a source of oil.

Chestnut

The sweet or Spanish chestnut (*Castanea sativa*) is a native tree of southern Europe, believed to have been introduced into Britain by the Romans. The fruit consists of two to four compartmentalized seeds or burrs, covered with numerous needle-sharp branched spines and containing the seeds or

Table 1 Major types of nuts and seeds grown for human consumption

Almond	Pecan
Brazil	Pine nuts
Cashew	Pistachio
Chestnut	Walnut
Coconut	Pumpkin seeds
Hazelnut	Sesame seeds
Macadamia	Sunflower seeds
Peanut	

'nuts,' which are covered with a tough outer coat. The flesh of the nut is hard and inedible and is cooked, often by roasting or boiling, before being eaten. The cooking process changes the texture so that the chestnut becomes much softer than other nuts and more like a vegetable, largely as a result of its high carbohydrate content (see below).

Coconut

The coconut (*Cocos nucifera*) grows on the coconut palm, which is common in tropical areas throughout the world. The native origin of the palm is uncertain, as the nuts were easily dispersed between both islands and continents by ocean currents and by early explorers. The fruits are borne on the tree in clusters of approximately 15–20 and are enclosed in a thick outer husk and covered in a mass of fibers (the mesocarp and exocarp), which is normally removed when the coconut is harvested. The familiar hard shell of the coconut is the endocarp, or inner layer, of the mature ovary of the fruit, and within the shell is the actual seed, covered with a thin brown seed coat. The white coconut 'meat,' which can be eaten either fresh or desiccated, is actually part of the endosperm (storage tissue) of the seed. Coconut 'milk,' which is found in the unripe nut and is drunk or used in cooking, is the liquid form of the endosperm, which solidifies as the fruit ripens. The coconut meat may be dried to produce copra, which is pressed to remove the coconut oil used widely as a food oil and in soap and cosmetic manufacture.

Hazelnut (Cobnut; Filbert)

The most widely grown hazelnut (*Corylus avellana*) is a native of Europe, although approximately 10 different species of *Corylus* grow throughout Europe, North America, and Asia. There is evidence that these nuts were cultivated in Ancient Greece and collected by Mesolithic peoples. The shell of the hazelnut is the matured ovary wall of the flower and the edible nut meat within this is the matured embryo.

Macadamia Nut

The macadamia nut (*Macadamia integrifolia*, smooth-shelled; *Macadamia tetraphylla*, rough-shelled) is native to eastern tropical Australia but was subsequently introduced to Hawaii, which is now the leading producer of these nuts, and also to parts of Africa and South America. It is the smooth-shelled variety that has been developed commercially. The edible kernel of the nut is the seed, consisting mostly of the cotyledons of the embryo. It is enclosed in a hard, thick, brown shell, which is itself encased in a fibrous husk that splits open when the husk dries. This occurs after the fruit falls, or when it is removed from the tree at maturity. After harvesting, the nuts are dried (to a moisture level of 1.5%), roasted (traditionally in coconut oil, or dry-roasted), and salted.

Peanut

The peanut (*Arachis hypogaea*), sometimes referred to as the ground nut or monkey nut, originated in South America.

Although referred to as a nut, it is in fact part of the legume family. The plant was introduced to Africa by early European explorers and to North America by the slave trade; it was also introduced to India and China. The name 'ground nut' derives from the fact that the flower withers after pollination to leave a stalk-like part of the plant, which pushes under the soil and carries the fertilized ovules in its tip. Underground, the tip continues to develop into the characteristic pod of the peanut, containing the seeds, or 'nuts.' The shape and size of the pod, as well as the number and color of the seeds, are variable, depending on the peanut cultivar. On a worldwide basis, two-thirds of the peanut crop is crushed for oil (arachis oil) and peanut products are used widely in both food processing, with peanut butter as an important product, and for animal feed. The peanut itself may be eaten fresh or roasted and salted.

Pecan

The pecan (*Carya illinoensis*) is a member of the walnut family, and the tree is classified botanically as a hickory. The tree is a native of North America, grown in the southern central states. After harvesting, the nuts are air-dried to remove 10–20% of their moisture. The nut is similar to the walnut, but with a more mild and sweet flavor. The pecan nut kernel is eaten fresh and in the US it is used widely in confectionery and baked goods.

Pine Nuts

Pine nuts or kernels are small edible seeds, which are extracted from the cones of various species of pine. The most commonly eaten variety is that from the European stone pine (*Pinus pinea*), which is native to northern Mediterranean regions. The small, oil-rich seeds are encased in a hard shell. The seeds are sometimes referred to as pignolia nuts, whereas the seeds of the pinyon pines (*Pinus edulis* and *Pinus monophylla*), which grow in the south-western US and in northern Mexico, are known as pinon nuts.

Pistachio Nut

The pistachio nut is the seed of the pistachio tree (*Pistacia vera*). It is a native of central Asia, Pakistan, and India, where it was cultivated 3000 years ago, and it has also been cultivated for many years in Mediterranean regions and more recently in California. The pistachio fruit is similar to a peach; the outer 'husk' (the exocarp and mesocarp of the fruit) encloses a hard but thin off-white shell (the endocarp). This splits open just before the nut matures to reveal the edible embryo, which consists mainly of two green cotyledons covered in a thin seed coat. The green nut kernels are highly prized and are eaten roasted and salted as well as in various Middle Eastern dishes.

Walnut

The walnut (*Juglans* spp.) is the common name given to approximately 20 species of trees in this family. The most important species is *Juglans regia* – the English or Persian walnut – which is believed to have originated in Ancient Persia, later

taken to Greece, and eventually distributed throughout the Roman empire. There are records of its growth in England in the sixteenth century. It was taken to America and called the English walnut to distinguish it from the native American black walnut (*Juglans nigra*) and the butternut (*Juglans cinerea*), both of which have much thicker, less brittle shells. The walnut fruit has an outer leathery husk and an inner furrowed stone, which is the shell of the nut, within which is the edible seed.

Seeds

Pumpkin Seeds

The large flat seeds of the members of the pumpkin family (*Cucurbita maxima*, *Cucurbita moschata*, and related species) can be dried and eaten raw, used in both sweet and savory cooked dishes, or roasted.

Sesame Seeds

The sesame plant (*Sesamum indicum*), which is a native of Africa, grows in tropical and subtropical regions and is now common in Asia. The seeds are small and off-white in color. They may be eaten whole or used in confectionery and baked goods and as a source of oil used in cooking. The seeds are also ground to a paste called tahini.

Sunflower Seeds

The sunflower (*Helianthus annuus*) is a member of the Compositae or daisy family. It is believed to have originated in North America, where it was cultivated by the native Indians, and was introduced to Europe in the sixteenth century. The flat seeds may be dehusked and eaten raw or cooked, but the plant is generally cultivated for the oil they contain, which is a rich source of polyunsaturated fatty acids (see below), and is

widely used for cooking and in margarine manufacture. The residual oil-cake is used for animal feed.

Macronutrient Content

Green nuts, as harvested, may contain 50% or more water, but these nuts must be cured or semidried for storage, so the moisture content of most nuts, as eaten, is low (1–6%). The exceptions are fresh coconut and chestnuts, with a moisture content of 45% and 52%, respectively. The water, macronutrient, and energy content of the nuts and seeds discussed in this article are shown in Table 2.

Fat

The total fat content of most nuts and seeds is high because, as the seed ripens, the fat store increases and its starch content declines. However, the amount of fat is quite variable, ranging from approximately 78% in the macadamia nut and 70% in the pecan to approximately 50–55% in nuts such as the almond, cashew, hazelnut, and pistachio, and as low as 3% in chestnuts. The fat content of the edible seeds is between 45% and 60%.

The different fatty acid fractions contained in these nuts and seeds are also quite variable, as shown in Table 3. The vast majority of nuts and seeds are rich in monounsaturated and polyunsaturated fatty acids. However, in some nuts, such as the peanut, hazelnut, and macadamia nut, monounsaturated fatty acids predominate, whereas in the walnut and in sunflower seeds polyunsaturated fatty acids predominate. The exception is the coconut, in which saturated fatty acids constitute the major fat fraction.

Carbohydrate

With the exception of the starch-rich chestnut (almost 37% carbohydrate), the carbohydrate content of most nuts is

Table 2 Water, macronutrient, and energy content of selected nuts and seeds (per 100 g, kernel only)

	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Energy	
					kJ	kcal
Almond	4.2	21.1	55.8	6.9	2534	612
Brazil	2.8	14.1	68.2	3.1	2813	682
Cashew	4.4	17.7	48.2	18.1	2374	573
Chestnut	51.7	2.0	2.7	36.6	719	170
Coconut	45.0	3.2	36.0	3.7	1446	351
Hazelnut	4.6	14.1	63.5	6.0	2685	650
Macadamia (salted)	1.3	7.9	77.6	4.8	3082	748
Peanut	6.3	25.6	46.1	12.5	2341	564
Pecan	3.7	9.2	70.1	5.8	2843	689
Pine nuts	2.7	14.0	68.6	4.0	2840	688
Pistachio (roasted, salted)	2.1	17.9	55.4	8.2	2485	601
Walnut	2.8	14.7	68.5	3.3	2837	688
Pumpkin seeds	5.6	24.4	45.6	15.2	2360	569
Sesame seeds	4.6	18.2	58.0	0.9	2470	598
Sunflower seeds	4.4	19.8	47.5	18.6	2410	581

Source: Data from Holland B, Unwin ID, and Buss DH (eds.) (1992) *Fruit and Nuts. The First Supplement to McCance & Widdowson's The Composition of Foods*, 5th edn. London: The Royal Society of Chemistry.

Table 3 Total fat and fatty acid composition of selected nuts and seeds (g per 100 g, kernel only)

	<i>Total fat</i>	<i>Saturated fatty acids</i>	<i>Monounsaturated fatty acids</i>	<i>Polyunsaturated fatty acids (total)</i>	<i>cis n-6 Polyunsaturated fatty acids</i>	<i>cis n-3 Polyunsaturated fatty acids</i>
Almond	55.8	4.7	34.4	14.2	13.3	0.1
Brazil	68.2	16.4	25.8	23.0	22.9	0.1
Cashew	48.2	9.5	27.8	8.8	— ^a	— ^a
Chestnut	2.7	0.5	1.0	1.1	1.0	0.1
Coconut	36.0	31.0	2.0	0.8	0.5	0
Hazelnut	63.5	4.7	50.0	5.9	5.4	0.1
Macadamia (salted)	77.6	11.2	60.8	1.6	— ^a	— ^a
Peanut	46.1	8.2	21.1	14.3	— ^a	— ^a
Pecan	70.1	5.7	42.5	18.7	16.0	0.7
Pine nuts	68.6	4.6	19.9	41.1	— ^a	— ^a
Pistachio (roasted, salted)	55.4	7.4	27.6	17.9	— ^a	— ^a
Walnut	68.5	5.6	12.4	47.5	— ^a	— ^a
Pumpkin seeds	45.6	7.0	11.2	18.3	— ^a	— ^a
Sesame seeds	58.0	8.3	21.7	25.5	23.6	0.4
Sunflower seeds	47.5	4.5	9.8	31.0	— ^a	— ^a

^aNo data available.

Source: Data from Holland B, Unwin ID, and Buss DH (eds.) (1992) *Fruit and Nuts. The First Supplement to McCance & Widdowson's The Composition of Foods*, 5th edn. London: The Royal Society of Chemistry.

relatively low at approximately 3–7%. However, peanuts, cashews, pumpkin, and sunflower seeds contain more carbohydrate (13–19%). In most nuts and seeds this carbohydrate is a variable mixture of starch and sucrose, although in some there are small quantities of glucose and fructose as well, and in sunflower seeds there are some oligosaccharides.

Protein

The protein content of nuts is quite variable, but most nuts are considered to be a good source of protein. It is low (2–3%) in the chestnut and coconut, between 8% and 15% for most other nuts, but high (18–26%) in the cashew, pistachio, almond, and peanut, so that the amount of protein in many nuts is about the same as in meat, fish, or cheese. Pumpkin, sesame, and sunflower seeds are also rich in protein.

However, the proportions of indispensable amino acids in any one particular type of nut or seed, and in fact all plant foods, differ from those needed in the human diet, with one or sometimes more 'limiting amino acids'. In most nuts and seeds, with the exception of pistachio nuts and pumpkin seeds, it is lysine that is the limiting amino acid. Thus, although the total amount of protein in nuts and seeds may be high, these foods must be complemented by other sources of plant protein, such as legumes, and/or animal sources of protein (meat, fish, eggs, milk, and cheese), to ensure that the overall protein quality of the diet is adequate.

Micronutrient Content

The vitamin and mineral contents of the nuts and seeds discussed in this article are shown in Tables 4 and 5, respectively.

In general, nuts and seeds are a good source of the B vitamins, including folic acid, and of the tocopherols (vitamin E), although some, such as almonds, hazelnuts, and sunflower

seeds, contain much more vitamin E than others. Nuts and seeds do not contain vitamin C, and many nuts have little or no vitamin A activity.

Nuts and seeds contain quite large amounts of many minerals. In particular, many nuts and seeds, especially sesame seeds, are good sources of calcium. They are also generally rich in potassium, magnesium, phosphorus, iron, and in trace elements such as copper, zinc, manganese, and others such as chromium. Brazil nuts are particularly rich in selenium.

Fiber Content

Compositional values for the total amount of fiber (nonstarch polysaccharides (NSP)), and the different fiber fractions where available, are shown in Table 6 for the nuts and seeds discussed in this article. It can be seen that nuts and seeds contain significant amounts of fiber, similar to the amounts found in vegetables and fruit. Although nuts and seeds do contain some soluble fiber, most of the fiber in these foods is of the insoluble type, much of which is cellulose. Of the insoluble non-cellulosic polysaccharides, arabinose predominates in most nuts, although the coconut contains large quantities of mannose. Most nuts and seeds are likely to contain quite large amounts of lignin, particularly those with a tough seed coat such as sesame seeds, although actual values are not available.

Toxins and Contaminants

Phytic Acid

Phytic acid (*myo*-inositol hexaphosphoric acid) is present in all seeds, where it is believed to act as a store of phosphate and trace elements for the developing plant embryo. The phytate content of the commonly eaten nuts and seeds is variable. In

Table 4 Vitamin content of selected nuts and seeds (per 100 g, kernel only)

	Carotene (μg)	Vitamin E (mg)	Thiamin (mg)	Riboflavin (mg)	Niacin (mg)	Vitamin B ₆ (mg)	Folate (μg)
Almond	0	23.96	0.21	0.75	3.1	0.15	48
Brazil	0	7.18	0.67	0.03	0.3	0.31	21
Cashew	6	0.85	0.69	0.14	1.2	0.49	67
Chestnut	0	1.20	0.14	0.02	0.5	0.34	N ^a
Coconut	0	0.73	0.04	0.01	0.5	0.05	26
Hazelnut	0	24.98	0.43	0.16	1.1	0.59	72
Macadamia (salted)	0	1.49	0.28	0.06	1.6	0.28	N
Peanut	0	10.09	1.14	0.10	13.8	0.59	110
Pecan	50	4.34	0.71	0.15	1.4	0.19	39
Pine nuts	10	13.65	0.73	0.19	3.8	N	N
Pistachio (roasted, salted)	130	4.16	0.70	0.23	1.7	N	58
Walnut	0	3.85	0.40	0.14	1.2	0.67	66
Pumpkin seeds	230 ^b	N	0.23	0.32	1.7	N	N
Sesame seeds	6	2.53	0.93	0.17	5.0	0.75	97
Sunflower seeds	15	37.77	1.60	0.19	4.1	N	N

^aNutrient present in significant quantities but no reliable information available on the amount.

^bEstimated value.

Source: Data from Holland B, Unwin ID, and Buss DH (eds.) (1992) *Fruit and Nuts. The First Supplement to McCance & Widdowson's The Composition of Foods*, 5th edn. London: The Royal Society of Chemistry.

Table 5 Mineral and trace element content of selected nuts and seeds (per 100 g, kernel only)

	Sodium (mg)	Potassium (mg)	Calcium (mg)	Magnesium (mg)	Phosphorus (mg)	Iron (mg)	Copper (mg)	Zinc (mg)	Manganese (mg)	Selenium (μg)
Almond	14	780	240	270	550	3.0	1.00	3.2	1.7	4
Brazil	3	660	170	410	590	2.5	1.76	4.2	1.2	1530 ^a
Cashew	15	710	32	270	560	6.2	2.11	5.9	1.7	29
Chestnut	11	500	46	33	74	0.9	0.23	0.5	0.5	Tr
Coconut	17	370	13	41	94	2.1	0.32	0.5	1.0	1 ^b
Hazelnut	6	730	140	160	300	3.2	1.23	2.1	4.9	Tr
Macadamia (salted)	280	300	47	100	200	1.6	0.43	1.1	5.5	7
Peanut	2	670	60	210	430	2.5	1.02	3.5	2.1	3
Pecan	1	520	61	130	310	2.2	1.07	5.3	4.6	12
Pine nuts	1	780	11	270	650	5.6	1.32	6.5	7.9	N ^c
Pistachio (roasted, salted)	530	1040	110	130	420	3.0	0.83	2.2	0.9	6 ^b
Walnut	7	450	94	160	380	2.9	1.34	2.7	3.4	19
Pumpkin seeds	18	820	39	270	850	10.0	1.57	6.6	N	6b
Sesame seed	20	570	670	370	720	10.4	1.46	5.3	1.5	N
Sunflower seeds	3	710	110	390	640	6.4	2.27	5.1	2.2	49 ^b

^aRange, 230–5300 μg per 100 g.

^bEstimated value.

^cNutrient present in significant quantities but no reliable information available on the amount.

Source: Data from Holland B, Unwin ID, and Buss DH (eds.) (1992) *Fruit and Nuts. The First Supplement to McCance & Widdowson's The Composition of Foods*, 5th edn. London: The Royal Society of Chemistry.

general, the oil seeds, such as sesame and sunflower, and a number of the tree nuts, have higher phytate levels than the leguminous peanut, although the oils expressed from the seeds do not contain phytate. The phytate content of the coconut and chestnut is particularly low.

Because of its molecular structure, phytic acid is a highly effective chelator, which forms insoluble complexes with mineral cations. Its presence in plant foods has led to concerns that it may reduce the bioavailability of various dietary minerals and trace elements, including calcium, magnesium, iron, zinc, and copper. Although nuts are rich in iron, there is

evidence that the addition of nuts to a meal can have a substantial inhibitory effect on iron absorption, presumably because of their phytate and polyphenol content. However, it appears that this can be overcome by the addition of a source of vitamin C to the meal, thereby underlining the need to mix different groups of foods within a meal, particularly when plant foods are the main source of nutrition.

The significance of dietary phytate intake to overall mineral nutrition is still uncertain. It is likely that in a mixed diet of animal and plant foods, dietary phytate may be of less significance than among people consuming diets where plant

Table 6 Total dietary fiber, as measured by the Englyst method, and fiber fractions in selected nuts and seeds (g per 100 g, kernels only)

	Total fiber	Fiber fractions			
		Cellulose	Noncellulosic polysaccharide		Lignin
			Soluble	Insoluble	
Almond	7.4 ^a	1.9 ^a	1.1 ^a	4.4 ^a	N ^b
Brazil	4.3	1.6	1.3	1.4	N
Cashew	3.2	0.6	1.6	1.0	N
Chestnut	4.1	1.1	1.3	1.7	N
Coconut	7.3	0.8	1.0	5.5	N
Hazelnut	6.5	2.2	2.5	1.8	N
Macadamia (salted)	5.3	1.4	1.9	2.0	N
Peanut	6.2	2.0	1.9	2.3	N
Pecan	4.7	1.2	1.5	2.0	N
Pine nuts	1.9	N	N	N	N
Pistachio (roasted, salted)	6.1	1.3	2.7	2.1	N
Walnut	3.5	1.1	1.5	0.9	N
Pumpkin seeds	5.3	1.1	1.7	2.5	N
Sesame seeds	7.9	N	N	N	N
Sunflower seeds	6.0	1.4	1.8	2.8	N

^aEstimated value.^bNutrient present in significant quantities but no reliable information available on the amount.

Source: Data from Holland B, Unwin ID, and Buss DH (eds.) (1992) *Fruit and Nuts. The First Supplement to McCance & Widdowson's The Composition of Foods*, 5th edn. London: The Royal Society of Chemistry.

foods are the sole source of nutrition (vegans). Available data suggest that the trace element status of most adult vegetarians is adequate, but because of increased requirements for growth, vegetarian children may be more vulnerable to the reduced bioavailability of minerals and trace elements, notably zinc, which could be a consequence of the ingestion of large amounts of phytate-containing plant foods.

Intolerances/Allergies to Nuts

Intolerances to nuts, or more specifically, allergies to nut proteins, occur in a relatively small minority of people. However, there is an evidence that such adverse reactions have become more common, and the severity of the reaction that occurs in these sensitive individuals means that they must be taken very seriously. Peanuts are the most commonly cited cause of these severe reactions, estimated to affect between 0.1% and 0.2% of the population, but allergic reactions to tree nuts, including Brazil nuts, almonds, hazelnuts, and cashews, and also to sesame seeds, have been reported.

Contaminants

Nuts and seeds may be subject to mold growth during storage if the conditions are inappropriate. Certain molds produce secondary metabolites, which are toxic to humans and

animals, known as the mycotoxins. Of these mycotoxins, the aflatoxins, notably aflatoxin B₁, are produced by three closely related species of mold: *Aspergillus flavus*, *Aspergillus parasiticus*, and *Aspergillus nomius*. These molds may contaminate various food commodities in tropical and subtropical regions, including tree nuts, but one of the most important crops to be affected is the peanut. Aflatoxins are acutely toxic to the liver and may also be involved in the etiology of human liver cancer in certain parts of the world. Ochratoxins, which are produced by other *Aspergillus* species, have also been found to contaminate nuts.

Some species of mold are able to proliferate within growing crops even before they are harvested, forming an endophytic relationship with the plant. This relationship has been found to exist between *A. parasiticus* and peanuts. It appears that when the plant is growing normally, no aflatoxin is produced by the mold, but when the plant is stressed, as occurs in drought conditions, then the mycotoxin may be produced. The concentrations of aflatoxins produced in this way are lower than would ensue from poor postharvest storage, but the economic consequences still may be considerable.

There are regulatory limits for the aflatoxin levels in foods. In the UK, the sale of nuts for direct consumption is prohibited if the aflatoxin content exceeds 4 µg kg⁻¹ or 10 µg kg⁻¹ for nuts, which are to be subjected to further processing before being sold. A proportion of nuts imported into the UK, especially peanuts, are contaminated with aflatoxin. In 1994, 3% of samples examined under a European surveillance program were found to exceed the UK limit. Nonetheless, such findings should be kept in perspective: The numbers are low and their significance in public health terms, relative to other diet-related risks, is small.

Role in the Diet

Nuts and seeds can make a useful contribution to the dietary intake of macronutrients, notably protein and unsaturated fatty acids, micronutrients, dietary fiber, and energy. Although these commodities play a relatively minor role in the average Western diet, they are more important in the diets of Western vegetarians, especially vegans. Even on a worldwide basis, the nutritional contribution of nuts and seeds is relatively small: Plant foods are estimated to supply approximately 65% of edible protein, but only 8% of protein and 4% of total dietary energy is estimated to derive from pulses, oil crops, and nuts.

In the UK, average weekly household consumption of nuts and their products, as recorded by the National Food Survey, is approximately 14 g per capita, with only 11% of households purchasing these commodities; there are no separate data for nuts eaten as out of home snacks. Data from the Dietary and Nutritional Survey of British Adults indicate that average weekly intake of people consuming unsalted nuts and nut mixes is 63 g per week, but again only 12% of the adults surveyed were consuming these commodities. Therefore, even for nutrients, which are present in relatively large amounts in nuts, such as vitamin E, magnesium, and copper, these foods only provide approximately 1% of the average daily intake in the UK.

See also: Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids; Metabolism. Fiber: Physiological and Functional Effects. Folic Acid. Food Safety: Mycotoxins – Occurrence and Toxic Effects. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Protein: Quality and Sources. Trans-Fatty Acids: Health Effects, Recommendations, and Regulations. Vegetarian Diets

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OBESITY

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Childhood Obesity

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Obesity is increasing in prevalence among children in virtually all developed countries. In the United Kingdom, 8.5% (twice the rate of 10 years ago) of 6-year-old and 15% (three times the rate of 10 years ago) of 15-year-old children are obese. Childhood obesity is also increasing in prevalence among the affluent in less well-developed countries. Given that it has been estimated that in Western countries one-third of obese adults were obese in childhood, and that both adult and adolescent obesity carry significant risk of health complications, obesity in childhood is currently seen as a concern for families, communities, and nations.

Body Composition in Childhood and Definition of Childhood Obesity

Obesity is an excess of body fat. However, the percentage of body weight that is fat varies normally throughout childhood (Table 1). The infant is born with modest amounts of fat. More than 50% of the energy in breast milk comes from fat, and young infants lay down fat very rapidly so that in the 4 or 5 months that it takes a normal infant to double birth weight, the weight of fat in the body has tripled. By 6 months of age, infants are increasing weight-bearing activity and fat

deposition slows relative to lean tissue growth. From 1 year onward, there is a natural process of slimming with less fat than lean tissue deposited so that the child of 5 years often has a lower percentage body weight as fat than at any other time in life. This is followed by the 'adiposity rebound,' when fat deposition accelerates only to slow again with the onset of the pubertal growth spurt in males. In pubertal girls, very brief slimming early in the female growth spurt is followed by vigorous fat deposition particularly around the breasts and hips.

Table 1 Percentage of body weight as fat at different ages in childhood

Age (years)	% Body weight as fat	
Birth	11 ^a	
0.3	25 ^a	
1.0	24 ^a	
	<i>Males</i>	<i>Females</i>
5	12.5 ^b	15.3 ^b
10	17.6 ^b	16.0 ^b
15	11.4 ^b	23.3 ^b

^aFomon SJ (1974) *Infant Nutrition*. 2nd edn, Philadelphia: WB Saunders, p. 69.

^bWiddowson EM (1974) Changes in body proportions and compositions during growth.

Assessment of Overweight and Obesity in Childhood

Precise methods of estimating body fat are complicated and expensive. There are no accepted age-related 'norms' for percentage body weight as fat in childhood. For these reasons, anthropometric indices involving weight and height are widely used to estimate relative fatness. Such methods are relatively simple, noninvasive, well tolerated, and can be used in clinical practice and large population studies. However, they provide only indirect measures of fatness.

Weight can be related to height and age in various ways. Until recently, there was no consensus definition of overweight/obesity from weights in relation to height and age. In adults, body mass index (BMI; weight in kg (height in m)⁻²) is used as a proxy for fatness. A BMI > 25 kg m⁻² (overweight) is associated with a significant increase in the risk of mortality and with an increased prevalence of complications of obesity. In childhood, BMI varies with age in a nonlinear fashion. Given that at different ages children tend to retain their growth positions in relation to those of their peers, the International Obesity Task Force has defined childhood overweight and obesity as those points on the BMI centile, or standard deviation, for age distribution charts that, if followed to the age 18, would meet the adult cutoff points for overweight and obesity (BMI, 25 and 30 kg m⁻²). This definition involves no direct assessment of body fat or lean body mass for age. It needs evaluating against other evidence of excessive body fat and the prevalence of complications of obesity, particularly as it presumes a constant prevalence of obesity in childhood at every age, which clinically seems unlikely. Nevertheless, the method does allow the opportunity to compare relative fatness between different studies and to demonstrate changes in population distribution of BMI for age over time.

Waist circumference is widely used in adult assessment of obesity because high waist circumference is associated with increased abdominal fat and increased risk for the morbid complications of obesity in adult life. Consensus regarding cutoff points for normal waist circumference measurements in childhood has not been reached, but high (compared with age-related populations) waist circumferences do seem to predispose to developing obesity comorbidities.

Risk Factors for Childhood Obesity

There is no clear evidence that obese individuals eat more or exercise less than their nonobese peers. Methods of measuring energy intakes and outputs are not precise when used over time and in community settings. The range of normal requirements and normal basal metabolic rates is large and obscures the energy imbalances of individuals. However, for the individual, obesity occurs when energy intake (food) exceeds the energy expenditure (basal metabolism, physical activity, growth, counteracting infection, maintaining body temperature, and thermodynamic action of food).

Familial Obesity

Most studies from developed countries show that approximately 80% of obese children have at least one parent, and 40% have both parents, overweight or obese. Twin and

adoption studies indicate that genetic factors play a role in this family predisposition to obesity, although lifestyles almost certainly also influence familial similarities in habitus. In most cases of familial obesity, there is no recognized genetic explanation or apparent Mendelian inheritance, suggesting a genetic susceptibility expressed in an obesogenic environment.

Socioeconomic and Environmental Deprivation

Although it is the affluent who tend to become obese in countries undergoing industrialization, it is children from socioeconomically deprived environments in Europe and from families in which child care and nurture are poor irrespective of income who show the greatest predisposition to obesity.

Early Feeding

There is no consistent evidence that breast feeding protects children from later obesity. Any associations between breast feeding and a low prevalence of obesity may simply indicate that both obesity and a low prevalence of breast feeding are common in socioeconomically deprived communities. Furthermore, breast feeding is not a passive process but one that involves maternal emotions and close mother-child contact. The process of feeding and recognizing readiness to feed may teach a mother subtle subconscious understanding of her child's needs. Thus, the process of breast feeding may have positive influences on mothers' attitudes to child nurture – attitudes that are less readily acquired through formula feeding. Likewise, studies of early weaning, although occasionally showing evidence of an association with later obesity, are certainly not consistent in finding relationships between weaning practices and later overweight. Early feeding studies can never be double-blind controlled, and differences may only reflect common aspects of nurture rather than specific effects of a particular infant feeding procedure.

Diet and Dietary Change

Studies from several countries suggest that the childhood obesity epidemic has developed despite secular trends toward lower energy intakes by children. These estimates may have failed to account for recent increases in food eaten outside the home in the United Kingdom and other countries. The eating habits of most families in industrialized countries have changed during the past 30 years in ways that seem likely to make it easy for individuals to overeat. Foods are readily available and children have money to buy them. Much advertizing of snack foods is aimed at children. Manufactured foods have varied forms and packaging. Small differences in flavor or appearance may reduce the satiety effect usually associated with eating large amounts of the same food. Most snacks aimed at children and the well-advertised prepared-before-sale meals are energy dense and high in saturated fats, refined carbohydrates, and sugar. In addition, the portion sizes in restaurants and of confectionery items have increased. It is too easy to eat without being aware of energy intake.

Physical Activity

Trends to lower energy expenditure, as well as dietary change, must have significance for the development of obesity.

Opportunities for vigorous physical activity in sport have declined in many schools and communities, but the increase in long periods of almost complete inactivity (such as when watching television) may be having greater effects on children's nutritional status than the loss of relatively brief periods of intense activity. Studies in the United States and Mexico indicate that in adolescent boys obesity increases in proportion to the hours spent watching television.

Characteristics of Obese Children

Children without Recognizable Pathology

Obese prepubertal children are relatively tall for age (many in the upper quartile and most in the upper half of the population distribution for height). Advanced growth may be associated with advanced maturity of bones (advanced bone age), early onset of puberty, and cessation of growth with only average stature in adult life. However, some children remain tall and obese into adult life, and others slim dramatically with the adolescent growth spurt. It is not clear whether obesity drives accelerated maturation or whether obesity is one manifestation of a predisposition to exuberant growth of both lean and fat tissue also expressed by early puberty.

Obesity Associated with Recognized Medical Condition

There are conditions in which obesity is part of a recognized genetic defect, clinical syndrome, or acquired pathological condition (Table 2). Together, these conditions account for only a very small proportion of obese children. With the exception of very rare single gene defects in leptin metabolism, obesity is a secondary feature in these conditions and presentation is usually for some other aspect of the condition. Single gene defects affecting leptin are associated with progressive gross obesity from early life and may respond with dramatic fat loss with leptin treatment. Where obesity is only a part of a spectrum of abnormalities, common associated features are short stature, developmental delay, and craniofacial and other bony abnormalities.

Chromosomal abnormalities are more frequent causes of a predisposition to obesity. Prader-Willi syndrome, due to deletion or uniparental disomy of part of the long arm of chromosome 15, is associated with characteristic facies, small hands and feet with tapering fingers, hypogonadism, early hypotonia, difficulty feeding, and initially failure to thrive. From the second year of life many of these children show voracious appetite, progressive obesity, and negative behavior (stealing food and refusing to follow a diet). Many also commonly have psychodevelopmental problems with moderate mental retardation that exacerbates the difficulties maintaining normal

Table 2 Specific conditions associated with obesity in childhood

<i>Conditions</i>	<i>Inheritance</i>	<i>Clinical example</i>
<i>Congenital conditions</i>		
Congenital obesity	Single gene defect affecting leptin metabolism	Congenital leptin deficiency Leptin receptor defect Prohormone convertase-1 defect Melanocortin-4 receptor defect Peroxisome proliferators activated receptor POMC deficiency
Inherited syndromes associated with childhood obesity	Autosomal dominant Autosomal recessive	Biemond's syndrome Alstrom's syndrome Bardet-Biedl syndrome Biemond's syndrome (some) Carpenter's syndrome Cohen's syndrome
Inherited syndromes affecting mobility	X-linked recessive X-linked recessive Polygenic inheritance	Borjeson-Forssman-Lehmann syndrome Duchenne muscular dystrophy Spina bifida
Inherited disorders of growth	Autosomal dominant	Achondroplasia
Chromosomal abnormalities	Deletion or uniparental disomy for q11-q13 fragment of chromosome 15 Trisomy 21 Abnormalities of sex chromosomes	Prader-Willi-Labhart syndrome Down's syndrome Klinefelter's syndrome Turner's syndrome
<i>Acquired conditions</i>		
	Hormonal abnormalities	Hypothyroidism Growth, hormone deficiency Cushing's syndrome Polycystic ovarian syndrome
	Hypothalamic damage	Hydrocephalus Meningoencephalitis
	Drug treatment	Steroid treatment Sodium valproate

weight for height and age. Gross obesity commonly leads to early death associated with hypoventilation (Pickwickian syndrome) and complications of type 2 diabetes mellitus.

Down's syndrome children are also prone to develop obesity in late childhood and adolescence. This is generally unrelated to recognized pathophysiological explanations for the obesity, although the syndrome is associated with an increased incidence of autoimmune thyroiditis and hypothyroidism (which exacerbates obesity).

Obesity may be an associated feature of other pathology in childhood. Endocrine problems, such as hypothyroidism and Cushing's syndrome, lead to obesity, but linear growth retardation does also, which often draws attention to the problem before obesity is severe. Hypothalamic damage (e.g., hydrocephalus and meningoencephalitis) and problems leading to immobility (e.g., spina bifida and Duchenne's muscular dystrophy) may also predispose to obesity. Nonpathological childhood obesity is usually associated with normal intelligence, relatively tall stature before puberty, and no overt abnormalities, so brief assessment of growth, general health, and intelligence usually distinguishes obese children for whom investigation for possible underlying pathology is required.

Complications of Childhood Obesity

Childhood obesity used to be considered relatively free of serious medical complications compared with adult obesity, although psychological consequences were recognized as common. Today, many obese children and adolescents show evidence of significant pathophysiological changes. Thus, the increasingly gross obesity of children and adolescents in North America, western Europe, and some other affluent societies has become a matter of major public health concern.

Cosmetic Problems

Orthopedic Problems

Flat feet and knock knee, perhaps related to the excess weight and need to internally rotate the knees to accommodate fat thighs when bringing the legs together, are common and can lead to ungainly gait. Slipped upper femoral epiphysis is a more serious problem, which is particularly common in overweight young adolescents and may also be associated with hormonal abnormalities such as hypothyroidism.

Skin Problems

Intertrigo, seborrheic eczema, and thrush are common in the thick heavy skinfolds of severely obese children. Pink or pale cutaneous striae, distinct from the purplish striae resulting from thinning of subcutaneous tissues in Cushing's syndrome, are common on the abdomen and upper limbs and may be a source of embarrassment. Hirsuties (abnormal facial and body hair) occurs particularly in adolescent girls with polycystic ovarian syndrome, which is associated with obesity and insulin resistance. Acanthosis nigricans, a velvety, pigmented, thickening of the skin usually at the back of the neck, is another important marker for insulin resistance, affecting up to 90% of children with type 2 diabetes mellitus.

Psychological Problems

Some overweight/obese children maintain high self-esteem and have little concern about their body image. These children may excel in sports in which their excess weight and tall stature are advantageous. However, many obese and overweight children have low self-esteem, dissatisfaction with their body image, and difficulty with peer relationships. Often, they underachieve at school. For some obese children, psychological problems antedate the obesity. Low self-esteem and difficulty with peer relationships have led to withdrawal, inactivity, and seeking solace in food. For other obese children, however, obesity is the prime cause of their psychological problems. Studies using silhouettes of figures with different body builds show that most children perceive obese silhouettes very negatively, preferring those portrayed by slimmer figures as friends.

Severe Complications

Adult Obesity

The extent to which childhood obesity progresses to adult obesity depends on the ages of children and adults at the time of study, the severity of obesity, the duration of obesity, and the family history of obesity. In one study fewer than 20% of males younger than 17 years of age remained obese as 35-year-old adults, whereas 20–39.9% of females younger than 17 years of age were still obese at 35 years of age. The probability of being obese at age 35 increased with increasing age and increasing BMI in childhood at the time of study. Where there is a strong family history of obesity in adult life, it seems likely that the obese child will follow the family pattern. Progression from child to adult obesity still only accounts for a minority of obese adults, although with the increasing prevalence of childhood obesity, this may change as it is highly unlikely that equal proportions of fat and thin children become obese adults.

Type 2 Diabetes Mellitus and the Metabolic Syndrome

Although hyperinsulinemia has long been recognized from research studies in obese children, overt type 2 diabetes mellitus has been considered a rarity in childhood until recently. Studies in the United States show that among grossly obese children, type 2 diabetes mellitus is now disturbingly common, not only in adolescence but also in children younger than 10 years old. The problem is less common, but certainly present, in Europe also. Although hyperinsulinemia seems most prevalent in obese children from the Indian subcontinent, hyperinsulinemia and overt type 2 diabetes mellitus are also described in obese Caucasian children. 75% of UK children with type 2 diabetes mellitus are overweight and 50% have a family history of type 2 diabetes mellitus. Girls are proportionally more likely (3:2) to develop type 2 diabetes than boys.

The metabolic syndrome (insulin resistance syndrome; syndrome X) is a clustering of problems associated with resistance to insulin and/or hyperinsulinemia that includes obesity, high central (i.e., intra- and peri-abdominal) distribution of fat, hypertension, and dyslipidemia. Females with polycystic ovarian syndrome also show clustering of these features. The criteria for diagnosis of the insulin resistance

syndrome in childhood have not been defined, but some obese children show clustering of extreme values for the parameters of the metabolic syndrome. Hypertension, hyperinsulinemia, and dyslipidemia in obese children are indications for vigorous intervention to prevent morbidity and early mortality.

Pickwickian Syndrome

Very severe obesity may be associated with hypoventilation and upper respiratory obstruction with sleep apnea. (The sleepy fat boy in Charles Dicken's *Pickwick Papers* is the origin of the syndrome's name.) Underventilation leads to increased circulating carbon dioxide levels, which may precipitate pulmonary hypertension and sided heart failure. Rising circulating carbon dioxide levels may result in the respiratory center of the brain ceasing to respond to carbon dioxide buildup and instead responding to falling oxygen levels as stimulus to breathe. Thus, if affected individuals are given oxygen because of increasing cyanosis, the stimulus to breathe may be removed with potentially disastrous consequences.

Management

Goals

Ideally, the goal of fat reduction in obesity should be to restore normal body composition and retain it for the rest of life. However, evidence suggests that morbidity and mortality are reduced with even small reductions in excess fat. Thus, loss of some excess fat and the pursuit of healthy eating and activity may be beneficial even if normal fatness is not restored. Parents and children need realistic guidance on achievable goals and on the time required to achieve them. Fat reduction programs should be sustainable, able to maintain normal linear growth, and follow overall healthy lifestyle practices.

For young children, it may not be necessary to lose weight as the normal rates of weight and height gain mean that keeping weight stationary while linear growth occurs allows children to grow 'into their weight.' However, most children presenting for help with obesity are so overweight that it would require years of static weights for current weights to decrease to normal for their heights. Gradual weight reduction should aim for weight losses of approximately 500–1000 g month⁻¹. Dramatic weight losses suggest excessive energy deficit with perhaps reduced lean tissue deposition, shorter adult height, and potentially reduced peak bone mass. The fattest children are unlikely to ever achieve normal BMI for age and normal fatness, but they need to be encouraged that significant fat reduction will improve their self-image, ability to exercise, and reduce late complications of obesity.

Dietary Management

Treatment must alter energy balance so that energy intakes are less than energy expenditures in metabolism and activity. Diets should be adequate for protein and micronutrients. They should aim to change the quality and energy density of the food eaten more than to reduce the quantity of food eaten, although reducing the amount of snacking will probably be appropriate (Table 3). There is no consistent evidence that

reduction of any particular energy source is more effective than any other in promoting fat loss, so 'balanced diets' conforming to the 'healthy diet' principles of World Health Organization and many governments should be followed.

Physical Activity

More time is spent in relatively minor activity than in strenuous physical activity, so policies that increase energy expenditure in activity must include reductions in sedentary 'activities.' People, not only children, tend to eat more when they are inactive and relaxing rather than when they are occupied and active. Keeping children from being bored or from spending their leisure time watching television, when food can be consumed almost unnoticed, should reduce eating opportunities. Overweight children should be encouraged to take up hobbies in order to keep their minds off eating. Table 4 outlines how their physical activity can be increased without necessarily subjecting them to the often perceived misery of sports and gym (although these should also be encouraged). Embarrassment and fear of ridicule as well as the high energy expenditure required for activity on the sports field are exacerbated by mechanical difficulties associated with gross weight.

Television

It is important to reduce time spent watching television for most of these children. Energy utilization is very low when viewing, and much advertising is aimed at encouraging children to eat foods that are energy dense, high in fat, and of low satiety. Viewing as a family should be encouraged, with the television in the living room rather than in children's bedrooms, so parents are involved in their children's viewing and can advise on the significance and nature of advertisements. Viewing time should be limited, but wise negotiation rather than didactic action will probably be necessary to avoid intrafamily conflict. Indeed, parents should be involved in children's slimming regimens, particularly because so many parents are overweight. Many children who watch television express preference for other activities but indicate that they are not given the opportunities to participate in other activities. Children cannot be expected to implement slimming behavior if an obesogenic family lifestyle continues unchanged around them.

Very severe childhood obesity (particularly if accompanied by a life-threatening complication such as Pickwickian syndrome) may require more dramatic interference than described previously. Very low-energy diets have been used quite successfully for short-term weight reduction. However, such diets are intrusive, carry some risk for nutrition and growth, and unacceptable to many obese. No drugs are currently approved for treatment of obesity in childhood. Drug treatment has not been associated with notable successes in the past.

Prevention of Obesity in Childhood

The prevention of obesity involves creating lifestyle changes at the family, school, community, and national level.

Table 3 Management of childhood obesity: Dietary measures

<i>Purpose of action</i>	<i>Policy</i>	<i>Action</i>
Organize eating	Control number of eating events	Restrict eating to recognized meal and snack periods with perhaps two snacks only for children and three snacks for adolescents
	Eat meals, as a family whenever possible, at table rather than in front of the television	Where possible, eat meals prepared at home and served on a plate rather than ready-to-eat, microwaved individual meals
Be aware of the nutrient content of meals	Meals prepared at home	Where possible, prepare meals at home so that the cook at least is aware of the nutritional makeup of the meal
	Precooked/ready-to-eat meals	Read the nutritional information given on the packet and observe not only the content/100 g but also the weight (and thus nutrient content) of the food bought and fed to each member of the family
Reduce the energy content of the food intake	Portion sizes	Portion sizes can be reduced – using smaller plates may make this less obvious; avoid second helpings
	Change the form of food used to low-energy density versions	Use 'low-calorie' margarines, spreads, mayonnaise, yogurts, soups, baked beans, etc. Use semi-skimmed milk, sugar-free fruit squashes, etc. Grill and bake and boil without added fat rather than frying foods
	Avoid added fats and sugars	Do not add fats to vegetables when preparing them for table Avoid (or reduce) added sugar to stewed fruit dishes; sweeteners dissolved in boiled water can be used instead if necessary
Reduce energy content of drinks	Fruit juices, etc.	Eat whole fruit rather than fruit juices (which are usually many fruits compressed and often with added sugar) Use 'low-calorie' fruit squashes Preferably drink water Avoid added sugar
Increase satiety	Tea, coffee, etc.	Try to avoid sweeteners so as to accustom child to less sweet tastes
	Increase intake of foods that require chewing, that take time to eat, or that increase satiety	Increase vegetable, salad, and fruit intake Increase whole-meal cereal intake Encourage 'jacket' potatoes, boiled potatoes rather than chips, crisps, and mashed potatoes
	Take more time over meals	Eat as a family when possible to allow social interaction during eating, slower eating, and thus greater sense of satiety after the meal

Table 4 Management of childhood obesity: Increasing energy expenditure

<i>Purpose</i>	<i>Type of action</i>
Reduce sedentariness	Reduce time spent watching television Develop interests/hobbies that give children things to occupy them at home and that may give them activities outside the home
Increase activity in everyday life	Encourage children to participate in family life by helping parents around homes, doing simple domestic tasks, running up- and downstairs to fetch for other members of family, etc. Walk or cycle rather than go by car whenever possible Use public transport rather than car so at least have to walk to bus stop Use stairs rather than elevators and escalators when practical Walk up escalators Do short walking errands for family as much as possible Send child out into garden for activity when he or she comes home from school before doing homework, etc.
Increase family activity	Make a habit of going for walks, taking part in physical activity in garden or parks, etc. in leisure time Plan activities during holidays and weekends
Encourage and support child to participate in physical activity at school	Obese children may be very successful at swimming (but may be too self-conscious to wear bathing suit) Dancing and aerobics may be more acceptable than contact sports, especially for girls
Increase energy expenditure as heat	Reduce home heating a few degrees to increase need for energy to keep warm in cold weather Encourage family to become accustomed to relatively cool environments

Initiatives need to be affordable and sustainable so that those most at risk of obesity are reached and feel ownership of community programs. **Table 5** suggests changes needed to reduce the obesogenic factors in the current

Westernized environment. If the obesity epidemic is to be halted, governments and international industries have to work with communities to bring about effective change.

Table 5 Possible national and community measures to reduce epidemic of childhood obesity in Western societies

Purpose	Action
Reduce snacking on energy-dense foods	Act to reduce all advertising of energy-dense foods to children Possibly ban advertising to children on television Remove sweetened drinks and confectionery dispensing machines in schools Review foods on sale at school
Increase children's and parents' knowledge of nutrient content of foods	Programs to educate parents and children on interpreting nutrition labels on foods Consider indicating energy content of foods in terms of minutes/hours of activity necessary to balance energy intake from food Practical nutrition teaching in schools
Encourage intake of whole foods, fruits, and vegetables, and home-prepared foods so there is more awareness of content of foods eaten	Consider subsidizing fresh fruits/vegetables and whole-meal cereals and making them more accessible in deprived communities Teach families how to cook rather than purchase ready-to-eat meals
Reduce energy intakes generally	Review nutritional content of school dinners Develop policies to encourage and make consumption of whole foods, fruits, and cereals attractive and fashionable to children
Increase energy expenditure in activity	Increase play areas, safe parks, and playing fields in communities Consider opening school playing fields off hours and on holidays Develop safe integrated community transport systems so children can use public transport Develop bike paths
Increase energy expenditure in heat	Reduce environmental temperature of public places by a few degrees; encourage people to wear more clothes if they find this uncomfortable

See also: Adolescents: Nutritional Problems of Adolescents. **Appetite:** Psychobiological and Behavioral Aspects. **Breast Feeding. Children:** Nutritional Requirements. **Diabetes Mellitus:** Etiology and Epidemiology. **Nutritional Assessment:** Anthropometry; Clinical Examination. **Obesity:** Complications; Definition, Etiology, and Assessment; Prevention; Treatment. **Physical Activity:** Beneficial Effects. **Weight Management:** Approaches

Further Reading

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Complications

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Glossary

Adipokines Cytokines present in adipose tissue.

Adiposis dolorosa Painful fat syndrome.

Dyslipidemia Abnormal blood fats.

Hyperinsulinemia High blood insulin.

Visceral fat Intraabdominal adipose tissue.

Obesity is a serious chronic disease associated with complications and comorbidities that involve most systems of the body (Table 1). The common factor in all obese people is the presence of excess adipose tissue stores and an increased percent body fat. Even in the absence of complications and comorbidities, obesity increases the risk of early mortality. The estimates of obesity-related excess deaths in the USA each year range from 125 000 to 400 000. In addition to the medical complications, obesity is associated with psychological and social problems that may overshadow the medical problems in the quality of life for many obese people. This article will discuss the role of distribution of body fat in the complications of obesity, then will summarize the major categories of complications and comorbidities.

Role of Distribution of Body Fat in the Complications of Obesity

The distribution of excess adipose tissue contributes to many of the complications of obesity. Obese individuals may be divided into those whose excess fat predominantly is deposited in the upper body versus those with increased lower body obesity, or both upper and lower body obesity. Upper body obesity may be localized to the subcutaneous space versus the intraabdominal space (visceral fat). Waist circumference and the ratio of waist-to-hip circumferences correlate with the morbidity and mortality of obesity. Individuals with increased visceral fat, as measured by the cross-sectional area on computerized tomography (CT) or magnetic resonance imaging (MRI) scanning, are at greater risk for systemic complications of obesity compared to people with fat localized to abdominal subcutaneous depots or to the lower body. The mechanisms of these differences are not clear, but research has shown that visceral fat has a higher triglyceride turnover rate and releases greater amounts of fatty acids into the circulation than do other adipose tissue depots. Also, visceral fat has a higher concentration of adipokines (cytokines), substances that affect function of the immune system. Because blood vessels from the visceral fat drain into the portal vein, some investigators postulate that exposure of the liver to high levels of free fatty acids or these adipokines produces insulin resistance, which is known to be correlated with many of the complications of obesity described below. There are significant racial differences in deposition of visceral fat. Asians and Hispanics tend to selectively deposit fat in the

abdominal cavity with excess energy intake whereas Blacks have less visceral fat than other groups.

Metabolic and Organ System Complications of Obesity

Obesity is a syndrome that resembles premature aging. Multiple metabolic, hormonal, and organ system dysfunctions occur in aging. Similar changes occur in obesity, but at an earlier age. Below we will review generalized metabolic changes that occur with obesity, and then examine individual organ systems.

Metabolic Syndrome

The term 'metabolic syndrome' has been given to a cluster of abnormalities that classically includes insulin resistance, glucose intolerance, hypertension, and dyslipidemia. Several other abnormalities such as sleep apnea, gout, and pseudotumor cerebri have been associated with insulin resistance and the 'metabolic syndrome.' The classic abnormalities will be reviewed below and the other abnormalities later in the article.

Type 2 Diabetes

Type 2 diabetes mellitus (DM): a strong association of obesity with the prevalence of Type 2 DM is well documented. The risk of developing Type 2 DM increases with the degree and duration of obesity; as much as 50-fold with severe obesity. The US National Diabetes Commission reported that the risk of diabetes doubles for every 20% of excess body weight. The risk of Type 2 DM is greater with visceral obesity. Type 2 DM is frequently associated with other complications such as hypertension and dyslipidemia, resulting in additive risks for atherosclerosis and cardiovascular disease. Poor glycemic control in Type 2 DM may lead to severe microvascular complications, including nephropathy, retinopathy, and neuropathy. Weight loss is a very effective treatment for Type 2 DM and can prevent the onset of Type 2 DM in susceptible individuals. Type 2 DM, once extremely rare in children, has increased greatly in prevalence with the obesity epidemic.

Insulin Resistance and Hyperinsulinemia

Insulin resistance refers to the phenomenon of insensitivity of the body cells to insulin's actions. Insulin resistance is usually

Table 1 Complications of obesity

1. Metabolic complications
 - a. Metabolic syndrome
 - b. Noninsulin-dependent diabetes
 - c. Insulin resistance, hyperinsulinemia
 - d. Dyslipidemia
 - e. Gout
 - f. Abnormalities of hormones and other circulating factors:
 - 1) Growth hormone (GH)
 - 2) The hypothalamic-pituitary-adrenal (HPA) axis
 - 3) Cytokines
 - 4) Renin-angiotensin system
 - 5) Leptin
 - 6) Ghrelin
2. Diseases of organ systems
 - a. Cardiac and vascular diseases
 - 1) Coronary heart disease (CHD)
 - 2) Hypertension
 - 3) Congestive heart failure
 - 4) Cerebrovascular disease
 - 5) Thromboembolic disease
 - b. Respiratory system abnormalities
 - 1) Obesity-hypoventilation syndrome
 - 2) Sleep apnea
 - c. Digestive system abnormalities
 - 1) Gallbladder disease
 - 2) Hepatic disease
 - d. Reproductive system abnormalities
 - 1) Hormonal complications: males
 - 2) Hormonal complications: females
 - 3) Obstetric complications
 - e. Nervous system
 - 1) Pseudotumor cerebri
 - 2) Adiposis dolorosa
 - 3) Alzheimer disease
 - f. Immune system dysfunction
 - g. Skin diseases
 - h. Eye disease
3. Cancer
 - a. Breast
 - b. Uterus
 - c. Gallbladder
 - d. Colon
 - e. Prostate
 - f. Others
4. Mechanical complications of obesity
 - a. Arthritis
 - b. Increased intraabdominal pressure
5. Surgical complications:
 - a. Perioperative risks: anesthesia, wound complications, infections
 - b. Incisional hernias
6. Psychosocial complications
 - a. Psychological complications
 - b. Social complications
 - c. Economic impact

associated with hyperinsulinemia. Different tissues may have differential insulin sensitivities. For example, adipose tissue may be more sensitive to insulin than muscle tissue, thus favoring the deposition of fatty acids in adipose tissue and diminished fatty acid oxidation in muscle. There is a reduced efficiency of insulin to inhibit hepatic glucose production and

stimulate glucose use in skeletal muscle and adipose tissue that leads to hyperglycemia.

Hyperinsulinemia is an independent marker that predicts the development of atherosclerosis. A causal relationship between hypertension and hyperinsulinemia has not yet been well established. Hypertension associated with hyperinsulinemia could be due to increased renal sodium retention, increased intracellular free calcium, increased sympathetic nervous system activity, or increased intraabdominal pressure because of increased visceral fat deposition.

The mechanisms of insulin resistance with increasing obesity are not clear, but increased production of cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) are thought to play a role. Basal insulin levels increase with the degree of overweight, perhaps due to increased insulin secretion or reduced clearance by the liver. A reduced receptor number or post insulin-receptor defects may play a role in insulin resistance. Both basal hyperinsulinemia and insulin resistance decrease with weight reduction.

Dyslipidemia

Obesity, particularly visceral obesity, is associated with increased serum levels of cholesterol, triglycerides, low-density lipoproteins (LDLs), very-low-density lipoproteins (VLDLs), apolipoprotein B, and reduced levels of high-density lipoprotein-cholesterol (HDL-c). There is a change in the composition of LDLs to increased small, dense LDLs, which are more atherogenic. The lower amounts of HDL-c compromise antiatherogenic function. Every 10% increase in relative bodyweight is associated with a 12 mg dl⁻¹ increase in serum cholesterol concentration. The correlation of serum cholesterol with body mass index (BMI = kg m⁻²) is greater for men than women. Increased serum triglycerides with weight gain may be due to increased intake of fats, hyperinsulinemia, and impaired removal of triglycerides into tissues because of low levels of lipoprotein lipase activity. Insulin resistance promotes lipolysis and increased circulating free fatty acids, which enhance the formation of VLDLs in the liver. Dyslipidemia contributes to increased atherosclerosis in obesity. Weight reduction usually reduces triglycerides, changes the composition of serum cholesterol to less small, dense LDLs, and increases HDL-c. These changes, if maintained over time, may reduce atherosclerosis.

Gout

Serum uric acid and the prevalence of gout correlate positively with BMI. High serum uric acid levels correlate with insulin resistance and an increased risk of atherosclerotic cardiovascular disease in obesity. Serum uric acid levels may temporarily increase with acute weight loss, but usually decrease with large amounts of weight loss. The lower uric acid levels are maintained with continued weight loss.

Abnormalities of Hormones and Other Circulating Factors

Multiple different hormones are altered in obesity. Below are a few that are felt to be important in altering the physiology and contributing to the complications of obesity.

Growth Hormone

Obesity is typically accompanied by a decrease in growth hormone (GH) levels and an increase in GH-binding protein (GHBP) levels. GH is released by the anterior pituitary and affects lipid, carbohydrate, and protein metabolism. An inverse relation exists between GH levels and percent fat mass. GH is lipolytic in adipose tissue. Animal studies show enhanced catecholamine-induced lipolysis and increased beta adrenoreceptors in adipocytes of GH-treated animals. The rises of GH after meals, with sleep, and in response to secretagogues such as arginine or levodopa are blunted in obese people. GH stimulates secretion of insulin-like growth factor-1 (IGF-1). However, IGF-1 is increased in obesity, suggesting a difference in sensitivity to GH. The defects in GH and IGF-1 are reversed by weight reduction.

The Hypothalamic–Pituitary–Adrenal Axis

The hypothalamic–pituitary–adrenal (HPA) axis may be abnormal in obesity, with similarities to patients with Cushing's syndrome such as insulin resistance, impaired glucose homeostasis, hypertension, and lipid abnormalities. High levels of emotional or physical stress are thought to increase cortisol secretion or turnover, and thereby increase visceral obesity. Another potential mechanism involves the peripheral metabolism of cortisol. The enzyme 11 beta-hydroxysteroid dehydrogenase-1 (11 beta-HSD-1), which converts steroid precursors to cortisol, is expressed in adipose tissue. With increasing obesity, more cortisol is derived from cortisone in adipose tissue due to the increased activity of this hormone. Urine studies in obesity also show an increase in the ratio of tetrahydrocortisol to tetrahydrocortisone, indicating a relative increase in the pathways leading to cortisol formation.

Adipokines (Cytokines)

Adipose tissue secretes a number of cytokines such as TNF- α , ILs, plasminogen activator inhibitor-1 (PAI-1), and retinol-binding protein 4 (RBP-4) that may play a role in fat metabolism and insulin resistance. Adipokine secretion is higher with obesity. TNF- α has been shown to alter basal and glucose-stimulated insulin secretion and produce insulin resistance in isolated cell lines. Adipocytes also produce IL-6, 10, and 11, which stimulate C-reactive protein, a systemic marker of inflammation. All of these ILs are increased in obesity. IL-6 and PAI-1 have been postulated to play an etiologic role in the increased risk of thromboembolism observed in obese patients. Plasma IL-8 is increased in normoglycemic obese subjects, and is related to fat mass and TNF- α levels. Circulating IL-8 is also acutely up-regulated by hyperinsulinemia. An increase in circulating IL-8 may be one of the factors linking obesity with greater cardiovascular risk. Adiponectin is the only adipokine whose circulating concentration decreases with increased fat mass and, in line with this, is inversely correlated with the metabolic syndrome.

Renin–Angiotensin System

Several components of the renin–angiotensin system are expressed by the adipose tissue. Angiotensinogen levels are increased and have been linked to hypertension and increased cardiovascular risk in obesity.

Leptin

Leptin is a cytokine made predominantly in the adipose tissue. Leptin binds to receptors in the hypothalamus to signal the state of adipose tissue stores and act by inhibiting neuropeptide Y. Serum leptin levels correlate positively with body fat stores, and are higher in obese people, denoting leptin resistance in obesity. Females have higher serum leptin levels than males, but this association does not appear to be due to estrogen levels. Leptin is found in greater concentrations in abdominal subcutaneous fat compared to visceral fat, and some studies link leptin to regulation of energy expenditure.

Ghrelin and Obestatin

Ghrelin and obestatin come from the same prohormone, preproghrelin. Ghrelin is a potent GH secretagogue that is produced mainly by the stomach and is present in several forms in serum. Administration of ghrelin increases food intake, and ghrelin levels increase with dieting and weight loss. However, serum ghrelin has a negative correlation with percent body fat, so levels in obese people are lower than in lean. Obestatin is thought by some to have an anorexic effect but its function and the interplay with ghrelin is not well understood.

Diseases of Organ Systems

Atherosclerotic and Arteriosclerotic Vascular Diseases

Diseases of the vascular system make the greatest contribution to the increased mortality associated with obesity. In both sexes, the excess mortality due to vascular disease increases linearly with BMIs greater than 25 kg m⁻². The vascular complications of obesity can be categorized into four major groups:

1. Coronary heart disease (CHD)
2. Hypertension
3. Congestive heart failure
4. Cerebrovascular disease

Coronary Heart Disease

Longitudinal studies show a positive correlation of BMI with CHD, and obesity is an independent predictor of CHD. However, in the presence of other risk factors such as hypertension, lipid abnormalities, and insulin resistance, all of which are increased by obesity, the risk of atherosclerotic CHD increases dramatically. Weight loss reduces all of these risk factors associated with cardiovascular disease, but there are only few long-term studies of changes in cardiovascular mortality due to weight loss. A very low-fat diet (10% of total calories as fat) has been shown to reduce the size of atherosclerotic plaques in coronary arteries.

Hypertension

The prevalence of hypertension among overweight adults in the USA is 2.9 times higher than that of nonoverweight individuals. Every 10 kg increase in bodyweight is associated with increases of 3 and 2 mm Hg in systolic and diastolic blood pressures, respectively. Persistent hypertension can

contribute to the development of left ventricular hypertrophy, coronary ischemia, and stroke.

The etiology of the association between hypertension and obesity is unclear. Some of the mechanisms offered to explain the association of obesity and hypertension are:

1. Hyperinsulinemia due to insulin resistance leading to increased renal reabsorption of sodium.
2. Sodium and fluid retention due to a decreased renal filtration rate, increased intraabdominal pressure, or increased plasma renin activity.
3. Increased sympathetic nervous system activity.
4. Increased cytokines and inflammation.

Except in long-standing cases, weight reduction is usually accompanied with a decrease in blood pressure. The reductions in blood pressure with weight loss are not dependent on decreases in salt intake. Many studies have shown that even modest weight losses, in the range of 5–10% of initial body-weight, may produce reductions or even normalization of blood pressure in obese individuals.

Congestive Heart Failure

Total blood volume increases with excess body weight. Higher oxygen consumption in obesity and increased blood flow to the splanchnic bed and adipose tissue increase cardiac output. Also, the transverse diameter of heart, thickness of the posterior wall, and thickness of the interventricular septum increase with body weight. Left ventricular mass is a stronger predictor of morbidity and mortality than blood pressure. A combination of these factors may result in the congestive heart failure seen in severely obese people. The heart rate, stroke volume, blood volume, cardiac output, and left ventricular work return toward normal with weight reduction. One study that compared weight loss by dieting to treatment with anti-hypertensive drugs demonstrated a greater improvement in cardiac hypertrophy with weight loss, despite similar reductions in blood pressure.

Cerebrovascular Disease

Obesity-related atherosclerosis, arteriosclerosis, and hypertension increase the risk of cerebrovascular disease and strokes. Obesity is an independent risk factor for strokes, even in the absence of other comorbidities.

Thromboembolic Disease

The risks of venous stasis, deep vein thrombosis, and pulmonary embolism are increased in obesity, particularly in persons with abdominal obesity. Lower extremity venous disease may result from increased intraabdominal pressure, impaired fibrinolysis, and the increase in inflammatory mediators described above.

Respiratory System

Obesity is associated with reduced lung volume, altered respiratory patterns, and an overall reduction in the compliance of the respiratory system, including a diminished vital capacity and total lung capacity. More severe obesity is associated with the obesity–hypoventilation syndrome, which is characterized

by excessive daytime sleepiness and hypoventilation. The increased work required to move the chest wall, a decrease in arterial oxygenation in the lungs, and a diminished sensitivity of the respiratory center to the stimulatory effect of carbon dioxide are postulated to contribute to the obesity–hypoventilation syndrome.

The obesity–hypoventilation syndrome may be associated with, or exacerbated by, obstructive sleep apnea, a syndrome characterized by repeated collapse of the upper airway and cessation of breathing with sleep. Obstructive sleep apnea occurs when the tongue obstructs the glottis and prevents entry of air into the trachea. Up to 50% of massively obese people have sleep apnea. The risk of arrhythmias and sudden death increases during apneic episodes. Weight reduction usually reduces the severity of sleep apnea, and massive weight reduction, such as that after gastric bypass surgery, eliminates the disease in most patients.

Digestive System

Gallbladder Disease

The risk of gallbladder disease, particularly gallstone formation, is increased in obesity, and occurs with greater frequency in women. The prevalence of gallbladder disease in obese individuals increases with age, bodyweight, and parity. The etiology of increased gallstones is unclear, but genetic factors play a role. Increased cholesterol production, which leads to increased excretion of cholesterol in bile, is known to occur in obesity and correlates with increases in body weight. Many obese people skip meals and the reduced number of meals may result in less frequent emptying of the gallbladder. The resulting bile stasis may contribute to gallstone formation. Although long-term weight loss and maintenance may reduce the occurrence of gallbladder disease, the risk of gallstone formation actually increases during the active weight loss phase. The etiology of this increase is thought to be the mobilization of cholesterol from adipose tissue during rapid weight loss. This increased load of cholesterol in the circulation produces supersaturation of the bile, leading to gallbladder sludge in approximately 25% of patients and to symptomatic disease in approximately 1–3%. Treatment with ursodeoxycholic acid reduces or eliminates the risk of gallstone formation during weight loss.

Hepatic Disease

Abnormalities in hepatic function are commonly reported in obese people. The frequency of fatty liver has been reported to be as high as 94% in very obese subjects, many of whom have elevated liver function tests. A small number of very obese subjects will develop micronodular cirrhosis. Weight loss results in disappearance of the excess fat and normalization of liver function tests.

Reproductive System

Hormonal Complications: Males

Obese men have elevated levels of plasma estrone and estradiol that correlate with the degree of obesity. Plasma total testosterone and free testosterone (the biologically active

moiety) are reduced in obese men, and the reductions correlate negatively with the degree of obesity. The reduced levels of free and total testosterone are not generally accompanied by hypogonadism or a decrease in libido, potency, or sperm count in obese men. Free and total plasma testosterone levels normalize on significant weight reduction. Also, estrogen levels are normalized if the individuals attain normal weight, but not if the weight loss is modest and significant obesity persists.

Hormonal Complications: Females

Obese women have normal levels of total plasma estradiol, but reduced levels of sex hormone-binding globulins (SHBGs). Thus, free estradiol (the biological active moiety) is significantly elevated. The high levels of free estradiol are postulated to increase the risks of endometrial and breast cancer and to reduce fertility. Estrone, derived in adipose tissue from androgen precursors, is also increased in obesity. Obesity in women is associated with the polycystic ovary syndrome (PCOS), characterized by hyperestrogenism, hyperandrogenism, polycystic ovaries, oligomenorrhea or amenorrhea, hirsutism, and infertility. Women with PCOS also have insulin resistance and are at high risk for developing impaired glucose tolerance and DM. Weight loss usually normalizes SHBG and estradiol levels for individuals with simple obesity, but weight loss may not restore fertility to patients with severe PCOS.

Obstetric Complications

Obesity increases the risk of complications during pregnancy and childbirth. Increased bodyweight, hypertension, and fluid retention during pregnancy can lead to toxemia of pregnancy. Heavier women have a longer duration of labor and a greater frequency of abnormal labor and caesarian sections.

Nervous System

Pseudotumor Cerebri

This syndrome is characterized by increased intracranial pressure, headaches, blurred vision or loss of vision, and papilledema. It is most common in massively obese individuals and may be seen in association with sleep apnea or with the obesity-hypoventilation syndrome. It may be associated with retinal hemorrhage or loss of vision from severe papilledema. Some investigators believe that increased intra-abdominal pressure with massive obesity is an etiologic factor for pseudotumor cerebri. Major weight loss, particularly after obesity surgery, results in dramatic improvement.

Adiposis Dolorosa

This is a syndrome of unknown etiology characterized by pain in subcutaneous adipose tissue. Adiposis dolorosa occurs predominantly in postmenopausal women (female to male ratio of approximately 30:1), and has been described over all areas of the body. The painful areas of fat may occur as subcutaneous lumps on physical examination, but more commonly there are no differences from normal adipose tissue. The disease usually begins gradually with mild pain and tenderness of the area involved, but may progress to severe pain, particularly with movement or exercise. Intravenous infusions

of lidocaine are reported to relieve pain, short term or even permanently. The mechanism involved in the relief of pain from lidocaine is unknown.

Alzheimer Disease

Obesity has been linked to an increased prevalence of Alzheimer disease. The etiology of this increase is unknown.

Immune System

Animal studies have shown an increased rate of infection and mortality in obese dogs compared to lean animals experimentally infected with canine distemper virus. Cell-mediated immune response is impaired in obese individuals. Maturation of monocytes into macrophages after *in vitro* incubation is significantly less for obese compared to lean subjects. Impaired cell-mediated immune response in children was demonstrated to be due to subclinical deficiencies of zinc and copper. The impairment in the immune response was reversed after 4 weeks of zinc and copper supplements. As described above, there are changes in numerous cytokines with obesity. The role of these changes in immune function is not clear.

Skin

Obese people may have several disorders of the skin. The most common is stasis changes of the skin of the lower legs in massively obese people. The etiology of this finding is venous stasis, edema, and breakdown of the skin. Fragilitas cutis inguinalis is a condition of fragile skin in the inguinal area of obese people. This condition is diagnosed by stretching the skin of the inguinal area. A linear tear appears at right angles to an applied force that is insufficient to tear the skin of a normal person. This condition is unrelated to the sex and age of the person.

Acanthosis nigricans, seen occasionally in obesity, is characterized by darkening of the skin in the creases of the neck, axillary regions, and over the knuckles. An association between acanthosis nigricans and insulin resistance is reported in persons who have circulating antibodies to the insulin receptors. Because acanthosis nigricans also may be associated with highly malignant cancers such as intraabdominal adenocarcinomas, physicians should be alert to this possibility and not attribute the condition simply to the presence of obesity.

Eye Disease

Obesity is associated with an increased prevalence of cataracts. Persons with abdominal obesity are at a greater risk than those with lower body obesity. Insulin resistance may be involved in the pathogenesis of cataract formation, and diabetes is a well-known risk factor.

Cancer

Obesity increases the risk of many cancers, including breast, colon, prostate, endometrium, cervix, ovary, kidney, gallbladder, liver, pancreas, rectum, brain, esophagus, and non-

Table 2 Deaths from cancer in women by BMI

Type of cancer	BMI (kg m ⁻²)					P for trend
	18.5–24.9	25.0–29.9	30.0–34.9	35.0–39.9	≥ 40.0	
All cancers						
Deaths per 10 000 women ^a	329.3	339.75	382.62	419.59	522.51	
RR (95% CI) ^b	1	1.08	1.23	1.32	1.62	<0.001
Breast cancer						
Deaths per 10 000 women ^a	39.1	51.13	60.65	67.56	84.86	
RR (95% CI) ^b	1	1.34	1.63	1.7	2.12	<0.001
Uterine cancer						
Deaths per 10 000 women ^a	10.68	15.68	26.05	30.16	60.83	
RR (95% CI) ^b	1	1.5	2.53	2.77	6.25	<0.001
Ovarian cancer						
Deaths per 10 000 women ^a	27.88	31.44	31.85	44.49	–	
RR (95% CI) ^b	1	1.15	1.16	1.51	–	<0.001
Colorectal cancer						
Deaths per 10 000 women ^a	38.67	43.28	53.81	56.14	63.11	
RR (95% CI) ^b	1	1.1	1.33	1.36	1.46	<0.001

^aDeaths per 100 000 women.^bRR, Relative risk of death compared to the reference group (BMI = 18.5–24.9 kg m^{-2}).Source: Adapted with permission from Calle EE, Rodriguez C, Walker-Thurmond K, and Thun MJ (2003) Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *New England Journal of Medicine* 348(17): 1625–1638.

Hodgkins lymphoma. **Table 2** shows the increased risk of selective cancers.

Although there are many theories about how obesity increases cancer risk, the exact mechanisms are not known. The mechanisms may be different for different types of cancer. Also, because obesity develops through a complex interaction of heredity and lifestyle factors, researchers may not be able to tell whether the obesity or other factors led to the development of cancer.

Mechanical Complications of Obesity

Arthritis

Obesity is frequently complicated by degenerative arthritis (DJD). Increased bodyweight leads to trauma of the weight-bearing joints and speeds the development of osteoarthritis in obesity. Knee and hip joints are particularly affected. However, obese patients have increased DJD of the hands, perhaps due to cytokines produced by adipose tissue, which may damage the cartilage in joints. Flattening of the arc of the planter surface of the feet (flat feet) occurs more frequently in obese people, presumably due to the stress of carrying excess body weight. Flat feet may lead to unsteady gait and aches and pains after walking. Increased fat deposition, particularly in the abdominal region, can change the natural curvature of the spine, causing lordosis and resulting in backache in obese people.

Intraabdominal Pressure

In severely obese people, the excess visceral fat is thought to increase intraabdominal pressure. Animal research shows that

experimentally induced acute increases in intraabdominal pressure to the levels seen in the abdomens of very obese people cause increases in pleural pressure, intracranial pressure, and central venous pressure. The investigators postulated that in humans, increased intraabdominal pressure may contribute to hypertension, insulin resistance and Type 2 DM, obesity--hypoventilation syndrome, pseudotumor cerebri, incisional hernia, and urinary incontinence. Massive weight loss following obesity surgery normalizes the increased intraabdominal pressure and reduces or eliminates all the symptoms listed above.

Surgical Complications

Obese patients are at an increased risk of surgical and peri-surgical complications. These risks include an increased risk of complications and death from anesthesia, longer operating times, delayed wound healing, increased postoperative wound infections and pneumonia, and a higher frequency of incisional hernias after surgeries involving the abdominal wall. Many surgeons recommend weight reduction before elective surgery, but there are few data to document that acute weight reduction improves the outcome of surgery.

Psychosocial Complications

Psychological Complications

Obesity is associated with negative emotions, low self-esteem, decreased marital satisfaction, and body image disparagement. All of these conditions and beliefs show improvement with weight reduction.

Dieting efforts correlate positively with the prevalence of eating disorders, particularly binge eating. A correlation of eating disorders with abuse of drugs and alcohol has been shown. In strictly dieting female college freshmen who were not alcohol abusers at baseline, the frequency of alcohol abuse was reported to increase after a year compared to nondieters.

more health problems, health-care costs for the obese are higher than for lean individuals.

See also: Adipose Tissue: Structure, Function and Metabolism

Social Complications

Obesity carries a social stigma that dramatically affects the quality of life for obese individuals, particularly for women. Factors contributing to the social bias against obese people are beliefs that obesity is merely due to overeating and therefore obese people lack will power. Many members of the general public, and even health professionals, ignore the evidence for the medical and genetic contribution to obesity, believe that obese people are responsible for their own plight, and believe that they do not deserve sympathy for their disability. Despite similar intelligence (as judged by IQ values and the Scholastic Aptitude Test scores), a significantly lower number of obese females were admitted to certain colleges compared to non-obese females. The choice of mates is adversely affected by obesity. Obese individuals tend to marry mates with less education and from a lower socioeconomic class. It is more difficult for an obese person to find a job or to be promoted once hired, so lower earnings and a lower socioeconomic status are correlated with obesity. Obese employees are viewed as less competent, less productive, inactive, disorganized, and less successful by employers, regardless of actual productivity.

The bias against obesity has been shown to begin in early childhood. Obese children are considered lazy, stupid, slow, and self-indulgent by both children and adults. Because of these societal attitudes, many obese children and adolescents have lower levels of self-esteem than do their nonobese counterparts.

Economic Impact

In the USA, the direct cost of obesity has been estimated at almost 150 billion dollars per year. The indirect costs of early retirement and increased risk for disability requiring financial support are also considerable. Because obese people have

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Definition, Etiology, and Assessment

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Glossary

Body composition Relative proportion of tissue types within an individual. At its simplest level the body can be divided into the relative proportion of fat and fat-free mass.

Body mass index (BMI) Index of weight independent of height used as a proxy measure of body fatness; calculated as $\text{weight}(\text{kg})/\text{height}(\text{m})^2$.

Metabolic syndrome A cluster of risk factors (including abdominal obesity) associated with increased risk of cardiovascular disease and diabetes.

Nutrition transition Population-level shifts in diet and physical activity patterns often associated with modern lifestyles; in the latter stages characterized by high-fat/high-sugar/low-fibre diets and lower levels of activity.

Obesity Excess accumulation of body fat, which can be associated with a range of co-morbidities.

Definition

Obesity is the excess accumulation of body fat or adiposity. It is commonly and most easily assessed by individuals' body weight independent of their height; the body mass index (BMI), calculated as $\text{weight}(\text{kg})/\text{height}(\text{m})^2$. The World Health Organization (WHO) classifies individuals with a BMI of 25–29.9 kg m^{-2} as overweight and over 30 kg m^{-2} as obese (Table 1). These classifications are themselves based on increased mortality and morbidity associated with excess body weight for height. Although the WHO recommends the use of these International Classification cut-offs for all countries there is evidence that the relationship between BMI and health may vary for different populations partly as a consequence of different associations with body fat and fat distribution. Lower BMI cut-offs for Asian populations have been suggested although the relationships are also not uniform across ethnic

groups. Additional cut-off points are therefore suggested as a useful public health reference in certain settings (Table 1).

Women are predisposed to higher body weight for the purposes of reproduction and in all populations mean BMI is higher for females compared to males. Data from 2010 collated by the International Association for the Study of Obesity (IASO) indicate that globally more than 1 billion individuals were overweight and 475 million obese. Rapid increases in BMI and the prevalence of obesity have occurred across all world regions; data from 199 countries compiled by the Global Burden of Metabolic Risk Factors of Chronic Diseases Collaborating Group indicate that between 1980 and 2008, mean BMI increased by 0.4 kg m^{-2} per decade for men and 0.5 kg m^{-2} per decade for women worldwide. These figures mask large variations in rate of change; certain regions, such as Oceania, experienced rates of increase of over 2 kg m^{-2} per decade during this time frame. The prevalence of overweight and obesity is now so high that the condition is increasingly recognized as a pandemic, with projections of further increases particularly within lower- and middle-income countries in the next decades. In children, overweight and obesity are assessed by age and sex specific cut-offs that predict adult BMI (Table 2). Globally, over 200 million school-aged children are classified as overweight and obese.

Table 1 International classification of adult overweight and obesity according to body mass index (BMI)

Classification	BMI (kg m^{-2})	
	Principal cut-off points	Additional cut-off points
Normal range	18.50–24.99	18.50–22.99 23.00–24.99
Overweight	25.00–29.99	25.00–27.49 27.50–29.99
Obese	≥ 30.00	
Obese class I	30.00–34.99	30.00–32.49 32.50–34.99
Obese class II	35.00–39.99	35.00–37.49 37.50–39.99
Obese class III	≥ 40.00	

Source: Modified from WHO Expert Consultation (2004) Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 363: 157–163.

Health Impacts

The WHO classifies overweight or obesity as the fifth leading risk factor for death, responsible for 7% of deaths globally. It is estimated that at 40 years of age, an obese person will live up to 7 years less than someone of normal weight. Obesity is associated with a range of comorbidities, many of which fall under the heading of the 'metabolic syndrome,' which is characterized by insulin resistance and dyslipidemia as well as by excess body weight and by increased body fat in the abdominal region. The risks of diabetes, hypertension, and dyslipidemia all increase with increasing body weight from a

Table 2 International cut-off points for body mass index (BMI) for overweight and obesity by sex between 2 and 18 years

Age (years)	BMI 25 kg m ⁻²		BMI 30 kg m ⁻²	
	Males	Females	Males	Females
2	18.41	18.02	20.09	19.81
2.5	18.13	17.76	19.80	19.55
3	17.89	17.56	19.57	19.36
3.5	17.69	17.40	19.39	19.23
4	17.55	17.28	19.29	19.15
4.5	17.47	17.19	19.26	19.12
5	17.42	17.15	19.30	19.17
5.5	17.45	17.20	19.47	19.34
6	17.55	17.34	19.79	19.65
6.5	17.71	17.53	20.23	20.08
7	17.92	17.75	20.63	20.51
7.5	18.16	18.03	21.09	21.01
8	18.44	18.35	21.60	21.57
8.5	18.76	18.69	22.17	22.18
9	19.10	19.07	22.77	22.81
9.5	19.46	19.45	23.39	23.46
10	19.84	19.86	24.00	24.11
10.5	20.20	20.29	24.57	24.77
11	20.55	20.74	25.10	25.42
11.5	20.89	21.20	25.58	26.05
12	21.22	21.68	26.02	26.67
12.5	21.56	22.14	26.43	27.24
13	21.91	22.58	26.84	27.76
13.5	22.27	22.98	27.25	28.20
14	22.62	23.34	27.63	28.57
14.5	22.96	23.66	27.98	27.87
15	23.29	23.94	28.30	29.11
15.5	23.60	24.17	28.60	29.29
16	23.90	24.37	28.88	29.43
16.5	24.19	24.54	29.14	29.56
17	24.46	24.70	29.14	29.69
17.5	24.73	24.85	29.70	29.84
18	25	25	30	30

Source: Modified with permission from Cole TJ, Bellizzi MC, Flegal KM, and Dietz WH (2000) Establishing a standard definition for child overweight and obesity worldwide: International survey. *British Medical Journal* 320: 1240–1243.

BMI as low as 21 kg m⁻². One of the closest associations between obesity and disease is with type II diabetes, where up to 90% of individuals with the condition are classified as overweight. Hypertension is up to five times higher in individuals who are overweight or obese. Excess body fatness is convincingly associated with a range of cancers and reducing obesity was one of the key recommendations of the 2007 World Cancer Research Fund report on cancer prevention. Other important comorbidities associated with body fatness include gall-bladder disease, nonalcoholic fatty liver disease, sleep apnea, and osteoarthritis. Increasingly, obesity is associated with psychological effects and stigma, which have important implications for quality of life. Owing to the wide range of associated morbidities, the direct and indirect costs of obesity are substantial and the health-care costs of treatment alone confer a massive burden on societies across the world. Middle- and lower-income countries are projected to experience the greatest rise in obesity rates but may lack the resources to cope with associated health costs.

Etiology

Obesity is primarily caused by an imbalance between energy intake and expenditure, leading to weight gain. The components of the diet and the amount of energy expended are thus important determinants. From a substantial review of the evidence in 2007, the World Cancer Research Fund concluded that there was convincing or probable evidence that a sedentary lifestyle (including excess television viewing) and the consumption of energy-dense foods, sugary drinks, and fast foods were associated with increased risk of weight gain, overweight, and obesity.

There is increasing recognition of the role played by societal and environmental forces in influencing both sides of the energy balance equation and obesity is no longer viewed solely as preventable by the individual. The rapid rise in obesity rates across the globe has been attributed to correspondingly rapid changes in population demographics and dietary behavior. The so-called 'nutrition transition' from traditional forms of diet and activity to more 'Western' diets and lifestyles, characterized by increased energy density and high fat is likely to be a particularly important driver of obesity rates. Increasing urbanization is occurring across the globe, often with corresponding increases in obesity-inducing behavior such as a reduction in physical activity and access to highly processed urban food supplies.

Patterns of overweight and obesity are not uniform within populations and are strongly related to socioeconomic status. As countries become wealthier this pattern changes so that in the countries with the highest income, obesity rates are the highest amongst the poorest members of society whereas in lower-income countries the prevalence of obesity is highest amongst the highest socioeconomic class. This pattern reflects the changing distribution of obesity risk factors that occurs as countries progress through the nutrition transition.

The relative contributions of genetics and the environment to the etiology of obesity have been evaluated in several studies with inconsistent results and estimates of the inheritance of obesity range from 64% to 84%. Only very few individuals have genetically determined obesity relating to single-gene effects but complex gene–environment interactions are likely to predispose certain individuals to obesity within the context of particular diet and activity patterns. Investigation of the polymorphisms that underlie these interactions may help to understand the etiology of obesity and inform potential treatment and prevention strategies. However it is important to recognize that changes to the environment, rather than to genes, underlie the current worldwide obesity epidemic and addressing these environmental causes will be the most important component of effective prevention strategies.

Assessment

One of the goals of assessment of overweight/obesity is to decide whom to treat. Three main issues must be evaluated: whether treatment is indicated, whether treatment is safe for the patient, and whether the patient is ready and motivated to lose weight. In addition, routine assessment of eating and

activity patterns in adults as well as in children must be considered. Recognition of excessive weight gain relative to linear growth is essential throughout childhood. Motivation for weight loss in obese individuals is a key component of effective treatment but is often lacking partly because it depends on the acceptance and recognition that obesity is a medical disorder. Motivation for change may also be reduced if the obese individuals are yet to develop comorbidities and do not yet conceive that their health is compromised.

Proper identification and classification of obesity through body composition assessment are important steps to initiate before beginning weight-loss treatment. Dietary management, physical activity, surgery, pharmacotherapy, and psychosocial and familial support must be considered together as part of obesity assessment. Before beginning a weight-loss program, patients should be evaluated for the number and severity of cardiovascular risk factors, conditions that may require treatment in addition to weight-loss strategies.

Measures of Body Fatness

Although BMI is a useful indicator of risk that is widely used at a population level, it is only a proxy measurement of body fatness and does not accurately reflect adiposity at an individual level. In addition, individuals with the same BMI may have very different body shapes depending on the distribution of fat; visceral fat in the abdominal region is associated with greater metabolic abnormalities. Many different techniques can be used to assess body fatness, each with its own advantages and disadvantages.

Cadaver Analysis

The only direct measure of body fat but clearly not widely applicable. The main use of cadaver studies is to validate methods that can be used to study patients *in vivo*. All remaining techniques are indirect assessments that require certain assumptions to calculate body fatness.

Imaging Techniques

Total adipose tissue and its distribution can be quantified using imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Both methods produce high-resolution cross-sectional images from signals resulting from exposure of the subject to an X-ray source (CT) or electromagnetic field (MRI). Total body fat volume, total fat mass, and percentage fat mass can be estimated and visceral fat depots can be measured with good accuracy. These techniques are very expensive and may be problematic for people with claustrophobia. Dual-energy X-ray absorptiometry (DXA) is a widely used imaging technique that utilizes the principle that transmitted X-rays at two energy levels are differentially attenuated by bone mass and soft tissue mass. It is possible to obtain abdominal fat estimates with DXA, although these cannot be separated into subcutaneous and visceral components.

Densitometry

Total body fat can be assessed by measuring body density based on the Archimedes principles of water displacement, assuming two body compartments (fat and fat-free tissue) of distinct densities. Body density was traditionally measured using the technique of under-water weighing, which is both expensive and not widely acceptable to patients. Air displacement techniques are becoming more popular although these techniques also require a participant burden and may be problematic for individuals with claustrophobia.

Bioimpedance Analysis

This predictive technique of assessing body fatness is becoming widely used for individuals and research studies. The measurement is based on the relative impedance of tissues to a small electric current that is passed through the body. The impedance is converted into an estimate of total body water, which is used to calculate fat-free mass and then fat mass by difference from body weight. Because the estimate of total body water is crude, the estimation of fat mass by this technique is relatively weak.

Anthropometry

In addition to height and weight measurements for the calculation of BMI, other anthropometric assessments can provide useful predictions of body fatness and metabolic risk. Body fat can be estimated by measuring skinfold thickness directly using a caliper at different sites on the body. These often include the upper arm (biceps and triceps), under the scapular (subscapular), and above the iliac crest (suprailiac). The raw data provide a direct estimate of body fat, with an increasing number of measurement sites correcting for differences in fat distribution. Data can also be used to predict body density and thence fatness through prediction equations. These equations are population-specific however and may not be widely generalizable, suggesting that the raw skinfold thicknesses are often more useful.

Waist circumference, or the ratio of waist to hip circumference, has recently been recognized as a simple measure that has important risk prediction properties. Waist circumference is only minimally related to height and is a good predictor of visceral and total fat mass as well as disease risk. It is relatively easily measured within the primary care and research settings, which makes it a useful addition to the assessment of obesity by BMI. WHO guidelines specify that the circumference should be measured at the midpoint between the lower margin of the last rib and the top of the hip bone. Waist circumference cut-points associated with increased risk have not been fully defined as yet.

See also: Biochemical Indices. Body Composition. Dietary Intake Measurement: Methodology. Nutritional Assessment: Anthropometry; Clinical Examination. Obesity: Childhood Obesity; Complications; Genetic Origins; Prevention; Treatment

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Genetic Factors

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Glossary

Candidate gene study Candidate gene studies are hypothesis-driven and rely on the current understanding of the biology and pathophysiology that underlies the susceptibility to obesity. Genes, for which there is evidence for a role in regulation of the energy balance in animal models or in extreme/monogenic forms of obesity, are tested for association with obesity-related traits at the population level.

Common obesity The prevalent form of obesity present in the general population.

Genome-wide association study Genome-wide association studies interrogate the entire genome, unconstrained by prior assumptions. They aim to identify previously unsuspected genetic loci associated with a disease or trait of interest and as such to expand our understanding of the underlying physiology. Genome-wide association studies screen the whole genome at higher resolution levels than genome-wide linkage studies and

thus, are able to narrow-down the associated locus more accurately.

Genome-wide linkage study Genome-wide linkage studies are hypothesis-generating and through surveying the whole genome, aim to identify new, unanticipated genetic variants associated with a disease or trait of interest.

Genome-wide linkage studies rely on the relatedness of study participants and test whether certain chromosomal regions co-segregate with a disease or trait across generations.

Locus Cluster of genetic variants that are highly correlated (in high linkage disequilibrium) and thus represent the same entity.

Monogenic obesity Obesity is caused by a mutation in a single gene and is a dominant feature. Monogenic forms of obesity are often early-onset and extreme.

Syndromic obesity Obesity is one of several clinical manifestations in a syndrome, often caused by a single gene defect or chromosomal abnormality.

Introduction

Rapid globalization of the westernized lifestyle is the main cause of the current obesity epidemic. However, not everyone in the present-day obesogenic environment becomes obese, many are resistant to this environment and remain of normal weight or even lean. This large inter-individual variation suggests that the response to the obesogenic environment is, at least in part, controlled by genetic factors. More specifically, the obesogenic environment increases the risk of obesity, but more so in those who are genetically susceptible. Indeed, obesity in its common form arises through the joint actions of multiple genetic and environmental factors. However, in very few cases, obesity is almost solely owing to genetic defects and the environment has hardly any influence.

Here, we review the current insights into the genetics of obesity and how research of both rare and common forms contribute to a greater understanding of the biological pathways that control body weight. We discuss the immediate implications of genetic mutations and variations on health of the individual and of the general population and how they may provide the basis for more targeted prevention and treatment.

Rare Forms of Obesity

In a fraction of the population, obesity is due to a single gene defect or a chromosomal abnormality. This typically results in extreme and early-onset obesity, which has often a Mendelian

inheritance pattern. We distinguish syndromic forms, in which obesity is one of several clinical manifestations, and monogenic forms, in which obesity is the dominant feature. Although these forms are rare, insight in the genetic basis of these diseases may result in life-saving therapies for the patients and they may also contribute to a better understanding of the physiological pathways that cause common obesity.

Syndromic Obesity

At least 20 clinically defined syndromes have been reported in which patients have extreme obesity along with a range of other abnormalities such as developmental delay, generalized brain dysfunction, and dimorphic features. The genetic cause of many of these syndromes has been identified and include single gene defects, chromosomal and imprinting abnormalities, and X-linked disorders, but the physiological link between these genetic abnormalities and the increase risk of morbid obesity often remains to be established. The most common syndromes are Prader–Willi syndrome and Bardet–Biedl syndrome.

The Prader–Willi syndrome is one of the commonest syndromes with an estimated birth incidence of 1 in 15 000–25 000, and is characterized by reduced fetal movement, increased prematurity, hypotonia at birth, hypogonadism, mental retardation, temporary feeding problems and poor weight gain in infancy, and hyperphagia that usually develops between 12 and 18 months leading to morbid obesity. Prader–Willi syndrome is the result of loss of expression of the paternally derived chromosomal region

15q11–q13, which harbors a number of genes, with the critical region spanning the promoter and first exon of the small nuclear ribonucleoprotein polypeptide N (*SNRPN*) gene. Most patients (75%) have deletion or disruption of 15q11–q13, whereas most others (22%) have maternal uniparental disomy (i.e., two maternally derived chromosomal regions). Hyperphagia and obesity do not seem to differ between these two major forms. The mechanism by which this gene or chromosomal region contributes to the obesity phenotype remains to be established.

The Bardet–Biedl syndrome has an estimated birth incidence of 1 in 150 000 live births in North America and Europe, although 10-fold higher incidences have been reported for consanguineous and isolated populations. Bardet–Biedl syndrome is a highly heterogeneous disorder, characterized by retinal degeneration, renal dysfunction, mental retardation, polydactyly, and hypogonadism. The majority of patients develop obesity in late childhood, which is often accompanied by reduced physical activity, type 2 diabetes, hypertension, and dyslipidemia. The Bardet–Biedl syndrome has been associated with at least 15 genes (*BBS1–15*), most of which affect the function of the primary cilium and the intraflagellar transport process. The contribution of mutations in each genetic locus varies across populations. *BBS1* and *BBS10* contribute the most (~40%) in patients of Northern European descent. For more than 20% of BBS patients, the genetic cause remains unknown. Also the mechanistic link between the BBS genes and obesity remains to be more firmly established.

Others syndromes that feature early-onset obese have been listed in Table 2 of the chapter on Childhood Obesity.

Monogenic Obesity

The first patients with single gene defects that led to extreme and early-onset obesity without any other major abnormalities were identified in the mid-1990's. To date, more than 200 cases with monogenic obesity have been reported. Most of the mutations locate in genes that encode ligands and receptors implicated in the leptin–melanocortin pathways known to play a critical role in the regulation of body weight through controlling energy sensing, food intake and appetite. The key genes involved in these pathways are leptin and the leptin receptor, pro-opiomelanocortin and its derived hormones, the melanocortin 4 receptor and also proprotein convertase subtilisin/kexin type 1 (Table 1). Many of the mutations in these genes cause partial or complete loss of function of the encoded protein.

Leptin is an adipocyte-derived hormone that circulates at levels proportional to body fat content. The primary site of leptin action is in the hypothalamus where it informs the brain about the status of the body's long-term energy stores. At least 12 patients have been identified with homozygous frameshift non-sense and missense mutations in the leptin (*LEP*) gene, resulting in the inability to produce leptin. Patients with mutations in the leptin receptor (*LEPR*) have been reported more frequently (in up to 3% of early-onset seriously obese patients). The clinical phenotypes for both types of patients are very similar; they have a normal birth weight, but exhibit rapid weight gain soon thereafter owing to a profound

hyperphagia. Patients also demonstrate hypogonadotropic hypogonadism and absence of normal onset of puberty. Subcutaneous injection of recombinant human leptin is an effective treatment of those with congenital leptin deficiency.

Leptin stimulates pro-opiomelanocortin (POMC), another key protein that is post-transcriptionally processed to produce a number of hormones in the hypothalamic-pituitary-adrenal axis, such as α -melanocyte-stimulating hormone (α -MSH), adrenocorticotrophic hormone (ACTH), and β -endorphin. These POMC-derived neuropeptides are physiological agonists of the melanocortin-4 receptor. Normally, the hypothalamic synthesis of α -MSH is stimulated by increased leptin levels, and the signal generated by α -MSH at the MC4R promotes energy expenditure and decreases food intake. At least five patients with congenital POMC deficiency owing to homozygous or compound heterozygous mutations have been reported. They show hypocortisolemia, leading to hypoglycemia, susceptibility to the effects of infection, pale skin, and red hair. Individuals with heterozygous POMC mutations showed a markedly increased prevalence of overweight and obesity.

The melanocortin-4 receptor (MC4R) is a seven-transmembrane G-protein coupled receptor that is expressed predominantly in the brain and encoded by the *MC4R* gene. The first patients with *MC4R* mutations were reported in 1998. To date, over a 100 different heterozygous and homozygous *MC4R* mutations have been reported in obese individuals from various ethnic backgrounds. Mutations occur throughout the coding sequence and many of the missense and frameshift mutations lead to a complete or partial loss of function. Up to 6% of individuals with severe, early-onset obesity carry pathogenic mutations in *MC4R*, making *MC4R* deficiency the commonest form of monogenic obesity. Patients with *MC4R* deficiency exhibit hyperphagia, increased fat and lean mass, greater bone mineral density, and accelerated linear growth. The severity of the clinical phenotype is proportional to the functional implications of the mutation on the receptor.

Prohormone convertase 1 (PC1/3), encoded by *PCSK1*, is expressed in neuroendocrine cells, and converts prohormones into functional key hormones that are involved in the regulation of central and peripheral energy metabolism. So far, four extremely obese individuals with compound heterozygous mutations in *PCSK1* have been reported. *PCSK1* mutations lead to impaired POMC processing, resulting in hyperphagia and severe obesity. Furthermore, patients have a severe, small-intestinal absorptive dysfunction in neonatal life, which is likely due to impaired processing of propeptides within the enteroendocrine cells and nerves in the gut. Because of impaired processing of proinsulin to insulin, their plasma levels of proinsulin and 32,33 split proinsulin are elevated.

Mutations in the single-minded homolog 1 (*SIM1*), brain-derived neurotrophic factor (*BDNF*), and its receptor, TrkB, have also been reported to cause monogenic obesity, but so far only a single case for each of these has been identified. These three genes are also part of the central leptin–melanocortin pathways.

Interestingly, so far no human monogenic mutations have been reported for genes involved in (peripheral) energy expenditure.

Table 1 Overview of the co-occurrence of genes in which mutations lead to extreme and early-onset obesity and of genes in which common variants contribute to obesity in the general population from large-scale candidate gene studies (genes in alphabetical order)

Gene	Presumed role in obesity-susceptibility	Monogenic obesity	Candidate gene studies ^a
β -Adrenergic receptor 3 (ADRB3)	ADRB3 is part of the adrenergic system, which is known to play a key role in energy metabolism. ADRB3 is primarily expressed in adipose tissue where it is involved the regulation of lipolysis and thermogenesis through activation of the sympathetic nervous system.	So far, there is no evidence of ADRB3 mutations that lead to monogenic obesity.	A large-scale meta-analysis, found the ADRB3 Arg64Trp variant to be associated with BMI in East Asians only, with Arg64-allele carriers (MAF: 18%) having a 0.31 kg m ⁻² higher BMI compared to the Arg64Arg homozygotes, whereas no associations were observed in Caucasians.
Brain-derived neurotrophic factor (BDNF)	BDNF is believed to act primarily in the hypothalamus, downstream of the leptin-pro-opiomelanocortin signaling pathway. A role of BDNF in the regulation of energy homeostasis comes from mutant mice, which show a reduced BDNF expression in the hypothalamus, hyperphagia, obesity and hyperactivity.	Although no mutations in humans have been described, a <i>de novo</i> chromosomal inversion at chr11p, a region encompassing BDNF, was detected in an 8-y-old girl who was hyperphagic, severely obese and hyperactive. Furthermore, in patients with the WAGR syndrome, those with BDNF haploinsufficiency had a higher BMI and all had developed obesity in childhood.	The minor allele homozygotes of the Val66Met (rs6265) BDNF variant (MAF: ~20%) were found to have a significantly lower BMI (-0.76 kg m ⁻²) than Val66-allele carriers in a large-scale study. The Met-allele has been shown to impair intracellular trafficking and reduce activity-dependent secretion of BDNF in hippocampal neurons. Note: Recently, GWAS have identified common variants in BDNF robustly associated with BMI and obesity.
Leptin (LEP)	Leptin is an adipocyte-derived hormone that circulates at levels proportional to body fat content. The primary site of leptin action is in the hypothalamus where it informs the brain about the status of long-term energy stores.	At least 12 patients have been identified with homozygous frameshift non-sense and missense mutations in LEP, resulting in the inability to produce leptin. Patients have a normal birth weight, but exhibit rapid weight gain soon thereafter owing to a profound hyperphagia. Patients also demonstrate hypogonadotropic hypogonadism and absence of normal onset of puberty.	So far, no convincing associations have been reported between common variants in LEP and obesity-susceptibility traits.
Leptin receptor (LEPR)	Leptin signals through the long isoform of the leptin receptor that contains motifs to activate downstream signaling events.	Up to 3% of extreme and early-onset obese patients are believed to have mutations in the leptin receptor. The clinical phenotype is very similar to those who have LEP mutations.	So far, no convincing associations have been reported between common variants in LEPR and obesity-susceptibility traits.
Lactase (LCT)	LCT is expressed in intestinal epithelial cells and encodes the lactase enzyme, which contributes to the digestion of the milk sugar lactose. Because lactase non-persistence individuals have a more restricted diet compared to those with lactase persistence it has been speculated that this may affect their BMI.	So far, there is no evidence of LCT mutations that lead to monogenic obesity.	The C/T ₋₁₃₉₁₀ variant, which has been associated with lactase persistence in adulthood, was found to be associated with increased BMI, in particular in Finnish adults.
Melanocortin 4 receptor (MC4R)	MC4R is predominantly expressed in the brain and plays a key role in the regulation of food intake and energy homeostasis.	Up to 6% of individuals with severe, early-onset obesity carry pathogenic mutations in MC4R, making MC4R deficiency the commonest form of monogenic obesity. Patients with MC4R deficiency exhibit hyperphagia, increased fat and lean mass, greater bone mineral density, and accelerated linear growth.	Large-scale candidate gene studies, including meta-analyses, have shown that rare alleles of the two most common MC4R variants, V103I (MAF: 2–3%) and I251L (MAF: 1–2%), which each result in a non-synonymous change with potential functional implications, are associated with a 20% and 50% reduced risk of common obesity, respectively. Note: Recently, GWAS have identified common variants near MC4R robustly associated with BMI and obesity.

Melanotonin receptor type 1 B (<i>MTNR1B</i>)	Because melatonin is involved in the regulation of circadian rhythms, which contribute to metabolic disorders when disturbed, it has been speculated that variation in the <i>MTNR1B</i> (which encodes the MT2-receptor) could contribute to obesity-susceptibility.	So far, there is no evidence of <i>MTNR1B</i> mutations that lead to monogenic obesity.	Each additional Glu24-allele of the Gly24Glu polymorphism (MAF: 9%) was associated with a 20% increased risk of obesity, and with increased BMI ($+0.50 \text{ kg m}^{-2}$) and waist circumference ($+1.2 \text{ cm}$) in a large population-based sample.
Prohormone convertase 1/3 (<i>PCSK1</i>)	<i>PCSK1</i> encodes an enzyme, expressed in neuroendocrine cells, that converts pro-hormones into functional key hormones that are involved in the regulation of central and peripheral energy metabolism.	Mutations in <i>PCSK1</i> lead to a PC1/3 deficiency, resulting in a syndrome characterized by extreme childhood obesity. Four extremely obese individuals with compound heterozygous mutations in <i>PCSK1</i> have been reported.	Two nonsynonymous <i>PCSK1</i> variants, N221D (rs6232) and the Q665E-S690T pair (tagged by rs6235), were found to be associated with obesity in adults and children. Each additional minor allele (MAF: 4–7%) of the N221D variant increased the risk of obesity by 1.34 fold, whereas each additional minor allele (MAF: 25–30%) of the Q665E-S690T pair increased the risk by 1.22 fold.
Pro-opiomelanocortin (<i>POMC</i>)	Pro-opiomelanocortin is post-transcriptionally processed to produce a number of hormones in the hypothalamic–pituitary–adrenal axis, such as α -melanocyte-stimulating hormone (α -MSH), adrenocorticotrophic hormone (ACTH) and β -endorphin. These <i>POMC</i> -derived neuropeptides are physiological agonists of the MC4R.	At least five patients with congenital <i>POMC</i> deficiency owing to homozygous or compound heterozygous mutations have been reported. They show hypocortisolemia, leading to hypoglycemia, susceptibility to the effects of infection, pale skin and red hair.	So far, no convincing associations have been reported between common variants in <i>POMC</i> and obesity-susceptibility traits. Note: Recently, GWAS have identified common variants near <i>POMC</i> robustly associated with BMI and obesity.

MAF: Minor allele frequency.

^aNote that this column only lists genes for which the association studies are convincing and consistent; i.e., derived from large-scale ($n > 5000$) studies or meta-analyses.

Common (Polygenic) Obesity

Unlike monogenic and syndromic forms of obesity, which are almost solely due to rare genetic defects with large effects, common obesity arises from the intricate interplay between environmental factors and a large number of common genetic variants that each have small effects.

Evidence of a Genetic Contribution to Common Obesity

The first evidence of a genetic contribution to common obesity was provided by family and migrant studies that rely on the relatedness between family members or between members of the same ethnic group to estimate the role of genes to obesity risk. Family studies have shown that individuals with a family history of obesity have a 1.5 to 5 times higher risk of being obese compared to the general population, with higher familial risks reported for those whose obese relative is more closely related and more obese.

A classical example of how ethnic origin might increase obesity-susceptibility is that of the Pima Indians. Of the Pima Indians who live in Arizona, 69% are obese compared to only 33% of the white Americans living in the same obesogenic environment. This observation suggests that despite the fact that Pima Indian and white Americans live in the same environment, their genetic background makes Pima Indians more susceptible to obesity. Interestingly, Pima Indians living in the restrictive environment of the Sierra Madre Mountains in remote Mexico have a much lower obesity prevalence (i.e., 13%), illustrating how genetic susceptibility and lifestyle interact.

As members of the same family or of the same ethnic group not only share their genetic background but also a similar

lifestyle and environment, these types of family and migrant studies do not distinguish the contribution of a genetic component from that of a shared environmental component.

Quantifying how much of the variation in obesity risk is explained by genes and environment is precisely what heritability studies aim to do. Heritability studies have shown that genetic factors contribute typically between 40% and 70% to the inter-individual variation in common obesity, but estimates as low as 5% and as high as 90% have been reported. Twin studies (heritability = $h^2 = 40\text{--}90\%$) often report higher estimates than family ($h^2 = 20\text{--}50\%$) or adoption ($h^2 = 20\text{--}60\%$) studies. Longitudinal twin studies have suggested that the heritability of obesity-susceptibility increases throughout childhood and adolescence until the onset of adulthood, after which the genetic contribution decreases again.

Identifying Obesity-Susceptibility Genes

The two approaches most used to identify genetic loci for obesity-related traits are the hypothesis-driven approach by using candidate gene studies, and the hypothesis-generating approach by using genome-wide screening studies (**Box 1**).

Candidate Gene Studies

Hundreds of genes have been examined in candidate gene studies (**Box 1**) for their presumed role in the regulation of energy homeostasis observed in animal studies or because mutations in the respective genes lead to monogenic obesity. Variants, typically single nucleotide polymorphisms (SNPs), in such candidate genes are tested for association with body mass index (BMI), obesity risk, or other body composition traits.

Box 1 Genetic epidemiological approaches to identify genes for common traits and diseases

The candidate gene approach

The candidate gene approach, performed since the early 1990's, is a hypothesis-driven approach and relies on the current understanding of the biology and pathophysiology that underlies the susceptibility to obesity. Genes for which there is evidence for a role in the regulation of the energy balance in animal models or in extreme/monogenic forms of obesity are tested for association with obesity-related traits at the population level.

The genome-wide screening approach

The genome-wide screening approach is a hypothesis-generating method that, through screening genetic variation across the whole genome, aims to identify new, unanticipated genetic variants associated with a disease or trait of interest. It is expected that the newly unidentified loci will provide insights into new pathways and biology that underlie obesity-susceptibility. The genome-wide screening approach has been implemented in linkage and association studies.

Genome-wide linkage studies

Genome-wide linkage studies, available since the mid-1990's, rely on the relatedness of study participants and test whether certain chromosomal regions co-segregate with a disease or trait across generations. Because of the rather low resolution, genome-wide linkage studies will identify broad intervals that harbor many genes.

Genome-wide association studies

Genome-wide association studies (GWAS), available since 2005, screen the whole genome at much higher resolution than genome-wide linkage studies and are thus able to better narrow-down the associated locus. GWAS test for association of ~ 2.5 million single nucleotide polymorphisms (SNPs) and obesity-related traits. For each SNP, association is tested (as in **Figure 1**) and p -values are presented according to their chromosomal location in a so-called Manhattan plot (**Figure 2**). GWAS do not rely on familial relatedness and can therefore achieve larger sample sizes than typical family-based studies. A key feature of genome-wide association studies is the two-staged study design; i.e., observations from the discovery stage are followed-up in replication stage to firmly establish the newly discovered genetic associations, which are considered significant if P -values reach a significance threshold of $< 5 \times 10^{-8}$.

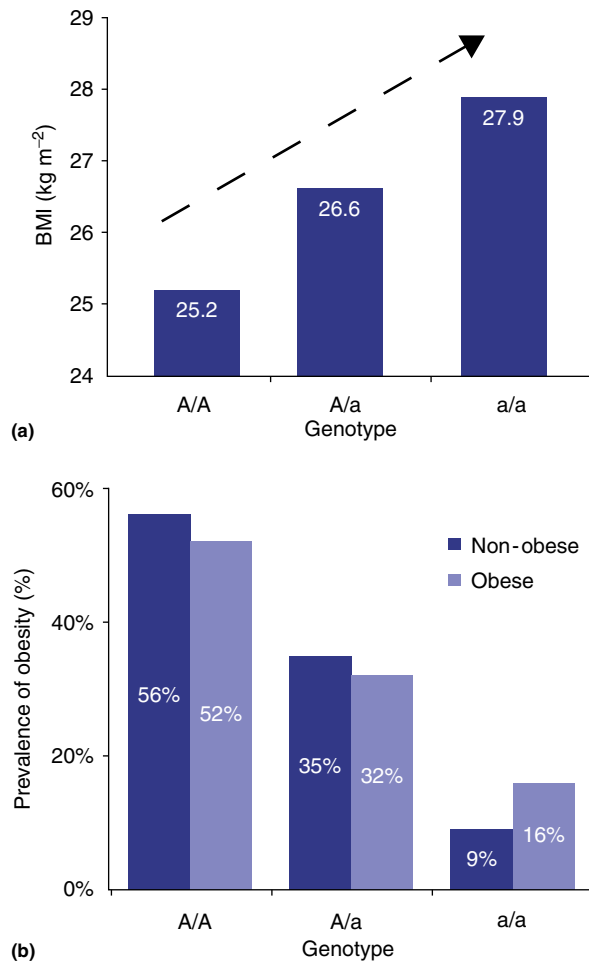


Figure 1 Example of association between a bi-allelic SNP (A/a) and BMI (panel a) or obesity risk (panel b), assuming an additive effect of the a-allele. This example shows that each additional a-allele increases BMI and risk of obesity.

SNPs are bi-allelic – one copy is inherited from each parent – such that an individual can be homozygous for the major allele (e.g., A/A), heterozygous (A/a) or homozygous for the minor allele (a/a). A candidate gene study examines whether either of the two alleles is associated with an increased risk of obesity (dichotomous) or with higher levels of an obesity-related trait (continuous; e.g., BMI) (Figure 1).

Despite the large number of candidate genes studied, very few have been firmly established as obesity-susceptibility genes (Table 1). The main reasons for the limited success of the candidate gene approach are that: (1) sample sizes used are often too small ($n < 1000$) to identify the expected small to modest effects; (2) the genetic variants studied did not capture all variation in the gene; and (3) the candidacy was based on flawed biological insights. In recent years, however, an increasing number of candidate gene studies have tested for associations in larger populations ($n > 5000$) and more often the initiative has been taken to perform meta-analyses of all available published data. Such studies have provided more convincing associations for nonsynonymous variants in genes encoding the β -adrenergic receptor 3 (ADRB3), *BDNF*, melanocortin 4 receptor (*MC4R*), melanotonin receptor type 1 B

(*MTNR1B*), prohormone convertase 1/3 (*PCSK1*), and for a functional variant near the lactase (*LCT*) gene.

Although large-scale candidate gene studies have sufficient power to identify small effects, they are also powered to refute associations. As such, large-scale studies ($n > 5000$) have found no evidence of association with obesity-related traits for variants in or near: ectonucleotide pyrophosphatase/phosphodiesterase (*ENPP1*, Lys121Gln); fatty acid binding protein 2 (*FABP2*, Ala54Thr); interleukin 6 (*IL6*, 174G > C); serotonin 5-HT-2C receptor (*HTR2C*, 759C/T); ghrelin receptor (*GHSR*); lipin 1 (*LPIN1*); liver pyruvate kinase (*PKLR*); and nitric oxide synthase 1 adapter protein (*NOS1AP*). For many other variants in candidate genes, however, the association results remain ambiguous because they have not yet been studied at a large-scale and comprehensive level.

Taken together, large-scale candidate genes studies have identified common variants, mostly nonsynonymous or functional, in at least six genes (*MC4R*, *ADRB3*, *PCSK1*, *BDNF*, *MTNR1B*, and *LCT*). However, larger studies and meta-analyses will be required to firmly confirm or refute the associations observed for many other variants in genes with a biologically plausible link to obesity.

Genome-Wide Linkage Studies

Following the first genome-wide linkage study (Box 1) on body fat percentage in Pima Indians, published in 1997, the number of chromosomal loci linked to obesity-related traits has grown exponentially. To date, at least 280 loci from more than 70 genome-wide linkage studies have been reported to show suggestive linkage with obesity-related traits. These loci typically harbor hundreds of genes and so far none have been narrowed down sufficiently to pinpoint the causal gene or variant. A meta-analysis of 37 genome-wide linkage studies with data of more than 31 000 individuals from 10 000 families of European origin could not locate a single obesity or BMI locus with convincing evidence, despite sufficient power to identify loci with even small effects. Taken together, genome-wide linkage has not been a fruitful approach to identify genetic variants for common obesity.

Genome-Wide Association Studies (GWAS)

The genome-wide association approach (Box 1) has dramatically increased the pace of gene discovery for a wide variety of common conditions. The two-staged design of GWAS, which requires that loci reaching highly significant associations at the first stage (Figure 2) are confirmed in second stage analyses, has provided high credibility to the identified loci. Only loci for which association with the trait reach a significance of $< 5 \times 10^{-8}$ are considered as established. To date, more than 1200 genetic loci for over 200 diseases and traits have been identified, of which at least 50 loci are unambiguously associated obesity-related traits (Table 2, Figure 3).

GWAS Discoveries and their Effects on Obesity-Susceptibility

Discoveries for Body Mass Index

Since 2007, four consecutive waves of large-scale genome-wide association studies for BMI have been performed, with each new wave including more samples than the previous (Table 2, Figure 4).

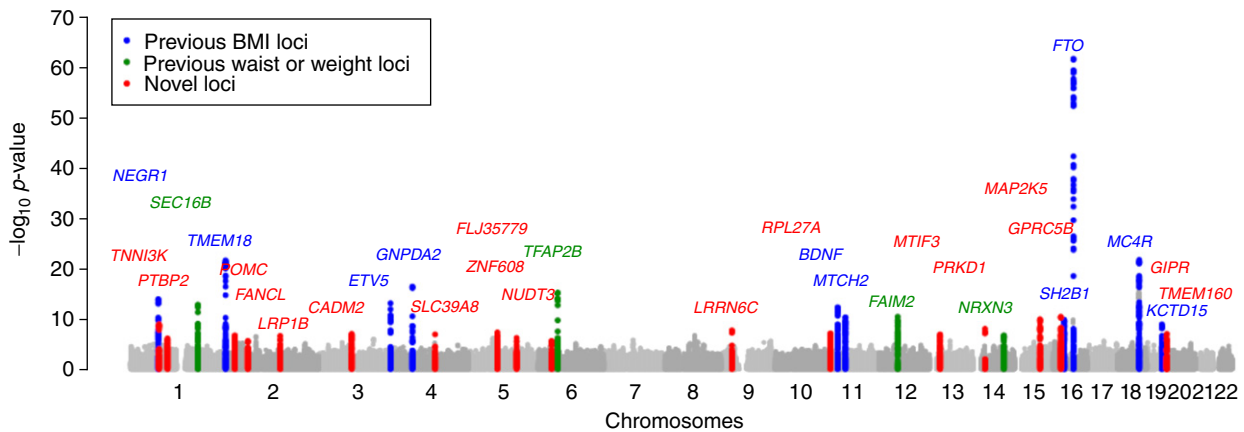


Figure 2 Manhattan plot of the association between genome-wide data and BMI in the meta-analysis of the GIANT consortium. The $-\log_{10} P$ -values for the association of each single nucleotide polymorphism with BMI are shown on the y-axis. The SNPs are plotted on the x-axis according to their chromosomal location. The SNPs that had previously been shown to associate with BMI are shown in blue (BMI) or green (weight and waist circumference). The SNPs that were taken forward from the discovery stage and that were replicated as new BMI hits are shown in red.

Table 2 Genetic loci associated with obesity-related traits (BMI, waist circumference, WHR adjusted for BMI, extreme obesity) identified through genome-wide association studies (loci sorted according to timing of discovery)

BMI	Waist circumference	WHR adjusted for BMI	Extreme and early-onset obesity
In FTO	In FTO	In <i>RSP03</i>	In FTO
Near MC4R	Near MC4R	Near <i>VEGFA</i>	Near MC4R
Near <i>NEGR1</i>	In TFAP2B	In <i>TBX15</i>	In <i>NPC1</i>
Near <i>TMEM18</i>	Near MSRA	Near <i>NFE2L3</i>	Near <i>MAF</i>
Near <i>KCTD15</i>	In NRXN3	Near <i>GRB14</i>	Near <i>PTER</i>
In <i>SH2B1</i>		Near <i>LYPLAL1</i>	Near MSRA
Near <i>GNPDA2</i>		In <i>DNM3</i>	
In <i>MTCH2</i>		Near <i>ITPR2</i> & <i>SSPN</i>	
In <i>SEC16B</i>		Near <i>LY86</i>	
Near <i>ETV5</i> & <i>DGKG</i>		Near <i>HOXC13</i>	
In <i>BDNF</i>		Near <i>ADAMTS9</i>	
Near <i>BCDIN3D</i> & <i>FAIM2</i>		In <i>ZNRF3</i>	
In TFAP2B		In <i>NISCH</i>	
In NRXN3		In <i>CPEB4</i>	
Near <i>RBJ</i> & <i>POMC</i>			
Near <i>GPRC5B</i>			
In <i>MAP2K5</i>			
In <i>QPCTL</i> & Near <i>GIPR</i>			
In <i>TNNI3K</i>			
In <i>SLC39A8</i>			
Near <i>FLJ35779</i>			
In <i>LRRN6C</i>			
Near <i>TMEM160</i>			
Near <i>FANCL</i>			
In <i>CADM2</i>			
Near <i>PRKD1</i>			
Near <i>LRP1B</i>			
Near <i>PTBP2</i>			
In <i>MTIF3</i>			
Near <i>ZNF608</i>			
Near <i>RPL27A</i> and <i>TUB</i>			
In <i>NUDT3</i>			

Loci in **bold** indicate those that were identified for multiple obesity-related traits.

The first wave comprised of two studies, each with ~ 4800 white Europeans in the discovery stage, that independently identified SNPs in fat mass and obesity associated gene (*FTO*) to be robustly associated with BMI. After the discovery of the

FTO locus, scientists realised that larger samples were needed to identify more genetic loci. As such, the genomic investigation of anthropometric traits (GIANT) consortium was formed, which led to the second wave of discoveries. This

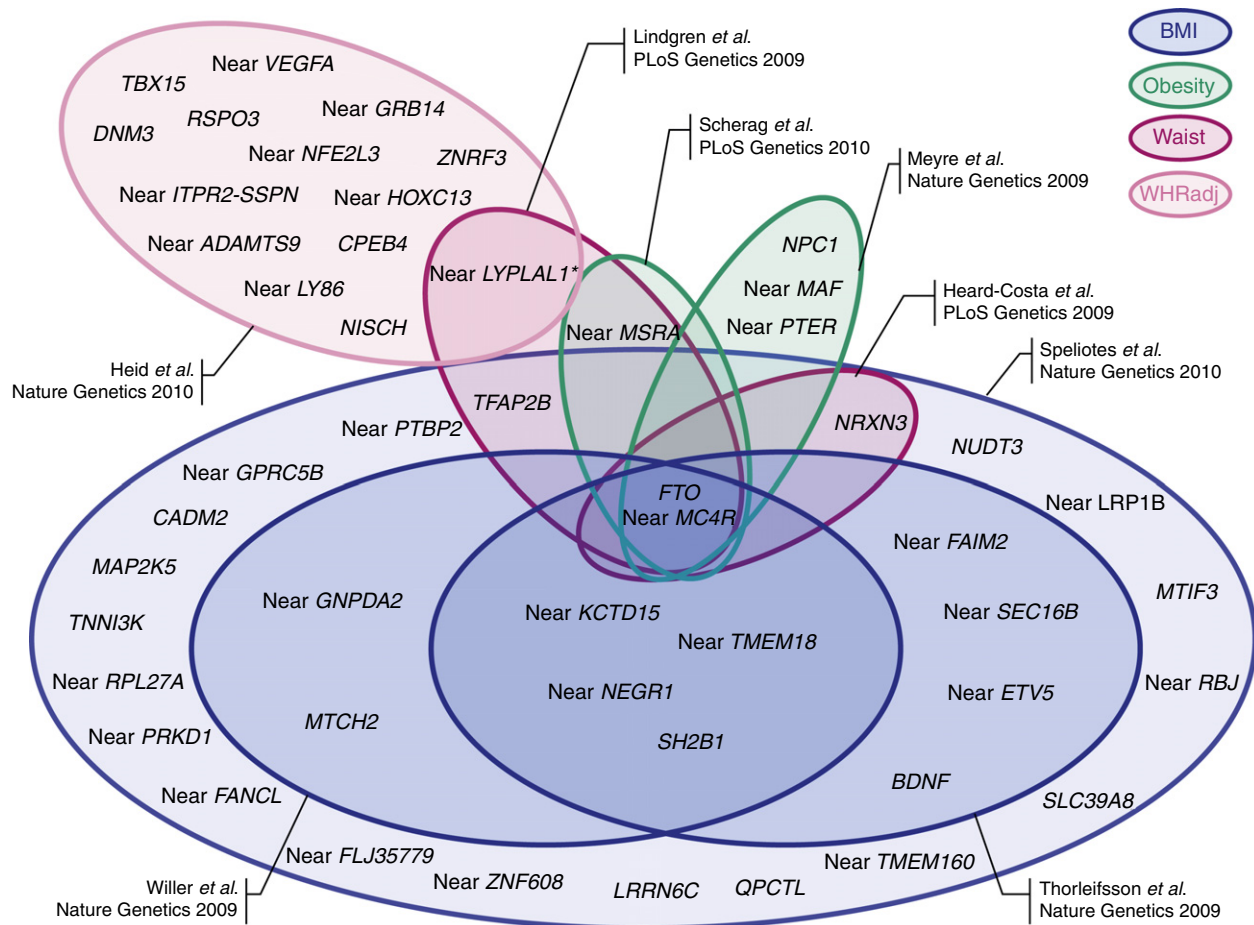


Figure 3 Obesity-susceptibility loci discovered in four waves of genome-wide association studies for BMI (blue), three waves of genome-wide association studies for waist circumference and WHR (pink), and two waves of genome-wide association studies for extreme and early-onset of obesity (green). Each Venn-diagram represents the loci of one study, except for studies that discovered only one locus, i.e., and the near-MC4R loci, for which no Venn-diagram was drawn. Reproduced from Frayling TM, Timpson NJ, Weedon MN, *et al.* (2007) A common variant in the *FTO* gene is associated with body mass index and predisposes to childhood and adult obesity. *Science* 316: 889–894; Scuteri A, Sanna S, Chen W-M, *et al.* (2007) Genome-wide association scan shows genetic variants in the *FTO* gene are associated with obesity-related traits. *PLoS Genetics* 3: e115; Hinney A, Nguyen TT, Scherag A, *et al.* (2007) Genome wide association (GWA) study for early onset extreme obesity supports the role of fat mass and obesity associated gene (*FTO*) variants. *PLoS ONE* 2: e1361; Loos RJ, Lindgren CM, Li S, *et al.* (2008) Common variants near *MC4R* are associated with fat mass, weight and risk of obesity. *Nature Genetics* 40: 768–775, and Chambers JC, Elliot P, Zabaneh D, *et al.* (2008) Common genetic variation near *MC4R* is associated with waist circumference and insulin resistance. *Nature Genetics* 40: 716–718, with permission from PLOS, AAAS and Nature.

wave consisted of a meta-analysis of seven GWAS, including 16 876 white Europeans. Besides confirming the *FTO* locus, a locus near *MC4R* was found to be unequivocally associated with BMI. In the third wave, sample sizes of the meta-analyses doubled and two independent GWAS meta-analyses (from GIANT and deCODE) of each ~32 000 individuals identified a total of 10 new BMI loci, besides confirming the previous two loci (Figures 3 and 4). In the fourth wave, the GIANT consortium increased its discovery stage to 123 865 individuals, or almost four times larger than in the third wave, and identified another 20 loci, besides confirming the 12 previous BMI loci (Figures 3 and 4).

Of the 32 established BMI loci, the firstly identified obesity-susceptibility locus, in *FTO*, was the easiest to be discovered as it has the largest, yet small, effect on obesity-susceptibility (Figure 5). Furthermore, the *FTO* risk-allele is highly prevalent

(46%) in populations of white European descent. Each additional *FTO* risk-allele increases BMI by ~0.39 kg m⁻² (equivalent to 1.125 kg in body weight for a person of 1.70 m tall (Figures 4 and 5)) and increases the risk of obesity by 20%.

As the sample size with each new wave increased, the statistical power to detect variants with smaller effects and/or lower minor allele frequencies increased (Figure 5). The effect sizes of the loci identified following the *FTO* discovery range from as low as 0.06 up to 0.31 kg m⁻² per risk-allele for BMI (or 170–895 g for a 1.70 m-tall person) and from 1.02 to 1.13 odds for risk of obesity. The frequencies of the risk alleles range from 4% to 87%.

The 32 BMI loci combined explain only 1.45% of the phenotypic variation in BMI, or 2–4% of genetic variation based on an estimated heritability of 40–70%. As a

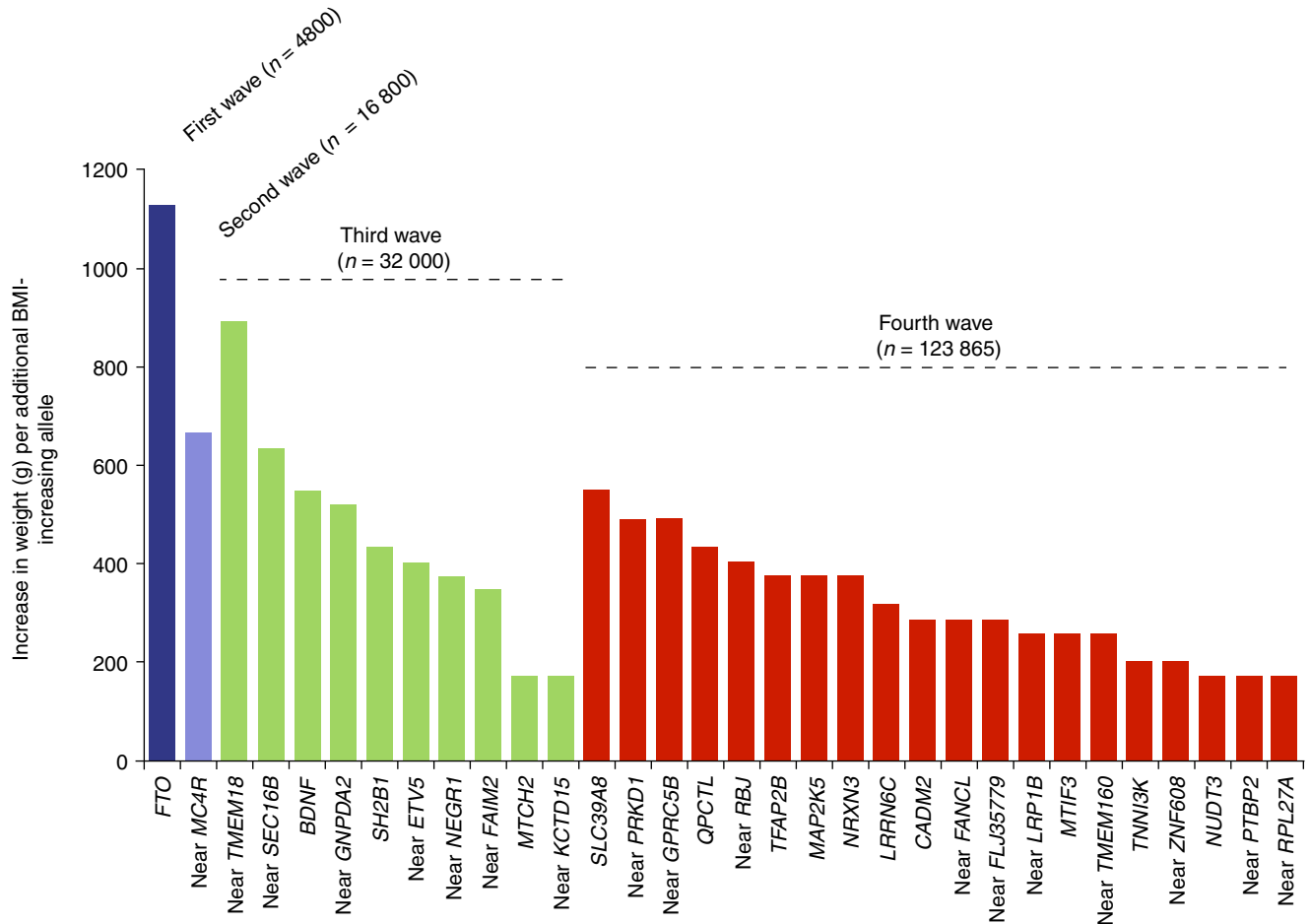


Figure 4 Per-allele effect size for each of the 32 genetic loci for BMI. The BMI loci are ordered according to effect size, stratified by wave of discovery. Adapted from Speliotes EK, Willer CJ, Berndt SI, *et al.* (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nature Genetics* 42(11): 937–948, with permission from Nature.

consequence, if the 32 loci were to be used as a genetic test for obesity, they would have a very low predictive value (AUC_{ROC} : 0.574).

Discoveries for Waist Circumference and Waist-to-Hip Ratio (WHR)

To better understand the pathogenesis of fat distribution, genome-wide association studies have been performed to identify genetic loci for waist circumference and waist-to-hip ratio (WHR).

The first two GWAS that focussed on central obesity both examined waist circumference as the main outcome (Table 2). Because waist circumference correlates highly with BMI, all five loci identified in these two GWAS were also identified by GWAS for BMI and obesity, suggesting that they are likely involved in overall adiposity rather than in fat distribution *per se*.

A subsequent larger GWAS, including more than 77 000 men and women, examined WHR adjusted for BMI, to focus more specifically on fat distribution, rather than on overall adiposity. This GWAS identified 14 loci associated with WHR of which seven showed significantly more pronounced effects in women than in men (Figure 6). None of these loci overlap with loci identified for BMI or obesity risk, suggesting that

they specifically affect fat distribution rather than overall adiposity.

The magnitude of the effects observed for the loci is similar to that of the BMI loci. Yet, despite the highly significant association results, these 14 loci together explain only a fraction (1.03%) of the phenotypic variation of WHR, although slightly more in women (1.34%) than in men (0.46%).

Discoveries for Extreme and Early-Onset Obesity

It has been speculated that individuals with early-onset or morbid obesity may be enriched for variants that predispose to obesity in the general population. As such, a GWAS for extreme obesity may have more statistical power to identify common obesity-susceptibility loci. Alternatively, if extreme obesity is a different phenotype from common obesity, then such GWAS may identify different loci that are specific to the extremely obese.

Two GWAS studies, each of ~1200 normal-weight controls and ~1200 obese cases, identified a total of six loci robustly associated with extreme or early-onset obesity (Table 2). Of these, three loci had been previously identified for BMI and waist circumference, whereas the remaining three loci seemed to be specific to extreme obesity.

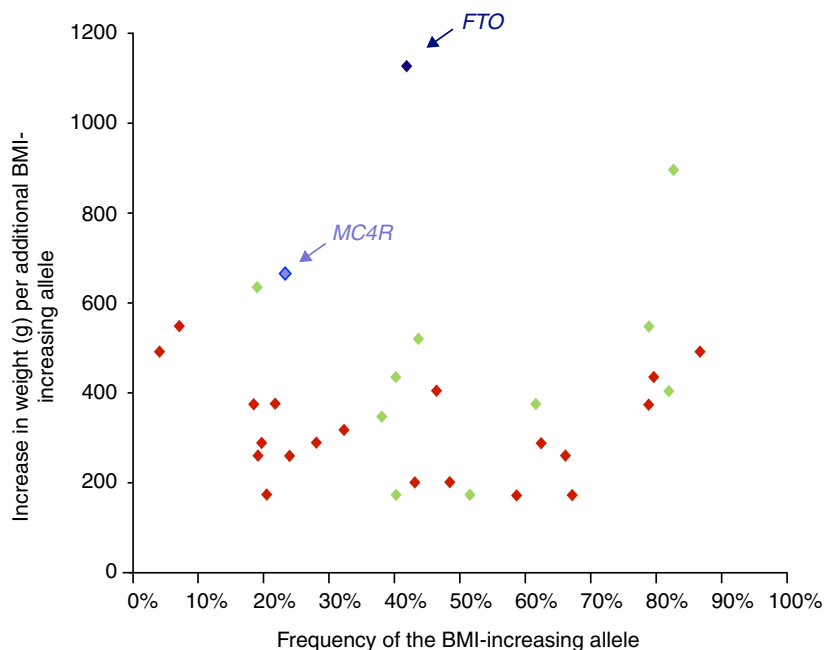


Figure 5 Per-allele effect size (y-axis) for each of the 32 genetic loci for BMI against the frequency of the BMI-increasing allele. Loci identified in the this wave are in green, and those in those in the fourth wave are red. Adapted from Speliotes EK, Willer CJ, Berndt SI, *et al.* (2010) Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. *Nature Genetics* 42(11): 937–948, with permission from Nature.

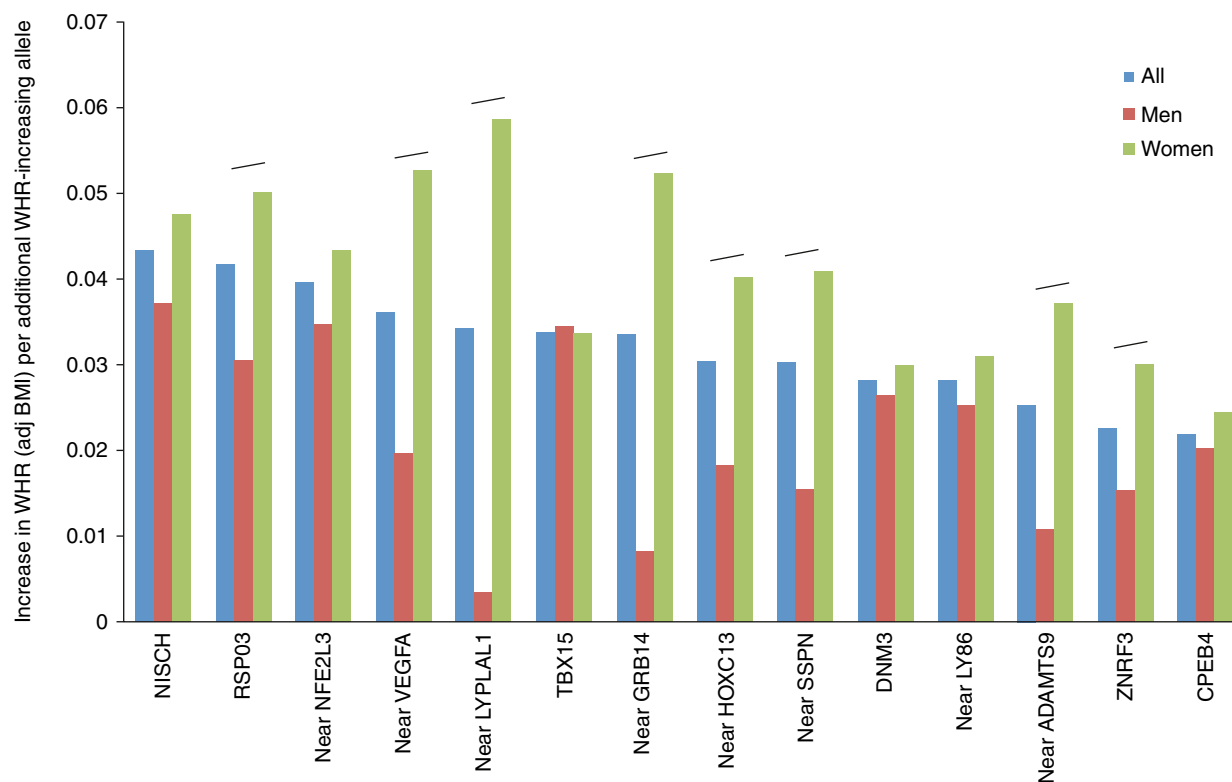


Figure 6 The per-allele effect size for each of the 14 genetic loci for WHR. The WHR loci are sorted by overall effect size. Effects are also shown for men and women separately. The lines above the bars indicate a significant difference in per-allele effect between men and women. Reproduced from Heid IM, Jackson AU, Randall JC, *et al.* (2010) Meta-analysis identifies 13 new loci associated with waist-hip ratio and reveals sexual dimorphism in the genetic basis of fat distribution. *Nature Genetics* 42(11): 949–960, with permission from Nature.

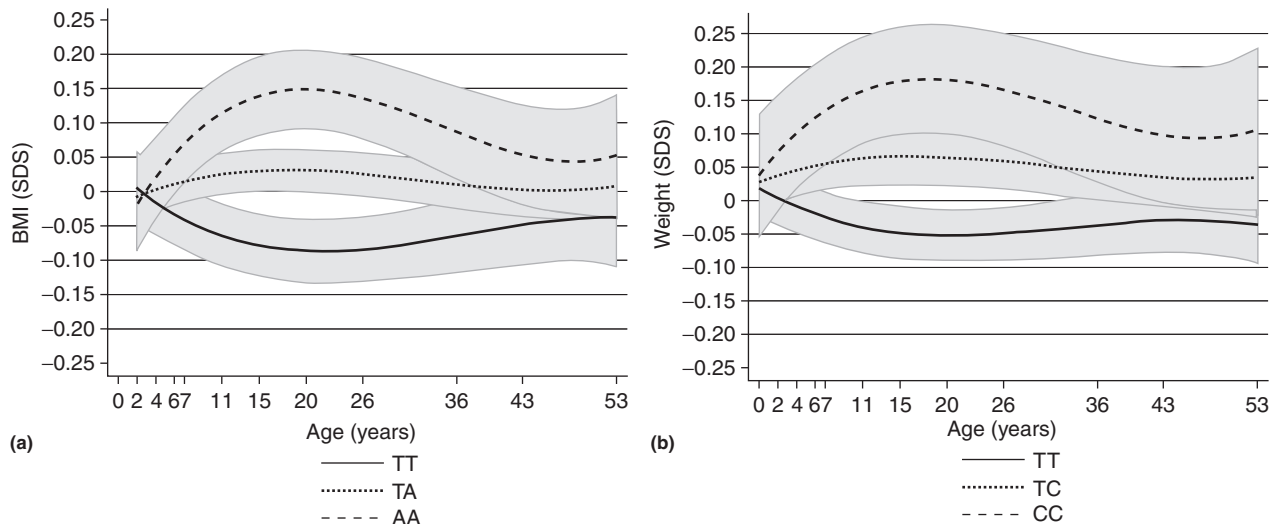


Figure 7 Linear prediction of mean and 95% prediction interval from additive genetic models for weight SDS based on weight were measured or self-reported repeatedly at 11 time-points between ages 2 and 53 years by *FTO* rs9939609 (panel a) and *MC4R* rs17782313 (panel b) genotype in 1240 men and 1239 women born in 1946. Reproduced from Hardy R, Wills AK, Wong A, *et al.* (2010) Life course variations in the associations between *FTO* and *MC4R* gene variants and body size. *Human Molecular Genetics* 19(3): 545–552, with permission from Oxford.

Discoveries in Populations of Nonwhite European Origin

So far, GWAS for obesity-related traits have been predominantly performed in individuals of white European origin. Because of differences in genetic architecture as well as differences in obesity-susceptibility, GWAS in individuals of non-white origin may identify novel loci that are ethnic-specific and that may also play a role in obesity-susceptibility across different ethnicities.

A large-scale GWAS in Koreans ($N=8842$) confirmed the locus in *FTO* and identified a variant in *C12orf51* to be associated with WHR. This obesity-susceptibility locus seems specific to East Asians as it is not polymorphic in individuals of white European or African descent. Relatively small GWAS in populations of African ancestry ($N=1931$) and in Indian Asians ($n=2684$), each independently confirmed the locus near *MC4R*, but did not identify new, ethnic-specific loci for obesity-related traits.

Although only a few GWAS in nonwhites have been performed, several nonGWAS studies in populations from various genetic backgrounds have aimed to replicate the obesity-susceptibility loci identified in whites. As such, the loci in *FTO* and near-*MC4R* have been consistently replicated in East Asians, in South Asians, and in individuals of African and African American origin. The replication of obesity-susceptibility loci that were identified in large-scale GWAS ($n>30\,000$) has been rather inconsistent and will likely require larger studies or meta-analyses to convincingly confirm or refute their role in obesity-susceptibility in other ethnicities.

Discoveries in Children and Adolescents

Almost all obesity-susceptibility loci have been identified through GWAS in adults. However, several follow-up studies have examined whether these loci affect obesity-susceptibility already during childhood and adolescence, which may provide insight in the etiology of obesity through the life course. None of the obesity-susceptibility loci affect weight at birth,

but there is strong evidence that the locus in *FTO* and near *MC4R* affects BMI already early in life. A birth cohort study showed that the effect of these two loci on BMI increases with age from childhood through adolescence, reaching its largest influence at age 20, after which it weakens again through adult life (Figure 7).

Most, but not all, of the remaining obesity-susceptibility loci have been shown to affect childhood obesity risk, typically with effects similar to those observed in adulthood. Additional large-scale studies will be needed to more firmly confirm associations for these loci in children and adolescents.

Translation of New Discoveries

Despite the overwhelming significance of associations and repeated replications, the explained variance and predictive value of the currently identified obesity-susceptibility loci is too low to be used for genetic profiling and personalized management of obesity, such that the efficacy of GWAS has been questioned by some. However, others argue that the true value of GWAS lies in the translation of the new loci into biology. It is anticipated that the newly identified loci will shed light on the physiology governing the regulation of energy balance and fat distribution and that they will point towards novel causal pathways and, subsequently, to the identification of therapeutic targets.

So far, only *FTO* has been examined extensively for its role in obesity. Studies in rodents have shown that *Fto* is expressed ubiquitously, particularly in the hypothalamic nuclei that are involved in the regulation of energy homeostasis. Mice that carry one or two extra copies of the gene display increased *fpo* expression, and increased energy intake and adiposity. Studies in humans have supported a central neuronal role for *FTO* as the BMI-increasing allele was found to be associated with increased appetite and energy intake, and reduced satiety. However, this neuronal hypothesis was challenged as the complete loss of *Fto* in mice led to a significant reduction in adipose tissue and lean body mass, as well as increased energy

expenditure and systemic sympathetic activation, suggesting a peripheral role for *FTO*. More experimental studies will be required to fully understand the pathways through which *FTO* confers increased obesity-susceptibility.

Most of the other loci have not been studied in an experimental context. For many loci pinpointing the causal gene or variant is challenging, as some loci harbor multiple genes, whereas others harbor no genes at all. Comprehensive resequencing and fine mapping will be required to unambiguously identify the causal variants before physiologists can start exploring the functional relevance of the locus in relation to the risk of obesity. Of interest is that at least three (*MC4R*, *POMC*, *BDNF*) of the 32 BMI loci harbor genes that have been previously linked to monogenic obesity. Many loci harbor genes that are highly expressed in the brain and hypothalamus, supporting a role for the nervous system in body weight control. Furthermore, mice knockout models have supported a role for diet-induced obesity for the *SH2B1* gene.

Unlike the BMI loci, those identified for WHR contain genes that seem predominantly involved in adipocyte metabolism and central regulation of energy homeostasis. The WHR-locus near *ADAMTS9* was previously found to be associated with risk of type 2 diabetes and with insulin resistance in peripheral tissues.

Of interest is that apart from the *FTO* and near-*MC4R* loci, none of the loci identified for extreme and early-onset obesity overlap with those identified for BMI, suggesting that extreme obesity and BMI are, at least in part, different phenotypes that

are caused by different genes, and thus potentially different physiological pathways.

Gene–Lifestyle Interaction

Environmental and genetic factors do not act strictly independently or just additively, but they also interact with each other in their causeway to disease. The discovery of loci robustly associated with obesity-susceptibility has increased the interest in examining gene–lifestyle interaction; i.e., whether lifestyle can attenuate or exacerbate the strength of association between a genetic locus and obesity-susceptibility.

Most gene–lifestyle interaction studies have so far focussed on the *FTO* locus showing rather consistently that daily physical activity reduces the effect of *FTO* on obesity-susceptibility by ~30%. No such interaction was observed for the locus near *MC4R*. In a large-scale population-based study ($n=20\,000$), 12 obesity-susceptibility loci were combined in a genetic predisposition score. Although each additional BMI-increasing allele increased BMI by 0.154 kg m^{-2} (or 445 g for 1.70 m tall person), this increase was significantly more pronounced in sedentary individuals (0.205 kg m^{-2} or 592 g per allele) than in physically active individuals (0.131 kg m^{-2} or 379 g per allele) (Figure 8). These findings hold an important public health message as they challenge the deterministic view of the genetic predisposition to obesity, showing that even the most genetically predisposed individuals will benefit from adopting a healthy lifestyle.

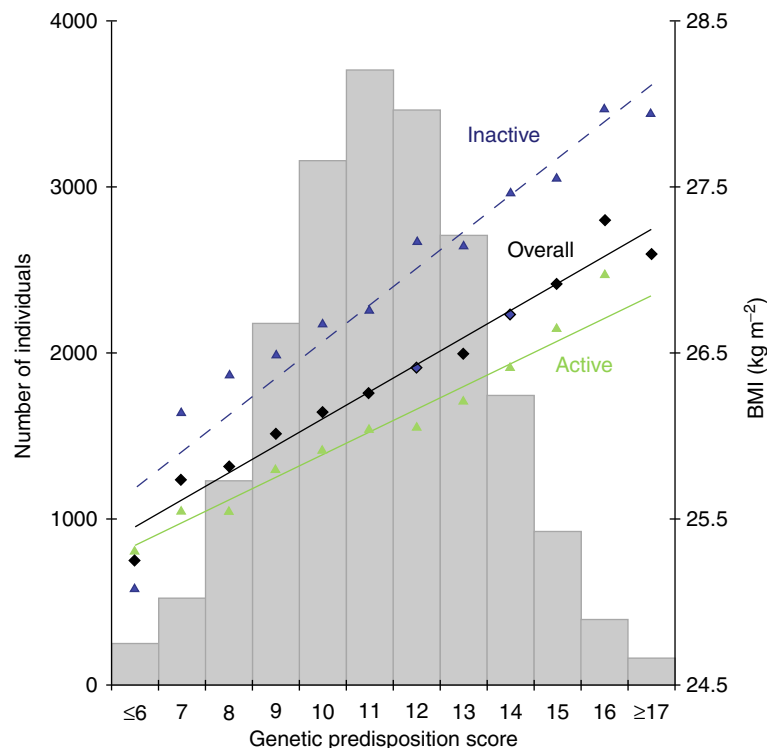


Figure 8 Association between the genetic predisposition score (sum of BMI-increasing alleles from 12 BMI loci) with BMI in all individuals (solid black line), in sedentary individuals (dashed gray line) and in physically active individuals (solid gray line). Reproduced from Li S, Zhao JH, Luan J, *et al.* (2010) Physical activity attenuates the genetic predisposition to obesity in 20,000 men and women from EPIC-Norfolk prospective population study. *PLoS Medicine* 7(8): pii: e1000332, with permission from PLOS.

Table 3 Future directions in gene discovery

<i>Continued and alternative use of genome-wide association studies</i>	
Increased discovery by efficient use of available genome-wide association data	<i>For example, follow-up of a larger number of SNPs ($n > 5000$) from the discovery stage</i>
Genome-wide association studies in populations of different ethnic backgrounds	
Genome-wide association studies in children and adolescents	
Genome-wide association studies of more refined traits of obesity-susceptibility	<i>For example, body fat percentage, leptin, ...</i>
Genome-wide association studies of intermediate traits of obesity-susceptibility	<i>For example, physical activity, food intake, ...</i>
Genome-wide gene-lifestyle interaction analyses	
<i>Identification of low-frequency variation</i>	
Genome-wide association of copy number variants (CNVs)	
Genome-wide association after implementation of the detailed reference data obtained through sequencing	<i>For example, through imputation of data from the 1000 Genomes project</i>
Deep-sequencing efforts using new generation sequencing technology	<i>For example, targeted sequencing for fine-mapping of established loci</i>
	<i>For example, whole genome or exome sequencing for discovery of new loci.</i>
<i>Follow-up of existing loci to identify the causal gene or variant</i>	
For example, by taking advantage of difference in genetic background across ethnicities	
<i>For example, using 1000 genomes data</i>	
<i>For example, by targeted sequencing</i>	

Future Directions

As the established loci explain only a fraction of the heritability, it has been speculated that more loci remain to be discovered and that the established loci may harbor low-frequency variants, not currently captured by the genome-wide genotyping arrays, that have larger effects. Various approaches have been proposed to identify more genetic loci and to pinpoint the causal variants (Table 3).

GWAS may continue to identify genetic loci for obesity-susceptibility for another few years when applied to understudied populations, to refined outcomes, and to intermediate traits. Also GWAS in the context of gene-lifestyle interaction might reveal loci sensitive to environmental triggers. Furthermore, it is believed that low-frequency variants, which cannot be identified with traditional GWAS, remain to be uncovered with new technologies.

Conclusion

Over the past two decades genetic insights in rare and common forms of obesity have shed new light onto the biology that underlies body weight control. Various approaches have been used to identify genes or genetic variants that increase

obesity-susceptibility. Although candidate gene studies have provided immediate clues of the pathways involved, recent genome-wide association studies have identified a plethora of loci of which the physiological role remain to be explored. The use of the obesity-susceptibility loci to develop personalized approaches to prevent or treat obesity lies in a future further ahead of us.

See also: Obesity: Childhood Obesity

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Prevention

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Obesity remains one of the major public health and economic problems throughout the world with the World Health Organization (WHO) estimating that approximately 1.5 billion people worldwide were overweight in 2008 and of these over 200 million men and nearly 300 million women were obese. Prevalence rates continue to rise rapidly in all areas of the world, including low-income countries, and obesity-associated illnesses are now so common that they have replaced the more traditional public health concerns, such as undernutrition and infectious disease, as the most significant contributors to global ill health.

The health impact of obesity is considerable, and obesity impacts on both quality and length of life. Overweight and obesity are associated with a wide range of chronic conditions, such as diabetes, hypertension, cardiovascular disease (CVD), and certain cancers, as well as non-life-threatening but painful conditions, such as arthritis, back pain, and breathlessness. Obesity also places enormous financial burdens on governments and individuals, and accounts for a significant proportion of total health care expenditure in developed countries. Analyses suggest that obesity is fast approaching cigarette smoking as the major preventable cause of mortality.

In recent years, our understanding of the epidemiology and causation of obesity has improved dramatically and there is an acceptance that urgent action is required to address the problem. There is also a limited, but growing, body of successful, large-scale obesity prevention initiatives from across the world. These experiences, together with understandings gained from smaller-scale obesity prevention initiatives and experiences from the management of other epidemics of noncommunicable diseases, provide useful guidance on effective planning and implementation of obesity prevention programs.

Principles of Obesity Prevention

Rationale for Obesity Prevention

There are a number of reasons why prevention is likely to be the only effective way of tackling the problem of overweight and obesity. First, obesity develops over time, and once it has done so, it is very difficult to treat. A number of analyses have identified the limited success of current obesity treatments (with the possible exception of surgical interventions) to achieve long-term weight loss. Second, the health consequences associated with obesity result from the cumulative metabolic and physical stress of excess weight over a long period of time and may not be fully reversible by weight loss. Third, the proportion of the population that is either overweight or obese in many countries is now so large that there are no longer sufficient health care resources to offer treatment to all. It can be argued, therefore, that the prevention of weight gain (or the reversal of small gains) and the maintenance of a healthy weight would be easier, less expensive, and potentially more effective than to treat obesity after it has fully developed.

Objectives of Obesity Prevention

There remains a great deal of confusion regarding the appropriate objectives of an obesity prevention program. It is often assumed that to be effective, any intervention to address the problem of excess weight in the community should result in a reduction in the prevalence of overweight and obesity. However, such an objective is unrealistic and may be counterproductive. Most communities are experiencing significant increases in the average weight of the population as a result of a sizeable energy surplus resulting from reduced energy expenditure combined with an increased energy intake. This is leading to rapidly escalating rates of overweight and obesity. To reverse this trend will require not only the removal of this energy surplus but also the creation of a negative energy balance that will need to be maintained by the whole population for a significant period of time. Few (if any) interventions are capable of reducing energy intake, or increasing energy expenditure sufficiently, or are sustained long enough and with sufficient reach to achieve this effect. More appropriate objectives would relate to a reduction in the level of weight gain or the maintenance of weight stability in adults and the achievement of appropriate growth and development in children. The achievement of these objectives would result in a slowing in the rate of increase, followed by stabilization, and then an eventual decline in the level of overweight and obesity in the community.

However, even the goal of weight stability within a population may be difficult to achieve in the short term because it would require the maintenance or reestablishment of energy balance in a time of significant energy surplus. Therefore, it may be necessary to identify more sensitive short- and medium-term outcomes to evaluate obesity prevention programs. Such process outcomes may relate to the achievement of appropriate changes in energy intakes or outputs, food or physical activity behaviors, or changes to the environment that are significant enough to positively impact upon the achievement of energy balance.

Importance of Weight Gain Prevention in Adults

There are a number of important reasons why it is preferable to focus on weight gain prevention as the key individual and population objective of obesity prevention initiatives in adults (**Box 1**). The association between elevated body mass index (BMI) and increased risk of ill health is clear and consistent. However, research has demonstrated that weight gain *per se* is also associated with increased health risk, and that this risk is independent of absolute BMI (provided a person is not underweight). A number of studies have shown strong relationships between weight gain and increasing levels of diabetes, hypertension, gall bladder disease, and coronary heart disease. Therefore, a large weight gain in a lean individual may carry equivalent risk in maintaining a stable but slightly elevated BMI in an overweight individual. The combination of an elevated BMI and ongoing weight gain, however, leads to greatly magnified levels of risk.

Box 1 Why focus on weight gain prevention?

- Weight gain in adulthood carries an independent risk of ill health.
- Risk for chronic disease begins to increase from low BMI levels and significant weight gain can occur within normal limits.
- Extended periods of weight gain are difficult to reverse.
- Weight gain in adulthood is mostly fat gain.
- The relationship between absolute BMI and health risk varies with age and ethnicity, but no such variations occur in the relationship between weight gain and ill health.
- A focus on weight gain prevention avoids exacerbation of inappropriate dieting behaviors.
- Weight maintenance can serve as a first stage goal for weight treatment programs.
- The message is equally relevant to all sections of the adult population.
- It avoids further stigmatization of people with an existing weight problem.
- It avoids reference to poorly understood terms such as 'healthy weight.'

Who Should Obesity Prevention Strategies Target?

Deciding where to invest limited time and resources in obesity prevention is a difficult task but finite health resources make this a necessity. WHO has identified three distinct but equally valid and complementary levels of obesity prevention (Figure 1). The specific 'targeted' approach directed at very high-risk individuals with existing weight problems is represented by the core of the figure, the 'selective' approach directed at individuals and groups with above average risk is represented by the middle layer, and the broader universal or populationwide prevention approach is represented by the outer layer. This replaces the more traditional classification of disease prevention (primary, secondary, and tertiary), which can be confusing when applied to a complex multifactorial condition such as obesity.

Universal prevention is the domain of public health, whereas selective and targeted prevention are predominantly dealt with in community and health care service settings. Community settings include schools, colleges, worksites, community centers, and shopping outlets.

Whole Community

Overweight and obesity are public health problems of relevance to the whole community and require strategies that focus on populationwide change rather than attempting to address individuals or small groups in isolation of the community in which they live. An effective population strategy needs to both improve population knowledge about obesity and its management and reduce the exposure of the community to obesity-promoting factors in the environment. Action at a population level requires coordination at a central level and the investment of resources to be maintained over a long period of time to achieve population change.

Family Focus

There are numerous reasons why children should be a major focus of any obesity prevention strategy. There is strong



Figure 1 Levels of obesity prevention intervention. Adapted from Gill TP (1997) Key issues in the prevention of obesity. *British Medical Bulletin* 53(2): 359–388, with permission from BMJ.

evidence that a high proportion of overweight or obese children will become obese adults. Childhood obesity also has immediate effects on health, and weight-related conditions are becoming more prevalent and their effect more pronounced as the rates of childhood obesity increase. However, children grow rapidly and increase the level of lean body mass as they age, and so reducing or keeping fat mass constant allows the normalization of weight over time. Thus, childhood (particularly younger children) is a period during which prevention efforts have a higher chance of success.

However, children also have little direct control over the environment in which they live. Parents and other caregivers mostly control decisions regarding the food available and the opportunities for activity. In addition, the behaviors of parents and other siblings have a profound effect on the diet and physical activity behaviors of children. For this reason, it is preferable to focus childhood obesity prevention efforts on the family environment rather than directly on children.

High-Risk Groups

There are a number of groups that appear to be at higher risk of developing overweight and obesity (Table 1). These groups warrant special attention and include the following:

- Those with a family history of weight problems.
- Socially disadvantaged and isolated communities.
- Certain ethnic groups.
- Smokers who have recently quit smoking.
- Those who have recently lost weight.

In addition, there are certain times in a person's life when the person is more prone to weight gain (Table 1). These age groups could be considered for selective prevention

Table 1 Identifying at-risk groups for obesity

<i>Critical ages and life stages</i>	<i>Reason for increased risk</i>
Prenatal	There is strong evidence that <i>in utero</i> development has permanent effects on later growth and energy regulation.
Adiposity rebound (5–7 years)	BMI begins to increase rapidly after a period of reduced adiposity during preschool years. Food and activity patterns change as a result of exposure to other children and school. Early and rapid weight rebound often precedes the development of obesity.
Adolescence	Period of increased autonomy that is often associated with irregular meals, changed food habits, and periods of inactivity during leisure combined with physiological changes that promote increased fat deposition, particularly in females.
Early adulthood	Early adulthood usually correlates with a period of marked reduction in physical activity, increased alcohol consumption, and poor diets.
Pregnancy	Excessive weight gain during pregnancy often results in retention of weight after delivery, particularly with early cessation of breast feeding. Inappropriately large as well as inadequate weight gain may both contribute to weight problems for the developing child.
Menopause	In Western societies weight generally increases with age, but it is not certain why menopausal women are particularly prone to rapid weight gain. The loss of the menstrual cycle does affect food intake and reduce metabolic rate slightly.
<i>High-risk groups</i>	
Family history of weight problems	There is no longer any doubt that given the same environment some individuals are more prone to depositing fat. The basis of these differences in individual susceptibility to obesity is yet to be fully elucidated, but is believed to involve a number of physiological processes associated with fat deposition, oxidation, and involuntary energy expenditure.
Recent migrants and refugees	Recent migrants and refugees often find it difficult to maintain traditional diet and physical activity patterns and may replace them with inappropriate foods.
Socially or economically disadvantaged	In many developed countries, there is an inverse association between income and education level and obesity, which is most pronounced among women. It is argued that cheaper foodstuffs are usually high in fat and energy dense and those with less financial resources spend more time in sedentary activities such as watching TV.
Shift workers	Disrupted sleep patterns and altered daily routine have been associated with poorer quality diets, reduced opportunities to exercise and weight gain in those working night shifts.
Recent successful weight reducers	Successful weight reduction is usually followed by the regain of one-half to one-third of the weight loss over the following year. It is believed that biological and behavioral processes act to drive body weight back to baseline levels.
Recent past smokers	Smokers are usually thinner than nonsmokers because smoking tends to depress appetite, increase the basal metabolic rate, and, after each cigarette, induce a surge in heart and metabolic rate. The effect on metabolism of smoking 24 cigarettes per day has been estimated at approximately 200 kcal per day.

Source: Adapted with permission from Gill TP (1997) Key issues in the prevention of obesity. *British Medical Bulletin* 53(2): 359–388.

interventions. These times include the following:

- Prenatal
- Adiposity rebound (5–7 years)
- Adolescence
- Early adulthood
- Pregnancy
- Menopause

Those with an Existing Weight Problem

In developing weight gain prevention strategies, it is important not to neglect those with an existing weight problem who could benefit from more intensive efforts to help prevent further weight gain.

Key Elements of a Weight Gain Prevention Plan

Weight gain and obesity develop when the energy intake from food and drink exceeds energy expenditure from physical activity and other metabolic processes. It is often assumed that the prevention of weight gain should focus solely

on attempting to alter these behaviors within individuals and communities. However, research has consistently shown that numerous and diverse factors, including environmental and social factors, influence the behaviors that lead to excessive weight gain. Addressing aspects of the obesogenic (obesity-promoting) environment, as well as individuals' eating and physical activity patterns, is considered to be critical to the success of any obesity prevention program.

The 2003 WHO report on diet, nutrition, and the prevention of chronic disease undertook a detailed review of the literature and identified a range of key factors that either increase or decrease the risk of weight gain and the development of obesity (Table 2). These factors were rated on the quality of evidence available to support their contributory role. This analysis serves as a very useful guide as to the focus of weight gain prevention initiatives.

Diet and Physical Activity Behaviors

The WHO analysis and subsequent reviews have identified a number of key dietary and physical activity behaviors,

Table 2 Summary of the strengths of evidence of factors that may promote or protect against weight gain and obesity

<i>Evidence</i>	<i>Decreases risk</i>	<i>Increases risk</i>
Convincing	Regular physical activity High dietary fiber intake	High intake of energy-dense foods ^a Sedentary lifestyle
Probable	Home and school environments that support healthy food choices for children Promoting linear growth	Heavy marketing of energy-dense foods and fast food outlets Adverse social and economic conditions in developed countries (especially for women) Sugar-sweetened soft drinks and juices
Possible	Low glycemic index foods Breast feeding	Large portion sizes High proportion of food prepared outside of home Rigid restraint/periodic disinhibition eating patterns
Insufficient	Increased eating frequency	Alcohol

^aEnergy-dense foods are high in fat/sugar and energy-dilute foods are high in fiber and water, such as vegetables, fruits, legumes, and whole grain cereals.

Source: Adapted with permission from World Health Organization (2003) *Joint WHO/FAO Expert Report on Diet, Nutrition and the Prevention of Chronic Disease*, WHO Technical Report Series 916. Geneva: WHO.

amenable to change, that could conceivably influence energy balance sufficiently to contribute to the prevention of weight gain and obesity. Behaviors that reduced the risk of obesity included regular physical activity, high dietary fiber intake, and possibly breast feeding, and low glycemic index diets. Behaviors that increased the risk of obesity included a high intake of energy-dense foods, a high intake of sugar-sweetened drinks and juices, time spent in sedentary behaviors, and possibly large portion sizes, a high intake of fast foods, and a restrained eating pattern.

The area of dietary and physical activity antecedents to weight gain and obesity is still incompletely understood and new research findings, which help clarify our understanding, are being presented on a regular basis. In addition, different behaviors are more prevalent or pronounced in different regions of the world. It is therefore difficult to give definitive recommendations on the most important and useful behaviors to target in obesity prevention strategies. However, strong evidence exists to support the inclusion of some key behaviors.

Reducing Energy Intake

Reducing the Intake of High Energy-Dense Foods (i.e., foods high in fat/sugar)

There is a high level of agreement that the overconsumption of energy-dense foods is a major contributor to excess energy intake and weight gain and that the restriction of energy-dense food items is a useful strategy for the prevention of weight gain. However, discussion continues as to whether fat or refined carbohydrate is the major contributor to energy density in the modern diet and thus should be the target of programs to control weight. The debate is being fueled by dietary data from many developed countries showing that dietary fat intakes have leveled out or declined slightly and intakes of carbohydrates have increased dramatically. However, research has shown that dietary fat (along with water and fiber) is a major contributor to the energy density of foods and that *ad libitum* low-fat diet plans are an effective dietary approach to weight gain prevention or moderate weight loss. There is also strong evidence that excess refined carbohydrate, particularly

high glycemic index carbohydrate, contributes to weight gain and its restriction aids weight loss and improves cardiovascular risk factor profiles.

Increasing the Intake of High-Fiber, Energy-Dilute Foods (especially vegetables and fruits)

There is less evidence on the effectiveness of increasing the intake of energy-dilute foods such as vegetables and some fruits in the diet. Such a strategy would assist weight gain prevention only if the inclusion of such foods leads to a reduction in the intake of more energy-dense alternatives and thus creates a reduction in energy intake. Few studies have addressed this issue in a comprehensive manner, but the additional health benefits of these foods make such a strategy low risk in nutritional terms.

Reducing the Consumption of Sugar-Sweetened Soft Drinks and Juices

Evidence is accumulating from a variety of studies that energy consumed as sweetened drinks is less well compensated for than energy consumed as solid food. Many, but not all, longitudinal studies have indicated that sweetened drinks (soft drinks or sodas) are associated with weight gain in both children and adults. Recent work has also demonstrated that the simple strategy of reducing the intake of sweetened drinks can be effective in preventing or limiting inappropriate weight gain.

Reducing the Level of Food Prepared Outside Home

The proportion of food purchased and consumed at food outlets outside home has increased dramatically in recent decades in both developed and developing nations. In the United States, approximately 40% of the household food budget is spent on food eaten away from home, and much of this is spent at fast food outlets. A number of analyses has linked increased consumption of fast food with increased risk of obesity. Although only a limited number of studies has evaluated the effect of reducing the consumption of fast food, it would seem to be a valuable strategy with few nutritional negatives.

Reducing Portion Sizes

The portion size of packaged foods and snacks, as well as restaurant serving sizes, has increased rapidly in recent times and has been identified as an important factor in the consumption of excess energy. Evidence suggests that people will consume the portion of food they are provided rather than respond to satiety signals to stop eating and leave food. Also, as the serving size increases, the ability of consumers to estimate accurately how much they have consumed decreases. Reducing portion sizes is a simple but immediately effective mechanism for reducing energy intake.

Increasing Energy Expenditure

Regular Physical Activity

Although it is difficult to obtain accurate assessments of physical activity, there is little doubt that energy expenditure from activity has decreased in the past 50 years in most countries throughout the world. In contrast to popular belief, participation rates in organized leisure-time physical activity have not declined in recent times and may have increased in many countries. This supports the contention that the greatest contributor to this reduction in energy expenditure is associated with substantial changes in occupational and incidental physical activity. Changes in employment patterns and work practices together with a reliance on motorized transport and the removal of almost all manual labor from our daily lives have led to a dramatic reduction in daily physical exertion.

Studies that have examined the association between physical activity and weight gain and the impact of increasing physical activity on weight gain prevention have been limited by the ability to accurately measure physical activity across the whole day and to engage people in sufficient levels of physical activity to prevent weight gain. However, there is sufficient evidence to support an important role for increasing physical activity in any weight gain prevention strategy, although questions remain about how much exercise is necessary and what type of exercise is appropriate to promote. The issue of the amount of extra time that people should spend in moderate physical activity to prevent weight gain remains hotly debated, but it is clearly substantially more than the 30 min on 5 days or more each week recommended by experts to reduce CVD risk. The type of exercise that should be the focus of weight gain prevention strategies is also under review. It has been suggested that the most effective ways to include regular physical activity in daily living are through increased incidental activity, increased participation in active recreation, and increased use of active transport.

Reduced Time Spent in Sedentary Behaviors (especially TV watching)

Changes in societal structures and improvements in technology have allowed a reduction in time spent at work or on domestic chores, leaving a greater proportion of the day for leisure. At the same time, most of the entertainment options developed to fill this time, such as watching television, playing video games, and using computers, are sedentary activities that require very little energy expenditure. These forms of entertainment, which initially complemented other forms of leisure activity, are

occupying more hours of the day and are displacing more active pursuits and games. As a consequence, a number of studies have identified clear links between time spent in this sedentary behavior and weight gain. However, it is important to make a distinction between a lack of physical activity and sedentary behavior because their mechanisms for impacting on body weight may be different and a person with a high level of physical activity can also have a high level of sedentary behavior. Although the precise pathway by which sedentariness influences weight gain is not known, it is believed to involve both a reduction in physical activity and physiological reductions in energy expenditure together with an increase in dietary energy intake through inappropriate food intake that is often stimulated by and accompanies sedentary activities.

Some studies in children have shown that programs that seek to reduce time spent in sedentary behaviors are more effective in controlling weight than programs that aim to increase physical activity alone. In some cases, a simple program to reduce the amount of time spent watching television was sufficient to significantly limit inappropriate weight gain in children.

Creating Supportive Environments

The external physical, social, political, and economic environments in which people exist have a profound effect on their attitudes and behaviors. Each day, people interact with a wide range of services, systems, and pressures in settings such as schools, the workplace, home, restaurants, and fast food outlets. In addition, laws, policies, economic imperatives, and the views of governments, industry, and society as a whole influence these settings. Each of the features of this complex system, which shapes the environment in which we live, has the capacity to inhibit or encourage appropriate dietary and physical activity patterns. The availability of open space, access to public transport, the design of suburbs, access to buildings, the perceived level of safety, provision of lighting, and many other factors influence our capacity and desire to be more physically active in our daily lives. Similarly, advertising pressures, access to appropriate food choices, school food policies, and nutrition information, and labeling all potentially influence food selection. Today, there is also a large commercial drive to promote obesogenic behaviors (cars and food are the two most advertised products on television). The Foresight Program of the UK Government Office for Science recently produced a complex conceptual model with 108 variables known as the obesity systems map to illustrate the complexity and interaction of the broad range of variables that drive the development of obesity.

Trying to motivate people to make healthy choices when the external environment works against such behaviors is a recipe for failure. **Figure 2** illustrates the role that the social environment plays in assisting or inhibiting personal behavior choices made by individuals, which ultimately has an impact on their health. Great success is likely to be achieved by creating a supportive environment and then promoting the healthy dietary and physical activity choices within such an environment.

Identifying Effective Obesity Prevention Interventions

Identifying the most efficacious programs to prevent obesity from the published literature has proved problematic.

A number of systematic reviews have assessed the current scientific literature of reported programs addressing the prevention of obesity in both children and adults and have identified only a limited number of evaluated programs to assess. Reviews of childhood obesity prevention initiatives indicated that certain approaches appear to be associated with greater success. An intensive intervention in small groups was a successful management strategy in children, as was involving all the family. Reducing levels of inactivity was successful at both treating and preventing weight gain. Some interventions which increased time spent in formal physical activity were successful in controlling weight gain but generally multi-component programs which addressed a range of strategies were deemed to hold the most potential. The Ensemble Prévenons l'obésité des Enfants program is a multicomponent, community-based childhood obesity program which has been implemented in a number of local government regions in France and throughout Europe and has produced promising reduction in measured obesity rates.

However, most reviews conclude that there was simply too small a body of research conducted in a limited number of settings to provide firm guidance on consistently effective interventions. To address the limitation, some researchers have proposed a system that allows the integration of the available information from the literature together with other forms of evidence including experience from past public health and health promotion action to identify the target groups and settings most likely to have produced effective action on obesity. This is achieved by producing a classifi-

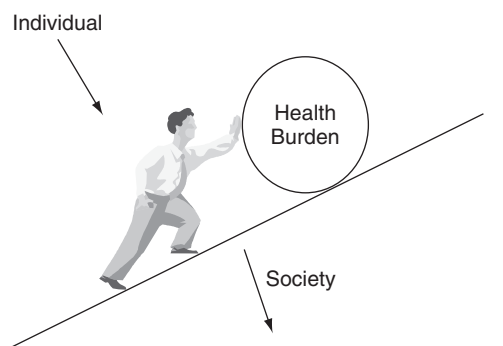


Figure 2 Influence of societal and environmental factors on development of obesity. Reproduced from House of Commons Health Committee (2004) *Obesity: Third Report of Sessions 2003–04. Volume 1. Report Together with Formal Minutes*. London: The Stationery Office Ltd.

cation system which is based on potential for change rather than demonstrated effectiveness. To achieve this, interventions are selected and assessed in terms of how ‘promising’ they may be in addressing population weight gain, using a health gain/risk framework. This allows the selection of interventions to be based on the best available evidence, while not excluding untried but promising strategies (see [Table 3](#)). The return or health gain can be defined in terms of demonstrated or modeled efficacy (from previous studies), potential population reach, and likely uptake (estimated). Uncertainty or risk can be defined in terms of the level of information or evidence to support the effectiveness of the intervention.

Lessons from Other Prevention Efforts

Although the number of successful large-scale obesity prevention programs is limited, there is a wealth of information from past public health programs that can be used to address other chronic diseases and risk factors. The International Obesity Task Force identified 10 key principles on which efforts to prevent obesity at a population level should be based. These are presented in [Box 2](#) and are drawn from experiences addressing CVD, smoking, alcohol and drug problems, dental disease, road accidents, and other public health issues.

Box 2 IOTF principles for the development of population obesity prevention initiatives

1. Education alone is not sufficient to change weight-related behaviors. Environmental and societal intervention is also required to promote and support behavior change.
2. Action must be taken to integrate physical activity into daily life, not just to increase leisure time exercise.
3. Sustainability of programs is crucial to enable positive change in diet, activity, and obesity levels over time.
4. Political support, intersectoral collaboration, and community participation are essential for success.
5. Acting locally, even in national initiatives, allows programs to be tailored to meet real needs, expectations, and opportunities.
6. All parts of the community must be reached, not just the motivated healthy.
7. Programs must be adequately resourced.
8. Where appropriate, programs should be integrated into existing initiatives.
9. Programs should build on existing theory and evidence.
10. Programs should be properly monitored, evaluated, and documented. This is important for dissemination and transfer of experiences.

Table 3 Matrix for determining the ‘promise’ of an intervention

Certainty of effectiveness* (Risk)	Potential population impact (Return)		
	Low	Moderate	High
Quite low	Least promising	Less promising	Promising
Medium	Less promising	Promising	Very promising
Quite high	Promising	Very promising	Most promising

Source: Adapted from Gill TP, King L, Webb K, and NSW Centre for Public Health Nutrition (2004) *Best Options for Promoting Healthy Weight and Preventing Weight Gain in NSW*. Sydney: NSW Health.

Although much has yet to be elucidated about the development of obesity and its effective management and prevention, there is a consensus that action to address the problem must not be delayed. Efforts to prevent weight gain need to be well designed, comprehensive, and appropriately resourced and evaluated so that the knowledge base improves with each new program.

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Treatment

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Introduction

Increasing body weight is associated with increasing health risks (Table 1). Randomized control trials (RCTs) demonstrate that weight reduction reduces these health risks and confirm the value in treating overweight and obesity.

Obesity is a chronic disease of multiple etiologies characterized by an excess of adipose tissue. Recent research has begun to unravel the biochemical and genetic factors implicated in its etiologies. As a result of the factors that determine its severity, health risks, and response to therapy, treatment must be tailored to specific needs. The ability of a treatment to

maintain long-term weight reduction is as important as its ability to cause the initial weight loss. In several studies, inability in maintaining the lowered weight is the cause of the treatment failure.

Nevertheless, a successful program should also lead to an improvement in the quality of life, self-esteem, social functioning, anxiety, and depression.

Several professional, governmental, and other organizations have drawn up guidelines for obesity management. These strategies for providing care to the obese patient provide useful evidence-based guidance for clinical management.

Table 1 Obesity-associated diseases and conditions

<i>Disorder</i>	<i>Associated diseases and conditions</i>
Cardiovascular	Coronary heart disease Hypertension Cerebrovascular disease Deep vein thrombosis Pulmonary embolism
Respiratory	Obstructive sleep apneas Obesity hypoventilation syndrome Breathlessness
Gastrointestinal	Nonalcoholic steatohepatitis Cirrhosis Gallstones Colorectal cancer Hiatus hernia/gastroesophageal reflux
Renal	Proteinuria
Reproductive	Primary ovulatory infertility Development of gestational diabetes Increased risk of neural tube defects
Musculoskeletal	Osteoarthritis Gout Nerve entrapment
Genitourinary	Endometrial cancer Prostate cancer Stress incontinence
Metabolic and endocrine	Artherogenic lipid profile Insulin resistance Type 2 diabetes mellitus Polycystic ovary syndrome Postmenopausal breast cancer Hirsutism
Skin	Acanthosis nigricans Lymphoedema Sweat rashes

Health Risks due to Overweight/Obesity

Increasing body fatness is accompanied by profound changes in physiological function. These changes are, to a certain extent, dependent on the regional distribution of adipose tissue. Generalized obesity results in alterations in total blood volume and cardiac function whereas the distribution of fat around the thoracic cage, and abdomen restricts respiratory excursion and alters respiratory function. The intraabdominal visceral deposition of adipose tissue, which characterizes upper body obesity, is a major contributor to the development of hypertension, elevated plasma insulin concentrations and insulin resistance, hyperglycemia, and hyperlipidemia. The alterations in metabolic and physiological function that follow an increase in adipose tissue mass are predictable when considered in the context of normal homeostasis.

Ethnicity has an impact on body fat distribution and adipose tissue metabolism. Currently overweight is defined as a Body Mass Index (BMI) $> 25 \text{ kg m}^{-2}$ and obesity as a BMI $> 30 \text{ kg m}^{-2}$. The evidence for this is drawn from large population studies that suggest people with a BMI of 19–25 kg m^{-2} have the lowest mortality. However, there have been proposals to define race-specific standards according to ethnic background. Specifically, Asians have greater visceral fat and associated morbidity than do Caucasians. A BMI as low as 23 kg m^{-2} may be associated with weight-related diabetes or insulin resistance in these groups. For any given weight category, the presence of certain complications moves the individual into a higher health risk category. Evaluation of such risks should be part of the intervention program.

Patient Selection

Obesity and overweight are chronic conditions. Short-term programs are likely to be ineffective, with rapid weight regain once treatment is stopped. Treatment programs must be for

the longer term and include measures to prevent relapse. Preventing further weight gain in those at risk should also form part of obesity management and help ensure an appropriate use of resources. Those at risk will include moderately overweight subjects and those who have upper body obesity. Weight loss is indicated in adults with a BMI of more than 25 kg m^{-2} and/or abdominal girth of more than or equal to 102 cm in males and more than or equal to 88 cm in females. Additional important treatment areas include weight gain in infancy, adolescence, and pregnancy. A family history of obesity or associated diseases, fat distribution, and risks for coronary heart disease are individually important factors that may influence treatment mode.

Treatment Aims and Realistic Weight Loss Goals

Treatment aims to improve health and well-being, and decrease the risks of ill health later in life, through reducing the amount and possibly distribution of body fat. The success or failure of any treatment program may be judged by an arbitrarily chosen weight or percentage weight loss. Hence, the evidence that modest degrees of weight loss produce significant health gain influences the success or failure of any treatment. With this background, it is logical to redefine successful treatment in terms of a decrease in the severity of obesity rather than a return to normal weight. Even weight stabilization without weight loss represents a modestly successful outcome compared with the natural history of obesity, which is progressive weight gain. A weight loss of between 5% and 10% of the initial body weight is associated with clinically useful improvements in terms of blood pressure, plasma cholesterol, and a significant improvement in diabetic control (see Table 2). Weight loss should be approached incrementally with new weight goals negotiated with the patient if the original target is achieved. Goals for older patients (>65 years) will be different from those who are young; data suggest that a population becomes heavier with age, whereas the risk from obesity does not increase proportionately.

Table 2 Benefits of 10 kg weight loss

Condition	Health benefit
Mortality	Fall of more than 20% in total mortality Fall of more than 30% in diabetes-related death Fall of more than 40% in obesity-related cancer death
Blood pressure	Fall of 10 mm Hg systolic blood pressure Fall of 20 mm Hg diastolic blood pressure
Diabetes	Fall of 50% in fasting glucose Reduces risk of developing diabetes by 50%
Lipids	Fall of 10% in total cholesterol Fall of 15% in LDL cholesterol Fall of 30% in triglycerides Rise of 8% in HDL cholesterol

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Source: Adapted with acknowledgment from the Scottish Intercollegiate Guidelines Network (SIGN) (1996) Obesity in Scotland: Integrating prevention with weight management. A national clinical guideline recommended for use in Scotland. Edinburgh.

Dietary Treatment of Obesity

The primary determinant of weight loss is energy deficit. Short-term weight loss has been achieved by energy reduction in diets of varied macronutrient composition. Obesity is a chronic and relapsing disease; hence, it is the long-term efficacy of these dietary strategies in maintaining lowered weight (and minimizing the risk of diet-related chronic diseases) that is of fundamental importance.

Types of Dietary Treatment

There are several dietary strategies available both in a clinical and commercial setting. These diets vary greatly in the degree of caloric restriction, relative amounts of macronutrients (protein, carbohydrate, and fat), medical supervision, scientific basis, and cost. These diets can be broadly divided into:

- Low-calorie diets (LCDs) ($\geq 3400 \text{ kJ day}^{-1}$ (800 kcal day^{-1}), typically $3400\text{--}6300 \text{ kJ day}^{-1}$ (800–1500 kcal day^{-1}))
- Very low-calorie diets (VLCDs) ($< 3400 \text{ kJ day}^{-1}$ (800 kcal day^{-1})).

Traditionally, LCDs that incorporate various methods for restricting food intake have been recommended for weight management.

Such treatment requires a period of supervision for at least 6 months. A review of 48 RCTs shows strong and consistent evidence that an average weight loss of 8% of the initial body weight can be obtained over 3–12 months with a LCD and this weight loss causes a decrease in abdominal fat, the adipose tissue deposition that is associated with the highest disease risk. VLCDs have been shown to reduce weight at a greater rate in the first 2–3 months compared to LCDs but have not been associated with superior maintenance of lost weight after a year. A review of weight loss trials of LCD and VLCD with available follow-up during 2–7 years showed that long-term weight loss in most trials is in the range of 2–6 kg.

Low-Fat, High-Carbohydrate Diets

Low-fat, high-carbohydrate diets have played a central role in the dietary management of overweight and obesity. Generally, these strategies aim to provide a macronutrient composition of 25–35% energy from fat, 45–60% from total carbohydrate, and 15–20% from protein, thereby moving individuals toward national dietary guidelines (COMA reports). A review of controlled clinical trials demonstrated that a 10% reduction of dietary fat leads to $\sim 3\text{--}4 \text{ kg}$ weight loss in normal overweight subjects and $\sim 5\text{--}6 \text{ kg}$ weight loss in the obese. Evidence from a recent systematic review suggests that a low-fat diet is equally as effective in achieving long-term weight loss in overweight and obese subjects as alternative dietary strategies. Low-fat, high-carbohydrate diets may have a role in weight maintenance. Combined with physical activity and behavioral strategies, the American Diabetes Prevention Program and the Finnish Diabetes Prevention Trial demonstrated maintenance of modest weight loss (3–4 kg) with a marked reduction in the risk of developing type 2 diabetes mellitus over a 4-year study period.

Low Glycemic Index Diets

The Glycemic Index (GI) is a dietary concept originally developed for the therapy of diabetes, which has recently become popular despite scant evidence of its effectiveness in weight management. The GI is a property that describes the effect of carbohydrate from a given food on postprandial blood glucose. It is measured by comparing the blood glucose response of the test food with that of a reference food (usually white bread). Low-GI foods are more slowly absorbed leading to an attenuated and prolonged insulin and metabolic response to foods; it is suggested that more moderate blood glucose and metabolic response may sustain satiety and energy balance to a greater extent than larger metabolic shifts would.

Epidemiological analyses link low-GI load diets to a more favorable lipids profile and reduced incidence of type 2 diabetes mellitus and cardiovascular disease. Evidence from interventional studies supports the benefits of low-GI diets in reducing the risks of coronary heart disease and diabetes but there are no long-term studies that have evaluated its weight-loss efficacy. Therefore, it is appropriate to promote the constituents of a low-GI diet (increased legumes, wholegrain cereals, and fruit consumption) as part of a well-balanced hypocaloric diet for the long-term management of obesity and its metabolic complications.

High-Protein, Low-Carbohydrate Diets

High-protein diets have recently been popularized as a means of rapid weight loss despite the lack of objective evidence in long-term efficacy and safety. Typically, these diets offer wide latitude in protein food choices, and are restrictive in other food choices (mainly carbohydrate). Animal protein rather than plant protein is advocated leading to a higher intake of total fat – mainly saturated fat and cholesterol. Many of the popular high-protein diets promote protein intake of 28–64% of dietary energy, which exceeds established requirement of 10–15%, and severely limit carbohydrate dietary energy to 3–10%. A recent popular high-protein, low-carbohydrate diet, the Atkins diet, provides on average 27% energy from protein, 5% energy from carbohydrates, and 68% energy from fat. The diet results in the avoidance of important staple foods, such as bread, pasta, rice, potatoes, and cereals, as well as foods high in sugars. Consumption of fruits, vegetables, whole grains, and low-fat dairy products, and foods associated with lowering blood pressure, protecting against cancer, and heart disease are all limited.

The initial weight loss in high-protein diets is high due to fluid and glycogen loss related to low carbohydrate intake, overall caloric restriction that is encouraged by structured eating plans, restricted range of foods allowed, and limited tolerance of high-protein foods. This often promotes a misconception about weight loss by suggesting that it is not related to total energy intake but is due to exclusion of certain foods.

A recent systematic review of the efficacy of low-carbohydrate, high-protein diets demonstrates that the amount of weight loss is principally associated with decreased caloric intake rather than reduced carbohydrate content. Researchers have yet to establish whether individuals can maintain long-term weight loss with a high-protein, low-carbohydrate diet because of the short duration of these studies, and long-term adverse effects are also unknown. Possible negative effects include increased risks of

cardiovascular disease, renal disease, cancer, osteoporosis, and compromised vitamin and mineral status.

Energy Prescribed Diet

This dietary strategy determines the daily energy requirement for weight loss by calculating energy expenditure, adjusting for physical activity, and subtracting an energy deficit to induce weight loss – usually 2100–2520 kJ (500–600 kcal) for 0.05 kg weight loss. As a result the prescribed diet will often be in excess of 3400–6300 kJ (800–1500 kcal). The popularity of this approach relates to the findings of improved compliance in those advised on a 2520 kJ (600 kcal) deficit diet compared to a traditional fixed energy intake of 5040 kJ day⁻¹ (1200 kcal day⁻¹).

Formulas and Meal Replacements

Meal replacements are another category of calorie-controlled diets. These include nutritional fortified shakes, snack bars, and low-calorie frozen meals. An entire meal or snack is replaced with a portion-controlled prepackaged meal or drink that provides approximately 840–1260 kJ (200–300 kcal), although formulations and nutrient content vary. Meal replacements are designed to be eaten with additions of conventional foods that supply dietary fiber, other nutrients, additional calories, and water. Most weight loss programs that use meal replacements recommend replacing two meals and one snack a day to lose weight and then replacing one meal per day to maintain weight loss. This strategy generally provides 5040–6729 kJ⁻¹ (1200–1600 kcal day⁻¹) and the regular meal should meet the recommendations of a healthy diet.

A recent meta-analysis that summarized the efficacy of this approach compared to conventional energy-restricted diets suggests that it is an effective weight-loss strategy both in the short and long terms in a clinical trial setting. There is no information about the efficacy outside a clinical trial where meal replacement products need to be purchased, and are frequently discontinued at an early stage.

Very Low-Calorie Diets

Very low-calorie (VLCDs) diets are formula foods; they are designed to provide larger and more rapid weight loss than the standard low-calorie diets. They are commonly given in liquid form to completely replace usual food and snack intake providing in the region of 1890–3400 kJ day⁻¹ (450–800 kcal day⁻¹). To reduce the potential risks from loss of lean body tissue, VLCDs are enriched in protein of high biologic value and also include the full complement of recommended daily allowance for vitamins, minerals, electrolytes, and fatty acids. However, diets providing such low-energy intakes are often associated with a feeling of fatigue, constipation, nausea, and diarrhea. A most serious complication associated with VLCD is the development of symptomatic cholelithiasis associated with the rapid weight loss (1–2 kg week⁻¹).

Owing to the potential adverse effects of these diets, they are generally reserved for short-term treatment in individuals who are moderately to severely obese (BMI > 35 kg m⁻²) and who have failed at more conservative approach to weight loss, in particular, in those with medical conditions that may respond to weight loss such as obstructive sleep apnea, type 2 diabetes mellitus, or before surgical procedure.

Weight regain is common with the reintroduction of food. Studies show that in the long term, VLCDs are no more effective than more modest dietary restriction.

Commercial Slimming Organizations and Products

Such organizations are profit-making ventures. However, they have been shown to be economical, practical, and an effective way of providing care for a large number of moderately obese people in the community. Weekly meetings serve to encourage and reinforce active participation by members, who learn through the exchange of ideas within the group. Weight losses achieved by commercial groups are comparable with those seen in general practice or hospital outpatient clinics. When behavioral techniques are added to the basic program of balanced diet, the results are further improved.

Over recent years, there has been increasing use of weight loss-related web sites on the Internet, which are directed mainly at females. The content and structure of these web sites vary widely. They often lack professional contact and the expertise to deal with medical complications.

Behavior Treatment

Behavior therapy provides an important approach to losing and maintaining weight. The focus is on behaviors related to body weight, namely food intake and physical activity. It serves to identify the abnormal eating behaviors and lifestyle developed over the years and helps to unlearn them and allow body weight to return to normal. The behavior techniques used include self-monitoring, stimulus control, and, recently, cognitive therapy, which involves identifying and changing negative thoughts.

The key difference between behavioral methods and other forms of treatment is that the individual must take responsibility for initiating and maintaining treatment rather than relying on external forces.

There are several elements of behavioral treatment (see Table 3). Evidence from RCTs confirms that behavioral strategies reinforce changes in diet and physical activity in obese subjects to produce weight loss of 10% over 4 months to 1 year. Longer term follow-up shows a return to baseline in the absence of continuing behavioral intervention.

Eating patterns and behaviors are, to a greater extent, acquired by learning, and for this reason there has been much interest in modifying the behavior within the family setting. Obese children are more likely than nonobese children to become obese adults. Behavior therapy seems to be effective in arresting this process in some children.

Exercise and Physical Activity

Exercise produces fat loss in obese and normal weight subjects, although losses rarely exceed 5% of body weight. For any given weight loss, fat-free mass (FFM) is better preserved in exercising than nonexercising subjects: this is likely to be important in the long term because FFM is the best predictor of resting metabolic rate, which is the largest contributor to daily energy expenditure for all but active athletes.

Table 3 Elements of behavioral treatment

<i>Element</i>	<i>Intervention strategy</i>
Self-monitoring	Observe, record, and provide feedback on: <ul style="list-style-type: none"> ● Food consumption (food diary) ● Physical activity (activity diary, and pedometer) weight record
Goal setting	Realistic weight loss goals Separate short-term from long-term goals Focus on health benefits
Stimulus control	Identify and modify environmental barriers: <ul style="list-style-type: none"> ● Healthy eating, and normalize eating pattern ● Increasing daily energy using activities
Problem solving	Handling emotional issues and social events: <ul style="list-style-type: none"> ● Examine situation ● Choose a solution and implement it ● Evaluate the outcome
Cognitive change	Changing inaccurate belief about weight loss <ul style="list-style-type: none"> ● Examine thoughts and feelings ● Challenge inaccurate ones ● Use positive self-affirmations

There are other beneficial effects of exercise that are independent of its effects on weight loss. Regular exercise reduces blood pressure, improves insulin sensitivity, both in association with or independent of weight loss. Favorable effects on the atherogenic lipid profiles have also been reported with exercise and physical training in obese subjects. Such benefits are substantial and should be emphasized to all patients; however, persuading an obese person to participate in regular physical activity and to maintain exercise as a part of daily routine is not easy.

One of the most consistent findings in studies of physical activity is enhanced weight maintenance for at least 2 years from the start of the intervention. It is not necessary to increase maximal oxygen uptake in the obese to derive benefit from exercise: Metabolic evidence of fitness is achieved with less vigorous exercise.

Physical activity recommendations suggest 30 min of moderate activity on at least 5 days in a week. This level of activity is associated with improved fitness and protection from cardiovascular diseases. When using exercise solely as a strategy for weight reduction, longer duration of daily activity of a moderate intensity lasting 45–60 min is required.

Reduction in the time spent in sedentary behaviors (such as television watching) is an important strategy for increasing physical activity and energy expenditure. Similarly, encouraging findings have been observed in children and adolescents advised to include more lifestyle activity (e.g., walking vs car use) compared with those with traditional programs of activity.

Drug Treatment of Obesity

Rationale

Diet restriction even when combined with behavioral therapy and increased exercise is often unsuccessful in achieving

weight loss and maintenance in obese subjects. Obesity is not a single disorder but a heterogeneous group of conditions with multiple causes. Although genetic differences are of undoubted importance, the marked rise in the prevalence of obesity is best explained by behavioral and environmental changes that have resulted from technological advances. In such circumstances, it is appropriate to consider pharmacological treatment as an adjunct to the other treatment modalities.

In broad terms, a pharmacological agent can cause weight loss by reducing energy intake or absorption and by increasing energy expenditure. Current drug treatment of obesity is directed at reducing energy/food intake either by an action on the gastrointestinal system or via an action through the central nervous system control of appetite and feeding.

Selection of Patients

Pharmacological treatments of obesity have had a controversial history and are still regarded with skepticism and suspicion by some medical practitioners. This results from experiences with older agents that turned out to have serious side effects and were withdrawn as a result. Current agents approved for use have been shown to be safe and effective both in weight reduction and in the improvement of comorbidities of obesity. Nevertheless, it is important that doctors who prescribe such drugs are fully familiar with the mode of action and potential risks.

Several sets of guidelines have been developed for the use of drugs in the treatment of obesity. In UK, The Royal College of Physicians' guidance on the use of antiobesity drugs suggests that it may be appropriate to consider the use of drugs after at least 3 months of supervised diet, exercise, and behavioral management. Exceptionally, this period may be shortened when the clinician judges that drug treatment is justified at an earlier stage due to overriding medical circumstances. **Table 4** lists the criteria that should be applied to judge the suitability of a patient for drug treatment.

The criteria applied to the use of an antiobesity drug are similar to those applied to the treatment of other relapsing disorders. It is important to avoid offering antiobesity drug

therapy to patients who are seeking a 'quick fix' for their weight problem. The initiation of drug treatment will depend on the clinician's judgement about the risks to an individual from continuing obesity. It may be appropriate after at least 3 months of supervised diet, exercise, and behavioral management, or at a subsequent review, if a patient's BMI is equal to or greater than 30 kg m^{-2} and weight loss is less than 10% of the presenting weight. In certain clinical circumstances, it may also be appropriate to consider antiobesity drug treatment for those patients with established comorbidities whose BMI is 27 kg m^{-2} or greater if this is permitted by the drug's licence (see **Figure 1**). An antiobesity drug should not be prescribed for a patient whose BMI is less than that specified in the product licence for the drug – the licence indication does not presently take account of the morbidity from obesity seen in certain populations at a lower BMI.

The experience from the use of antiobesity drugs during 12–24-month RCTs indicate that approximately 50% of the actively treated patients respond as judged by 5–10% reduction in body weight maintained over 12 months. The weight loss occurs in the 'responder' group within 12 weeks. This indicates a suitable time period when a response to drug treatment can be identified and a decision taken to continue the medication. Continuing the assessment of drug therapy for efficacy and safety is essential. If the drug is efficacious in helping a patient to lose and/or maintain weight loss, and there are no serious side effects, it may be continued. If not, it should be discontinued. Once a weight loss target has been achieved, there should be an opportunity for renegotiation of a new target, if indicated, and/or long-term monitoring with reinforcement.

Types of Drugs

The two categories of antiobesity medication currently licensed for use in obese subjects are:

1. Those that act on the gastrointestinal system (pancreatic lipase inhibitors) as malabsorption agents to inhibit nutrient absorption.
2. Those that act on the central nervous system primarily to reduce hunger perception.

Drugs Acting on the Gastrointestinal System

Orlistat

Orlistat is a gastric and pancreatic lipase inhibitor that reduces the absorption of dietary fat in a dose-dependent manner. At the therapeutic dose of 120 mg three times a day, it blocks the absorption approximately about 30% of dietary triacylglycerol resulting in an energy deficit of 850 kJ day^{-1} ($200 \text{ kcal day}^{-1}$) for an individual on an average diet of 9240 kJ day^{-1} ($2200 \text{ kcal day}^{-1}$) with 40% of calories from fat.

Adverse effects of orlistat are predominantly related to its gastrointestinal action of fat malabsorption and can be associated with a modest reduction in fat-soluble vitamins (A, D, E, and K). However, clinical deficiency has not been reported in clinical trials. Nevertheless, it is recommended that patients taking orlistat receive vitamin supplements. Patients may complain of loose or liquid stool, fecal urgency, anal leakage, and infrequently fecal incontinence due to undigested fat.

Table 4 Criteria for selecting obese patients suitable for obesity drug treatment

- Drug treatment may be appropriate where diet and exercise have not achieved acceptable weight loss relative to medical risk
- In such patients, drug treatment may be appropriate for:
 - Those whose BMI is more than 30
 - Those with established comorbidities whose BMI is more than 27, if the drug license permits
- Weight-lowering drugs should be targeted at those at high risk from obesity, not obesity alone

The following groups will have priority for drug treatment

- Patients with established comorbidities such as type 2 diabetes, hypertension, and dyslipidemia
- Patients who are physically restricted by their weight either because of breathlessness or arthritis
- Patients considered to be at high risk – for example, those with a family history of overweight or obese parents who died prematurely from CHD or developed type 2 diabetes with complications

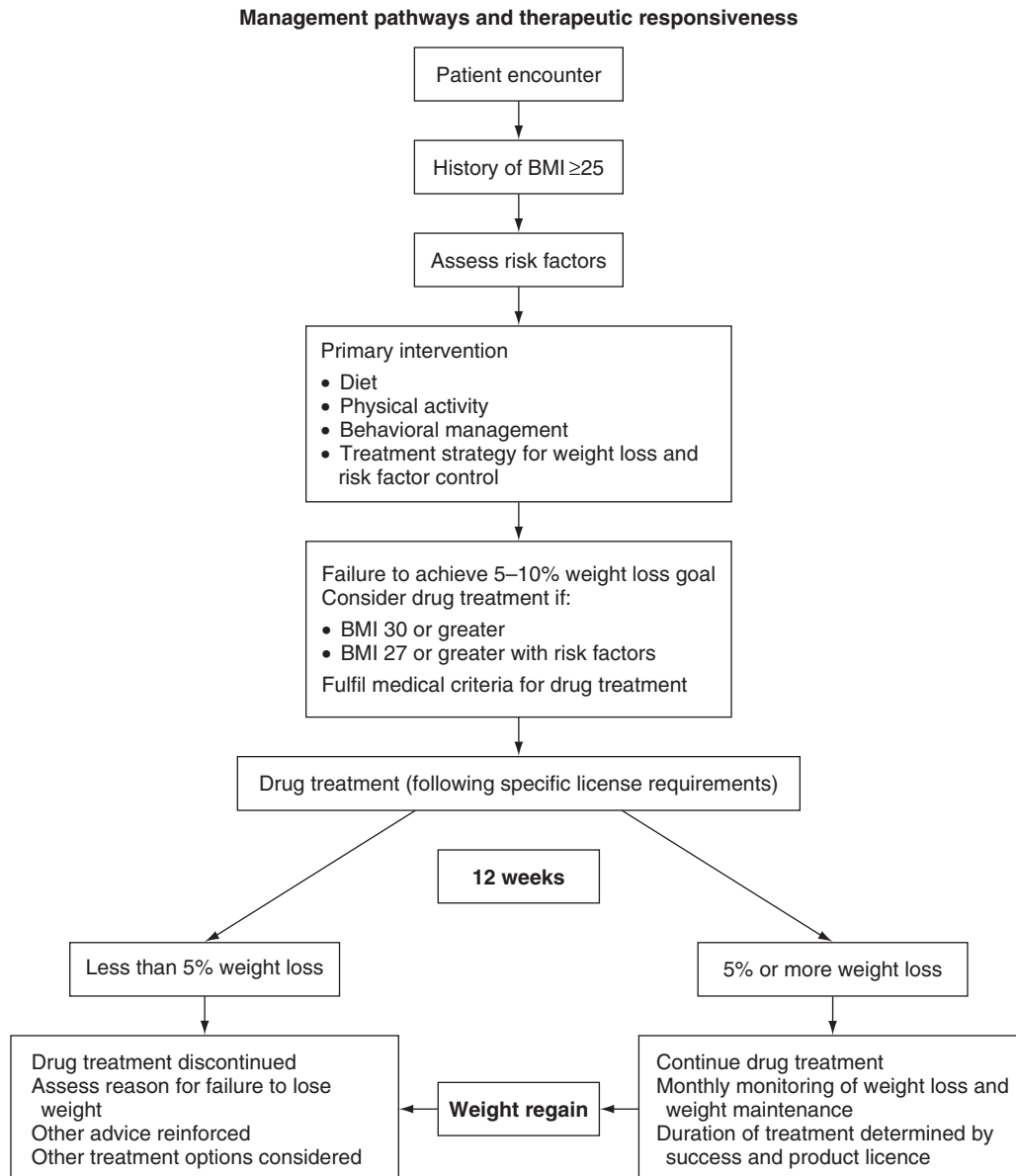


Figure 1 A management pathway for the appropriate prescription of an antiobesity drug. Adapted from Royal College of Physicians of London (2003) *Anti-Obesity Drugs. Guidance on Appropriate Prescribing and Management RCP*. London: Royal College of Physicians of London.

These adverse effects become less common with longer duration of treatment suggesting that patients learn to avoid high-fat meals to avoid these side effects hence enforcing behavioral change. This may well contribute to the therapeutic effects of orlistat treatment. Orlistat is minimally absorbed (less than 1%) and systemic events are negligible.

Drugs Acting on the Central Nervous System

These drugs are commonly referred to as appetite suppressants, which is only one of their actions. Some of these agents have been proven to enhance satiety and slow gastric emptying; and an increase in energy expenditure has also been suggested. They act by increasing the neurotransmitter activity in the brain centers that regulate food intake.

Sibutramine

Sibutramine enhances the sensation of satiety after a meal by its central action as a serotonin and/or epinephrine reuptake inhibitor. Sibutramine is a β -phenethylamine and is well absorbed following oral administration. It undergoes extensive first-pass metabolism in the liver to produce two pharmacologically active metabolites that have long elimination half-lives of 14–16 h.

Side effects commonly reported in clinical trials include dry mouth, constipation, anxiety, rhinitis, and insomnia but these rarely led to withdrawal from the study. The noradrenergic actions of the drug may cause an increase in blood pressure and heart rate in some patients or prevent the expected fall in these parameters with weight loss. It should not be given in patients with uncontrolled hypertension. It should neither be

Table 5 Comparison of actions and indications for use of sibutramine and orlistat

	<i>Sibutramine</i>	<i>Orlistat</i>
Mode of action	Promotes satiety Enhancing effect on thermogenesis	Dietary fat malabsorption
Indication	Adjunct to diet in obese patients with BMI ≥ 30 kg m ⁻² without comorbidities or BMI ≥ 27 kg m ⁻² with comorbidities	Adjunct to diet in obese patients with BMI ≥ 30 kg m ⁻² without comorbidities or BMI ≥ 28 kg m ⁻² with comorbidities
Suitable for	Those with uncontrollable appetite Frequent snackers Nocturnal eaters Those with need for immediate weight loss for medical reasons Those without contraindication to its use (specifically cardiac abnormalities or elevated blood pressure, i.e., > 145/95 mm Hg)	Those who have lost at least 2.5 kg through diet and lifestyle modification Patients requiring longer term behavioral changes whose dietary assessment suggests high-fat intake Patients with impaired glucose tolerance Those with elevated LDL cholesterol Chronic malabsorption Cholestasis Pregnancy, breast feeding
Specific contraindication	Patients with low HDL cholesterol Tourette syndrome Cardiovascular disease Congestive cardiac failure Hypertension Hyperthyroidism, pheochromocytoma Pregnancy, breastfeeding	
Duration of treatment	Not more than 1 year	Maximum of 2 years

HDL, high-density lipoprotein; LDL, low-density lipoprotein.

given concomitantly with monoamine oxidase inhibitors, nor other centrally acting anorexic drugs, or sympathomimetic agents, including cold remedies such as pseudoephedrine.

Phentermine and Diethylpropion

Published evidence of the use of phentermine and diethylpropion indicates short-term induction of weight loss that is frequently followed by weight regain on cessation of the drug. There are no recently published RCTs of the drugs demonstrating efficacy beyond 26 weeks. Both drugs remain restricted to 3 months' use in the terms of their product license.

Rimonabant (SR 141716)

Rimonabant is a selective central cannabinoid (CB1) receptor antagonist. It is an appetite suppressant in advanced development for obesity treatment. The rationale behind this drug is to reduce appetite by blocking cannabinoid receptors in the hypothalamus. The central cannabinoid (CB1) receptors are believed to play a role in controlling food consumption and the phenomena of dependence/habituatation.

Preliminary results from a 2-year international multicenter study confirm its effectiveness in weight reduction, reduction in waist circumference (a marker of the dangerous abdominal obesity), and improvements in lipids and glycemic profiles. The study also confirmed its good safety profile. The side effects reported were mainly mild and transient and most frequently involved nausea, diarrhea, and dizziness.

Rimonabant has potential as a treatment for smoking cessation because the central cannabinoid system is also involved in the body's response to tobacco dependence.

Prescribing Guidelines for Antiobesity Drugs

Antiobesity drugs should be prescribed in an appropriate clinical setting that includes systems for monitoring and follow-up of progress. The choice of antiobesity drug is largely dependent on the experience of the prescriber in using one or another agent (see [Table 5](#)). For the two agents currently recommended for use there are no good clinical studies that have directly compared them or have explored which particular patient will benefit more from one than the other. A drug should not be considered ineffective because weight loss has stopped, provided the lowered weight is maintained.

The Elderly and Children

There is limited information about the use of antiobesity drugs in patients older than the age of 75 years. In such circumstances, the accepted practice is to aim for weight maintenance rather than weight loss. Neither drug is licensed for use in children.

Surgical Treatment for Obesity

Surgical treatment is an appropriate intervention for the management of morbid obesity. Criteria for selection of patients suitable for surgery are listed in [Table 6](#).

Types of Obesity Surgery

At least 30 surgical techniques have been developed for the treatment of obesity. Superficial cosmetic removal of adipose tissue (liposuction) will not be considered because it has no

lasting benefit and it is not regarded as a treatment for obesity. Jaw wiring (intermandibular fixation) can restrict intake of food but it is no longer recommended for surgical treatment of obesity due to a lack of long-term efficacy.

The operative procedures currently used for the surgical treatment of obesity are outlined below.

Gastric Restriction

Gastric restriction can be achieved by gastroplasty or gastric banding. Gastroplasty techniques involve the fashioning of a proximal pouch of the stomach by vertical stapling and a constrictive band opening, thereby restricting the gastric volume to approximately 15–20 ml that empties into the remainder of the stomach.

Gastric banding involves the external ‘pinching off’ of the upper part of the stomach with a band usually made of Dacron. A modification of the gastric banding is an inflatable circumgastric band attached to a subcutaneous reservoir that allows access by a hypodermic syringe to inject or withdraw fluid, thereby tightening or enlarging the band-width. This operation can be performed laparoscopically, significantly improving the perioperative safety of operating for the severely obese patients.

Gastric restriction operations require strict dietary compliance because an intake of high caloric liquids or soft foods are not inhibited by the narrow outlet and may explain a failure to lose weight. The advantage of these techniques is very low operative mortality (<1%) and relative lack of long-term nutritional deficiencies. The reported excess weight loss after 3–5 years is between 40% and 60% but there is a slow regain thereafter.

Table 6 Criteria for patient selection

- BMI ≥ 40 kg m⁻²
- BMI ≥ 35 kg m⁻² with serious comorbidity demonstrated to be responsive to weight loss
- Failure to achieve weight loss with conventional means
- Able to lose weight before surgery
- Have no evidence of psychiatric disease or maladaptive eating behaviors
- Absence of endocrine disorders that can cause morbid obesity
- Psychological stability:
 - Absence of alcohol and drug abuse
 - Understanding of how surgery achieves weight loss
 - Preoperative psychological evaluation for selected patients

Gastric Bypass

A 20–30 ml pouch is created by staples and connected to the jejunum transected 50 cm from the ligament of Treitz (Roux-en-Y gastric bypass). It results in weight loss by both restrictive and malabsorptive mechanisms. Published evidence confirms this procedure produces greater weight loss compared to gastric restrictive techniques but more frequent adverse effects including ‘dumping’ and nutritional deficiency may accompany it. Its operative mortality is approximately 1%.

Biliopancreatic Diversion

Biliopancreatic diversion includes a gastric resection and diversion of the biliopancreatic juice to the terminal ileum to reduce the absorption of nutrients. In this operation, an entero–entero anastomosis is performed between the proximal limb of the transected jejunum and ileum, 50–100 cm proximal to the ileocecal valve.

Biliopancreatic diversion achieves up to 78% excess weight loss at 5 years. Nutritional deficiencies are relatively common (between 5% and 40% of patients for the longer term). In addition, alterations in bowel movements are frequent with 3–5 motions, commonly offensive, occurring each day.

Efficacy of Surgical Treatment for Obesity

Surgery is usually successful in inducing substantial weight loss in the majority of obese patients. This is achieved primarily by a necessary reduction in calorie intake.

In a review of RCT comparing different treatment strategies of obesity, surgery resulted in greater weight loss (23–28 kg more weight loss at 2 years) with improvement in quality of life and comorbidities.

The Swedish Obese Subjects (SOS) study demonstrated long-term beneficial effects on cardiovascular risk factors. The development of type 2 diabetes mellitus is most favorably influenced with a 14-fold risk reduction in those obese patients undergoing surgical treatment.

A Multidisciplinary Approach to the Management of Overweight and Obesity

Published evidence confirms that patients do better whatever the treatment when seen more frequently and for a greater length of time. Moreover, strategies that involve expertise

Table 7 Essential elements of an appropriate setting for obesity management

- Trained staff directly involved in the running of the weight loss program. These staff (medical, nursing, and other healthcare professionals) should have attended courses on the management of obesity and must be given the opportunity to continue their education
- Printed program for weight management that includes clear advice on diet, behavioral modification technique, physical exercise, and strategies for long-term lifestyle changes. Such a program may include a family and/or group approach
- Suitable equipment, in particular accurate and regularly calibrated weighing scale and stadiometer
- Specific weight-loss goals for patients with energy deficit being achieved by moderating food intake and increasing physical expenditure
- Documentation of individual patients’ health risks. This will include BMI, waist circumference, blood pressure, blood lipids, and cigarette smoking and comorbid conditions
- A clearly defined follow-up procedure that involves collaboration between the different settings of care, and provides regular monitoring and documentation of progress, along with details of criteria for judging the success of weight loss. This will allow a weight loss program to be properly supported, medical conditions to be monitored, and problems or issues to be addressed at the earliest opportunity. It is also advisable to have a checklist of possible adverse drug effects – for example, anxiety, disturbances of sleep, breathlessness, depression, and diarrhea

incorporating dietetic, behavioral, and exercise experts as well as physicians and surgeons are also more successful in sustaining weight loss. This underlines the importance of a multidisciplinary approach. Treatment programs should include a system for regular audit and the provision for change as a result of the findings. Any center that claims to specifically provide expertise in weight management should incorporate the essential elements outlined in **Table 7**.

Strategies for Weight Loss Maintenance

Preventing regain of fat losses is the major challenge of weight maintenance. A program to enable the individual to maintain their lowered weight must follow any successful weight loss. Published evidence suggests that a combination of dietary and physical activity modifications and reinforcement of behavioral methods are the most effective in the long term. These modifications need to be integrated and accepted as a way of life and the responsibility for following this must lie with the patient.

See also: Coronary Heart Disease: Prevention. Energy: Balance. Hunger. Hyperlipidemia: Overview. Obesity: Definition, Etiology, and Assessment; Prevention. Physical Activity: Beneficial Effects

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OLDER PEOPLE

Contents

**Nutritional Management of
Nutritional Requirements
Physiological Changes**

Nutritional Management of

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Glossary

Dysphagia Difficulty in swallowing.

Enteral feeding Tube feeding, with the tube passed through the nose into the stomach, or sometimes directly into the stomach or jejunum.

Iatrogenesis Any unintended and untoward consequence of well-intended health-care interventions.

Orexigenic Increasing or stimulating appetite, or a drug that has this effect.

Parenteral feeding Intravenous feeding.

Percutaneous endoscopic gastrostomy A procedure in which a feeding tube is passed through a small incision in the abdomen into the stomach.

Introduction

Overwhelming evidence implicates undernutrition as a major predictor of increased mortality in older adults. Undernourished elders admitted to acute care facilities are more likely to develop complications, resulting in increased length of stay and health-care costs. Rehabilitative efforts are less rewarding as patients often fail to return to baseline functional status and are more likely to require long-term placement or emergency readmission.

Free-living older persons with suboptimal nutritional status are at increased risk of dependence on care givers as a result of compromised activities of daily living. Additionally, convincing evidence exists linking undernutrition with an increased incidence of frailty, gait instability, falls, hip fractures, immune dysfunction, delayed wound healing, and decreased cognitive function. Nevertheless, nutritional assessment and dietary management are often overlooked when health professionals evaluate geriatric patients.

Some claim that as many as one-third of older adults in the US may be undernourished. However, early clinical detection and appropriate intervention occur in less than one-tenth of cases. Health-care providers must remain astutely aware that geriatric health maintenance mandates efficient nutritional evaluation, surveillance, and prompt intervention.

Diagnosis and Evaluation of Undernutrition

Anthropometry

Several anthropometric indices have been proffered for the evaluation of undernutrition in older adults. These include body weight less than 80% of the ideal body weight for height and age; weight loss exceeding 10% of baseline weight in the preceding 6 months or body mass index (BMI) less than 17. Erroneously, the normative references for most of these criteria have been developed for younger subjects. Within the older population the usefulness of these indicators is hampered by the lack of age-adjusted reference values. Reference values applicable in younger adults are not suitable for use in older persons with sarcopenia, age-related skin changes, and vertebral osteoporosis with height loss, which confound such norms. Within the older population, intentional weight loss resulting from dietary restriction should not discourage comprehensive nutritional assessment, as recent evidence indicates that both voluntary and involuntary weight loss in older persons portend similar adverse health outcomes.

Calculation of the BMI is considered to be one of the most objective anthropometric indices, as it permits correction of body weight for height. The BMI, calculated by dividing weight in kilograms by the height in meters squared, is based on the proven premise that weight in the younger adult increases proportionately with height. However, this concept is

false in older persons as height is significantly affected by age-related changes. Loss of height with aging occurs secondary to shortening of the axial skeleton due to age-related osteoporosis, degenerative disc changes, vertebral thinning, and kyphoscoliosis. Furthermore, using height as an anthropometric index is impractical in nonambulatory and bed-bound persons. Nevertheless, clinical use of the BMI in the older population has been preserved by the development of adapted nomograms. Such nomograms are based on the determination of the BMI using surrogate parameters of height adapted from the appendicular skeleton, which is relatively unaffected by age-related osseous changes. These parameters include total arm length, arm span, erect forearm length, and knee to floor height.

Skinfold thickness measurements are also used as anthropometric indices of total body fat in younger adults. However, the precise relationship between skinfold thickness and total body fat is unpredictable, as is the response of subcutaneous fat to undernutrition. Furthermore, in the older adult, the accuracy of this technique is confounded by age-related qualitative and quantitative changes in body fat. Altered compressibility of body fat has also been shown to occur with aging, rendering skinfold thickness measurements unreliable for use in older adults. Measurement of mid-arm circumference is another frequently used anthropometric index. However, several factors influence muscle bulk including exercise, disease, and genetic factors. In the older person this index is of doubtful clinical utility.

Since several factors confound the use of anthropometric indices, it is important to use serial measurements. These allow for quantification of response to intervention and also enhance accuracy of data interpretation by utilizing intra-subject comparison. More accurate methods of body composition analysis are available but are unlikely to be suitable for routine clinical use. These include computerized tomography, bioelectrical impedance, nuclear magnetic resonance imaging, *in vivo* neutron activation analysis, dual energy X-ray absorptiometry (DEXA) and direct photon absorptiometry (DPA). Because of alterations in body water with aging, the value of bioelectrical impedance is questionable. The DEXA technique is excellent but the migration of body fat to the abdomen with aging may result in an underestimation of body fat in older persons. DPA is based on analysis of tissue attenuation of photons transmitted at two different energy levels. This technique permits measurement of different tissue compartments. Both fat mass and fat-free mass can be measured using this technique. Currently, these methods are used almost exclusively for research purposes. Most of these emerging techniques are very expensive and have not been validated for use in clinical settings. Therefore, for practical clinical purposes, the most cost-effective nutritional parameter of proven clinical utility in older adults remains serial body weight measurements.

Biochemistry

Hypoalbuminemia is often used as an index of undernutrition. However, the diagnostic specificity of this index is poor. Serum albumin levels are determined by a complex

interplay between nutrient intake, total body albumin distribution, and pathological changes that alter the biosynthetic and catabolic rates of albumin. In the acutely ill or stressed older person, cytokine release suppresses albumin and pre-albumin synthesis. Additionally, the release of catabolic counter-regulatory hormones in stressful situations reduces albumin synthesis even further. Direct down-regulation of albumin gene expression also occurs in situations of acute stress. Paradoxically, undernutrition itself may result in a compensatory reduction in albumin catabolism, yielding inappropriately high albumin levels. Although serum albumin is a poor index of undernutrition, hypoalbuminemia is linked with frailty, excess comorbidity and increased mortality in older adults. Thus, the clinical relevance of hypoalbuminemia lies in the identification of a high-risk subset of older persons in whom early and aggressive nutritional intervention is crucial.

Several other biochemical indices are used as nutritional markers in the elderly. However, like albumin, they lack diagnostic specificity and have relatively long half-lives, which limit their value in the serial evaluation of undernutrition. Insulin-like growth factor 1 (IGF-1) is considered to have the greatest positive predictive value as it has been shown to correlate well with nutritional status even during periods of acute stress. Added advantages of this indicator are a relatively short half-life of 2–6 h and a rapid response to fasting and refeeding. Nonetheless, routine use of this assay in the evaluation of undernutrition is precluded by cost. Overall, for practical clinical purposes, the use of biochemical markers in routine nutritional geriatric management is cost-ineffective and unreliable.

Hematology

Anemia of chronic disease resulting directly from undernutrition is a recognized clinical entity. Studies have identified reduced erythropoiesis and alterations of erythrocyte function in undernourished persons that respond to nutritional repletion. Iron, vitamin B₁₂ and folate deficiency anemias may also result from inadequate micronutrient intake in undernourished persons.

Measurement of the total lymphocyte count (TLC) is helpful mainly in the stratification of the severity of undernutrition. A TLC of less than 1200×1061^{-1} indicates mild undernutrition whereas counts less than 800×1061^{-1} are usually found in severely undernourished persons.

Recognizing Causative Factors of Undernutrition

Age-related physiological reduction in appetite, 'anorexia of aging,' is well documented. Several factors have been implicated in the genesis of this phenomenon. Evidence suggests that the decrease in lean body mass, energy expenditure, and metabolic rate that occur with advancing age may partially account for the reduction of food intake in healthy older persons. Age-related reduction in olfactory and gustatory receptor sensitivity may compromise the hedonic qualities of meals, further reducing the desire to eat. Similarly, age-related alterations in hormonal and neurotransmitter-mediated

function may also play a role in suppressing food intake. Animal studies suggest that aging results in a reduction in the opioid feeding drive and an increase in the satiating effect of cholecystokinin. This may lead to the ingestion of smaller meals and prolonged periods of satiety between meals. More recently ghrelin, a hunger-inducing peptide hormone, has been shown to decrease with age. Similarly, older hypogonadal men have inappropriately high levels of leptin, a satiation-inducing peptide hormone.

The occurrence of a variety of pathological factors superimposed on the background of age-related physiological changes may further compromise nutritional status in the older adult (Table 1). Existing data suggest that as many as one-third of undernourished older persons suffer from untreated depression. Neuro-vegetative symptoms in depressed older persons often result in anorexia, social withdrawal, reduced motivation, and decreased activity, all of which can compromise nutritional intake. The use of appropriate antidepressants very often reverses these symptoms, resulting in an increase in food intake and restoration of adequate nutritional

Table 1 Common and uncommon causes of undernutrition in older persons

Reduced food intake
Anorexia
Ill-fitting dentures
Periodontal disease
Oropharyngeal disease
Orofacial dyskinesias
Psychosocial factors
Depression
Eating disorders
Bereavement
Social isolation
Low financial income
Physical/mental disability
Persistent tremors
Dyskinesia/dyspraxia
Arthritides
Parkinsonism
Cerebrovascular disease
Dementia
Behavioral disorders
Increased nutrient metabolism
Hyperthyroidism
Phaeochromocytoma
Wandering, agitation
Movement disorders
Hemiballismus
Reduced nutrient utilization
Malabsorption syndrome
Chronic inflammatory bowel disease
Gluten enteropathy
Gastroesophageal disease
Inflammatory disease
Neoplasms
Dysmotility
Multifactorial disorders
Chronic bronchitis, emphysema
Cardiac failure
Malignant disease
Substance abuse

status. Choice of antidepressants is crucial in the management of depressed, older undernourished persons. The popularity of selective serotonin reuptake inhibitors in younger persons has led to their increasing use in the older population. However, in older persons the efficacy of such agents in improving mood may be marred by adverse gastrointestinal effects, such as nausea, vomiting, and diarrhea, which may further compromise nutritional status. Thus, where such agents are used, careful monitoring of nutritional status is mandatory. Mirtazapine is a useful antidepressant that is unrelated to selective serotonin reuptake inhibitors, tricyclics, or monoamine oxidase inhibitors (MAOI). Mirtazapine belongs to the piperazino-azepine group of compounds. Available evidence suggests that Mirtazapine has an additional orexigenic and antiemetic effect, which may increase energy consumption. Electroconvulsive therapy is a viable option in depressed persons with severe anorexia. There is evidence that this treatment restores appetite following failure of pharmacological agents.

Minor dysphoric (anxiety, depression) changes may adversely affect nutritional status and warrant intervention. More than 30% of older community-dwelling persons live alone, usually as a result of bereavement or migration of younger family members. Meals are often eaten alone and the lack of social interaction during meal preparation and consumption can compromise the recreational and hedonic aspects of dining. Consequently, such elders are poorly motivated to prepare and eat meals. Particular attention should be paid to the recreational aspects of mealtimes, and older persons should be encouraged to socialize during meals. This can be accomplished in a variety of ways. Participation in dining clubs, where available, should be encouraged. Arrangements can also be made for older persons to dine at senior citizens' centers. Ambulant senior citizens should be encouraged to eat out, if this is preferred.

Effective nutritional intervention requires due consideration of financial and socioeconomic factors. Approximately one-third of the older population lives below the poverty line and many experience difficulty with the purchase of food items necessary to ensure a balanced diet. Inadequate transportation, limited mobility, and poorly accessible shopping facilities may be added limiting factors. Social and community agency services should be considered where relevant, and an attempt should be made to provide appropriate assistance.

A wide variety of prescribed drugs can cause anorexia, nausea, and other signs and symptoms of gastrointestinal distress in older persons, rendering medication review an important component of nutritional management. Digoxin, theophylline, and nonsteroidal anti-inflammatory agents are frequent culprits in this regard. Inquiry must also be made into the use and tolerance of self-prescribed medication. Offending drugs, once identified, must be discontinued. Iatrogenesis also contributes to undernutrition by way of therapeutic diets. Low-cholesterol and low-salt diets are often prescribed to older persons on the basis of data extrapolated from younger persons. There is currently little evidence to suggest that these diets are of any benefit to older persons when used as primary prevention strategies. Available data actually indicate increased mortality in older adults with

low-cholesterol levels. Evidence suggests that hypocholesterolemia may reflect increased cytokine expression in acutely ill and frail older adults. Thus, restrictive diets in older persons should be discouraged, as they often reduce palatability and consequently discourage food intake. Health professionals should also make enquiries regarding self-prescribed diets. Studies indicate that the older population is more susceptible to food fads and advertised commercial diets, which are often unbalanced and of dubious benefit. Prolonged ingestion of such diets can result in marked undernutrition.

A wide variety of medical illnesses require focused therapeutic intervention in order to maintain or restore adequate nutritional status. Degenerative and neurological diseases can significantly impair mobility and physical function. The use of adaptive appliances and cutlery in such cases may improve manual dexterity and preserve the ability to self-feed. In older persons with severely impaired function, who are unable to cook, meal delivery services ('meals on wheels') may be an acceptable alternative to home-cooked meals. Tooth loss is another important risk factor for undernutrition. Periodontal disease and edentulism are highly prevalent among the geriatric population and can impair masticatory ability. Older persons who have lost teeth, experience pain on mastication, or receive inadequate dental care should be carefully screened and offered appropriate therapy. The use of dentures may improve food intake. However, where dentures are poorly tolerated, alteration in the consistency of meals is helpful. Dysphagia occurs commonly in older persons with degenerative and vascular neurological conditions such as dementia, Parkinsonism, and cerebrovascular disease. A bedside swallowing evaluation should be an integral component of nutritional evaluation, followed by a modified barium swallow with fluoroscopy in cases where significant dysphagia is identified. In most cases oral food intake will remain possible, with appropriate modifications regarding swallowing technique, feeding precautions, and food consistency.

Health professionals often wrongly assume that older adults possess adequate knowledge of basic dietetic practice and nutritional studies. There is evidence to suggest that the nutritional attitudes and knowledge of undernourished older persons may be inadequate, particularly with regard to food preparation. Dietary education and counseling are crucial components of nutritional intervention in undernourished older persons who retain the responsibility for preparing their own meals. Such counseling should be targeted toward identifying deficits in basic dietary knowledge and the correction of poor nutritional practices.

Nutritional Assessment Tools

Arrays of nutritional screening tools have been developed to facilitate the identification of older persons at risk for undernutrition. The Nutrition Screening Initiative (NSI) in the US stemmed from a collaborative effort between family physicians, dietitians, and the National Council of Aging. This was a three-tiered tool formulated to assist in the detection of older persons at risk for nutritional compromise and subsequent direction of such persons toward the appropriate level of care which is still useful. The first level of screening is designed to be initiated by the patient or primary care giver. Persons identified to have an increased risk of undernutrition are then referred for evaluation by health care or social services personnel. This constitutes the second level of screening. The identification of factors that may warrant medical intervention will prompt referral to a physician for further evaluation. The NSI is of proven value as an epidemiological tool and serves to increase the awareness of patients and care givers to undernutrition. However, its usefulness within orthodox settings may be hampered by the number of personnel and services required, which may constitute a significant drain on available resources. Added drawbacks to the use of this tool for the individual patient are the lack of professional supervision at initiation and reliance on patient compliance in adhering to the specified clinical pathway protocol.

The Mini Nutritional Assessment (MNA) is a comprehensive and simple tool designed to evaluate the nutritional status of older persons. This is the first well-validated nutritional screening instrument and is recommended for use in people aged over 75 years. Cross-validation indicates that nutritional assessment using this tool will accurately evaluate and categorize nutritional status in approximately 75% of older persons without the need for further biochemical tests or clinical assessment. The MNA scoring system permits the stratification of older adults into three categories: well nourished, at risk of undernutrition, and undernourished. An advantage of the MNA is that it can easily be used by a wide range of health professionals in a variety of clinical settings that cater for both free-living and institutionalized older persons. Several other tools are of practical value in the clinical setting. Morley has developed a useful screening tool known by the acronym SCALES (Table 2). This uses basic biochemical and anthropometric indices to identify older adults at risk of undernutrition, and can be readily incorporated into serial evaluation of the older person in different clinical settings. The simple mnemonic MEALS ON WHEELS, also devised by Morley, may prove

Table 2 SCALES: screening tool for the early detection of patients at risk of protein-energy undernutrition

Parameter	Score 1 point	Score 2 points
Sadness	GDS 10–15	GDS > 15
Cholesterol	<4.65 mmol l ⁻¹ (180 mg dl ⁻¹)	<4.14 mmol l ⁻¹ (1660 mg dl ⁻¹)
Albumin	<40 g l ⁻¹ (4 g dl ⁻¹)	<35 g l ⁻¹ (3.5 g dl ⁻¹)
Loss of weight	<1 kg (2 lb) in 1 month	<2.7 kg (6 lb) in 6 months
Eating problems	Cognitive impairment or physical limitations	Cognitive impairment and physical limitations
Shopping problems	Inability to shop or prepare a meal	

Patients scoring over 3 are at risk. GDS, geriatric depression score.

Table 3 MEALS ON WHEELS: common causes of undernutrition in older persons

Medication (e.g., digoxin, theophylline, psychotropic drugs)
Emotional (depression)
Anorexia, alcoholism
Late-life paranoia
Swallowing disorders
Oral and dental disease
No money (absolute or relative poverty)
Wandering (dementia, behavioral disorders)
Hyperthyroidism, hyperparathyroidism
Entry problems (malabsorption)
Eating problems
Low-salt or low-cholesterol diets
Shopping and food preparation problems

useful in prompting consideration of the risk factors and common causes of nutritional compromise (Table 3).

More recently, the Council of Nutrition Appetite Questionnaire has been validated for the evaluation of appetite in older adults. A unique feature of this appetite assessment tool is the ability to predict significant weight loss during the next 6 months (Table 4).

Oral Nutritional Repletion

Appropriate treatment of the underlying causes of undernutrition should be accompanied by oral nutritional supplementation in persons who are able to eat. Objective quantitative baseline assessment of food intake is mandatory. This is best achieved by the maintenance of a food diary, in which the patient records all food items consumed over a 72-h period. Review of the food diary also permits evaluation of food preferences and eating patterns. The goal of nutritional supplementation should be the consumption of the recommended daily allowance of macronutrients and micronutrients. Several predictive equations have been derived for the purpose of determining the optimal energy intake for each individual. However, it remains unclear as to what extent corrections have been made for age-related physiological changes in nutritional requirements and energy expenditure. The Harris-Benedict equation is perhaps the best known and most frequently applied. Using this equation, the required daily energy intake in kilocalories is derived as follows:

$$\text{Men} : 66 + 13.7W + 5H - 6.8A$$

$$\text{Women} : 665 + 9.6W + 1.8H - 4.7A$$

where W is the weight in kilograms, H is the height in centimeters, and A is the age in years. Upward adjustment is required by factors ranging from 1 to 1.5, to compensate for increased activity or pathologically stressful conditions.

For practical clinical purposes, a total daily energy intake of 147 kJ kg^{-1} (35 kcal kg^{-1}) achieves efficient nutritional repletion. Recent dietary guidelines emphasize an overall healthy and balanced dietary pattern that includes a wide variety of fruits, vegetables, and grain products. Specifically, at least five daily servings of fruits and vegetables and six daily servings of grain products, including whole grains. Low-fat dairy products, fish, legumes, poultry, and lean meats are

Table 4 The Council of Nutrition Appetite Questionnaire

-
- 1 My appetite is**
 - 1 Very poor
 - 2 Poor
 - 3 Average
 - 4 Good
 - 5 Very good
 - 2 When I eat**
 - 1 I feel full after eating only a few mouthfuls
 - 2 I feel full after eating about a third of a meal
 - 3 I feel full after eating over half a meal
 - 4 I feel full after eating most of the meal
 - 5 I hardly ever feel full
 - 3 I feel hungry**
 - 1 Rarely
 - 2 Occasionally
 - 3 Some of the time
 - 4 Most of the time
 - 5 All of the time
 - 4 Food tastes**
 - 1 Very bad
 - 2 Bad
 - 3 Average
 - 4 Good
 - 5 Very good
 - 5 Compared to when I was younger, food tastes**
 - 1 Much worse
 - 2 Worse
 - 3 Just as good
 - 4 Better
 - 5 Much better
 - 6 Normally I eat**
 - 1 Less than one meal a day
 - 2 One meal a day
 - 3 Two meals a day
 - 4 Three meals a day
 - 5 More than three meals a day
 - 7 I feel sick or nauseated when I eat**
 - 1 Most times
 - 2 Often
 - 3 Sometimes
 - 4 Rarely
 - 5 Never
 - 8 Most of the time my mood is**
 - 1 Very sad
 - 2 Sad
 - 3 Neither sad nor happy
 - 4 Happy
 - 5 Very happy
-

Instructions: Complete the questionnaire by circling the correct answers and then tally the results based on the following numerical scale: A=1, B=2, C=3, D=4, E=5. The sum of the scores for the individual items constitutes the CNAQ score.

Scoring: If the CNAQ score is less than 28, there is an increased risk of significant weight loss over the next 6 months.

encouraged. Guidelines also suggest at least two servings of fish per week.

The current recommended daily allowance for protein is at least 1 g kg^{-1} body weight. However, acutely stressful or hypercatabolic conditions mandate an increase in protein intake to approximately 1.5 g kg^{-1} . Generally, compliance with these dietary guidelines achieves the dual purpose of ensuring optimal macronutrient and micronutrient intake.

This obviates the need for the routine prescription of pharmacological multivitamin preparations in undernourished persons, unless specific signs of micronutrient deficiency are evident.

Nutritional supplementation with regular or fortified usual food items is the ideal mode of nutritional repletion. This possesses the advantages of familiarity, palatability, and cost-effectiveness. Where the patient is reluctant or unable to consume the required total energy intake in natural food items, commercially formulated nutritional supplements are a reasonable alternative. The choice of preparation should be based on palatability and patient preference unless underlying medical conditions such as lactose or gluten intolerance have to be considered. Patients with malabsorption syndromes should be given hydrolyzed preparations to enhance nutrient absorption. Regardless of the preparation used, an attempt should be made to vary flavors, as age-related sensory-specific satiety may limit intake if only one flavor is used. Erroneously, nutritional supplements are often administered with meals. Recent evidence indicates that liquid supplements are more effective in increasing daily energy intake when administered at least 1 h before meals. Data show that when supplements are administered with meals, a suppressant effect on food consumption is evident. Thus, older adults on nutritional oral supplements should receive these between meals to maximize net energy intake. Ultimately, in persons with severe undernutrition, the focus should be on energy intake and patient food preference, not on optimal proportions of macronutrient and micronutrient intake. Frequently, efforts to ensure a balanced diet necessitate the use of food items that may compromise palatability and result in a counterproductive reduction in food intake.

Enteral Tube Feeding

Enteral or parenteral modes of nutrient delivery are often used in people who are unable to eat or swallow. In the presence of a functioning gastrointestinal tract, enteral feeding is more appropriate due to the lower incidence of complications, more efficient nutrient utilization, increased cost-effectiveness, and greater ease of administration. Additionally, small bowel hypoplasia and alterations in gastrointestinal secretions may result from prolonged parenteral nutrition. Nasogastric and nasoenteric tubes should be reserved for short-term nutritional support in persons who may be able to resume oral feeding within 14 days, in order to avoid the significant morbidity associated with the use of nasal tubes. In persons in whom prolonged enteral intake is anticipated, gastrostomy or jejunostomy tubes may be considered.

In patients who retain normal gastrointestinal absorptive function, regular meals may be puréed and delivered through large-bore feeding tubes. A variety of polymeric enteral feeding formulas are also available; these are of relatively low viscosity, rendering them particularly suitable for delivery through small-bore tubes, which are usually more comfortable and esthetically pleasing. In persons with malabsorption, hydrolyzed pre-digested formulas are available. Specific formulations also exist for people with special nutritional requirements due to diseases such as diabetes mellitus or renal or respiratory failure.

In older people, large-volume bolus tube feedings may be associated with a greater risk of aspiration. Thus, where possible, continuous infusions of feeds are preferred. To further reduce the risk of aspiration pneumonia, it is recommended that the patient is positioned in a 30° head-up incline during feedings. Feeds may be infused over a 24-h period or over 14–18 h with a nocturnal break. The latter infusion schedule is often advocated on the grounds that it mimics normal eating patterns more closely. In addition, the absence of a nocturnal feed-free period has been shown to obliterate the physiological diurnal variation in insulin, cortisol, and glucagon secretion. Maximal nutrient utilization is also encouraged by daytime feed infusions as gastric emptying occurs more rapidly during the day. Continuous infusion of enteral tube feeds should be initiated at a rate of 30 ml h⁻¹ using half-strength feeds. If tolerated, full-strength feeds may then be introduced at the same rate and increased by 25 ml h⁻¹ every 8–12 h until the recommended daily energy intake is achieved. Despite the popularity of enteral tube feeding, emerging evidence indicates that the medical risks of percutaneous endoscopic gastrostomy (PEG) tube feeding may outweigh the risks. Studies in older adults with dementia fail to demonstrate any reduction in comorbidity or mortality with PEG feeding. Similarly, available data fails to demonstrate any significant improvement in functional status, nutritional status, or quality of life with this method of feeding. Thus, health providers should set realistic goals for patients and family members who opt for PEG feeding. Ultimately, the indications and benefits of PEG tube placement are more likely to be based on personal psychosocial, cultural, or ethical preferences.

Parenteral Nutritional Repletion

In the older person with a nonfunctioning gastrointestinal tract, parenteral nutrition may be unavoidable. All patients receiving parenteral nutrition must be monitored closely for adverse effects. For short-term intravenous nutritional repletion, peripheral parenteral nutrition may be used. Low osmolality nutritional preparations, with a low risk of toxicity to soft tissue, are best suited for this purpose. There is a paucity of data regarding the safety and efficacy of most peripheral parenteral nutritional products for periods exceeding 14 days. Thus, where longer periods of intravenous feeding are required, total parenteral nutrition through a large central vein is indicated. Standard total parenteral formulations comprising 25% dextrose, 5% amino acids, electrolytes, and trace elements in optimal amounts are suitable for use in most patients. During prolonged parenteral nutrition, lipid emulsion supplements should be added to prevent deficiency of essential fatty acids.

Pharmacological Management of Undernutrition

Older patients with a poor response to treatment of underlying causes and nutritional supplementation may benefit from orexigenic agents (Table 5). Megestrol acetate is a synthetic progestogen approved for use by the Food and Drug

Table 5 Orexigenic agents

Megesterol acetate
Mirtazapine
Dronabinol (delta-9-tetrahydrocannabinol)
Corticosteroids
Loxiglumide (cholecystokinin antagonist)
Oxoglutarate
Anabolic agents (testosterone, anadrol)
Oxandrin
Growth hormone
Cyproheptadine

Administration (FDA) as an orexigenic agent in patients with Acquired Immune Deficiency Syndrome (AIDS) and cancer-related anorexia and cachexia. Recent evidence indicates that megesterol acetate is also an effective orexigenic agent in geriatric patients. Thromboembolic disease and adrenal suppression are rare complications, but patients should be monitored closely for these events.

Dronabinol (delta-9-tetrahydrocannabinol), the active ingredient of Cannabis sativa, is another FDA-approved orexigenic agent for use in patients with AIDS. Dronabinol is also an effective orexigenic and antiemetic in patients receiving cancer chemotherapy. Additional evidence indicates that dronabinol induces weight gain in persons with dementia, although research has yet to determine whether weight gain in such patients is due to increased energy intake or reduced agitation with improved behavior and consequently decreased energy expenditure. Side effects of dronabinol in older adults include delirium, euphoria, and increased somnolence. The latter two qualities may favor the use of dronabinol as an orexigenic agent in palliative care.

One-third of depressed older adults manifest with weight loss. Effective antidepressant therapy should result in weight gain in this subset of patients. Notably, the choice of antidepressant therapy may influence body weight reuptake. Selective serotonin (5-hydroxytryptamine, 5-HT) inhibitors, such as fluoxetine, can cause significant weight loss at the onset of therapy. Evidence in younger adults suggests that this is a transient phenomenon with baseline body weight being restored as treatment progresses. However, age-related changes in energy regulation and adaptation to chronic disease may delay or prevent return to baseline body weight in older patients. Mirtazapine has proved useful in the management of depressed patients with weight loss. Mirtazapine is a well-tolerated and effective antidepressant that inhibits presynaptic alpha2 adrenergic receptors and postsynaptic 5-HT2 and 5-HT3 receptors. Mirtazapine has been shown to induce an earlier increase in appetite and subsequent weight gain in older depressed persons with weight loss.

Several agents previously touted as effective orexigenic agents, such as human growth hormone, have fallen out of favor. The administration of human growth hormone to healthy older adults has been shown to increase muscle bulk. However, significant side effects such as carpal tunnel syndrome, gynecomastia, and hypoglycemia were noted; furthermore, the increase in muscle bulk failed to produce a parallel increase in muscle strength. Inadequate data regarding the safety and efficacy of growth hormone administration

preclude routine clinical use. Similarly, the role of insulin-like growth factor (IGF-I) in the management of undernutrition is questionable. Although the data suggest that exogenously administered IGF-I may enhance nitrogen retention, gluconeogenesis, and maintenance of normal gastrointestinal function, evidence-based outcome studies are lacking.

Abundant data exist regarding the role of anabolic steroids in the management of undernutrition. However, current evidence supports the restriction of testosterone therapy as an orexigenic agent to hypogonadal undernourished men. As a general rule, pharmacological treatment should be considered second-line therapy and reserved for patients who have failed to respond to nonpharmacological measures.

Managing Undernutrition in the Community Setting

With increasing emphasis on home health care, the number of community-dwelling persons requiring alternative modes of feeding has increased. Special consideration and appropriate modification of therapeutic regimens may be required in such cases to ease the care giver or personal burden.

If enteral tube feeding is provided at home, continuous infusion may limit the patient's mobility and functional independence. This method also has the disadvantage of requiring immediate access to technical support, in the event of mechanical failure of the infusion pump. Thus, care givers and patients may find intermittent bolus feeding a more convenient and less daunting task. To minimize the aspiration risk, intermittent bolus feeds should be administered, where possible, with the patient in a seated position. Patients should also be encouraged to remain seated for at least 1 h after feeds. Some active older people resent the social inconvenience and embarrassment of tube feeding during daytime hours, and may prefer overnight enteral infusions of hypercaloric feeds. Hypercaloric feeds contain twice the amount of equal volumes of regular enteral feeds, thereby permitting the provision of adequate nutritional support over shorter periods.

Parenteral nutrition within the home is fraught with all the hazards of intravenous therapy. Thus, availability of skilled services to monitor such therapy is critical. Additionally, adequate care giver and social support is mandatory for patients receiving this mode of nutritional repletion at home.

Health providers involved in home delivery of enteral and parenteral nutritional therapy will need to develop and implement comprehensive therapeutic programs incorporating skilled nursing and dietary services to ensure safe and effective treatment.

Managing Undernutrition in Long-Term Care Institutions

Therapeutic strategies for managing undernourished institutionalized older adults are similar to those used within the community, though perhaps due to readily available medical supervision, enteral and parenteral modes of feeding are used more often. The comparatively formal structure of the nursing home environment has the added advantage of encouraging closer supervision of therapy and stricter nutritional surveillance.

A major drawback to oral nutritional repletion in institutionalized older persons is the restricted variety of meals. This can usually be circumvented by involving the residents in menu development and, where feasible, granting permission for meals of the residents' choice to be supplied by family or friends. Residents of nursing homes are often less functional than their peers in the larger community and thus may be more dependent on assistance for their basic activities of daily living. When the ability to self-feed is compromised, it is imperative that all meals are supervised and assistance with feeding rendered where necessary. Many residents are persistent wanderers and may expend a considerable amount of energy in this exercise. In such patients an appropriate increase in their daily energy intake is required to prevent weight loss. Similar adjustments may be required for residents with persistent involuntary movements or severe agitation.

Long-term care institutions must preserve the social and recreational aspects of meals; all too often, mealtimes are reduced to clinical, sanitized, and isolated events. Within the nursing home environment mealtimes are best managed as a component of recreational therapy. Socialization and the preservation of each resident's dignity should be encouraged during meals. Nursing facilities should also attempt to mimic community resources by making food items available outside scheduled mealtimes, from vending machines and snack carts.

Nutritional surveillance programs are crucial to the success of established intervention strategies within nursing homes. Quality indicators, preferably employing anthropometric indices, should be defined to monitor the success of intervention strategies. Continuous quality improvement and total quality management programs must also be implemented as critical components of effective nutritional intervention strategies. Finally, the development of nutrition focus groups and the use of interdisciplinary intervention strategies directed at increasing nutritional intake and preventing undernutrition should be encouraged.

Micronutrient Deficiency

In older people at risk of nutritional compromise, micronutrient supplementation deserves special attention, in order

to forestall the development of micronutrient deficiency (Table 6). The clinical features of established vitamin deficiency are well recognized. The first recourse in the management of micronutrient deficiencies should be the provision of a well-balanced diet. In the presence of a functioning gastrointestinal tract, an adequate diet containing the recommended daily allowance of each micronutrient effectively prevents and corrects deficiency states. However, the failure to consume the required amount of food may warrant the use of oral pharmacological micronutrient supplements. Vitamin B₁₂ deficiency may be considered unique in this regard as, traditionally, replacement therapy has been administered parenterally. However, available evidence suggests that food-cobalamin deficiency may be the most common cause of vitamin B₁₂ deficiency in older adults. In this condition cobalamin cannot be extracted from ingested food, although free cobalamin (such as added in fortified foods and used in supplements) is readily absorbed as absorptive function is normal and intrinsic factor is present in adequate quantities. Thus, in persons with vitamin B₁₂ deficiency resulting from food-cobalamin deficiency, repletion may be adequately achieved by oral replacement therapy although initially an intramuscular dose or high oral doses (e.g., 500 µg day⁻¹) may be needed to replete liver stores.

There is a rising trend toward dietary supplementation with pharmaceutical preparations containing large doses of vitamins and minerals, based on conclusions drawn from the results of several studies. Available evidence derived from human and animal studies indicates that antioxidant micronutrients, mainly vitamins A, C, and E, may play a role in boosting immunity, preventing neoplastic disease, and preventing or retarding the progression of several degenerative diseases, such as atherosclerosis. Vitamins E and C have also been shown to have the positive effect of reducing low-density lipoprotein (LDL) cholesterol levels and increasing high-density lipoprotein (HDL) levels, in addition to lowering fasting plasma insulin and improving insulin efficiency. Epidemiological studies have suggested a protective role for antioxidants such as vitamin C, vitamin E, β-carotene, and glutathione in macular degeneration and cataracts. Nevertheless, evidence derived from other epidemiological studies suggests that antioxidants may lack significant

Table 6 Vitamins: Recommended daily allowances (RDAs) and clinical features of deficiency states

	<i>RDA</i>	<i>Deficiency states</i>
Vitamin A	500–625 µg RAE	Decreased immunity to infections. If severe, xerophthalmia, night blindness
Niacin	11–12 mg	Pellagra (dermatitis, dementia, diarrhea), glossitis, cheilosis
Pyridoxine (B ₆)	1.3–1.4 mg	Dermatitis, delirium, peripheral neuropathy, glossitis
Riboflavin (B ₂)	0.9–1.1	Glossitis, cheilosis, normochromic anemia
Thiamin (B ₁)	0.9–1.0 mg	Beriberi, Wernicke's encephalopathy, Korsakoff's psychosis
Cobalamin (B ₁₂)	2.0 µg	Megaloblastic anemia, optic atrophy, peripheral neuropathy, subacute combined degeneration of the cord, dementia
Ascorbic acid (C)	60–75 mg	Hyperkeratosis, petechial hemorrhages, mucosal bleeding, lethargy
Vitamin D	20 µg	Osteomalacia, osteoporosis
Vitamin E	12 mg	Peripheral neuropathy, ataxia, hemolytic anemia
Folate	320 µg	Megaloblastic anemia, cognitive dysfunction
Vitamin K	90–120 mg	Spontaneous hemorrhage, hypotherbinemia

NE, niacin equivalent; RAE, retinol activity equivalent. The lower values in each range are for women, and the higher, for men.

benefit. Studies are ongoing in an attempt to resolve this controversy.

In older adults reduced cutaneous synthesis and enteric absorption of vitamin D increases the risk of vitamin D deficiency. Reduced renal responsiveness to parathormone is an added risk factor. At least $10\text{--}15\ \mu\text{g day}^{-1}$ of vitamin D is required to prevent significant risk of osteoporosis in postmenopausal women. Institutionalized patients with reduced exposure to sunlight are at higher risk of vitamin D deficiency due to reduced cutaneous synthesis. The role of calcium supplementation in the prevention of osteoporosis is also well accepted. Additional evidence suggests that inadequate dietary calcium consumption may play a role in the genesis of colorectal cancer and hypertension.

Currently, the safety of large pharmacological doses of micronutrient supplements in humans remains to be established. In spite of this, a considerable proportion of the older population consumes large doses of these supplements as a primary preventive health measure. The risk of long-term supplementation with high doses of micronutrients, particularly in the presence of age-related changes, cannot be ignored, and few studies have addressed this issue specifically. Due caution must be exercised, even with the use of micronutrients such as vitamin D and calcium where clinical benefits have been clearly established. The complications of overenthusiastic calcium ($>2500\ \text{mg day}^{-1}$) and vitamin D ($>50\ \mu\text{g day}^{-1}$) supplementation include hypercalcemia, nephrocalcinosis, milk-alkali syndrome, ectopic calcification, and rebound gastric acidity. Calcium supplementation may also impair iron absorption. With regard to vitamin A, available data have identified an increase in absorption and reduced peripheral clearance of this vitamin in older adults, therapy increasing the risk of vitamin A toxicity. Similarly, older persons on long-term iron therapy, particularly in the absence of proven iron deficiency, are at increased risk for the development of secondary hemochromatosis.

On the basis of existing evidence, the use of pharmacological doses of vitamin and mineral supplements is probably best restricted to low-potency supplements and reserved for persons with established micronutrient deficiency who are unable to eat an adequate diet. Close monitoring of such patients for adverse effects is mandatory.

Obesity

More than one-third of people over the age of 60 years are obese and an additional one-third are overweight. Although the prevalence drops with age, the prevalence of overweight among men and women over 75 years is still considerable, over 50%. At all ages, African Americans have a higher prevalence of overweight and obesity.

With aging there is increasing upper and central body fat distribution. This trend is accelerated in women following menopause. In women aged 55–69 years, central obesity has been demonstrated to be correlated with greater coronary artery disease mortality as well as total mortality. Even with weight loss, the waist to hip ratio remained an important predictor of mortality in elderly women. Leptin is a hormone produced by fat cells. In women, leptin levels rise in middle

age in concert with the increase in fat mass and then fall in late old age as fat mass declines. In men, leptin levels increase progressively from 65 years onward. This may be due to age-related hypogonadism. In older men, testosterone replacement therapy decreased leptin levels.

As food intake declines with aging, obesity in old age is probably due to other factors. All three components of energy output – resting metabolic rate, thermic energy of feeding, and physical activity – decline with aging; thus the pathogenesis of obesity in old age appears to be predominantly due to altered energy output rather than to increased food intake.

While moderate degrees of overweight appear to confer minimal increased mortality in the older population, those above 130% of average body weight have an increased risk of death even at extreme ages. Most of the complications of obesity in older persons are similar to those seen in younger persons. Certain effects of obesity appear more commonly in older persons; for instance, functional decline is more common compared with younger persons. This is often associated with a ‘fear of falling.’ This syndrome is particularly common in older urban-dwelling adults and may lead to voluntary restriction of physical activity and consequent frailty. The prevalence of diabetes mellitus increases with age, due in part to the increased fat mass in middle age onward. Obesity markedly increases the prevalence of sleep apnea in older persons. Overweight increases the rate of progression of osteoarthritis and its effects on function. In nursing homes, obesity has been associated with an increase in pressure ulcers. Increasing weight increases claudication in older persons with peripheral vascular disease.

Management of obesity in older persons usually should focus on enhancing functional status and increasing physical activity as opposed to aggressive caloric restriction. Available evidence linking aggressive weight loss in older adults with increased mortality mandates close monitoring during treatment. Surgery for obesity is not appropriate in older adults as the risks of bariatric surgery outweigh the benefits. For similar reasons, the use of thermogenic and anorexic agents should be avoided. Thus, a combination of exercise, healthy eating, and behavior modification is the cornerstone of therapy in older persons. Older obese adults need to be carefully monitored for the development of sarcopenia, visceral protein depletion, and increasing frailty. Due attention should also be given to micronutrient supplementation.

See also: Antioxidants. Body Composition. Nutritional Assessment: Anthropometry. Nutritional Support: Infants and Children, Parenteral. Obesity: Definition, Etiology and Assessment

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Nutritional Requirements

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Human Aging and Nutrition

The World Health Organization (WHO) defines the 'elderly' as persons of 60 years of age and older. The elderly constitute a rapidly expanding segment of populations in both developed and developing countries. This is the combined result of ever-longer survival and dramatic reductions in fertility rates. Regardless of age, people must respond to their feelings of hunger and thirst by consuming foods and beverages. This eating and drinking behavior also serves to provide the nutrients to nourish the body. The amount of a nutrient that must be ingested and absorbed to maintain an adequate and appropriate body composition varies with age across the life span, depending on basic underlying physiological and metabolic processes specific to the chronological stage of life. Similarly, the degree to which we retain and conserve, or excrete or degrade, absorbed nutrients is influenced by chronological age and biological aging.

As a consequence of this new demographic reality, attention is being focused belatedly on gerontology and its nutritional biology; this, in turn, is reflected in very recent efforts to refine our knowledge of the amounts of various macro- and micronutrients that the aging body requires (nutrient requirements) and of the amounts that must be consumed in the diet to provide for sufficient uptake of these nutrients (nutrient recommendations).

Successful Aging, Normative Aging, and Frailty

From an epidemiologic and demographic, as well as an economic and humanitarian standpoint, the ideal contribution of life-long nutrition would be to a situation of 'compression of morbidity,' first enunciated by J. Fries. It strives to keep individuals functional, independent, and free of chronic illness, until the final moments of their lives, and thus reduces to a minimum the burden of disability and dependency suffered by older persons, their families, and the society that contributes to their maintenance.

A disclaimer has traditionally been appended to the official pronouncements of recommended nutrient intakes (RNIs); whether they are from national or international expert panels, the prescriptions are meant to apply to 'healthy' individuals. Nutrient needs in disease conditions are considered to be a clinical matter, and are related to the pathological processes in question.

When it comes to older persons, the exigency of being 'healthy' becomes immediately problematic. Advanced age is associated with increased susceptibility to chronic and degenerative illnesses. Most persons over 60 years of age have two or three diagnosed chronic illnesses, and are receiving multiple medications. Maintaining a rigid definition of 'healthy' for application of nutrient recommendations in later life

would exclude almost everyone from coverage by general nutrient-intake standards.

In fact, the older the cohort of individuals examined, the more heterogeneous are individuals of the same chronological age in their physical and cognitive functioning. Over the last two decades, general domains of classifications have come into usage to embrace the heterogeneity of aging populations: successful aging; usual aging; and frail aging. Successful aging has been defined as multidimensional, 'encompassing the avoidance of disease and disability, the maintenance of high physical and cognitive function, and sustained engagement in social and productive activities.' It may involve aspects of resilience and wisdom, as well. Usual aging involves an accumulation of ailments and loss of function that is 'typical' or 'normative' for older persons surviving to later life. Frailty is the far extreme of disability and dependency associated with major physical and cognitive decline in which disease and senescent processes become irreversibly established.

A prominent and optimistic school of thought suggests that exposures to behavioral and environmental factors that modify risk of disease and dysfunction determine one's position in these alternative outcomes in the aging process. In this view, more optimal nutrient intake, food selection, and lifestyle choices could reduce the heterogeneity, retaining more individuals in the successfully aged category for most of their life span. Others consider that genetic constitution may be as important in determining the course of aging as any positive or negative influences during our lifetime.

Overview of Specific Factors of Aging Influencing Nutritional Requirements

The discussion of nutrient requirements and recommended dietary intakes of nutrients in older persons has proceeded on both the theoretical and empirical level. Since the peak years for human reproduction occur before middle age, and well before older age begins, the forces of natural selection, governing fecundity in reproduction cannot exert themselves for the Darwinian selection of traits favoring longevity in the evolution for any traits related to longevity *per se* or physiological sustained function. Hence, there has been little or no evolutionary selection for nutrient requirements to achieve advanced age or for long-term survival. It is more for the preservation of comfort and function for those surviving to advanced age that the optimization of nutrition intakes for the elderly would apply, that is, for humanitarian and public health importance in the face of the physiological and anatomic changes of senescence.

As early as the 1970s, nutritional scientists advanced the proposition that requirements for different macro- and micronutrients changed with age. A large number of

conjectures based on an emerging scientific understanding of senescent physiology have been advanced. It has been suggested that the decreased physical activity and physical conditioning associated with the body composition changes attendant to aging sets the stage for alterations in requirements in both amounts and relative proportions of protein and the energy-yielding macronutrients. Decreased gastric secretory capacity has a negative influence on the absorption of calcium, iron, and vitamin B₁₂. Changing intestinal motility and digestive function evoked considerations of distinct increases and decreases of nutrients to compensate for the senescence of the intestinal tract, with particular interest in dietary fiber. Attention to compensatory intake for all of the nutrients involved in skeletal mineralization has come to the fore in relation to the recognized tendency to bone mineral loss with advancing age.

The immune and host defense system has been a major focus of gerontological nutrition. Increased intakes of both vitamin E and zinc, well above the normally recommended level, have stimulated certain immune functions in studies involving older volunteers. More recently, evidence for enhanced immune function in older individuals from physiological doses of zinc has been reported. Zinc may act to fortify the responsiveness of lymphocytes to stress. Impaired interferon and interleukin-2 responses may be the mechanisms whereby low zinc status impairs immune function in elderly humans. Additionally, vitamin D seems to play a role in the regulation of the aging immune system in the area of antibody and cytokine responses. Cognitive function declines with advancing age, and it has even been suggested that the adjustment of nutrient intake can favorably affect the retention of memory and cognitive function in older persons. Results of association studies and intervention trials have been mixed and inconclusive. The adequate intake (AI) of B-complex vitamins, particularly those related to homocysteine metabolism (vitamin B₁₂, folic acid, vitamin B₆, riboflavin), are associated with mental function in older age. It has also been suggested that older individuals need more *n*-3 fatty acids for preserving cerebral cellular anatomy related to cognition. A prospective trial of B-vitamin and *n*-3 fatty acids in older French adults found positive effects in the subsegment with a history of cerebral strokes. Implications for a role of vitamin D in preserved cognition with advancing age have emerged in human studies. Animal research suggests that both vitamin D and K may be important in conserving central nervous system function with aging. For several nutrient effects in immunity, cognition, and other areas, an interaction with a biomarker of a genetic polymorphism seems to be important.

Nutrient Intake Recommendations in Later Life

Comprehensive recommendations for macro- and micro-nutrients with differential attention to older persons have arisen from a collaboration between the US and Canada, and from expert panels serving the United Nations (UN) System. Each panel has set out its methodology and definitions and then presented tables of quantitative estimates. The recommendations for persons considered elderly in the respective systems are outlined below.

Definitions Surrounding Recommended Intakes of Nutrients

An important advance in establishing nutrient intake recommendations relates to the semantics. There has been a refining of the operational definitions of terms related to nutrient intakes. RNIs are set by the agencies of the UN System and are considered to be the intakes of nutrients required to satisfy the requirements of nearly all healthy persons of a given age, sex, and physiological condition, and should be universal for all regions of the globe. In 2006, a way to calculate (retrofit) the population-relevant estimated average requirement (EAR) to the UN System was published.

The Food and Nutrition Board of the Institute of Medicine in the US took a new approach in 1997 in which they applied the new dietary reference intakes (DRI) to micro- and macronutrient intakes. This work was undertaken jointly with Canada. It began with an assessment, where possible, of the EAR, defined as "the average daily nutrient intake level estimated to meet the requirement of half the healthy individuals in a particular life stage and gender group." The EAR is critical for an assessment of the risk of a nutrient deficiency problem at the population level. The traditional criterion used for decades, the recommended dietary allowance (RDA), is preserved. It is defined in the DRI process as "the average daily nutrient intake level sufficient to meet the nutrient requirement of nearly all (97 to 98%) healthy individuals in a particular life stage and gender group." When an EAR cannot be established from which to derive a formal RDA, the DRI process has a 'fall-back' category known as AI; this is defined as "a recommended average daily nutrient intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate." A new classification scheme involving a range of intakes was created specifically for energy, electrolytes, and liquids: the acceptable macronutrient distribution ranges (AMDRs).

For the first time, a specific and well-defined process to delimit levels of excess intake of nutrients and dietary substances was defined by the DRI process as the upper tolerable intake levels (UL). The UL is 'the highest average daily nutrient intake level likely to pose no risk of adverse health effects to almost all individuals in the general population.' It is considered that as intake increases above the UL, the potential risk of adverse effects increases. To date, the UN System's process has dealt much less explicitly with issues of excessive intake of nutrients and dietary substances.

Established Recommended Intakes for Older Persons

In earlier versions of the RDAs for the US population (up to the 10th edition in 1989), the nutrient recommendations for all healthy adults over 51 years of age were combined as a single value. For the UN System, the age threshold in the early editions was 50 years or older. Concerted efforts to refine our understanding of nutrient requirements for older adults have been made over the past two decades. This allowed the US-Canada DRI process to establish categories

Table 1 Nutrient intake recommendations for older males

	UL ^a	EAR ^a	RDA/AI ^a AMDR ^a	EAR ^b	RNI ^c
Macronutrients					
Water (l)	—	—	2.1 ^d	—	—
Carbohydrate (g)	—	100	120	—	—
Protein (g)	—	46	56	—	—
Total fat (g)	—	—	20–35	—	—
n-6 PUFA (g)	—	—	14 ^d	—	—
n-3 PUFA (g)	—	—	1.6 ^d	—	—
Dietary fiber (g)	—	—	30	—	—
Vitamins					
Vitamin A (RAE)	3000	625	900	430	600 (μg RE)
Vitamin D (μg)	4000	—	20	—	15
Vitamin E (mg α-tocopherol)	1000	12	15	8	10 (mg α-TE)
Vitamin K (μg)	—	—	120 ^d	—	65
Vitamin C (mg)	2000	75	90	38	45
Thiamin (mg)	—	1.0	1.2	1.0	1.2
Riboflavin (mg)	—	1.1	1.3	1.1	1.3
Niacin (mg)	35	12	16	12	16
Vitamin B ₆ (mg)	100	1.4	1.7	8.0	1.7
Biotin (mg)	—	—	30 ^d	—	—
Pantothenic acid (mg)	—	—	5 ^d	—	5
Folic acid (μg)	1000	320	400	320	400
Vitamin B ₁₂ (μg)	—	2.0	2.4	2.0	2.4
Choline (mg)	3500	—	550 ^d	—	—
Elements					
Sodium (g)	2.3	—	1.2 ^d	—	—
Potassium (mg)	—	—	4.7 ^d	—	—
Chloride (g)	3.6	—	1.8 ^d	—	—
Calcium (mg)	2000	—	1200	1083	1300
Phosphorus (mg)	3000	580	700	—	—
Magnesium (mg)	(350)	350	420	—	230
Iron (mg)	45	6	8	10	14 ^e
Zinc (mg)	40	9.4	11	5.8	7.0 ^f
Iodine (μg)	1100	95	150	93	130
Copper (mg)	10	0.7	0.9	—	—
Fluoride (mg)	10	—	4 ^d	—	—
Manganese (mg)	11	—	2.3 ^d	—	—
Chromium (μg)	—	—	30 ^d	—	—
Selenium (μg)	400	45	55	28	34
Molybdenum (μg)	2000	34	45	—	—

^aIn DRIs 70 years plus is considered as 'older'.^bIn UN System (WHO/FAO/IAEA) 65 years plus is considered as 'older'.^cThe EARs estimated retrospectively from factors published by Allen L, de Benoist B, Dary O, and Hurrell R (2006) *Guidelines on Food Fortification with Micronutrients*. World Health Organization and Food and Agricultural Organization of the United Nations. Geneva: WHO.^dRecommendation in the form of adequate intake.^eAssumes a 10% bioavailability of iron from the diet.^fBased on the assumption of a moderate bioavailability of zinc.

The figures in bold denote recommendations specifically modified for ageing (see text). UL, upper tolerable upper intake level; EAR, estimated average requirements; RDA, recommended dietary allowance; AI, adequate intake; AMDR, acceptable macronutrient distribution range; RNI, recommended nutrient intake; PUFA, polyunsaturated fatty acids; RAE, retinol activity equivalents; RE, retinol equivalents; α-TE, alpha-tocopherol.

for men and women aged 70 years and older. For the WHO/Food and Agriculture Organization (FAO) process, a specific estimation for individuals over 65 years has been provided in the 2004 micronutrient recommendations.

Given the magnitude of the theoretical considerations regarding senescence and aging physiology that have been raised by various authors, what is really surprising is the paucity of specific instances in which the recommended intakes of nutrients for men or women in the 'elderly category' are considered to be different from persons in the next youngest age

category. Composite tables for men (**Table 1**) and women (**Table 2**) are given for all of the nutrients and dietary substances expressed in the US–Canada DRIs and in the UN system for RNIs.

Macronutrients

In the DRI system, a universal, individual protein requirement was established as 0.80 g of good-quality protein per kilogram

Table 2 Nutrient intake recommendations for older females

	UL	EAR ^a	RDA/AI ^a AMDR ^a	EAR ^b	RNI ^c
Macronutrients					
Water (l)	—	—	2.6 ^d	—	—
Carbohydrate (g)	—	100	120	—	—
Protein (g)	—	38	46	—	—
Total fat (g)	—	—	20–35	—	—
<i>n</i> -6 PUFA (g)	—	—	11 ^d	—	—
<i>n</i> -3 PUFA (g)	—	—	1.3 ^d	—	—
Dietary fiber (g)	—	—	21	—	—
Vitamins					
Vitamin A (RAE)	3000	500	700	430	600 (μg RE)
Vitamin D (μg)	4000	—	20	—	15
Vitamin E (mg α-tocopherol)	1000	12	15	6.2	7.5 (mg α-TE)
Vitamin K (μg)	—	—	90 ^d	—	55
Vitamin C (mg)	2000	60	75	38	45
Thiamin (mg)	—	0.9	1.1	0.9	1.1
Riboflavin (mg)	—	0.9	1.1	0.9	1.1
Niacin (mg)	35	11	14	11	14
Vitamin B ₆ (mg)	100	1.3	1.5	1.3	1.5
Biotin (mg)	—	—	30 ^d	—	—
Pantothenic acid (mg)	—	—	5 ^d	—	5
Folic acid (μg)	1000	320	400	320	400
Vitamin B ₁₂ (μg)	—	2.0	2.4	2.0	2.4
Choline (mg)	3500	—	425 ^d	—	—
Elements					
Sodium (g)	2.3	—	1.2 ^d	—	—
Potassium (mg)	—	—	4.7 ^d	—	—
Chloride (g)	3.6	—	1.8 ^d	—	—
Calcium (mg)	2000	—	1200	1083	1300
Phosphorus (mg)	3000	580	700	—	—
Magnesium (mg)	(350)	265	320	—	190
Iron (mg)	45	5	8	6.9	11 ^e
Zinc (mg)	40	6.8	8	4.1	4.9 ^f
Iodine (μg)	1100	95	150	79	110
Copper (mg)	10	0.7	0.9	—	—
Fluoride (mg)	10	—	3 ^d	—	—
Manganese (mg)	11	—	1.8 ^d	—	—
Chromium (μg)	—	—	20 ^d	—	—
Selenium (μg)	400	45	55	22	26
Molybdenum (μg)	2000	34	45	—	—

^aIn DRIs 70 years plus is considered as 'older'.^bIn UN System (WHO/FAO/IAEA) 65 years plus is considered as 'older'.^cThe EARs estimated retrospectively from factors published by Allen L, de Benoist B, Dary O, and Hurrell R (2006) *Guidelines on Food Fortification with Micronutrients*. World Health Organization and Food and Agricultural Organization of the United Nations. Geneva: WHO.^dRecommendation in the form of adequate intake.^eAssumes a 10% bioavailability of iron from the diet.^fBased on the assumption of a moderate bioavailability of zinc.

The figures in bold denote recommendations specifically modified for ageing (see text). UL, upper tolerable upper intake level; EAR, estimated average requirements; RDA, recommended dietary allowance; AI, adequate intake; AMDR, acceptable macronutrient distribution range; RNI, recommended nutrient intake; PUFA, polyunsaturated fatty acids; RAE, retinol activity equivalents; RE, retinol equivalents; α-TE, alpha-tocopherol.

of body weight per day independent of age. No evidence for altered protein requirements with older age has been found. Moreover, it is recommended that the contribution of protein to total energy intake should not exceed 30%. The US Food and Nutrition Board also established an amino acid pattern in 2002. It specifies the density (mg g⁻¹ protein) of seven indispensable (essential) amino acids (histidine, isoleucine, leucine, lysine, threonine, tryptophan, and valine) and for two amino acid combinations (methionine + cysteine, phenylalanine + tyrosine). This pattern is

universal from age 1 year to the extremes of older age without modification.

It has long been recognized that energy recommendations cannot be made on a group basis, as each individual has his or her own daily energy requirement dependent on the amount of energy one is forced to expend with metabolic reactions, food processing, and physical exertion. In the DRI process, this is recognized in an effort to individualize the estimation of energy intake. Estimated energy requirement (EER) is based on the amount of energy needed to maintain

energy balance in relation to one's total energy expenditure. The DRI process for the US and Canada has published general EER equations (multidimensional nomograms) by which a reasonable estimate of an individual energy requirement can be calculated. There are general equations for adult men and women (over 19 years of age), based on consideration of physical activity level, weight, and height. In addition, there is an age term in the general EER, which is attached to a negative (minus sign) term in the equation. This signifies that energy requirements decline as a function of advancing years.

Although dietary fiber is not considered to be an 'essential' nutrient, the DRIs give a recommended level for intake. Curiously, in light of the active discussion of the role of fiber for the elderly in colonic function, the recommendations for intake by men decline from 38 to 30 g day⁻¹ and in women from 25 to 21 g day⁻¹ after 50 years. These are continued throughout the 70-year period, as well. This is a consequence of the fiber recommendations being pegged to total average energy intake.

Water

It is recommended in the DRI as an AI that males over the age of 70 require 2.6 l and females 2.1 l of water per day; this is a decline from the 51–70-year age group, where the daily water intake recommendations were 3.7 l and 2.6 l, respectively. It is further suggested that males and females over 70 years of age derive 81% of their daily water allowance from beverages and 19% as the metabolic water from foods. This is consistent throughout adulthood from age 19 years. Hence, there is no consideration of a higher requirement for water intake with older age. With respect to the electrolytes, no differences in AIs exist across the ages in adulthood.

Micronutrients

A number of recommendations (RDAs or AIs) change with advancing age in the DRI system; this is indicated by the bold type in **Tables 1** and **2**. The change in recommendations occurs at either age 50 or 70 years. In women over 50 years, the RDA for dietary iron decreases from 18 to 8 mg day⁻¹; there is no change in requirement for the 70-year plus age group. This lower value is the recommendation for adult men of all ages. The fact that the menopause allows women to replete iron stores depleted by an adulthood of monthly menstrual blood loss accounts for this lower RDA in older women.

The senescence of the skeletal system and the reduction of bone mineral content with age are a major nutritional concern in gerontological nutrition. In recent revisions of the recommendations, evidence for the need for increases in both vitamin D and calcium for older persons has led to changes in the estimates of requirements for these nutrients in later life. Within the DRI system the RDA for vitamin D for males and females over 70 years has recently been raised from 15 to 20 µg. The RDA for adults over 51–70 years is now 15 mg, as it is for young adults as well. Similar increases in vitamin D intake with age are recommended by the FAO/WHO and

represent a progression from 5 µg in young adulthood to 10 µg after mid-century to 15 µg after 65 years. With respect to calcium, the recommended levels increase from 1000 mg for younger adults to 1200 mg at age 50 years and beyond in the DRI system, and from 1000 to 1300 mg in the FAO/WHO standards. These are justified based on the higher propensity for skeletal fractures after 70 years of age associated with epidemiological evidence of widespread vitamin D deficiency in this age group, and evidence showing a reduction in bone loss with daily calcium intakes exceeding 1000 mg after midlife.

With respect to chromium, it is interesting that the estimation of an AI declines with advancing age. The AI for persons over 70 years in the DRI is the same as that for individuals between 51 and 70 years, but it is 5 µg day⁻¹ higher for the 19–50 years age range. This reduction is tied to the lower energy demands for individuals over 50 years of age.

The upper tolerable UL for phosphorus in the DRI system is 3000 mg day⁻¹ for both men and women over 70 years as compared to 4000 mg day⁻¹ for adults in the 19–70 years age group. This lower tolerance is explained by the greater prevalence of impaired renal function in advanced old age. Recently, moreover, the DRI has lowered the UL for calcium to 2000 mg beyond age 50 years, which is 500 mg below the level for ages 19–50 years.

Magnesium intake recommendations in the FAO/WHO guidelines decline for individuals over 65 years by 30 mg day⁻¹ compared to those in the 51–65 years age group. An anomalous finding for the magnesium RDA in the DRI system, which applies to all adult age groups, is that the UL for magnesium has been set at 350 mg. This is only 30 mg higher than the 320 mg daily recommended for older women, and is 70 mg lower than the 420 mg daily intake recommended for older men.

Dietary Guidelines for Function, Health, and Disease Prevention

If indeed the motivation for our dietary intake is hunger and thirst, and the primary evolutionary purpose is fulfilling nutritional needs, the manner in which we eat has important consequences for function, health, and disease prevention. The discipline of nutritional epidemiology has emerged over the past 30 years to assess associations between the selection of foods in the diet and probability and risk of suffering from poor physical function or ill health. Various features of eating behavior, from the size of portions to the number of repasts consumed in a day, to the frequency of consumption of foods with protective or noxious characteristics, have been implicated in function and health. Since aging represents an independent risk factor for disease and dysfunction, the effects of the dietary pattern become ever more manifest as an individual ages. Recent epidemiological research has shown that compliance or behavior concordant with healthy eating guidelines are associated with lower later life incidences of certain cancers, cataracts, diabetes, hypertension, stroke, and cardiovascular diseases, as well as overall survival. Moreover, there is intense interest in whether and how dietary pattern in addition to nutrition influence the maintenance of memory and cognitive function with aging.

These suggestions and recommendations have been codified and promoted by various entities focused on specific pathological situations, such as guidance for a healthy cardiovascular system by the American Heart Association or cancer prevention by the World Cancer Research Fund. In its Technical Report 916, Diet, Nutrition and the Prevention of Chronic Diseases, an expert committee of the UN agencies provides generic guidelines for reducing the risk of six important noncommunicable diseases. National bodies have also established dietary guidelines for healthful eating. They are often projected to the public in the form of emblems such as plates, rainbows, or pyramids, with visual representation of the relative proportion of various food-groups to consume, with lesser intakes of foods with salt, saturated fats, and high-energy density foods, and greater intakes of grains, fruits, and vegetables. More elaborated justifications and instructions can be found in the published forms in paperback editions or online on the Internet. Such is the case for the Dietary Guidelines for Americans in its 2011 edition.

The notion is that one adheres throughout life, even from childhood, to the tenets of these guidelines. Robert Russell and colleagues constructed a food guidelines pyramid, which specifically focused on the health of the elderly with more generous allowances in some areas (e.g., vitamin E, dietary fiber, liquids) and more extreme restrictions (e.g., sodium, solid fats), but it was based more on a potpourri of published findings about nutrient and food associations than any systematic review with agency backing.

Barriers to Meeting Recommended Nutrient Intakes and Healthful Dietary Intake Patterns by Older Persons

The late Professor Doris Calloway, in the early 1970s, commented: “People eat food, not nutrients.” This highlights the paradoxes in considering and enumerating the objectives of dietary intake at the level of the chemical composition, whereas most members of the general public are uninformed as to the nutrient composition of the foods and beverages in their diets.

Elderly persons face a number of challenges in meeting their RNI. In the first instance, they are likely to be those with the least sophisticated or available knowledge of the nutrients required and the food sources to provide them. The social, economic, and physiological changes imposing on the lives of persons surviving to advanced age pose logistical problems for their selecting and purchasing a diet. Economic dependency and the limited incomes of older persons may restrict their access to high-quality foods. Social isolation, depression, and impaired mobility, as well as chewing difficulties, may limit the variety of items included in the diet with advancing age. In some circumstances, it may be that free-living and independent elders are relatively less able to optimize their nutrient intake and dietary pattern compared to the more dependent individuals served or fed in institutional settings.

The exigencies of consuming a healthful diet for the prevention of chronic diseases, emphasizing a plant-based diet rich in whole grains, fruits, and vegetables, limits the nutrient selection that would be obtained from an even wider variety of

foods and food groups. Specific essential fatty acids, and certain minerals (calcium, zinc, selenium) and some vitamins are far less nutrient dense in foods of vegetal origin, setting a dilemma between consuming for nutrient adequacy and prevention of degenerative disease. Two nutrients with accentuated requirement levels in later life – vitamin D and calcium – have so few rich dietary sources that the elderly may be able to afford or assimilate, that supplemental forms will most likely be required.

Future Considerations

The DRI recommendations are specifically derived for the populations of the US and Canada in North America. The RNIs of the UN System are meant to be universal across the entire world. With respect to meeting nutrient intakes, increased selection of fortified foods by older individuals may contribute to the closing of any intake gaps or deficits. Fortified food consumption, however, has a number of caveats. The need for iron is one nutrient requirement that decreases with advancing age, at least for postmenopausal women. It may actually be that both sexes would benefit from a lesser burden of exposure to the oxidant effects of iron with advancing age. Similarly, the folate requirement has been set with interest in reproductive matters (prevention of neural tube defects), which are of no biological relevance in later life. Some, as yet inconclusive, evidence that colonic and prostatic tissues may receive dangerous proliferative stimulation from folic acid, the pharmaceutical form of the vitamin, has been reported. On the safety side of the equation, however, the effective upper tolerable levels for certain nutrients in later life may prove to be lower than those for younger members of the adult population.

The slight majority of the living elderly are currently to be found in the low income, largely tropical regions of the world in which 80% of the global population reside; this shift is due to rise rapidly over the next two decades. With specific reference to low-income societies, a number of caveats apply to the estimation of nutrient intake recommendations for the elderly across the world. If the “applies only to healthy individuals” disclaimer were applied to the developing world, then virtually no older people would qualify as eligible for coverage by any nutrient recommendations system. However, rather than abandon the effort for nutrient intake guidance, an attempt should be made to take into account the influences of life-long climatic issues (heat, humidity) and ecological factors (parasites, recurrent infections) on nutrient needs in later life.

Nature *versus* nurture issues will also continue to be debated with regard to nutrient requirements, especially in later life. The revelation of the human genome (complete genetic code), has given rise to the issues of ‘nutrigenomics’ and ‘nutrigenetics’; theoretically, it could soon be possible to understand individual variation in needs for and tolerances of essential and nonessential nutrients and dietary bioactive substances. The significance of this potential for the already aged person, however, is likely to be limited for two reasons. First, the accumulative effects of nutrient imbalance will already have been established. Second, the economic and

intellectual wherewithal to access and execute such individualized prescriptions for nutrient intakes and dietary patterns will likely escape the majority of older persons with limited financial means. Hence, further refinements in recommended intakes for older persons are likely to remain at the public health level for this segment of the population, and will involve establishing evidence that increased intakes of specific nutrients and will have health-protective effects or function-enhancing properties.

See also: Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Calcium. Chromium. Folic Acid. Older People: Nutritional Management of. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Riboflavin. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements. Vitamin D: Physiology, Dietary Sources, and Requirements. Vitamin E: Physiology and Health Effects. Zinc: Physiology, Dietary Sources, and Requirements

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Physiological Changes

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The Aging of the Population and its People

The maximal human life span is approximately 120 years. Approaching this degree of longevity, however, was not a prominent feature in the evolutionary phases of our species, *Homo sapiens*. The imperative was to survive the various mortal hazards long enough to reproduce and provide initial care for the offspring. The twenty-first century has ushered in an unprecedented longevity. The life expectancy of infants born today in Western Europe or Japan is more than 75 years. The most rapidly increasing population segment in the world today is the centenarian. By the year 2020, there will be more than 1 billion people older than 60 years of age, constituting 13.3% of the global population, and three-quarters of them will be living in developing countries.

Many people are living a long time, but not all of them are healthy and functional throughout their lifespan. Chronic disability and the cost of health services and custodial care are a growing burden on the economies of developed and developing countries alike. To understand the pathological aspects of advancing age, the normative pattern of changes in physiological function in older persons is an essential benchmark.

The Nature of Senescence

Aging has been described as “a series of time-related processes that ultimately bring life to a close,” that is, a process of physiological ‘wearing out.’ Physiology is the basis of human functionality as well as of our susceptibility to disease. The late gerontologist, Nathan Shock, established the principle of a progressive decline in physiological reserves as a consequence of ‘normal’ aging, recognizing that the rate of decline differed markedly among the body’s organ systems. In fact, one cannot really separate the concept of the physiology of older persons from the physiology of the aging process itself. Similarly, the high prevalence of chronic diseases in older persons challenges our ability to discriminate ‘normative’ senescence from pathophysiological changes.

The origin of physiological changes in older persons begins within the domain of cellular senescence. The extension to tissue and organ levels originates in what we interpret to be the physiological changes of human aging. Major advances in our cellular and molecular understanding of basic aging processes have been made in recent years.

Cellular Senescence

In most tissues, with the notable exception of neural tissue, healthy cells are replicating cells, which are capable of mobilizing at least 20 enzymes and proteins that must be

preassembled to initiate deoxyribonucleic acid (DNA) synthesis for cell division. An irreversible state of growth arrest known as replicative senescence is the fundamental basis of cellular aging. Such senescent cells remain viable and metabolically active, but their genomic function and protein expression are distinct from that of normal, proliferating cells. Iron accumulates in senescent cells, possibly contributing to the greater oxidative stress and cellular dysfunction seen in senescent cells. Senescent cells also express proinflammatory enzymes, an internal process that could possibly contribute to the aging process; intercellular adhesion molecules, which are part of the inflammatory response, are overexpressed in association with senescent cells and aging tissue.

Telomeres and Telomerase

Telomeres are small units composed of the tandem DNA repeats and associated proteins, which cap the end of linear chromosomes and are responsible for maintaining chromosome length. They provide stability to the chromosome and protect against DNA loss associated with cellular replication. The mechanism of replicative arrest of senescent cells has been related to changes in the function of telomerase, a nuclear enzyme that synthesizes and maintains the telomeres. Shortening and uncapping of these structures, related to the number of past cell divisions, renders the DNA strand incapable of replication.

Apoptosis

Another factor involved in aging at the cellular level is the orderly ‘retirement’ of cells. For every cell that divides in, another would somehow have to make space for the extra cell in order to maintain numerical stability in the organ. This is achieved by a process of programmed cell death, known as ‘apoptosis.’ Cell senescence disrupts these apoptotic processes. Necrosis, by contrast, is cell death due to injury or noxious stimuli. Diseases of aging may favor the necrotic process.

Mitochondrial Senescence and Oxidative Stress

The intracellular mitochondria, organelles involved in energy metabolism, are central to the process of cell senescence. They are also involved in regulating thermogenesis, calcium buffering, and integrating apoptosis. With aging, mitochondria become less efficient, partly due to mutations in the cell nucleus, derepressing the expression of proteins that compete with mitochondrial function. This disrupts energy metabolism for the cell and makes the mitochondria more porous, releasing reactive oxygen species into the rest of the cell. The mitochondrial production of reactive oxygen species is

inversely proportional to longevity in animals. The oxidative activity also damages the mitochondria themselves. Mitochondria have their own DNA strands, and these accumulate mutations with age. In tissues dependent on progenitor (stem) cells, mitochondrial DNA mutations can disrupt replication.

Free radicals and reactive oxidative species can produce mutations in nuclear material and oxidize proteins and lipids throughout the cells. Aging involves an accumulation of oxidative damage at the cellular level, if not an increase in its intensity as well. The thiol-containing antioxidant mechanisms, typified by glutathione but represented by a number of sulfur-containing species, represent an important buffer against intracellular free radicals, but decline with age due to downregulation of their synthetic enzymes. Confirming the cellular trend to oxidative stress in aging cells, clinical biomarkers of oxidation, and antioxidant mechanisms reveal that systemic oxidative stress increases with aging characterized by lower concentrations of vitamins E and C and carotenes as well as lower activities of Cu–Zn-superoxide dismutase, catalase, and glutathione peroxidase.

Physiological Changes Occurring in Tissues and Organ Systems with Human Aging

Physiology has classically been organized around organ systems. According to this convention, the important features of the age-associated changes are enumerated and synthesized, with implications for human nutrition.

Integumentary Tissues

The integumentary tissues (skin, hair, and nails) cover and protect the body. Two of the more classical and reproducible manifestations of aging can be seen in this system. The depigmentation of hair to gray or white is an almost universal aging effect given its sufficient survival. Wrinkling of the skin, due to alteration in connective tissue composition, is another consequence of aging; it should be assessed by the changes in skin texture only in the nonsun-exposed regions of the body. Beyond the cosmetic consequences of the aging integumentary tissues, wound healing is a health-relevant consideration. Healing of wounds is slower with increasing age, but the resulting scars have the same tensile strength. Reduced recruitment of vessels of the microvascular is a function of aging.

The skin is an endocrine organ. Vitamin D is produced from the conversion of 7-hydroxy-cholesterol to cholecalciferol in the dermis of the skin. The efficiency of vitamin D decreases with age, such that older persons need a longer exposure to solar radiation to produce a given quantity of the vitamin.

Pulmonary and Respiratory System

Compliance of the chest wall changes with age, which gets stiffer and less compliant. The muscular force of the diaphragm is reduced with advancing years. The combination of these two factors reduces the maximal amount of air that can

be moved into and out of the lungs. This diminution in the so-called forced vital capacity of the lungs occurs as one gets older. There is less compliance, less recoil, and greater dead space. The original lung capacity, however, is sufficient to allow for sufficient gas exchange throughout life in the absence of underlying pulmonary disease. Nonetheless, the longitudinal Framingham Heart Study found an association between decrease in lung capacity and all-cause mortality.

The hygiene of the respiratory airways is somewhat compromised by a decreasing function of the microcilia of the bronchial epithelial cells. Because this mechanism is used to clear microbial pathogens, it has a direct influence on host defenses. Finally, because the basis of the respiratory system is an exchange of gases (oxygen, carbon dioxide, trace gases) with the bloodstream, any cardiovascular changes involving the side chambers of the heart will influence the overall gas exchange efficiency for the body.

Cardiovascular and Circulatory System

For this system, it is necessary to separate the aging effects on the cardiac muscle and its apparatus from the aging of the vessels of the circulatory system, which transports blood to and from the heart. A characteristic of aging is a diminished resting cardiac output, which can have the combined bases of lower force of the cardiac muscle and a lesser oxygen demand for metabolism with diminished active cell mass. Aging of the myocardium reduces its capacity for cellular repair and replacement. With aging, elevations of noradrenaline (norepinephrine) associated with downregulation of β -1 receptors mimic the process of the failing heart. The compliance of the arteries emanating from the heart decreases with age. Stiffening of these vessels produces a progressive rise in the systolic blood pressure.

It is the circulation through smaller blood vessels and the generation of new vessels (neovascularization) that is a major concern with advancing years. The process of angiogenesis, through which new blood vessels are formed, is impaired during aging. The integrity of endothelial cells lining the vessels, the cascade of coagulation factors, and growth factors and neurochemical mediators, and their respective receptors are all altered by aging in the neovascularization processes.

Oral Cavity and Alimentary Tract

The digestive tract is subject to functional changes with aging. Beginning in the oral cavity, loosening and loss of teeth is a frequent companion of aging. Saliva secretion decreases leading to relative degrees of xerostomia or dry mouth.

Reduced parietal cell function develops in older persons, but prior *Helicobacter pylori* infections are now thought to be a major cause of hypochlorhydria in later life. An important nutritional consequence of reduced gastric acid secretion is a lesser biological availability of iron. Because iron stores are generally replete in both men and women in later life, this has little practical nutritional impact. The reduced secretion of gastric intrinsic factor, however, contributes to vitamin B₁₂ deficiency, which is an important nutritional problem of older persons.

The capacity of the liver for biliary secretion and the pancreas for digestive enzyme and bicarbonate secretion begins adult life with a >90% excess of the necessary minimum. Secretory function declines with increasing age, but rarely falls below the minimal reserve capacity. The metabolic and detoxifying capacity of the human liver also has a reserve capacity and is not usually compromised by normal aging.

Intestinal motility is reduced with aging as a result of functional changes in the visceral nerves. With decreased transit, the residence time of the chyme on the absorptive surfaces is longer, compensating for any senescence in the mucosal uptake itself. The reduction in motility produces the most noticeable and notorious of the manifestations of intestinal health in older persons, namely reduced frequency of defecations.

Musculoskeletal System

Bone mineral content declines with age; this aging process is known as 'osteopenia.' (It should be distinguished from the related pathological process in which bone architecture is altered, producing 'osteoporosis.') From the peak in the third and fourth decades, a 30% average decline in bone mineral density occurs through the ninth decade. In women, there is well-characterized acceleration of the rate of bone mineral loss immediately following the menopause. Decreasing levels of anabolic hormones may be associated with musculoskeletal atrophy and decrease in function that is observed in older women. This change in skeletal mineralization with aging is not associated with any apparent change in vitamin D nutrition as reflected in circulating levels of the vitamin.

The joints of the body undergo changes with the senescence of replacement of the cartilaginous substance, complicated by the pathological effects of cumulative use over the life span.

Recently, increasing attention has been given to the loss of muscle strength and substance with increasing age. Sarcopenia loss of lean body mass skeletal muscle mass replacement by fat mass decreased creatinine-to-height ratio in normative aging in healthy subjects diminished grip strength is a function of age. Reduction in muscle mass (sarcopenia obesity) is an important determinant of physical function and metabolic rate.

Renal and Urogenital System

Renal creatinine and inulin clearance decreases with aging have been demonstrated for decades. These functional changes in filtration are associated with changes in the glomerular structure in the kidney. Circulatory senescence decreases blood flow to the kidneys, which further reduces the efficiency of renal clearance. The reserve capacity of these organs is such, however, that age-associated glomerular decline per se does not compromise the net excretion of nitrogenous waste.

Urine flow at the outlet is another aging consideration. The male urogenital system undergoes a characteristic aging change in the hypertrophy of the prostate gland, associated with decreased secretion of prostatic fluid. The anatomical consequence is a constriction in the passage through which urine flows from the bladder.

Gonads and Reproductive System

It has been aptly stated by Harman that: "It is clear that aging results in alterations of endocrine physiology, which in turn appear to contribute to development of the senescent phenotype." Aging is associated with a decrease in pituitary hormone secretions. This decline explains, in part, the reduction in gonadal hormone production with aging. Primary aging of the testes and ovaries themselves accounts for the remainder of the changes. As the ovaries have a finite number of eggs, ovulation can only continue through the number of cycles that correspond to the original store of ova. Menopause ensues with the characteristic cessation of estrogenic hormone secretion. In both sexes, gonadal androgenic hormone production declines with consequent effects on libido.

Endocrine Systems and Metabolism

As stated above, the pituitary gland is the hub of endocrine regulation. Important among the decline stimulation within the axis is that growth hormone (GH) secretion declines with increasing age, a condition termed 'somatopause.' The changes in the GH/insulin-like growth factor axis with aging produce changes in function, metabolism, and body composition analogous to the pathological GH deficiency seen in younger adults. Another change with age is the efficiency with which physical activity stimulates the secretion of GH.

The availability of hormones is not the only variable in endocrine signaling. Cellular and intracellular receptor function is complementary. An attractive explanation for the dis-ordering of hormonal axes is oxidative damage to cell membranes, compromising the function of receptors.

Basal and resting metabolism and diet-induced thermogenesis are all reduced with increasing age. Changes in body composition, and the replacement of lean tissue with fat and the increasing visceral distribution of fat, as well as decreasing physical activity, influence these metabolic changes of aging. Basal metabolic rate declines in aging more than can be attributed to body composition changes and intracellular mitochondrial senescence may explain part of this discrepancy. For practical purposes, the standard oxygen consumption value equivalent to one metabolic equivalent, that is, $3.5 \text{ ml min}^{-1} \text{ kg}^{-1}$, is not appropriate for elderly people.

Hematopoietic and Immune System

The formation of new red and white blood cells and platelets is one of the most proliferation-dependent physiological processes of the body. The various classes of circulating white cells are the underpinning of the host defense system, together with tissue macrophages, hepatic proteins, and the alimentary tract's mucosa.

Hematological Aging

The blood-forming organ is the bone marrow. Aging is associated with fatty infiltration of the marrow spaces in the long bones, but enough marrow remains to support the turnover of erythrocytes and red blood cell lines. The circulating red blood cell mass neither changes normally with advancing age nor does the normative peripheral white cell count or platelet

number. As noted, iron stores tend to be abundant in later life; nutritional problems influencing red blood cell production are based on alterations in gastric function (vitamin B₁₂ malabsorption), which result in a macrocytic (megaloblastic) anemia.

Immunological Aging

Circulating phagocytic white blood cells counts do not reduce with aging but aging does influence the innate host defense system. Mucosal barrier functions are influenced by aging of the gut in its interaction with microflora. Although not reduced in number, aged macrophages and neutrophils have blunted intracellular signaling by specific receptors, decreased metabolic functions, and impaired bacterial killing. Production of superoxide anion, chemotaxis, and orderly apoptosis of neutrophils is also disrupted by the disordered signaling. The tumor cell-destroying capacity of natural killer cells in the elderly is diminished.

More profound changes occur in the adaptive immune functions, which rely on the memory (T-cell) lymphocytic cell line. Life-long antigen exposure induces increases in the number of memory T-cells, but with enhanced reactivity against self-antigens, priming the individual for autoimmune disease. In healthy adults, immunoglobulin A concentration increases by 0.2 g l^{-1} per decade throughout life. The T-lymphocytes, however, respond more poorly to ongoing antigen assault in later life. Thymic involution associated with neural and hormonal changes of aging is an impediment to T-cell maturation in older persons. The basis of intrinsic function deficits of memory cells, however, has been ascribed to defective signaling and includes hyporesponsiveness to mitogen-stimulated proliferation and decrease in genetic suppression, allowing increased stimulation of inflammatory cytokines; the balance between pro- and anti-inflammatory cytokines shifts with aging, favoring the inflammatory pole, especially with the greater expression of interleukin 6. This has a negative systemic effect on bone metabolism, as well as dysregulating overall immune function.

Aging of mitochondria in the immune cell lines produces increased intracellular reactive oxygen species burdens. Finally, there is diminished programmed death (apoptosis) of immune cells and dysregulation of apoptosis-dependent functions.

Central and Peripheral Nervous System

The integration of all senses and origins of all systemic coordination is a function of the brain and central nervous system. This is the one system in which proliferation of the primary cells (neurons) is not an issue after early childhood, although the supportive, nerve-tending (glial) cells continue to depend on replication and apoptosis for normal function.

Central Nervous System

The neurons of the brain continue to divide only through to the second year of life. Thereafter, the goal is to preserve the number and health of the cerebral nerve cell mass. Myelination of axons of nerve cells must be maintained throughout life. This is the function of the supporting cells

(oligodendrocytes), which for more than 40 years continue to differentiate into myelin-producing cells. Free radicals pose a threat to these axon-tending cells, whose metabolic demands for producing the brain's cholesterol and maintaining its array of myelin sheaths render them particularly vulnerable to stress.

Positron emission tomography imaging of the aging brain has revealed and mapped the plethora of changes in blood flow and neurotransmitter metabolism that occurs with advancing years.

Special Senses

The special senses related directly to the cranial nerves (vision, hearing, taste, and smell) experience age-related change. With respect to vision, the most typical of all biological aging changes is presbyopia, or the loss of accommodation function for the ocular lens with loss of capacity of the associated musculature. The consequence is loss of near-vision, which leads to the need for reading glasses or bifocal spectacles. A more important aging change related to the lens is the opacification that leads to cataract formation. The eye is designed to translate light energy into visual images, but the energy of light, particularly the ultraviolet β -rays of solar energy, damages ocular tissue. Thus, there is as a strong environmental component to the disarranging of the laminar stacking of the fibrillar proteins of the lens, which imparts its clear, transparent basis; consumption of diets high in antioxidant vitamins has been associated with the delay in cataract formation.

Age-related hearing loss is a feature of biological aging. It affects the cochlear neural structures and leads to loss of acuity, especially for higher pitched tones. It is speculated that apoptosis of the most vital neural cells drives this hearing loss, based on mutations in the mitochondria due to life-long free-radical stress.

Taste and smell acuity decline with aging, both in sensitivity and in accuracy of recognition. Because these combined senses account for the recognition of flavors, their diminution with age could affect appetite and reduce the enjoyment of meals.

Cognitive Function

The intellectual, reasoning, and memory functions of the cerebral cortex decline with increasing age. This has been a universal observation in general elderly populations. The debate is whether this is a consequence of neurodegenerative diseases (pathological change) or a biological correlate of aging (senescence). Continued intellectual stimulation has been posited as an approach to retard cognitive decline, and a role for B-complex vitamins and antioxidants has been advanced.

Peripheral Nervous System

Vibratory perception in the peripheral extremities is the classical index of peripheral nervous decline with aging. Less well appreciated is the effect of aging on pain perception, in which there can be a numbing of sensation or, less commonly, an accentuation of perception. Pain perception from the visceral organs is often dulled, which can have adverse implications for the early detection of organic diseases. All of the peripheral nerve dysfunction can result from the compensatory sprouting

of axonal limbs to compensate for the loss of motor neurons. This is well directed at first, but with further aging, the synaptic connections are poorly directed and motor function suffers as a consequence.

Drug Metabolism

The metabolism of drugs and pharmacological agents is not the purview of any single organ system. Older persons tend to be prescribed increasing numbers of medications with advancing age. Important changes in drug metabolism occur with aging. Metabolism and disposition of drugs change with age. This involves age-associated decrease in the function of some, but not all, cytochrome P-450 enzymes. Among the pharmacokinetic and pharmacodynamic changes that occur with advancing age are reductions in renal and hepatic clearance and an increased effective half-life of lipid-soluble drugs. The older population shows increased sensitivity to some psychotropic drugs and anticoagulants, with the frail elderly being more susceptible than healthy elders.

Synthesis and Conclusion

The number of older people is increasing in all regions and all societies of the world. Advancing age produces senescent changes in cellular function that are reflected in a declining capacity of all physiological systems. The increased prevalence of disease in older population aging is a major risk factor for disease but does not necessarily lead to age-related diseases.

All physiological systems are intrinsically interrelated in maintaining the health and function of the organism. Aging is associated with a loss of complexity in the dynamics of many physiological systems. It has been speculated that the basis for the syndrome of frailty in older persons may result from a reduced ability to adapt to internal and external stresses of

daily life due to the loss of dynamic coordination among the interrelated physiological systems.

The alterations in physiological functions with aging have important implications for absorbing, retaining, and utilizing nutrients. The extent to which dietary patterns and nutrient intakes are accelerating or retarding the rates of functional decline is a matter of ongoing investigation in gerontological nutrition and physiology.

See also: Aging. Brain and Nervous System: Biology, Metabolism, and Nutritional Requirements. Cytokines: Nutritional Aspects. Older People: Nutritional Management of; Nutritional Requirements. Osteoporosis: Nutritional Factors. Vitamin K

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OMEGA-3 POLYUNSATURATED FATTY ACIDS

Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases

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Abbreviations

AA	Arachidonic acid	HDL	High-density lipoprotein
AI	Adequate intake	IL	Interleukin
ALA	α -Linolenic acid	LA	Linoleic acid
CAD	Coronary artery disease	LC-PUFA	Very-long-chain polyunsaturated fatty acid
CHD	Coronary heart disease	5-LO	5-Lipoxygenase
COX-2	Cyclooxygenase-2	NF-κB	Nuclear factor-Kappa-B
CRP	C-reactive protein	PAI-1	Plasminogen activator inhibitor factor-1
CTSS	Cysteine protease cathepsin S	PG	Prostaglandin
DHA	Docosahexaenoic acid	PLAVR	Plasminogen activator urokinase receptor
EFA	Essential fatty acid	PPARα	Peroxisome proliferator-activated receptor alpha
EPA	Eicosapentaenoic acid	PUFA	Polyunsaturated fatty acid
EPG	Ethanolamine phosphoglyceride	TNF	Tumor necrosis factor
FADS	Fatty acid desaturase	VLDL	Very-low-density lipoprotein

Glossary

Allele Alternative form of a genetic locus; a single allele for each locus is inherited from each parent (e.g., at a locus for eye color the allele might result in blue or brown eyes).

Gene The fundamental physical and functional unit of heredity. A gene is an ordered sequence of nucleotides located in a particular position on a particular chromosome that encodes a specific functional product (i.e., a protein or RNA molecule).

Gene expression The process by which a gene's coded information is converted into the structures present and operating in the cell. Expressed genes include those that are transcribed into mRNA and then translated into protein and those that are transcribed into RNA but not translated into protein (e.g., transfer and ribosomal RNAs).

Genetic polymorphism Difference in DNA sequence among individuals, groups, or populations (e.g., genes for blue eyes versus brown eyes).

Genotype The genetic constitution of an organism, as distinguished from its physical appearance (its phenotype).

Nutrigenetics Refers to an individual's specific response to diet due to genetic variants or polymorphisms (i.e., individuals responding differently to the same diet by having different levels of, for example, serum cholesterol and blood pressure because of genetic variation).

Nutrigenomics Refers to the role of nutrients in gene expression (i.e., polyunsaturated fatty acids suppress fatty acid synthase (mRNA) gene expression).

Polymorphism Difference in DNA sequence among individuals that may underlie differences in health. Genetic variations occurring in more than 1% of a population would be considered useful polymorphisms for genetic linkage analysis.

Introduction

Approximately 80 years ago (1929–1930) Burr and Burr were the first to discover the importance of linoleic acid (LA)

18:2 ω -6 and alpha-linolenic acid (ALA) 18:3 ω -3 in restoring the effects caused by the fat-free diet in deprived animals. They coined the term 'essential fatty acids' (EFA). Although healthy skin and successful growth, reproduction, and lactation were

obtained in mammals fed with LA as the only source of EFA, ALA was found to permit growth, but was unable to prevent the skin lesions of EFA deficiency, or support reproduction. Omega-6 fatty acids are the predominant polyunsaturated fatty acids (PUFAs) in all diets, especially the US and other Western diets (Table 1). The major omega-6 fatty acid in Western diets is LA, representing approximately 90% of all the PUFA in North American Diets. Today we know that LA and ALA and their long fatty acid derivatives, arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) respectively are essential for normal growth and development of human beings, and in the prevention and management of chronic diseases.

Over the past five years major advances have taken place in the understanding of mechanisms by which omega-6 and omega-3 fatty acids carry out their metabolic effects at the molecular level. Genetic variants in the LA and ALA metabolic pathways indicate the need to consider their genetic variants in the determination of estimating dietary requirements and in influencing gene expression. Genetic variants most likely account for the conflicting results of epidemiologic studies relative to the effects of omega-3 fatty acids in cardiovascular disease and cancer. Therefore this article focuses on Nutrigenetics – how genetic variation influences dietary response and

on Nutrigenomics – how omega-6 and omega-3 fatty acids influence gene expression.

Eicosanoid Metabolism and Biological Effects of Omega-6 and Omega-3 Fatty Acids

The two families of omega-6 (LA) and omega-3 (ALA) fatty acids are physiologically and metabolically distinct, they cannot be synthesized in the human body, and they must be obtained from the diet. Figure 1 shows the metabolism of LA and ALA into very-long-chain polyunsaturated fatty acids (LC-PUFA) through a series of desaturases and elongases. Both LA and ALA use the same enzymes (desaturases and elongases) and compete with each other for enzyme availability. During evolution there was a balance in the intake of LA and ALA with a ratio of ω -6/ ω -3 = 1, whereas today in Western societies the ratio is approximately 16/1 ω -6/ ω -3 due to the high intake of vegetable oils-corn oil, sunflower, safflower, soybean, and linseed oil, which are high in LA (Figure 2). LA is found in high amounts in grains with the exception of flaxseed, perilla, rapeseed, and walnuts that are rich in ALA. The green leaves of plants, particularly wild plants are higher in ALA than LA. A low LA/ALA ratio, that is, an increase of ALA leads to higher EPA levels, which competes with AA, and increases the production of anti-inflammatory prostaglandins and leukotrienes.

The PUFA composition of phospholipids has been shown to be associated with normal growth and development, as well as in the outcome of chronic diseases such as coronary heart disease (CHD), hypertension, cancer, diabetes, mental health, neurodegenerative diseases, arthritis, allergies, and other autoimmune diseases, because both omega-6 and omega-3 PUFAs are processed to powerful promoters of eicosanoids such as prostaglandins and leukotrienes, influence gene expression, and telomere length. Figure 1 shows the

Table 1 n -6/ n -3 ratio in various populations

Population	n -6/ n -3
Paleolithic	0.79
Greece before 1960	1.00–2.00
Current Japan	4.00
Current India, rural	5–6.1
Current UK and northern Europe	15.00
Current US	16.74
Current India, urban	38–50

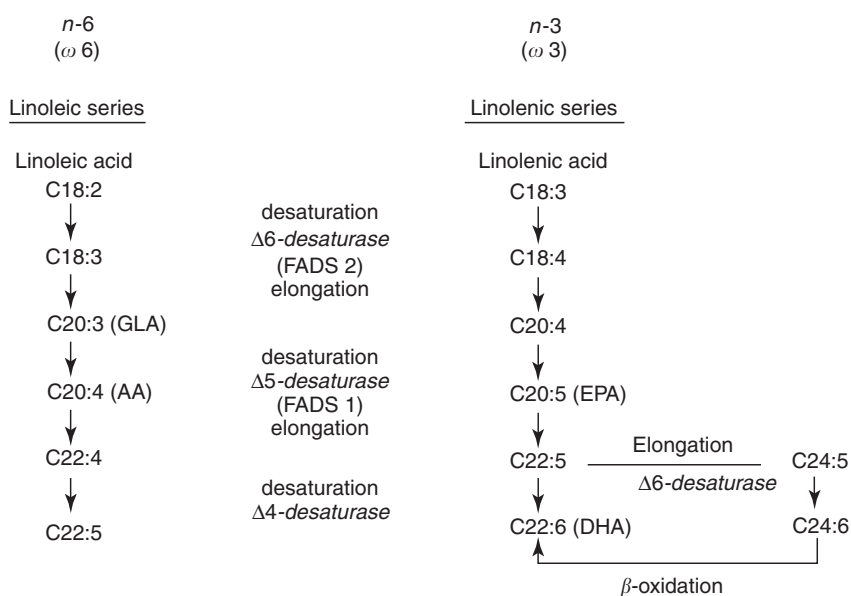


Figure 1 Desaturation and elongation of ω 3 and ω 6 fatty acids.

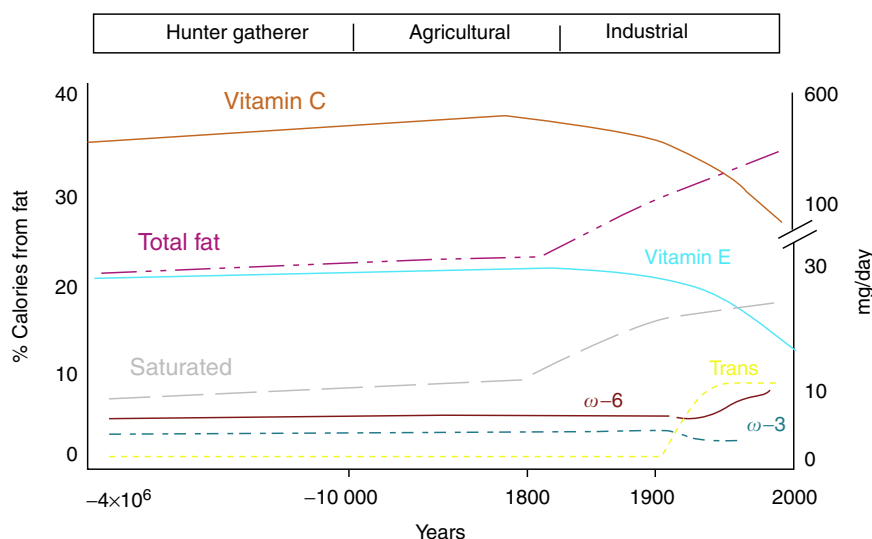


Figure 2 Hypothetical scheme of fat, fatty acid ($\omega 6$ and $\omega 3$, trans and total) intake (as percent of calories from fat) and intake of vitamins E and C (mg day^{-1}).

Table 2 Effects of ingestion of EPA and DHA from fish or fish oil

Decreased production of prostaglandin E_2 (PGE_2) metabolites
A decrease in thromboxane A_2 , a potent platelet aggregator and vasoconstrictor
A decrease in leukotriene B_4 formation, an inducer of inflammation, and a powerful inducer of leukocyte chemotaxis and adherence
An increase in thromboxane A_3 , a weak platelet aggregator and weak vasoconstrictor
An increase in prostacyclin PGI_3 , leading to an overall increase in total prostacyclin by increasing PGI_3 without a decrease in PGI_2 , both PGI_2 and PGI_3 are active vasodilators and inhibitors of platelet aggregation
An increase in leukotriene B_5 , a weak inducer of inflammation and a weak chemotactic agent

metabolic pathways of omega-6 and omega-3 fatty acids. The key enzymes in this pathway are the delta-5 and delta-6 desaturases, which are encoded by fatty acid desaturase (FADS) 1 and (FADS) 2, respectively. They are the rate limiting enzymes in the synthesis of the long chain PUFA, AA, EPA, and DHA from their dietary precursors LA and ALA.

Competition between the omega-6 and omega-3 fatty acids occur in prostaglandin formation that are metabolically and physiologically distinct and have suppressing properties. EPA competes with AA, for prostaglandin and leukotriene synthesis at the cyclooxygenase and lipoxygenase level (Figure 1). Table 2 shows the changes that occur when humans ingest fish or fish oil.

Omega-3 fatty acids modulate prostaglandin metabolism, decrease triglycerides, raise high-density lipoprotein (HDL), and in high doses lower cholesterol, and have antithrombotic and anti-inflammatory properties that account for decreasing the risk for CHD and other chronic conditions, i.e., cognition in the elderly (Table 3). Recent studies show that EPA and DHA attenuate the rate of decrease in telomere length, which may account for their beneficial effects on aging and CHD, whereas LA is the most potent nutrient in decreasing telomere

length leading to cell apoptosis and aging, and increasing the risk for CHD. In the early phase of inflammation, excessive amounts of interleukins and lipid mediators are released and these play a crucial role. Proinflammatory eicosanoids of AA metabolism are released from membrane phospholipids in the course of inflammatory activation. EPA is released to compete with AA for enzymatic metabolism inducing the production of less inflammatory and chemotactic derivatives (Table 2). During the resolution phase of inflammation EPA produces Resolvins E1 and E2 and DHA produces Resolvins D1-D2 and neuroprotectin D1.

Genetic Variation: Nutrigenetics

Genetic Variants, FADS1 and FADS2 in Estimating Nutritional Requirements of Omega-3 and Omega-6 Fatty Acids

The FADS1 and FADS2 gene cluster involved in the metabolic pathway of LA and ALA as well as the enzymes involved in the production of eicosanoids, 5-LO, and cyclooxygenase from the AA and EPA, are polymorphic. Recent studies on their polymorphisms indicate that the minor alleles of the genetic variants in FADS1 and FADS2 are associated with higher LA and lower AA levels in red cell membrane and plasma phospholipids, which may influence the estimation of dietary requirements particularly during pregnancy and lactation as well as the infant's IQ whereas an increase in the activity of the desaturases increases the AA to LA ratio and the risk for CHD. Furthermore genetic variants in the 5-LO and COX-2 genes have been associated with increased risk for CHD and cancer.

The levels of LC-PUFA in plasma serum or red cell membrane phospholipids depend on dietary intake and endogenous metabolism (Figure 1). There have been many indications for considerable inter-individual variation in the capacity for endogenous formation of LC-PUFAs. For example, more than 20 years ago there was a rather close correlation of omega-6

Table 3 Effects of *n*-3 fatty acids on factors involved in the pathophysiology of atherosclerosis, inflammation, and aging

<i>Factor</i>	<i>Function</i>	<i>Effect of n-3 fatty acid</i>
Arachidonic acid	Eicosanoid precursor; aggregates platelets; stimulates white blood cells	↓
Thromboxane A ₂	Platelet aggregation; vasoconstriction; increase of intracellular Ca ²⁺	↓
Prostacyclin (PGI _{2/3})	Prevent platelet aggregation; vasodilation; increase cAMP	↑
Leukotriene (LTB ₄)	Neutrophil chemoattractant; increase of intracellular Ca ²⁺	↓
Fibrinogen	A member of the acute phase response; and a blood clotting factor	↓
Tissue plasminogen activator	Increase endogenous fibrinolysis	↑
Platelet activating factor (PAF)	Activates platelets and white blood cells	↓
Platelet-derived growth factor (PDGF)	Chemoattractant and mitogen for smooth muscles and macrophages	↓
Oxygen free radicals	Cellular damage; enhance LDL uptake via scavenger pathway; stimulate arachidonic acid metabolism	↓
Lipid hydroperoxides	Stimulate eicosanoid formation	↓
Interleukin 1 and tumor necrosis factor	Stimulate neutrophil O ₂ free radical formation; stimulate lymphocyte proliferation; stimulate PAF; express intercellular adhesion molecule-1 on endothelial cells; inhibit plasminogen activator, thus, procoagulants	↓
Interleukin-6	Stimulates the synthesis of all acute phase proteins involved in the inflammatory response: C-reactive protein (CRP); serum amyloid A; fibrinogen; α_1 -chymotrypsin; and haptoglobin	↓
CRP	An acute phase reactant and an independent risk factor for cardiovascular disease	↓
Endothelial-derived relaxation factor	Reduces arterial vasoconstrictor response	↑
Insulin sensitivity		↑
VLDL		↓
HDL	Decreases the risk for CHD	↑
Lp(a)	Lipoprotein(a) is a genetically determined protein that has atherogenic and thrombogenic properties	↓
Triglycerides and chylomicrons	Contribute to postprandial lipemia	↓
Telomeres	Have anti-aging effects whereas LA promotes shortening of telomeres and aging	↑
Resolvin E1–E2 (EPA)	Anti-inflammatory important in the resolution of inflammation	↑
Resolvin D1–D2 (DHA)	Anti-inflammatory important in the resolution of inflammation	↑
Neuroprotectin (DHA)	Protects brain; important in patients with strokes or trauma	↑
PPAR	Upregulates the expression of genes involved in lipid metabolism and downregulates the expression of genes involved in inflammation and suppresses NF- κ B	↑

VLDL: very-low-density lipoprotein.

and omega-3 fatty acids content in mature milk in human beings even though the main dietary sources were different. Thus it appears that some women have a higher ability to synthesize and secrete milk LC-PUFAs of both the omega-6 and omega-3 series, than others. In addition there was a tracking of plasma LC-PUFA levels in the absence of tracking of dietary intake patterns, suggesting that there is inter-individual variation in the ability to endogenously synthesize LC-PUFAs among children, which persists over time and could most likely be due to genetically determined differences in metabolic turnover. Changes in PUFA conversion have been shown with stable isotope studies.

Genetic Variants of the FADS1 and FADS2 Gene Cluster Influence Omega-6 and Omega-3 Fatty Acids Composition in Both Plasma and Red Cell Membrane Phospholipids During Pregnancy and Lactation

AA, EPA, and DHA play central roles in infant growth, neural development, and immune function. The maternal status of

AA, EPA, and DHA during gestation influences maternal to infant transfer via the placenta and breast milk provides fatty acids to infants after birth. FADS1 and FADS2 single nucleotide polymorphisms influence plasma phospholipid and erythrocyte ethanolamine phosphoglyceride (EPG) omega-6 and omega-3 fatty acids during pregnancy and their breast milk during lactation. Minor allele homozygotes of rs 174553 (GG), rs 99780 (TT), and rs 174583 (TT) had lower AA but higher LA in plasma phospholipids and erythrocyte EPG and decreased omega-6 and omega-3 fatty acids product to precursor ratio at 16 and 36 weeks gestation $P < 0.001$. Breast milk fatty acids were influenced by genotype, with significantly lower 14:0, AA and EPA, but higher 20:2 ω -6 in the minor allele homozygotes of rs 174553 (GG), rs 99780 (TT), and rs 174583 (TT) and lower AA, EPA, DPA 22:5 ω -3, and DHA in the minor allele homozygotes GG of rs 174575. The results indicate a robust association between minor alleles of the 4 SNPs and lower AA and other omega-6 fatty acids relative to precursor LA. Similar results were found for the omega-3 series. Genetic variation in the FADS1 and FADS2 gene cluster

is important for the composition of fatty acids provided to breastfed infants in mother's milk.

Genetic Variants in Omega-6 and Omega-3 Fatty Acids Metabolism and IQ

Children's intellectual development is influenced by both genetic and environmental experiences. Breastfed children attain higher IQ scores than children not fed breast milk. Breastfeeding is thought to influence brain development through nutritional processes involving fatty acids. The predominant LC-PUFA present in human milk but not in cow's milk, are DHA and AA. Substantial amounts of DHA and AA accumulate in the human brain during the first postnatal months and infants who are breastfed have higher concentrations of DHA and AA than infants fed unsupplemented formulas. Randomized controlled clinical trials comparing the neurodevelopment of infants fed DHA supplemented versus unsupplemented formula have produced inconsistent results. A search in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database for genes involved in LC-PUFA metabolism that might moderate the effect of breastfeeding on IQ led to FADS2. FADS2 gene expression is regulated through end product inhibition and dietary LC-PUFA such as those available in breast milk. Two SNPs (rs 174575 and rs 1535) were selected as candidate biomarkers, to test the hypothesis that the cognitive advantage associated with breastfeeding in human beings is related to genetic differences in LC-PUFA metabolism, which was replicated in two birth cohorts. It was found that the difference in IQ test scores between breastfed and not breastfed to be 5.6 IQ points in one birth cohort and 6.3 IQ points in the other cohort. Analysis revealed that the rs 174575 interacted with breastfeeding to influence IQ in both cohorts. There was a dominant effect of the C allele in response to breastfeeding, with those carrying the C allele having a statistically significant 6.4-IQ-point advantage relative to children not breastfed. In contrast, GG homozygous neither gained an advantage from breastfeeding nor suffered a disadvantage from not being breastfed. The interaction between children's 174575 genotype and breastfeeding suggests that C carrying children benefit from breast milk more than GG homozygotes. There were no significant IQ differences among children fed breast milk as a function of maternal genotypes. Therefore these results suggest that the rs 174575 influence of breastfeeding effects on IQ involves genetic differences in children's LC-PUFA metabolism rather than rs 174575 differences among lactating women in their milk composition. Among GG homozygote children the IQ advantage associated with breastfeeding was nil. But children who were C-carriers the difference in IQ was 6.8 IQ points with C-carriers having the advantage. This advantage corresponds to a moderate effect size that is associated with many important life outcomes. This very important finding needs to be replicated in much larger cohorts and populations. The molecular mechanism by which rs 174575 may influence cognitive development is not known (Table 4). The rs 174575 C allele is linked with the major alleles of FADS1 and FADS2 SNPs, which are associated with more efficient fatty acid processing, possibly due to increased transcriptomal activity or a more active protein (Table 5).

Table 4 Adequate intake (AI) for adults

<i>Fatty acid</i>	<i>Grams per day (2000 kcal diet)</i>	<i>% Energy</i>
LA	4.44	2.0
(upper limit) ^a	6.67	3.0
ALA	2.22	1.0
DHA + EPA	0.65	0.3
DHA to be at least ^b	0.22	0.1
EPA to be at least	0.22	0.1
TRANS-FA		
(upper limit) ^c	2.00	1.0
SAT		
(upper limit) ^d	–	<8.0
MONOs ^e	–	–

^aAlthough the recommendation is for AI, the Working Group felt that there is enough scientific evidence to also state an upper limit (UL) for LA of 6.67 g day⁻¹ based on a 2000 kcal diet or of 3.0% of energy.

^bFor pregnant and lactating women, ensure 300 mg day⁻¹ of DHA.

^cExcept for dairy products, other foods under natural conditions do not contain *trans*-FA. Therefore, the Working Group does not recommend *trans*-FA to be in the food supply as a result of hydrogenation of unsaturated fatty acids or high-temperature cooking (reused frying oils).

^dSaturated fats should not comprise more than 8% of energy.

^eThe Working Group recommended that the majority of fatty acids are obtained from monounsaturates. The total amount of fat in the diet is determined by the culture and dietary habits of people around the world (total fat ranges from 15% to 40% of energy) but with special attention to the importance of weight control and reduction of obesity. ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MONOs, monounsaturated fatty acids; SAT, saturated fatty acids; TRANS-FA, *trans*-fatty acids.

Note: If sufficient scientific evidence is not available to calculate an estimated average requirement, a reference intake called an adequate intake is used instead of a recommended dietary allowance. The AI is a value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of healthy people. The AI for children and adults is expected to meet or exceed the amount needed to maintain a defined nutritional state or criterion of adequacy in essentially all members of a specific healthy population.

Genetic Variants in FADS1 and FADS2 and CHD Risk

Desaturase activity is assayed in vitro or in animals by measurement of the rate of conversion of radiolabeled precursor fatty acids to their respective products, but ethical and practical reasons prevent this possibility in humans. Instead a product to precursor ratio (e.g., AA/LA or EPA/ALA) as a surrogate measure to estimate desaturase activity is well established. In an ongoing case-control study with or without angiographic evidence of coronary artery disease (CAD), both AA/LA and the ratio of EPA to ALA were higher in participants with CAD than in those without CAD, but in a multiple logistic regression model only a higher AA/LA resulted as an independent risk factor for CAD. Concentrations of high sensitivity C-reactive protein (hs-CRP) increased progressively across tertiles of AA/LA. Graded increases in hs-CRP concentrations and CAD risk were related to the carriership of FADS haplotypes, including the alleles associated with a higher ratio of AA/LA.

Table 5 Adequate intake (AI) for infant formula/diet

<i>Fatty acid</i>	<i>Percent of fatty acids</i>
LA ^a	10.00
ALA	1.50
AA ^b	0.50
DHA	0.35
EPA ^c	
(upper limit)	<0.10

^aThe Working Group recognizes that in countries like Japan the breast milk content of LA is 6–10% of fatty acids and the DHA is higher, approximately 0.6%. The formula/diet composition described here is patterned on infant formula studies in Western countries.

^bThe Working Group endorsed the addition of the principal long chain polyunsaturates, AA and DHA, to all infant formulas.

^cEPA is a natural constituent of breast milk, but in amounts more than 0.1% in infant formula may antagonize AA and interfere with infant growth.

AA, arachidonic acid; ALA, α -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; LA, linoleic acid; MONOs, monounsaturated fatty acids; SAT, saturated fatty acids; *TRANS-FA*, *trans*-fatty acids.

Note: If sufficient scientific evidence is not available to calculate an estimated average requirement, a reference intake called an adequate intake is used instead of a recommended dietary allowance. The AI is a value based on experimentally derived intake levels or approximations of observed mean nutrient intakes by a group (or groups) of healthy people. The AI for children and adults is expected to meet or exceed the amount needed to maintain a defined nutritional state or criterion of adequacy in essentially all members of a specific healthy population.

A number of studies have shown that higher amounts of AA in adipose tissue are associated with higher risk of acute myocardial infarction. In populations eating a Western diet rich in omega-6 PUFA a high desaturase activity due to FADS1 and FADS2 polymorphism may promote an increased bioavailability of AA with prevailing synthesis of AA-derived proinflammatory eicosanoids leading to atherosclerosis and vascular damage. On the other hand, high desaturase activity in subjects on a diet rich in omega-3 fatty acids or receiving EPA and DHA supplementation could result in an opposite situation with a preferential synthesis of anti-inflammatory eicosanoids.

Genetic Variants in the 5-LO, the Role of Omega-6 and Omega-3 Fatty Acids in CHD

Because atherosclerosis involves arterial inflammation, investigators determined 5-lipoxygenase (5-LO) genotypes, carotid artery intima-media thickness, markers of inflammation, CRP, interleukin-6 (IL-6), dietary AA, EPA, DHA, LA, and ALA with the use of six 24-hour recalls of food intake. The results showed that 5-LO variant genotypes were found in 6.0% of the cohort. Mean intima-media thickness adjusted for age, sex, height, and racial or ethnic group was increased among the carriers of two variant alleles as compared with the carrier of the common (wild-type) allele. In multivariate analysis, the increase in intima-media thickness among carriers of two variant alleles was similar in this cohort to that associated with diabetes, the strongest common cardiovascular risk factor. Increased dietary AA significantly enhanced the apparent atherogenic effect of genotype, whereas increased dietary intake of omega-3 fatty acids EPA and DHA blunted this effect.

Furthermore, the plasma level of CRP of two variant alleles was increased by a factor of 2, as compared with that among carriers of the common allele. Thus, genetic variation of 5-LO identifies a subpopulation with increased risk for atherosclerosis. The diet-gene interaction further suggests that dietary omega-6 fatty acids LA and AA promote, whereas marine omega-3 fatty acids EPA and DHA inhibit leukotriene-mediated inflammation that leads to atherosclerosis in this subpopulation (**Figure 1**). The study constitutes evidence that genetic variation in an inflammatory pathway – in this case the leukotriene pathway – can trigger atherogenesis in humans. These findings could lead to new dietary and targeted molecular approaches for the prevention and treatment of cardiovascular disease according to genotype.

Genetic Variants in the 5-LO, Omega-6 Fatty Acids, and Breast Cancer

A number of epidemiologic studies and animal experiments suggest that omega-6 fatty acids increase the risk of cancer and omega-3s decrease. However not all studies have produced consistent results. The 5-LO pathway has been implicated in carcinogenesis and tumor progression in many types of cancer; lung, colon, prostate, kidney, bladder. Earlier epidemiologic studies on dietary fat intake and breast cancer did not find positive association between omega-6 and breast cancer risk. Those studies however did not take into account genetic predisposition related to omega-6 fatty acid metabolism. A recent study on genetic variants in the 5-LO gene (ALOX5) and 5-lipoxygenase-activating protein gene (ALOX5AP) in combination with dietary LA intake in a population-based multiethnic case-control study on breast cancer in Latin, African-American and White women in the San Francisco area, did not find significant main effects of ALOX5 and ALOX5AP genotypes on breast cancer risk that were consistent across race or ethnicity. There was a significant interaction between the ALOX5AP – 4900 A > G polymorphisms and dietary LA intake among women consuming a diet high in LA (top quartile of intake > 17.4 g day⁻¹), carrying the AA genotype. The AA genotype was associated with higher breast cancer risk, compared to genotype AG or GG. Among women consuming ≤ 17.4 g day⁻¹ of LA ALOX5AP – 4900 genotype was not associated with breast cancer risk. These findings indicate that studies on dietary fat intake and cancer should take into consideration both type of fat and genetic variants. Furthermore, in the U.S. 17.4 g day⁻¹ is the intake that a significant portion of the population ingests.

Genetic Variants of Cyclooxygenase-2 (COX-2) and the Protective Effect of Long Chain Omega-3 Fatty Acids in Cancer of the Prostate

Prostate cancer is one of the most common types of cancer in men. Increasing evidence points to chronic inflammation as one of the factors leading to cancer. Inflammation may result from bacterial or viral infections, intra-prostatic urine reflux, or diet. Dietary components that are potent anti-inflammatory agents are the omega-3 PUFAs. Studies have shown that genetic variants at the COX-2 gene modify prostate inflammation

through the COX-2 enzymatic pathway. COX-2 is a key enzyme in fatty acid metabolism and inflammation. In a case-control study of 466 men diagnosed with aggressive prostate cancer and 478 age- and ethnicity-matched controls nine COX-2 tag SNPs were genotyped. Dietary history was assessed with a semiquantitatively food frequency questionnaire. Increasing omega-3 intake was associated with a decreased risk of aggressive prostate cancer, and this inverse association was even stronger among men with genetic variants rs 4648310 (+ 8897 A/G) flanking the 3' region of COX-2. The patient with the lowest intake of omega-3s and the genetic variant had the most aggressive tumor whereas the omega-3 PUFAs were protective and this effect was modified by the genetic variant. This gene by diet (omega-3s) interaction clearly shows that the main dietary effect was modified by the genetic variant whereas men with the variant genotype AG or GG and low intake of omega-3s had much higher risk than men with the variant genotype and high intake of omega-3s. A study in Swedish men found similar inverse association between consumption of fatty fish and prostate cancer risk, an effect also modified by rs 5275 (+ 6364 A>G) SNP in COX-2.

Nutrigenomics: The Role of Omega-6 and Omega-3 Fatty Acids in Gene Expression

Anti-Inflammatory Aspects: Omega-3 Fatty Acids Downregulate the Expression of Genes Involved in Inflammation and Obesity

Animal experiments and human studies have shown that EPA and DHA have the ability to upregulate and downregulate genes in various tissues including adipose tissue and peripheral blood mononuclear cells (PBMCs) in humans. Clinical studies indicate that inflammation is at the base of many diseases including cardiovascular disease, aging, mental health, obesity, diabetes, and even cancer. EPA and DHA have been shown to have beneficial effects in these conditions but the exact mechanisms by which EPA and DHA suppress inflammation are still under investigation. Previous studies have focused on the ability of EPA and DHA to suppress interleukin-1 β (IL1 β) and IL-6 cytokines and to play an important role in the resolution of inflammation as well as through the production of resolvins E1 and E2 from EPA, and D1 and D2 from DHA, and neuroprotectins D1 from DHA. EPA and DHA activate peroxisome proliferator-activated receptor alpha (PPAR α), which upregulates the expression of genes involved in lipid metabolism and downregulates the expression of genes involved in inflammation by suppressing Nuclear factor-Kappa-B (NF- κ B).

In studying the effect of fish oil on adipose tissue it was noted that 1.8 g of EPA and DHA decreased the total omega-6/omega-3 ratio in the plasma phospholipids from 12.9 ± 1.1 to 5.6 ± 0.7 . Total fat mass and subcutaneous adipocyte diameter were lower in the group receiving 1.8 g of EPA and DHA than in the placebo group. In addition, with EPA and DHA supplementation, significant correlations were found between the adipocyte markers (adipocyte diameter and whole fat mass) and the main adipokines-plasma leptin and adiponectin as well as plasma atherogenic factors (plasminogen

activator inhibitor factor-1 (PAI-1), insulin, and triacylglycerol). There was no correlation between adipocyte diameter and plasma tumor necrosis factor- α (TNF- α) or plasma IL-6. As expected adipocyte diameter and fat mass percentage were correlated with atherogenic (cysteine protease cathepsin S or CTSS) and inflammation related genes (the chemoattractant gene plasminogen activator urokinase receptor, or PLAVR), the macrophage surface marker CD11b, and the macrophage phagocytic activity marker CD68. There was no change in weight but there was significant loss in body fat mostly in the trunk and subcutaneous tissue, but not in visceral tissue. After fish oil treatment, PAI-1 was lower whereas leptin, IL-6, TNF- α , and serum amyloid A did not change significantly after 2 months with 1.8 g EPA and DHA supplementation. Epidemiologic studies have shown a correlation between adipocyte size and the omega-6 and omega-3 fatty acids content in subcutaneous abdominal adipose tissue, in a group of overweight patients who had undergone abdominal surgery, and metabolic studies indicate a beneficial role of EPA and DHA in lowering adiposity in humans.

Studies on the relationship between plasma omega-3 PUFA composition and weight status found that higher omega-3 PUFA intake was associated with a healthier BMI, waist circumference, and hip circumference. These findings suggest that omega-3 PUFA may play a role in weight status and abdominal adiposity in human beings. In earlier studies EPA and DHA supplementation reduced body fat mass and stimulated lipid oxidation in healthy adults. Others subsequently concluded that omega-3 fatty acid intake by itself or along with exercise increases weight loss. Caloric restriction is recommended for weight loss. Of interest is the fact that caloric restriction affects gene expression in a manner similar to EPA and DHA supplementation.

Summary and Conclusions

Fatty acid composition in red cell membranes and serum phospholipids plays an important role in cellular processes, and has been shown to be associated with the etiology of several complex diseases in humans. The metabolism of EFAs, LA, and ALA and their metabolic derivatives are controlled by enzymes encoded by polymorphic genes. Therefore the availability of PUFAs to various tissues is of major importance to health and depends on both dietary intake and endogenous production or metabolic turnover.

The genetic variants FADS1 and FADS2 lead to differences in the conversion of omega-6 and omega-3 fatty acids catalyzed by the delta-5 and delta-6 desaturases, which suggests that individuals may require different amounts of dietary PUFAs or LC-PUFAs to achieve comparable biological effects. Furthermore further studies addressing the biological effects of PUFAs and LC-PUFAs should include genotyping for FADS1 and FADS2 polymorphisms.

Variants in the human genes of delta-5 and delta-6 desaturase FADS1 and FADS2 that influence both serum and red cell membrane phospholipid levels of PUFA, have a frequency of 26% in the population. The minor alleles are associated with lower AA and higher LA and account for 28% of the variation in serum phospholipid AA and up to 12% of its

precursor fatty acids. Smaller percentage values were found for omega-3 fatty acids. These findings suggest that individuals may require different amounts of dietary LA, ALA, or AA, EPA, and DHA, for both normal development and in the prevention and management of chronic diseases.

The interaction between dietary factors and genetic variants could explain the differences noted in association studies. Considering that a low omega-3 intake in the presence of certain genetic variants leads to a more aggressive disease, an increase in omega-3 intake and a decrease in omega-6 leading to a balanced omega-6/omega-3 ratio may be a sensible recommendation to reduce disease risk in the general population. Nutrigenetics/nutrigenomics will continue to provide data on mechanisms of nutrients and gene interactions in both health and disease.

See also: Children: Nutritional Requirements. Coronary Heart Disease: Prevention. Early Origins of Disease: Fetal. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Metabolism. Growth and Development: Physiological Aspects. Nutrient–Gene Interactions: Health Implications; Molecular Aspects

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Organic farming, also known as biological or ecological farming, should follow a well-defined regulation and the mode of production must be controlled by a certification body. Its main constraints are a minimal use of off-farm inputs, the prohibition of synthetic chemicals (fertilizers, pesticides, drugs), the use of organic fertilizers and natural pesticides, long crop rotations, the maintenance of the organic matter and microbial life in the soil, the prohibition of genetically modified plants. For animal husbandry, organic regulations concern welfare, use of organic feeds and the limitation of therapeutic treatments (especially antibiotics and hormones). When necessary, derogations are granted. The conversion time from conventional to organic farming varies from 2 to 3 years. Organic farming involves obligations in relation to means of production but not in respect of the nutritional, health, or sensorial qualities of the products. Foods marketed under the label “organic” must contain at least 95% ingredients from organic agriculture, possibly with food additives from a very restrictive list. For processed foods, only biological and physical treatments (except irradiation) are allowed.

Certified organic agriculture represents currently approximately 2% of arable land worldwide, but this average hides large variations between continents and countries: for example, 0.4% in Africa, 1% in North America, 2.6% in Europe with a maximum of 25% in Austria. In the last case, the mountainous regions with predominance of grazing livestock are more conducive to organic farming. In contrast, in sub-Saharan Africa, farmers who have no access to chemical fertilizers and pesticides unknowingly practice a form of not-certified organic farming.

The organic market is increasing everywhere. The demand is mainly motivated by the protection of the environment, including water resources, encouraged by government measures, but also ethical reasons and health maintenance. Thus, fear of “chemical” and attraction to the “natural” are the main reasons of choosing organic foods. Productivity is much lower in organic agriculture, the yields being 30–50% lower than in conventional farming, especially in the case of cereals. As costs of production are higher, selling prices are also significantly higher (30–100% more in stores). The market for organic foods is, according to country, from 0.5 to 5% of food purchased, but demand is growing and most of the major consuming countries are dependent on imports.

Outside the motivations of consumers’ choice, ethical or ideological, which are not discussed in this article, it is important to know if the widespread belief of a better nutritional quality of organic foods is justified by scientific studies.

The chemical composition of foods varies depending on many factors which are not all directly linked to the mode of

production. These are, for crops, species and variety, ripeness at time of harvest, yield, climate, season, and for animal products, breed, age, adiposity, growth rate, feed intake. It is obvious that when these conditions are comparable, it is unlikely that tissue composition is different. The clearest example is that of milk, whose lipid composition depends strongly on the consumption of grass or fresh forage, regardless of the production system, organic or conventional.

The differences between organic and conventional products have been the matter of numerous comparative studies. However, most of these studies were not published in journals with peer review and therefore cannot be known or validated. Several systematic reviews have attempted to take stock. It is significant that most reviews published by organizations or associations of organic farming draw a positive balance in favor of organic foods, whereas independent academic or university publications claim that there is an overall lack of significant differences. The balanced data summarized below result from several US syntheses, two systematic reviews supported by the UK Food Standards Agency and a report published in 2003 by the French Agency for Food Safety (Afssa, now Anses) and updated in 2010 (approximately 100 new references added).

Numerous comparative studies failed to demonstrate a significant change in product composition attributable to the mode of production, either because the experimental design or characterization of food were not suitable or well defined, or because statistical interpretation was missing or impossible. This results in a lack of usable results for some constituents, particularly for some vitamins.

Essential or Beneficial Nutrients

Plant Products

A trend toward a higher dry matter content in some organic vegetables has sometimes been reported. This difference is not systematic and depends greatly on the cultivar and stage of ripeness at harvest. No significant differences were observed for carbohydrate, protein, and total lipids of vegetables and fruits, particularly on a dry matter basis.

Organic cereals are generally poorer in protein, which poses technological problems for bread production and requires the choice of adapted varieties of wheat. It seems however that the balance of essential amino acids is sometimes better in the organic wheat.

For minerals and trace elements, it can be concluded, from a large number of individual results on approximately 15

species of fruits and vegetables, that overall there is a lack of differences in levels. Occasionally magnesium and iron have a tendency to be somewhat higher in some organic vegetables, but no other differences were observed for calcium, potassium, sodium, copper, zinc, or selenium. Some extremely high levels of copper were found in potatoes, tomatoes, and grapes, due to repeated treatments with copper sulfate. The mineral composition of seeds is almost constant and no difference was recorded. Mineral and trace element contents of bread depend on the proportion of bran in the flour and not on the mode of production of wheat.

Too few studies have been done on vitamins A, E, and B group in vegetables and fruits, but the changes seem small. However, many studies have examined vitamin C, especially in tomatoes. A trend toward higher levels in some organic fruits and vegetables seems to be confirmed but is not systematic: In 43 individual comparative results, 19 are higher, 18 are equal, and six are lower. This trend must be tempered by taking into account other factors that affect even more the level of vitamin C, especially the freshness of the product.

Several studies have shown the presence of larger amounts of phytochemical components such as polyphenols and other phenolic compounds in organic plants. In the absence of phytopharmaceutical treatment, this increase can be explained by a defense reaction of the plant unprotected against attacks from insects or fungi. The production of secondary metabolites, including molecules with beneficial antioxidant action, is then promoted. Low availability of soil nitrogen could also have the same effect. Thus, the levels of polyphenols, especially flavonoids, are sometimes 20–40% greater in organic vegetables and fruits, most of the results concerning tomato. However, this superiority is not systematic because among 70 individual validated data across all products, 31 are higher in organic agriculture, 23 are equal, and three are lower. In contrast, levels of carotenoids in organic fruits and vegetables are mostly smaller or equal, especially for lycopene in tomatoes. Finally, herbicide treatments may also cause a defensive reaction of the plant, increasing the production of secondary metabolites, including phenolic compounds.

Several studies have shown that the antioxidant power of organic apples is 10–15% greater than that of conventional apples, but it remains to show that these small differences have any nutritional significance.

A recent systematic review on the compared nutritional quality of conventional or organic foods, supported by the UK Food Standards Agency, has found significant differences only for nitrogen (protein in grains and nitrate in vegetables), lower in organic produce, and phosphorus, lower in conventional mode (but phosphorus is not a limiting factor in the human diet). Despite the use of very strict criteria for exclusion of publications, this review is currently the most comprehensive.

Animal Products

The diet of organically farmed animals is not very different from that of conventional animals, particularly for pigs and poultry, which eat mainly grains and oil-seed cakes. The only difference is that most feeds (95–100%) must come from organic farms. However, the chemical composition of

these feeds, mainly seeds, depends little on production mode. A supplement of minerals, trace elements, and vitamins is allowed.

For most constituents, the composition of meat depends little on the mode of production. Only the lipid composition varies according to breed and age. Thus, comparisons must be made on animals of the same age because the degree of adiposity varies accordingly. For example, an “industrial” 40-day-old chicken cannot be compared to an 80-day-old organic chicken. Feed can affect the fatty acid contents, especially of unsaturated fatty acids in meat, and more in pigs and poultry than in ruminants. Thus, the meat of cattle mainly fed with grass or fresh forage, preferred in organic husbandry, is richer in polyunsaturated fatty acids (PUFA) $\omega 3$ and sometimes in CLAs (conjugated linoleic acid) than the meat of cattle fed with corn silage and concentrate. It is the same for the composition of eggs. However, similar compositions are obtained for extensive grazing livestock or for hens with an outdoor run.

Several recent and validated studies have focused on the composition of milk, which for most of its constituents (protein, lactose, minerals, and trace elements) is fairly constant. Only vary $\omega 3$ PUFA levels and CLAs that depend heavily on the proportion of grass in the diet. Identical results, even better, can be obtained by extensive livestock grazing or by incorporation of clover or flax seeds. Only two trace elements from milk, iodine, and selenium, are influenced by dietary intake and their levels are increased by the use of a mineral supplement. Selenium content of feed strongly depends on the soil concentration.

Undesirable Components

Nitrate is abundant in vegetables (but not in fruit, cereals, milk, or meat). Nitrate levels in vegetables vary according to several factors: sunlight, rainfall, and especially nitrogen fertilization. The levels are lower in autumn or in greenhouse production and higher in spring and summer. They increase when nitrogen is provided by rapidly available soluble fertilizers. Organic farming uses mostly organic fertilizers such as guano, meat, or blood meals, which are rapidly assimilated by the plant and thus also lead to high levels of nitrate. Nevertheless, the published comparative studies show that organic vegetables (lettuce, spinach, rucola, carrot, beet etc.) are usually less rich in nitrates than conventional vegetables.

Nitrates do not have a good reputation because of past accidents of methemoglobinemia in infants with poor food hygiene, notably contaminated feeding bottles in which microorganisms accelerate the reduction of nitrate to nitrite. Improvement of hygiene leads now to a very low risk. In adults, the formation of carcinogenic nitrosamines has long been suspected. In fact, many studies have shown that nitrate of vegetables, which represent approximately 75% of nitrate ingested, have no negative effect on health in adults and their carcinogenic effect has not been demonstrated. Note that water is also a vector of nitrates in the diet but the maximum limit (usually 50 mg l^{-1}) is not a threshold of toxicity for adults. In addition, several recent studies emphasize the beneficial effects of nitrate, especially in the immune

protection of the mouth and stomach, in the prevention of hypertension and cardiovascular disorders by the formation of nitrogen monoxide.

The fear of residues of synthetic pesticides in conventional plant products is by far the main reason for the choice of organic foods by consumers. It is true that the prohibition of their use in organic agriculture should logically lead to the absence of residues in food. Many surveys have been conducted on these residues in the United States, the United Kingdom, New Zealand, Netherlands, France, and elsewhere. For example, an annual report of the European Food Safety Authority (EFSA) combines the results of controls in the Member States on a large number of plant products. For 2009, no detectable traces of pesticide residues were found in 58% of samples (all origins taken together), traces were detected in 38% but at levels below the maximum residue level (MRL), and only 4% samples exceeded the MRL. These results overlap well with those of other national studies. Note that the tests are done on raw, unwashed, unpeeled products, and that exceeding the MRL does not mean that the dose ingested is toxic.

The MRLs are based on acceptable daily intakes (ADI) by applying a large safety factor (100 or more) and the ADI itself is calculated with another safety factor of at least 100, from the higher dose without effect observed in laboratory animals. These assessments are based on a calculation of risk, taking into account the possible cumulative effects of several molecules of the same chemical group having the same mechanism of action, and leading to a "reasonable certainty of no harm". There is an MRL for each pesticide and each plant species and values adopted have been harmonized in Europe in 2008.

If synthetic pesticides pose a distinct risk to the health (skin disorders and hematopoietic cancers) of the farmer poorly protected and highly exposed, this should not be amalgamated with the negligible risk to consumers who ingest doses of residues of the order of one million times lower. Based on studies published by international bodies, residues of synthetic pesticides are without any risk to the consumer and the expected marginal benefit of eating more organic products is insignificant. Thus, according to a study in the European Union, individual chronic exposure to pesticides would be between 0 and 0.2% of the acceptable daily intake (ADI). Other studies in the USA done by the FDA also show pesticide exposures below 1% of ADI, or approximately 10 000 times lower than the highest dose having no effect on the animal.

To be on the safe side, it is advisable, especially for infant feeding, to wash and peel vegetables and, if possible, fruits. It should be noted that there is a consensus on the beneficial health effects of consumption of fruits and vegetables, whereas nearly half contain detectable residues of synthetic pesticides. Arguments based on pesticide residues (and nitrates in the case of vegetables) should not therefore be used as a pretext for decreasing the recommended consumption of fruits and vegetables.

It is true that organic plant products do not generally contain residues of prohibited synthetic pesticides. However, surveys also sometimes reveal their presence at levels below the MRL, which is due to pollution, errors, faults, or derogations. Furthermore, organic products also contain residues of natural pesticides authorized as rotenone, pyrethrins, azadirachtin from neem oil, and particularly copper often heavily

used. These residues are not taken into account in official inspections, although their safety is not guaranteed. Thus, rotenone is neurotoxic and is now or in a near future banned, whereas azadirachtin from neem oil, permitted in some countries, is an endocrine disruptor. Copper excess poisons the soil and is not without health consequences.

Like the beneficial antioxidants, toxic secondary metabolites can be formed in the plant untreated, in defensive response to attacks by insects or fungi. The effect on human health of hundreds of natural toxins acting as insecticide or fungicide has not been well studied. Some are well known such as cruciferous glucosinolates (sometimes beneficial), glyco-alkaloids in potatoes and tomatoes and celery furanocoumarins. Others have not been identified or studied, and it may be important to be concerned about their effects on health.

Similarly, lipid transport proteins in Rosaceae (most of fruits) are proteins of plant defense and are responsible for severe allergies in children and adults. Studies have shown that they are more abundant in the skin of organic apples and plums whose consumption should not be recommended for allergy sufferers.

It would be logical that the levels of carcinogenic mycotoxins were higher in organic cereals not protected by anti-fungal. In fact, if cases of severe contamination by mycotoxins have been found in organic cereals, the difference with the conventional grains is not systematic. Thus, the presence of *Fusarium* mycotoxins in wheat depends on many factors and several recent studies in various regions of Europe (Germany, United Kingdom, Netherlands, Italy) showed that organic wheat is sometimes the least contaminated. The main factors are the year, the climate, and the storage conditions.

The risk of pollution by heavy metals, PCBs, or dioxins is not different in the two modes of production but depends on exposure to atmospheric deposition. All outdoor productions, animal or vegetable, are most at risk. This is usually the case for organic agriculture but also often for conventional agriculture, especially for grazing animals. Thus, milk from cows on pasture, whether organic or conventional, is less well protected than the milk from cows in barn because the consumption of grass and earth may be an important vector of various pollutants. It is the same for eggs from hens with outdoor run, often more contaminated than eggs from hens in cages.

For similar reasons, the risk of bacterial or viral contamination is greatest in outdoor production, from hydro-telluric sources (e.g., *Clostridium botulinum*) or contact with wildlife fauna (*Campylobacter*).

Microbial contamination of plants can be enhanced by the use of organic fertilizers compared to mineral fertilizers. Thus, the use of manure or composted manure increases the risk of contamination of fruits and vegetables by *Escherichia coli*, *Salmonella*, or *Listeria monocytogenes*. Poultry manure from organic livestock is often contaminated with *Campylobacter*, which may be an increased risk for eggs. However, according to several studies, these are only trends and, on the other side, it appears that the resistance of these bacteria to various antibiotics is lower in organic farming.

Cases of mastitis in dairy cows are more frequent in the absence of antibiotic treatment. However, milk is not sold then and a limited use of antibiotics in livestock is also

authorized in organic husbandry if required. Internal parasites in sheep are conditions prevalent in organic farming, but the new European regulation now allows the use of antiparasite treatments without limitation.

The use of hormones is universally prohibited in organic animal husbandry, but also in conventional husbandry in the European Union. Several countries (USA, Canada, Australia, South Africa, New Zealand, Mexico, Chile) authorize steroid hormones to increase meat yield in beef cattle (estradiol, progesterone, testosterone, zeranol, trenbolone acetate, and melengestrol acetate) or a protein hormone, rBST (recombinant bovine somatotropin), to increase milk production in dairy cows (prohibited in Canada based on concerns about the health effects including mastitis in treated animals). The Food Safety authorities of these countries ensure that the possible residues found in meat and milk do not present any health issue for the consumer (early puberty in girls, risk of breast cancer or allergy, endocrine disruption have been mentioned in countries where these treatments are not allowed) if the treatments are applied according to the regulation.

Health Effects (Clinical Trials)

Given the many factors that determine the nutritional and health qualities of agricultural products, it is difficult to demonstrate significant differences resulting specifically from the production system, organic or conventional.

Ten controlled clinical studies comparing organic and conventional foods were included in the systematic review supported by the UK Food Standards Agency. Most were carried out on subjects consuming vegetables and fruits, conventional or organic, and used, as a biomarker, the antioxidant status of blood plasma. None of them could demonstrate a positive effect of diet on this blood parameter but this does not suffice, however, to characterize the health status. There is no published long-term study comparing the health effects of a diet exclusively organic or conventional, using several criteria relevant to health. This deficiency is unfortunate, but the small differences found in the composition of foods would leave little chance of finding a significant effect. Moreover, such studies should focus on a very large number of subjects and avoid confounding factors such as differences in nutritional balance or behavior: consumers of organic foods are often more concerned with their health and may have different patterns of exercise, tobacco and alcohol use, and dietary supplements.

A recent attempt to model and compare the effects of pesticide exposure in Switzerland and the United States has evaluated the impact expressed as disability adjusted life years. The large difference in exposure between the two countries would only lead to a loss of a few minutes of life. Although this approach is questionable and such an estimate includes many biases and weaknesses, it is a confirmation of the findings of other authors.

Organoleptic Quality

Organoleptic characteristics of organic foods have not been the subject of many comparative tests. Those that have been

published do not highlight superior sensorial qualities due to the production method, as results are variable and contradictory. For fruit and vegetables, sensorial properties are mainly determined by the variety, ripeness and freshness. Organic farming often uses more hardy cultivars and, if production is local, the harvest can be later. For meat, the taste properties depend mainly on breed, age, and degree of adiposity. The mode of production, organic or conventional, is not a criterion of taste. For milk and eggs, no reproducible difference was obtained in the literature.

In line with the nutritional values and health attributes, regulation of organic agriculture does not imply any better organoleptic quality of its products, but only an obligation of means of production.

Conclusions and Prospects

Low consumption of organic foods cannot have a nutritional effect in the overall diet. Even if we admit a slight superiority of organic food for some nutrients in some foods, the difference would be insignificant in the global regime. For example, measures taken in France for a "One organic meal per week in collective catering", cannot have significant influence on the quality of diet throughout the week. The health impact is also negligible because the effect of organic vegetables or fruits on blood antioxidant status could not be demonstrated, nitrate vegetables are safe and chemical residues of conventional foods, including synthetic pesticides, are almost entirely below the maximum limit and do not pose a risk to health.

In most western countries, organic foods account for less than 2% of foods consumed. Despite a strong trend of increasing demand, this share will be limited if a larger supply does not bring down the selling prices of organic foods. Significant imports from distant areas are not desirable because they are incompatible with the ecological spirit of organic agriculture. Production in industrialized and emerging countries will also be limited by local availability of organic fertilizers and especially by heavy yield losses (e.g., 50% for wheat). In developing countries, particularly in several parts of sub-Saharan Africa, food production could be increased through a wide application of the principles of organic agriculture for food crops associated with animal husbandry. However, this production will be selfconsumed and may not be sufficient without recourse to a minimum of mineral fertilizers and plant protection products to reduce the very high crop losses.

The increasing demand for organic foods, currently hampered by higher purchase prices, could explode if a large increase in local production or imports led to falling prices. However, this would be a vicious circle as farmers' income could drop and then discourage their conversion. In addition, throughout the world, intensive conventional farming will be subject to environmental constraints, including the use of pesticides. Reductions of 30–50% of the doses used are already planned in some countries. Under these conditions, the difference in food quality and ecological effects perceived by consumers will decrease and will not be market-friendly with organic foods. Between the two extreme forms of agriculture, intensive conventional and organic farming, there are

several intermediate modes of production that provide good quality food and preserve the environment, without sacrificing the high productivity required to feed the future world.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Food Composition Data. Food Safety: Bacterial Contamination; Mycotoxins – Occurrence and Toxic Effects; Pesticides. Food Security. Phytochemicals: Classification and Occurrence; Health Effects

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OSTEOPOROSIS

Nutritional Factors

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Glossary

Dual energy X-ray absorptiometry (DXA) This noninvasive test uses the degree to which X-rays are attenuated as they pass through the human body to evaluate bone mineral content and density of the entire skeleton or at selected sites (typically the lumbar spine, hip, and cervical vertebrae).

Osteocyte Osteocytes comprise 95% of all bone cells and are derived from osteoblasts that have become embedded in mineralized bone. These cells respond to mechanical forces and play an important role in the regulation of bone turnover.

Osteopenia Osteopenia is a term used to denote low bone mass. It is diagnosed when the bone mineral density T Score (from a DXA test) is between -1.0 and -2.5 .

Osteoporosis A term used to denote a significant loss of bone. It is typically defined when the bone mineral density T Score (from a DXA test) is less than -2.5 .

Parathyroid hormone (PTH) Parathyroid hormone is secreted by the parathyroid glands in response to low serum ionized calcium. It functions to maintain circulating calcium concentrations within a narrow range.

Phosphatonin Phosphatonins are compounds that regulate phosphorus metabolism using PTH-independent mechanisms.

Introduction

Optimal dietary intake is essential for bone health. During childhood and the pubertal growth spurt, nutrients are needed to fully consolidate skeletal mass and to insure the attainment of a peak bone mass consistent with one's genetic potential. After peak bone mass is obtained, nutrition continues to play an essential role in skeletal health. If key nutrients are not consumed at required levels, mineral may be lost from bone or essential bone proteins may not be fully functional.

Osteoporosis and osteopenia are currently significant public health problems. Once a substantial amount of bone mineral and matrix is lost, bone becomes more susceptible to fracture. Osteopenia is defined when adult bone mineral density values are 1–2.5 SD below the mean peak value observed in a young adult. Osteoporosis is defined when adult bone mineral density falls 2.5 SD or more below that observed in a young adult. Recent estimates suggest that 10 million adults over the age of 50 years in USA have osteoporosis, and another 34 million have osteopenia and are at risk for osteoporosis and fracture. The increasing impact of low bone mass on public health can be demonstrated by the large increase in the age specific rate of hip fractures that has occurred over the last 20 years. Recognition of this chronic disease is an increasing concern globally as the population ages and mean lifespan increases. Because bone loss is not fully reversible, the most effective strategies for treating osteoporosis should focus on prevention with nutrition playing a key role.

Dietary Intake and Body Mass

A balanced diet is important to promote health and to maintain an appropriate body weight. An individual's body weight is one of the strongest determinants of bone mass because of the skeleton's responsiveness to the load that is placed on it. Individuals with small body frames or those who are excessively thin have an increased risk of osteoporosis due to a lower overall skeletal reserve to draw on for calcium needed to offset the annual loss of bone that occurs later in life. At the extreme end of this spectrum, individuals with anorexia nervosa are at risk of osteoporosis because of alterations in hormonal status and amenorrhea in addition to insufficient dietary intake of nutrients required for bone health.

Excess body weight is typically associated with a greater skeletal mass but obesity may have negative effects on bone health. Obesity has been associated with lower 25OHD and higher parathyroid hormone concentrations. These changes are thought to occur in part due to sequestration of vitamin D in adipose tissue. Given the global increase in obesity, more data are needed to identify the long-term impact of excess adiposity on bone health. Bariatric surgery as a treatment for morbidly obese individuals is becoming more common and leads to both a loss of body weight and bone mass. At present the long-term impact of this surgery on skeletal health has not been fully elucidated and it remains unclear if the amount of bone lost following surgery is solely a response to the decrease in body weight or if it is also associated with other adverse consequences of this surgery on bone health.

Table 1 Nutritional and lifestyle parameters that may influence bone health

Minerals	Vitamins/Hormones	Lifestyle and Environmental Factors	Dietary Components
Calcium	Vitamin D	Body mass index	Protein
Phosphorus	Vitamin K	Exercise	Soy/phytoestrogens
Magnesium	Vitamin A	Cigarette smoking	Fatty acids
Sodium	Vitamin C	Alcohol intake	Homocysteine
Zinc	Vitamin B ₁₂		
Copper	Vitamin B ₆		
Iron	Folate		
Boron			
Manganese			
Fluorine			
Potassium			
Silicon			

Although overall caloric intake impacts body weight, many individual nutrients and dietary components have been studied in relation to their impact on bone health (Table 1). Several of these key nutrients and components of the diet have key roles in bone health and skeletal homeostasis.

Calcium

Calcium is the most abundant mineral found in bone and comprises approximately 33% of bone mineral. Optimal calcium intakes are essential across the lifecycle to meet the daily intrinsic requirements of calcium required for skeletal growth and to offset urinary, dermal, and endogenous fecal calcium losses. When dietary intakes of calcium are not sufficient to maintain circulating calcium concentrations or when the losses of calcium from the body are excessive, bone calcium will be resorbed to maintain calcium homeostasis. Because calcium is essential for the structural integrity of bone, deficiencies or inadequate intakes of this mineral will have a detrimental impact on bone mass and quality.

Skeletal mass peaks at approximately age 20–30 years with the majority of this gain (nearly 50%) being accrued during the pubertal growth spurt. Thus, this period of skeletal accretion can be viewed as a window of opportunity to maximize skeletal mass. Calcium supplementation studies in children found increased bone mass following supplementation, an effect that is most pronounced when implemented during the prepubertal period. However, supplementation appears to primarily impact the tempo at which peak bone mass is achieved because gains in bone mass are not typically maintained after supplementation ends and this does not appear to result in a net difference in peak bone mass. Calcium supplementation has been found to have beneficial effects on bone mass and reduced fracture risk in adults and may have the greatest impact in individuals whose habitual dietary calcium intakes are below 400 mg (10 mmol) d⁻¹.

In 2010 the Institute of Medicine (IOM) updated the dietary recommendations for calcium and vitamin D (Table 2). Using newly available data, the committee was able to set a recommended dietary allowance (RDA) for calcium and vitamin D for all age groups except infants. In infants during the first year of life available data were only sufficient to

Table 2 2010 Dietary reference intakes for Ca and vitamin D

Group	Ca RDA (mg)	Vitamin D RDA (IU)
<i>Infant</i>		
Birth–6 months	200 (AI)	400 (AI)
7 months–1 year	260 (AI)	400 (AI)
<i>Children</i>		
1–3 years	700	600
4–8 years	1000	600
<i>Male Adolescents/Adults</i>		
9–18 years	1300	600
19–70 years	1000	600
> 70 years	1200	800
<i>Female Adolescents/Adults</i>		
9–18 years	1300	600
19–50 years	1000	600
51–70 years	1200	600
> 70 years	1200	800
<i>Pregnancy</i>		
14–18 years	1300	600
≥ 19 years	1000	600
<i>Lactation</i>		
14–18 years	1300	600
≥ 19 years	1000	600

Only adequate intake (AI) recommendations are available for those under the age of 1 year.

establish an adequate intake (AI). As in the previous recommendations, due to the importance of calcium in bone mineralization, the recommended daily allowance of calcium is highest ((1300 mg or 32.5 mmol) d⁻¹) between the ages of 9 and 18 years. Because of the known decreased efficiency of intestinal absorption coupled with increased losses of calcium that occur in older women as they progress through menopause, recommended calcium intakes were increased to 1200 mg (30 mmol) d⁻¹ in women aged 51 years and older but recommended intakes for men remain at 1000 mg until the age of 70 years at which time they also increase to 1200 mg (30 mmol d⁻¹). Recent dietary intake data suggest that the current recommended calcium intakes are in large part achieved by most age groups in North America and in many

European countries. An exception was noted for adolescent girls who do not ingest sufficient calcium at this key period of bone acquisition.

Because the majority of dietary calcium is obtained from dairy products, those with low dairy intakes or with other factors such as lactose intolerance, dieting, or altered appetite and food consumption patterns may need to rely more on fortified food products or calcium supplements. An increasing variety of calcium-fortified food products are now also available. Individuals with lactose intolerance may improve intake of calcium by use of lactose-free dairy products or lactase pills. Whenever possible, increased calcium intake should be obtained from dietary versus supplemental sources in order to obtain additional nutrients needed for bone health including protein, magnesium, zinc, phosphorus, and vitamin D.

Use of calcium supplements is currently common in the USA, particularly among older women. Several forms of calcium supplements are commercially available with calcium carbonate and citrate being the forms most commonly consumed. Existing supplemental forms differ slightly with respect to their relative calcium content per tablet and their absorability; however the magnitude of these differences is minor and may not be biologically significant. Several calcium supplements now also contain additional nutrients required for bone health including vitamins D and K. Because the fraction of calcium absorbed decreases as calcium intake increases, little additional benefit per dose is achieved when taking supplemental calcium sources containing more than 500 mg (12.5 mmol) per dose.

The increased availability of calcium-fortified food products may increase the likelihood of excessive intakes of calcium. The tolerable upper intake level (UL) for calcium ranges between 1000 and 3000 mg d⁻¹ depending on the age group in question. These limits for adults were based on data that found increased risk of kidney stones (mainly among postmenopausal women ingesting calcium supplements).

Vitamin D

Vitamin D is integral to bone health in large part due to the role it plays in calcium and phosphorus homeostasis. The active form of vitamin D stimulates calcium and phosphorus absorption and vitamin D along with parathyroid hormone, plays a regulatory role in renal calcium reabsorption and in calcium release from bone.

Vitamin D can be obtained either from the diet (as D₂ or D₃) or is produced in the skin (as D₃) following cutaneous exposure to sunlight. These two forms of vitamin D may have similar effects on target tissues at low doses but some data suggest that high doses of D₂ may be less effective than D₃. Circulating 25OHD is the best indicator of vitamin D status because this form is not tightly regulated and its production is indicative of available D₂ and D₃.

Lack of sufficient endogenous production of vitamin D in the skin is influenced by geographical location (northern latitudes have a shorter season over which the wavelength needed for vitamin D synthesis is available), increased use of sunscreen and cosmetics and skin care products containing sunscreen (sunscreens when applied properly may limit the

dermal production of vitamin D) and by lifestyle factors that decrease exposure to sunlight.

Calcium absorption is believed to be maximal when 25OHD concentrations are between 12 and 20 ng ml⁻¹ (30 and 50 nmol l⁻¹). A serum 25OHD level of 16 ng ml⁻¹ (40 nmol l⁻¹) was identified by the 2010 IOM committee as that desired for the population median and a concentration of 20 ng ml⁻¹ (50 nmol l⁻¹) was identified as sufficient to meet the needs of 'coverage' for the population. Recognizing the importance of vitamin D on skeletal health, the 2010 daily RDA for vitamin D was tripled over the earlier adequate intake of 200 IU to the new RDA value of 600 IU for those between the ages of 1 and 70 years. In those over the age of 70 years this was raised to 800 IU to account for the increased concerns of bone health in this age group and for the variability in the physiology of aging that may impact renal function, cutaneous synthesis of vitamin D, altered body composition, and increased PTH concentrations.

The UL for vitamin D was assessed using the indicators of hypercalcemia and related toxicity in adults and on retarded longitudinal growth in infants. Using these endpoints the UL was set between 1000–3000 IU for infants and children through 8 years of age after which time it is set at 4000 IU over the remaining life course.

Achieving the required level of vitamin D may be challenging from diet alone unless fortified food products are consumed. Vitamin D supplements may be useful in meeting requirements in those with low intake of dairy products. Several supplements are on the market containing various amounts of either D₂ or D₃.

Although both calcium and vitamin D have been found to have a positive impact on bone mass their independent effects on bone is challenging to isolate because supplementation studies typically administer both calcium and vitamin D. It is important to note that the recent calcium and vitamin D dietary reference intakes (DRI), as are all DRI recommendations, are based on the needs of healthy populations and are not meant to displace medical recommendations targeted to those with diseases that impact bone health. Because many nutrients are important in bone health and there is still much to be learned about individual nutrients and bone physiology, it is essential that a well-balanced diet, containing grains, fruits and vegetables, protein, and calcium-rich products be consumed. Many nutrients, in addition to calcium and vitamin D, have particular relevance to bone homeostasis as detailed below.

Magnesium

More than half of the magnesium found in the body is located in bone. In addition to its presence in bone, magnesium is important in calcium metabolism and bone health because it can influence parathyroid hormone secretion. Parathyroid hormone (PTH) is integral to bone health because it increases the production of the active form of vitamin D (1,25-dihydroxyvitamin D) and plays a role in the tubular reabsorption of calcium and phosphorus.

Although magnesium deficiency is associated with abnormalities in vitamin D metabolism, hypocalcemia, and impaired

PTH secretion, epidemiological studies linking magnesium intakes to measures of skeletal health have produced conflicting results. Relationships between magnesium status and bone mass may be more challenging to elucidate due to the lack of a highly sensitive indicator of magnesium status.

Studies have indicated that typical magnesium intakes in healthy adolescents may not be sufficient to maintain magnesium balance. Because dietary intakes fall below recommended levels in several age groups and due to the known relationships between magnesium and hormones integral to bone health, increased attention should be focused on optimal magnesium intakes in relation to bone homeostasis particularly during the period of maximal bone acquisition.

Zinc and Copper

Zinc and copper play important roles in bone metabolism and bone health in part due to the roles they play as cofactors for various enzymes required for the synthesis or modification of bone matrix constituents. Zinc is a cofactor for many enzymes in the body, including alkaline phosphatase. Alkaline phosphatase is synthesized by osteoblasts and is essential for bone mineralization. Zinc also plays a role in the osteoblast *via* its involvement in aminoacyl-tRNA synthetase. Copper is a necessary cofactor for lysyl oxidase, an enzyme that is involved in collagen cross-linking. Both copper and zinc are found as components of superoxide dismutase, and may protect bone from oxidative damage. Genetic defects that cause zinc deficiency (acrodermatitis enteropathica) or copper deficiency (Menkes' disease) result in growth retardation, stunting, and impaired bone growth.

Vitamin K

Many proteins integral to bone health are dependent on vitamin K for the carboxylation of gamma-carboxyglutamyl (Gla) residues. Osteocalcin, one such vitamin K-dependent protein, is the most abundant noncollagenous protein in bone. Osteocalcin contains three Gla residues that require vitamin K for carboxylation. The ability of osteocalcin to bind to the hydroxyapatite fraction of bone is dependent on its degree of carboxylation. Deficiency of vitamin K increases the fraction of undercarboxylated osteocalcin in the circulation. In addition to osteocalcin, other vitamin K-dependent proteins (including matrix Gla protein and protein S) are found in bone and cartilage. Continued research is needed to elucidate the impact of vitamin K deficiency on the risk of osteoporosis and fracture. Because of the known relationship between vitamin K and several crucial bone proteins, optimal status of this vitamin should be achieved to promote skeletal health.

Phosphorus

Phosphorus, like calcium, is an integral component of hydroxyapatite in bone. Bone contains 85% of the phosphorus found in the body, and together calcium and phosphorus comprise the major fraction of bone mineral. Although

sufficient phosphorus intake is necessary to support bone mineralization, phosphorus homeostasis can be maintained across a range of intakes and ratios of calcium to phosphorus in the diet.

Much attention has been focused recently on regulation of phosphorus homeostasis by PTH-independent mechanisms that are mediated *via* phosphatonins. To date, at least four phosphatonins have been identified. Of the phosphatonins identified to date, fibroblast growth factor 23 (FGF23) is believed to be the major phosphatonin that contributes to phosphate homeostasis. Synthesis of FGF23 increases in response to elevations in plasma phosphorus and calcitriol and phosphatonins suppress 1- α hydroxylase activity. These effects work in combination to reduce the body of excess phosphorus that is released during bone resorption. Their role and import in bone physiology may open up new possibilities for treatment of phosphorus-related diseases that adversely impact bone mass.

The phosphoric acid and phosphorus content of soda is often discussed in relation to bone health. The impact of these products on bone health is thought to be caused by their displacement of other more nutritive beverages (such as dairy products) from the diet. Because increased soda consumption may increase the risk of excess weight gain and displace more nutritive beverages from the diet, excessive soda intakes should be avoided.

Sodium and Potassium

Some dietary components influence the retention of nutrients required for optimal bone health. Sodium is one of the strongest determinants of urinary calcium excretion. Increased dietary sodium concentrations elevate urinary calcium losses. Every 2300 mg (100 mmol) increase in dietary sodium increases the urinary excretion of calcium by roughly 40 mg (1 mmol). Thus excessive intakes of sodium (such as those that may occur in individuals who consume large amounts of processed food, salt food heavily, or consume foods high in sodium) increase the obligatory losses of calcium from the body. During the growth phase this could potentially limit the amount of calcium that can be utilized for bone mineralization. The long-term impact of variation in sodium intake on bone mass and fracture risk has been difficult to quantify because of a lack of sufficient information on how dietary effects on urinary sodium loss are counterbalanced and because other dietary components may modify this response.

Dietary potassium may help buffer calcium losses in urine. Increased fruit and vegetable intake will assist in increasing dietary potassium intake while providing additional nutrients and antioxidants that have been linked to overall skeletal health.

Protein

Protein is essential for the formation of the organic matrix of bone and optimal intakes are required for normal skeletal development and growth. Protein intakes are also needed to maintain muscle mass and help limit the involuntary loss of muscle (sarcopenia) that can occur as aging progresses. The

importance of protein in bone health is well known, however conflicting reports exist on the relative impact of extremes of protein intake on bone health. Many proteins are rich in sulfur amino acids. The resulting protein-induced acid load must be buffered before excretion from the body. Calcium is a positive cation and can be utilized to buffer increased dietary acid loads from high protein intakes. On average, for every one-gram increase in dietary protein intake, urinary calcium excretion increases by approximately 1 mg.

Differences in habitual protein intakes have been related to bone mass and risk of fracture. Higher animal protein intakes are typically associated with greater bone mineral density as summarized by a recent meta-analysis. Urinary calcium excretion increases in response to acute increases in protein intake but intestinal calcium absorption also increases by an amount nearly comparable to that lost in urine. Insufficient intakes of protein can adversely impact muscle mass and function. In addition, low dietary protein intake has been associated with reductions in serum insulin-like growth factor 1 (IGF-1) concentrations. IGF-1 plays an essential role in skeletal health *via* its impact of osteoblast formation and bone growth. More research is required to address the relative impact of the quantity and type of protein (animal vs. vegetable) on skeletal health.

Phytoestrogens

Phytoestrogens are dietary components that have a chemical structure similar to that of endogenous estrogens. The primary phytoestrogens in the diet are obtained from soybean isoflavones (including genistein and daizein). These compounds appear to be able to weakly mediate some the genomic and nongenomic effects of estrogen and may function as agonists or antagonists depending on the tissue and type of estrogen receptor involved. To date, supplemental sources of these compounds have not been found to decrease fracture risk. Additional clinical data will assist in definition of the long-term impact of phytoestrogens on bone health and fracture risk.

Homocysteine

For some time it has been known that individuals with a genetic defect in homocysteine metabolism (homocystinuria) have an increased risk of early onset osteoporosis but less was known about the potential impact of circulating homocysteine concentrations on bone health among the general population. Much interest in this topic was generated by studies reporting significant relationships between elevated serum homocysteine and increased fracture risk in adults. The strength of the relationship observed is substantial and is similar to the relationship found between serum homocysteine concentrations and cardiovascular disease. At present the mechanisms responsible for the impact of homocysteine concentrations on fracture risk are not known. Increased homocysteine concentrations could possibly interfere with normal collagen production but the studies to date have not found a significant relationship between serum homocysteine concentrations and bone mineral density, and the impact of

elevated homocysteine concentrations on bone health may be indirect. Further research will assist in identifying the mechanisms and relationships between homocysteine and bone health and the degree to which this relationship is influenced by folate, vitamin B₁₂, and vitamin B₆ status.

Other Lifestyle Factors

Lifestyle choices such as smoking, alcohol abuse, and physical activity also impact overall bone health. Excessive alcohol intake is a risk factor for low bone mass. This finding may be a consequence of poor dietary quality in chronic alcoholics and may also be related to adverse effects of excessive alcohol intake on osteoblast function. Cigarette smoking also adversely impacts bone health. Smokers may be leaner, and female smokers may experience an earlier menopause and have lower postmenopausal estrogen levels. Smoking may also have adverse effects on bone cells themselves either directly or indirectly through an increase in oxidative stress.

Exercise is known to positively influence bone mass. During exercise the strain placed on bone stimulates the osteocyte to positively influence the balance in bone remodeling. Many studies have found positive associations between exercise and bone mass at a number of sites, especially the hip and the spine. The impact of exercise on bone mass is related to the intensity of the exercise and is associated with the degree to which it increases the habitual physical activity level of the individual. The impact of exercise on bone mass is also influenced by diet and may be most efficacious when calcium intake is optimal. Exercise not only impacts bone mass but also influences muscle strength, muscle mass, balance, and coordination. These improvements in muscle strength may also lead to improvements in posture, balance, flexibility, coordination, and gait stability that influence the risk of falls.

Targeting Groups at Risk

The World Health Organization has evaluated risk factors for fracture using global epidemiology data. Using these data an electronic web-based program called FRAX[®] has been developed that allows individuals to calculate their 10-year risk of major osteoporotic fracture using their individualized risk factors and other attributes that have been linked to osteoporotic fracture. Many of these identified factors are detailed in [Table 3](#).

Nutrient/Gene Interactions

Optimal nutrition is needed to supply the necessary substrates for bone but an individual's ability to utilize a given nutrient is influenced by their genetic makeup as a substantial amount of bone mineral acquisition (up to 80%) is genetically determined.

Many candidate genes have been associated or linked with the risk of osteoporosis or fracture, including genes coding for hormones (parathyroid hormone), receptors (including the parathyroid hormone, vitamin D, estrogen, glucocorticoid,

Table 3 Factors that have been associated with risk of osteoporotic fracture*Nonmodifiable Risk Factors**Height*

Shorter stature is associated with a lower net bone mass

Age

Risk of osteoporotic fracture in women doubles every 7–8 years after the age of 50

Sex

Women are at greater risk from lower peak bone mass and loss that occurs during menopause

Low Femoral Neck BMD

A 1 SD decrease in BMD increases fracture risk by approximately 1.5–2.6 fold

Prior Fragility Fracture

Is associated with a nearly twofold increased risk of subsequent fracture

Parental History of Fracture

Up to 80% of the variability in bone mass is due to genetic factors

*Modifiable Risk Factors**Weight*

Low weight (<127 lb or BMI <21 kg m⁻²) is a risk factor for low bone mass

Current Tobacco Smoking

Smoking is associated with leanness, earlier menopause and may be toxic to bone cells

Alcohol

Alcohol Intake greater than two units per day

Physical Activity

Sedentary behavior and lack of weight bearing activity

Dietary Intake

Low intake of Ca and vitamin D and a poorly balanced diet low in fruits/vegetables and protein and high in sodium

*Disease-Related Risk Factors**Medication Use*

Oral glucocorticoids, GnRH agonists, depot medroxyprogesterone acetate (MPS), aromatase inhibitors, heparin, anticonvulsants (phenytoin)

Diseases

Rheumatoid arthritis, lupus, hyperthyroidism, type 1 diabetes, ankylosing spondylitis, Cushing's, renal failure, total gastrectomy, gastrointestinal diseases (IBD and celiac), diseases that reduce mobility (stroke, Parkinson's, multiple sclerosis), organ transplant

Other Clinical Factors

History of fainting, falls, or dizziness

*Muscle weakness**Neuropathy of the lower extremities**Impaired vision*

and calcitonin receptors), cytokines, and growth factors (including the insulin-like growth factor 1, transforming growth factor B, epidermal growth factor, interleukin 4 and interleukin 6) and bone matrix proteins (such as osteocalcin, collagen type 1 ($\alpha 1$ and $\alpha 2$) and collagen type 11 ($\alpha 1$)). Although many of these genes have obvious roles in bone metabolism, other candidate genes (such as those coding for apolipoprotein E and methylenetetrahydrofolate reductase) have less obvious relationships to bone mass.

Diet may influence interactions between genotype and environmental factors. For instance, the impact of exercise on bone can be influenced by the habitual dietary calcium intake and the individuals' genotype (such as the vitamin D receptor genotype). Further research into the genetic control of bone mineral acquisition and loss will be invaluable in targeting groups at risk for low bone mass and may eventually be useful in setting genotype specific intakes of bone related nutrients to maximize skeletal health throughout the lifecycle. Newer work is utilizing genome-wide association studies (GWAS) to identify genes associated with bone mass in various pathways such as those involved in vitamin D metabolism.

These studies are shedding new light on genetic determinants of bone with the long-term goal of identifying those at risk so interventions and nutritional recommendations can be initiated before the development of osteoporosis.

See also: Bone: Nutritional Aspects of Bone. Calcium. Phosphorus: Physiology, Dietary Sources, and Requirements. Vitamin D: Physiology, Dietary Sources, and Requirements. Vitamin K

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Surgeon General's Report on Bone Health.

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Third Edition



ENCYCLOPEDIA OF HUMAN NUTRITION

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THIRD EDITION

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VOLUME 4



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Dr. Caballero is a recognized expert on the nutritional needs of children and adults, and on nutrient requirements in undernourished populations. For the past 10 years, he has focused on the problem of childhood obesity in the US and in developing countries, and explored the impact of dietary transition and globalization on health indicators. He is an active participant in key scientific committees advising the US government on issues of diet and health, including the Dietary Reference Intakes (DRI) Committee; the Expert Panel on Macronutrient Requirements; and the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences. He was a member of the Dietary Guidelines for Americans Advisory Committee, and is currently a member of the Scientific Advisory Board of the Food and Drug Administration (FDA) and of the International Life Sciences Institute (ILSI).

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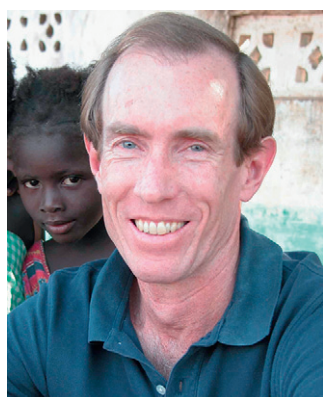
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PREFACE

By the turn of the twentieth century, nutrition science had completed a slow but remarkable historical transition, from a discipline focused on preventing nutrient deficiencies (and hence focused on identifying minimum nutrient needs) to one aimed at reducing disease risk and optimizing health, seeking to define an elusive optimal diet. But progress in our knowledge has not yet caught-up with that transition in focus, and our understanding of how diet constituents affect long-term disease risks is still not on a par with our knowledge of essential nutrients, their metabolism, and required intake levels. One reason is that the experiments needed to unravel diet–health interrelationships are more complex, costly, and in some cases unfeasible, compared with the classical studies that identified vitamins and other essential nutrients. Another reason is that, although the discovery of essential nutrients was based on a strong, unifying scientific paradigm (the concept of a compound essential for human life but which humans are unable to make), there is no single or unifying paradigm from which to explore diet–health relationships. In addition, our ability to timely process and integrate scientific discoveries is now continuously challenged by the massive volume of information of the digital era.

In that context, the need to provide accurate, succinct, and up-to-date information on a wide range of topics is more important than ever, and is the aim of this Encyclopedia. Currently, nutrition research and practice is fundamentally a multidisciplinary endeavor, so we aim to offer scientific information to a wide audience of researchers and professionals. In addition, the information revolution of the internet has

changed the consumer from a passive recipient of advice to an active participant in decisions involving health and related issues. Thus, although this work is not specifically targeted to the general public, we hope that the educated readers with a minimum scientific background should also be able to obtain from this book useful (and reliable) information on their topic of interest.

This third edition builds on the success of the previous one. We have included new articles or made extensive updates when needed, while keeping the proven core of established knowledge. The comprehensive index and extensive cross-referencing will allow readers to quickly identify specific topics, and to move deeper into related areas if desired.

We have a great debt of gratitude to the hundreds of authors who contributed to the large body of knowledge represented here. In turn, authors benefited from the valuable feedback of our distinguished Editorial Advisory Board. Of course, as editors we are ultimately responsible for the content, particularly for any errors. Finally, both the print and electronic version have the unmistakable production quality of the Major Reference Works division of Elsevier, and this is the result of the unrelenting enthusiasm and hard work of our editorial team.

We hope this work will be a valuable addition to the knowledge base of any person interested in the critical area of nutrition, diet, and human health.

Benjamin Caballero
Editor-in-Chief

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GUIDE TO USE OF THE ENCYCLOPEDIA

Structure of the Encyclopedia

The Encyclopedia is arranged as a series of entries in alphabetical order. Some entries comprise a single article, whilst entries on more diverse subjects consist of several articles that deal with various aspects of the topic. In the latter case the articles are arranged in a logical sequence within an entry.

To help you realize the full potential of the material in the Encyclopedia we have provided three features to help you find the topic of your choice.

Contents Lists

Your first point of reference will probably be the contents list. The complete contents list appearing in each volume will provide you with the volume number and page number of the entry. On the opening page of an entry a mini-contents list is provided so that the full details of the articles within the entry are immediately available.

Alternatively you may choose to browse through a volume using the alphabetical order of the encyclopedia as your guide. To assist you in identifying your location within the Encyclopedia a running headline indicates the current entry and article within that entry. Please see an example below:

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A

Adipose Tissue: Structure, Function and Metabolism 1
G Frühbeck and J Gómez-Ambrosi

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Cross References

All of the articles in the Encyclopedia have been cross referenced. The cross references, appear at the end the articles and they link together related articles.

Example

The following list of cross references appear at the end of the entry entitled Nutritional Assessment: Clinical examination.

See also: Dietary Intake Measurement: Methodology. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Nutritional Assessment: Anthropometry; Biochemical Indices

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PANTOTHENIC ACID

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Glossary

Adequate intake (AI) A term used (in the US and Canada) for the amount of a nutrient that is known to be adequate for the majority of individuals in a population group, but there is insufficient information to define an RDA or EAR accurately for that nutrient.

Coenzyme A (CoA); Acyl carrier protein (ACP) (see Pantothenic Acid) Carriers and activators of fatty acyl groups, enabling them to be transported across membranes, modified, and catabolized as energy sources.

Estimated average requirement (EAR) Similar to RDA and RNI, except that this is the mean (i.e., average) nutrient requirement of the individuals in a defined population group (USA and UK).

Microbiological assay A procedure to measure the concentration of a substance (e.g., a vitamin) in a body fluid or food extract, by the quantitative growth of a specific microorganism (usually a bacterium). The growth medium

contains all the necessary growth factors, except the substance being assayed.

Nutrient (e.g., vitamin) status Is commonly assessed by measuring the concentration of the nutrient in an accessible body fluid such as serum or urine, or else the functionality of an enzyme or biochemical pathway (functional status). Published 'normal ranges' enable the result to be classified as, for example, deficient, low, normal, or high.

Recommended dietary allowance (RDA) The amount of a nutrient (per day) that covers the needs of the majority (usually approximately 97.5%) of the individuals in a defined population group (e.g., adult males) in the US and Canada. The term 'Reference Nutrient Intake (RNI)' is used for a similar concept in the UK.

Upper level (UL) Maximum daily amount of a nutrient (for individuals of a defined population group) that is considered to be free from undesirable (e.g., toxic) effects in the long term.

Absorption, Transport, Storage and Status Measurement

Much of the pantothenic acid (formerly vitamin B₅; discovered by Williams, Elvehjem, and Jukes in the 1930s, see **Figure 1**) that is present in food eaten by animals or humans exists as derivatives such as coenzyme A (CoA) and acyl carrier protein (ACP). It is released as free pantothenic acid or

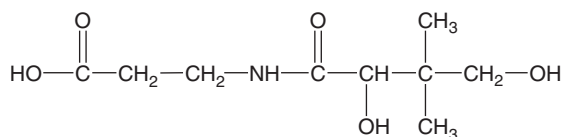


Figure 1 Structure of pantothenic acid.

pantetheine by pancreatic enzymes, and absorbed along the entire length of the small intestine by a combination of active transport and passive diffusion, of which the active transport process predominates at physiological intakes. This active transport process is dependent on sodium, energy, and pH, and is saturable: the K_m is approximately 17 μM and V_{\max} is approximately 1000 $\text{pmol cm}^{-2} \text{h}^{-1}$, with minor variations among species. The transport pathway is shared by biotin in colonic epithelial cells and is regulated by an intracellular protein kinase C-mediated pathway. Calmodulin is also implicated in cellular pantothenic acid transport pathways.

In mice, there is no evidence for adaptation of absorption to low or high intakes. However, studies on rats suggest that a limited secretion of enzymes that degrade CoA in the gut lumen may limit the availability of pantothenic acid from dietary CoA.

In humans, studies of urinary excretion of pantothenic acid after oral doses of either free pantothenic acid or the pantothenate in food have indicated an availability of approximately 50% from the food-derived vitamin. Urinary excretion of pantothenate was approximately 0.8 mg day^{-1} when a pantothenate-deficient diet was eaten, rising to $40\text{--}60 \text{ mg day}^{-1}$ at an intake of 100 mg day^{-1} . At intermediate intakes, in the range $2.8\text{--}12.8 \text{ mg day}^{-1}$, the urinary excretion rate varied between 4 and 6 mg day^{-1} . Excretion levels of less than 1 mg day^{-1} are considered low. Urinary excretion rates reflect recent intakes more closely than most other biochemical indices.

The contribution of the gut flora to absorbed pantothenate in humans is unknown, but there is evidence that bacterial synthesis of the vitamin may be important in animals, especially ruminants, because severe deficiency can only be achieved here by using antibiotics or antagonists, and recent evidence suggests that absorption of pantothenate synthesized by the flora of the human large intestine may make an important contribution to the pantothenate supply of this tissue. Clinical conditions such as ulcers or colitis can adversely affect pantothenate status and excretion rates, and dietary fiber may affect its absorption.

After a dose of ^{14}C -labeled pantothenate, approximately 40% of the dose appears in muscle tissue and approximately 10% in the liver, with smaller amounts elsewhere. The differential affinities of tissues determine their individual contents of the coenzyme derivatives, CoA and ACP, because there is no major surplus store of the vitamin anywhere in the body. Most organs, including placenta, exhibit evidence of a unidirectional active transport process for the intracellular accumulation of pantothenate, which again is dependent on sodium, energy, and pH. In placenta (and probably elsewhere), this transport process is also shared, and competed for, by biotin and some of its analogs.

The only tissues that have been shown to differ with respect to the transport mechanisms are red cells and the central nervous system. The uptake and efflux of pantothenate in red blood cells are unaffected by sodium, energy, or pH. They contain pantothenate, 4-phosphopantothenate, and pantotheine, but do not contain mitochondria, or carry out CoA-dependent processes. The functions of the pantothenate derivatives in red cells are unknown, but their formation results in higher concentrations of total pantothenate in red cells than in plasma, and red cell (or whole blood) total pantothenate is considered a better status index, and more predictably related to intake, than serum or plasma pantothenate. A concentration less than $1 \mu\text{mol l}^{-1}$ of pantothenate in whole blood is considered low; the normal range being $1.6\text{--}2.7 \mu\text{mol l}^{-1}$. Pantothenate in serum appears to be a very short-term marker and it is not well correlated with changes in intake or status.

Concentrations in body fluids are traditionally measured by microbiological assay using *Lactobacillus plantarum*. If CoA is present, enzymatic hydrolysis is needed to liberate free pantothenic acid for the microbiological assay. Other assay methods include gas chromatography (after conversion to a volatile derivative), radioimmunoassay (RIA), or enzyme-linked immunoabsorbent assay (ELISA).

Unlike several other B-vitamin precursors of enzyme cofactors, pantothenate is not entirely converted to coenzyme

forms inside the cell, and metabolic 'trapping' is therefore less dominant than it is for some other B vitamins. Free pantothenate in tissues seems more closely related to dietary pantothenate than the coenzyme forms are; the latter are protected during periods of dietary deficiency. Uptake of pantothenate from plasma into most tissues is proportional to the plasma concentration because the active transport process is not saturated at typical plasma concentrations of approximately $1 \mu\text{M}$ (or $1.6\text{--}2.7 \mu\text{M}$ in whole blood).

Pantothenate in acetyl CoA is required for the hepatic acetylation of drugs, and pantothenate deficiency can impair it; moreover, 20–60% of human populations are slow acetylators, varying with ethnicity.

Metabolism and Turnover

The primary role of pantothenic acid is in acyl group activation for lipid metabolism, involving thiol acylation of CoA or of ACP, both of which contain 4-phosphopantotheine, the active group of which is β -mercaptoethylamine. CoA is essential for oxidation of fatty acids, pyruvate and α -oxoglutarate, for metabolism of sterols, and for acetylation of other molecules, so as to modulate their transport. Acyl carrier protein (ACP), which is synthesized from apo-ACP and coenzyme A, is involved specifically in fatty acid synthesis. Its role is to activate acetyl, malonyl, and intermediate-chain fatty acyl groups during their anabolism by the biotin-dependent fatty acid synthase complex (i.e., acyl-CoA: malonyl-CoA-acyl transferase (decarboxylating, oxoacyl- and enoyl-reducing, and thioester-hydrolyzing), EC 2.3.1.85).

The liver has the highest concentration of pantothenate, followed by adrenal cortex, because of the requirement for steroid hormone metabolism in these tissues. Ninety-five percent of the CoA within each tissue is found in the mitochondria. However, the initial stages of activation of pantothenate and conversion to CoA occur in the cytosol, and CoA is then transported across the mitochondrial membrane. β -Oxidation within the peroxisomes is also CoA dependent and is reduced by pantothenate deficiency.

The pathways of conversion of pantothenic acid to CoA and to ACP are summarized in [Figure 2](#). There are three ATP-requiring reactions and one CTP-requiring reaction in the synthesis of CoA. The rate of CoA synthesis is under close metabolic control by energy-yielding substrates, such as glucose and free fatty acids (via CoA and acyl CoA), acting at the initial activation step, which is catalyzed by pantothenate kinase (ATP: pantothenate 4-phosphotransferase, EC 2.7.1.33). There are also hormonal effects of insulin, corticosteroids, and glucagon, which result in important changes in tissue distribution, uptake, etc. (e.g., in diabetics). The mechanisms are complex and not yet fully understood; however, insulin represses and glucagon induces this enzyme. Although prokaryotes and eukaryotes carry out the same biochemical reactions in the synthesis of CoA, genome studies have revealed important protein-sequence differences, and there is new interest in this pathway for the design of antibacterial drugs.

The recent discovery of a human neurodegenerative disorder has also stimulated research in this area. This rare

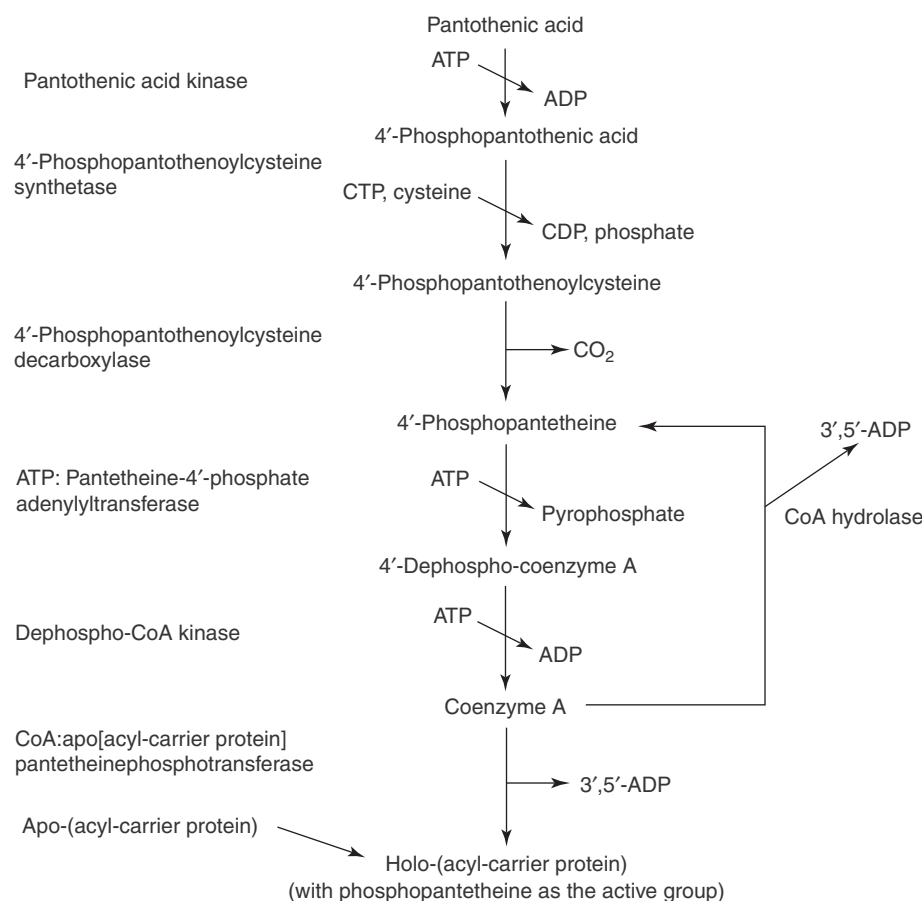


Figure 2 Synthetic pathway between pantothenic acid, coenzyme A, and acyl carrier protein.

genetic disease, formerly called Hallervorden–Spatz syndrome, results from mutations and hence a functional deficiency of pantothenate kinase, and is now known as pantothenate kinase-associated neurodegeneration (PKAN). Dystonia, involuntary movements, spasticity, optic atrophy, and iron deposits in the basal ganglia and globus pallidus occur, and although there is no cure, some palliative replacement is possible.

In humans, fasting results in a reduction of fatty acid synthase activity with loss of the coenzyme of ACP, which thus results in a shift away from fatty acid synthesis and toward breakdown. This interconversion of apo-ACP and holo-ACP is thus a very important process for the short-term regulation of fatty acid synthesis.

A deficiency of sulfur amino acids or copper overload (by interfering with sulfur amino acid function) can result in reduced CoA synthesis.

Free pantothenate in urine is the primary excretion route in humans; however, in other mammals its glucuronide or glucoside may be excreted, and pantothenate is very efficiently conserved in animals. Some bacteria can cleave it to pantoic acid and β -alanine. One breakdown product of CoA is taurine, via cysteamine, and is an essential nutrient for some carnivorous animals such as cats.

When dietary intakes are low, much of the circulating pantothenate, after filtration in the kidney tubules, is absorbed

by the same sodium-dependent active transport process that occurs at most other sites in the body. Secretion into breast milk is proportional to intake and to blood levels of the vitamin; therefore, dietary supplements taken by the lactating mother generally increase the breast milk content of the vitamin.

Metabolic Function and Essentiality

The biochemical functions, and hence the basis for the dietary requirement of pantothenic acid, arise entirely from its occurrence as an essential component of CoA and of ACP, because the vitamin cannot be synthesized *de novo* in mammals. Pantothenic acid was isolated as a chick, bacterial, and yeast growth factor, and as an antidermatitis dietary factor, in the 1930s. Use of omega-methyl-pantothenate as a vitamin antagonist in humans resulted in a syndrome of numbness and burning sensation in the extremities, increased sensitivity to insulin, impaired antibody production, and other symptoms.

In addition to the well-established roles of CoA in the degradation and synthesis of fatty acids, sterols, and other compounds synthesized from isoprenoid precursors, there are also a number of acetylation and long-chain fatty acylation processes which require CoA. The acetylation of amino sugars and many other functions of acetyl-CoA and succinyl-CoA

have been known since the 1980s. However, the addition of acetyl or fatty acyl groups to certain proteins in order to modify and control their properties is a more recent discovery. Acetylation of N-terminal amino acid residues occurs in at least half of all the known proteins in higher organisms. The recipients of these acetyl groups are most commonly methionine, alanine, glycine, threonine, or serine. The purposes of this are not entirely clear and may include modifications of hormone function, of ligand-binding and site recognition, of tertiary peptide structure, and of susceptibility to degradation. Acetylation can also involve the amino groups of the side chain of internal lysine residues, notably the histones in the cell nucleus, and the α -tubulin proteins of the cytoplasmic microtubules, which help to determine cell shape and motility. Its role in the synthesis of α -tubulin appears particularly important.

Proteins can also be modified by acylation with certain long-chain fatty acids, notably the 16-carbon saturated fatty acid, palmitic acid, and the 14-carbon saturated fatty acid, myristic acid. Although structurally very similar to each other, these two fatty acids seek entirely different protein locations for acylation and have different functions. They have mainly been explored in viral and yeast proteins, but proteins in higher animals, in organs such as lungs and brain, can also become acylated with palmitoyl moieties, and enable transport of protein through the Golgi apparatus. Protein acylations may control protein interactions, especially in cell membranes, and palmitoylated proteins are associated with plasma membranes. Signal transduction (e.g., of the human β_2 -adrenergic receptor) is one process that appears to be controlled by palmitoylation, and other palmitoylated proteins are structurally important, for example, in the protein-lipid complex of brain myelin. Acylation may be involved in the activation of some hormones and transcription factors. Clearly, these subtle protein modifications, all of which depend on CoA and hence on pantothenic acid, have wide-ranging significance.

Pantothenic acid is an essential component of the diet of all mammalian species studied, namely humans, bovines, pigs, dogs, cats, and rodents, as well as poultry and fish. Pantothenate deficiency signs in animals are nonspecific and vary between species and with age. Deficiency in young animals results in anorexia and impaired growth, and the requirement estimates based on maximum growth rates are between 8 and 15 mg kg⁻¹ diet. Rats fed a diet low in pantothenate also exhibit scaly dermatitis, alopecia, hair discoloration and loss, porphyrin-caked whiskers, spastic gait, anemia, leukopenia, impaired antibodies, sex organ disruption, congenital malformations, and adrenal necrosis. Deficient chicks have dermatitis, abnormal feather development, thymus involution, myelin degradation, locomotor abnormalities, neurological symptoms including convulsions, fatty liver, and hypoglycemia. Pigs exhibit dermatitis, intestinal problems, spastic gait, and abnormalities of dorsal root ganglion cells, and several species suffer nerve demyelination. Fish show fused gill lamellae, reproductive failure, clumping of mitochondria, and kidney lesions. Signs specific for pantothenate depletion are not well characterized for humans. A syndrome that included 'burning feet' has been described in tropical prisoner-of-war camps during World War II, and it was said to respond to pantothenic acid supplements;

however, this was likely to have been a more complex deficiency. A competitive analog of pantothenate, ω -methyl pantothenate, interferes with the activation of pantothenic acid and produces burning feet symptoms, Reye-like syndrome, cardiac instability, gastrointestinal disturbance, dizziness, paresthesia, depression, fatigue, insomnia, muscular weakness, loss of immune (antibody) function, insensitivity to adrenocorticotrophic hormone, and an increased sensitivity to insulin. Calcium hopantenate, another potential antagonist of the vitamin, has produced some similar effects. Large doses of pantothenate can reverse these changes. One of the earliest functional changes in mildly deficient rats was an increase in serum triacylglycerols and free fatty acids, resulting from impairment of β -oxidation. Paradoxically, CoA levels are relatively resistant to dietary pantothenate deficiency.

CoA is required for Golgi function, and hence protein transport; pantothenate deficiency therefore causes a reduction in the secretion of some proteins. Other metabolic responses to deficiency include a reduction in urinary 17-ketosteroids, a reduction in serum cholesterol, a reduction in drug acetylation, a general reduction in immune response, and an increase in upper respiratory tract infection.

Studies of wound healing and fibroblast growth have indicated that both pantothenic acid and ascorbic acid are involved in trace element distribution in the skin and scars of experimental animals and that pantothenic acid can improve skin and colon wound healing in rabbits. It is not yet known whether these observations are relevant to wound healing in humans. Reports that high-dose pantothenic acid supplements can alleviate some of the symptoms of rheumatoid arthritis or lupus erythematosus have yet to be confirmed.

Requirements

In the UK, National Food Survey records suggest that during recent decades, mean adult daily pantothenate intakes were in the range of 4–6 mg. Because there is little information on the minimum requirements in humans, the UK committee responsible for the revision of dietary reference values in 1991 concluded that intakes in the range 3–7 mg day⁻¹ are adequate (although no specific values for the reference nutrient intake, estimated average requirement, or lower reference nutrient intake for pantothenate were set). The US/Canada adequate intake (AI) for pantothenic acid was set at 5 mg day⁻¹ for adults, rising to 6 mg in pregnancy and 7 mg in lactation; at 4 mg for children aged 9–13 years; at 3 mg for 4–8 years, at 2 mg for 1–3 years and at 1.7–1.8 mg for 0–1 year. There was insufficient evidence to set an estimated average requirement (EAR), a recommended daily allowance (RDA), or a tolerable upper intake level (UL). As for most water-soluble vitamins, maternal blood levels decrease during pregnancy, and the mean daily output of the vitamin in breast milk is of the order of 1.7 mg. Infant formulas should contain at least 2 mg pantothenate per liter.

There are few studies in communities where intakes are likely to be low; indeed, pantothenic acid is so widely distributed in human foods that it is unlikely that any natural diets with a very low content will be encountered. Some variations in status among communities have been described,

Table 1 Pantothenate content of selected foods

Food	mg per 100 g wet wt	mg per MJ
<i>Meat, offal, and fish</i>		
Stewed minced beef	0.36	0.41
Grilled pork chop	1.22	1.58
Calf liver, fried	4.1	5.59
Lamb's kidney, fried	4.6	5.87
Cod, grilled	0.34	0.85
<i>Dairy products</i>		
Cow's milk, full cream	0.58	2.12
Cheese, cheddar	0.50	0.29
Yogurt (whole milk, plain)	0.50	1.50
Boiled chicken's egg	1.3	2.12
Human milk	0.25	0.87
<i>Fruits</i>		
Apples, eating, flesh and skin	trace	trace
Oranges, flesh	0.37	2.34
Pears, flesh and skin	0.07	0.41
Strawberries, raw	0.34	3.01
Dried mixed fruit	0.09	0.08
<i>Vegetables</i>		
Potatoes, boiled, new	0.38	1.18
Carrots, boiled, young	0.18	1.94
Brussels sprouts, boiled	0.28	1.83
Cauliflower, boiled	0.42	3.59
Onions, fried	0.12	0.18
<i>Grains, grain products, nuts</i>		
White bread	0.30	0.33
Wholemeal bread	0.63	0.68
Rice, boiled, white	0.20	0.34
Cornflakes	0.30	0.19
Baked beans in tomato sauce	0.18	0.51
Peanuts, plain	2.66	1.14

but these do not define requirements. In a group of adolescents in the USA, daily pantothenate intakes were approximately 4 mg; total blood pantothenate was in the 'normal' range of approximately 350–400 ng ml⁻¹, and intakes were correlated with red cell pantothenate ($r=0.38$) and with urinary pantothenate ($r=0.60$), both $P<0.001$. In adults, these correlations were less strong.

Dietary Sources and High Intakes

Pantothenate is widely distributed in food; rich sources include animal tissues, especially liver, and yeast, with moderate amounts occurring in whole grain cereals and legumes (see Table 1). It is stable during cooking and storage, although some destruction occurs at high temperatures and at pH values below 5 or above 7. Highly processed foods have lower contents than fresh foods, and one-fifth to two-thirds may be lost during freezing, canning, etc. Commercial vitamin supplements containing pantothenate usually use the calcium salt, which is crystalline and more stable than the free acid.

Synthesis by gut flora in humans is suspected, and the rarity of diet-induced deficiency has been attributed partly to the likely contributions from gut flora.

Pantothenic acid supplements may be beneficial for treatment of rheumatoid arthritis and for enhancement of athletic performance, specifically while running. Pantethine, the disulfide dimer of pantetheine, may have cholesterol-lowering properties and pantothenol may enhance wound healing in animal models. The mechanisms of these reported effects are unclear and they require verification. A homolog of pantothenate, pantooyl γ -aminobutyrate (hopantenate, see section on Metabolic Function and Essentiality), which can act as a pantothenate antagonist, has been used to enhance cognitive function, especially in Alzheimer disease. It acts on gamma-amino-butyric acid (GABA) receptors to enhance acetylcholine release and cholinergic function in the brain. Some of the side effects of valproic acid (used in the treatment of epilepsy and bipolar disorder) may be susceptible to alleviation by pantothenate and carnitine supplements.

There is little evidence for pantothenate toxicity at high intakes, even up to 100–500 times the normal intake; at daily intakes of approximately 10 g, there may be mild diarrhea and gastrointestinal disturbance.

See also: Biochemical Indices. Cofactors: Organic. Energy Metabolism. Fatty Acids: Metabolism. Lactation: Dietary Requirements

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PARASITISM

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Introduction

In common with all other animals, human beings are susceptible to a range of parasitic organisms. The most important and commonest of these have been with man for countless years and have become so well adapted that for most, man is their major if not only host. Although parasitic infections occur throughout the world, it is in the wet tropics and sub-tropics where they are found at their greatest prevalence and intensity. Most developing countries are also located in these areas and the consequent poverty, poor hygiene, and inadequate sanitation augment the favorable environmental conditions to enhance proliferation of these organisms. Only those that are known to interfere with host nutritional status will be discussed in this article.

Parasitic infections of the gastrointestinal (GI) tract are among the commonest diseases in the world (Table 1); and in many developing countries, there has been little improvement in prevalence rates for many years. In some South American and Asian countries, prevalence rates have fallen in the last decade as individual countries became richer and national control programs were introduced, but elsewhere, and particularly in sub-Saharan Africa, case numbers are increasing with the increasing population. Moreover in some situations, for example, with schistosomiasis, local prevalence has been increasing with expanding irrigation schemes. Their association with poverty ensures that these diseases occur in areas where poor child growth and malnutrition are common and where there are persistent health problems. Although there is no doubt that severe infections of any parasite can result in severe illness or even death of the host, such cases are rare even in areas of high prevalence and the norm is for low-to-moderate parasite numbers, which result in few, if any overt clinical symptoms. Nevertheless, by causing subtle reductions

in appetite, digestion and absorption; by increasing chronic inflammation; and by inducing nutrient loss, particularly of iron and protein, it is believed that such low-level but long-term infections contribute to the persistent poor nutritional state of many, especially children, in the developing world.

The most important parasites of man are from two main groups: the helminth worms and protozoans. Although several hundred different species have been described, the vast majority of infections are caused by relatively few.

Mechanisms of Parasite–Host Nutrition Interactions

GI parasites interfere with the nutrition of their host by one or more of the following mechanisms (Figure 1).

Loss of Appetite, Anorexia

Loss of appetite is a common feature in many illnesses and not only those involving the GI tract. It is now thought that much of the appetite loss in disease is mediated by one or more cytokines

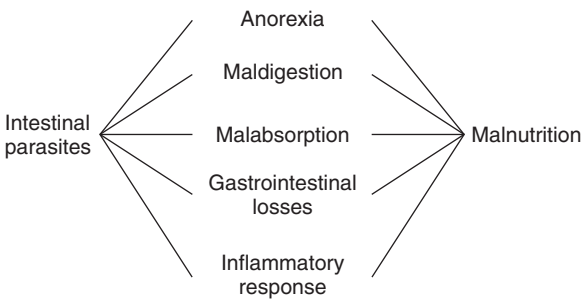


Figure 1 Mechanisms of parasite–host nutrition interactions.

Table 1 Estimated world prevalence of parasites important to human nutrition

Parasites	Approximate prevalence (millions)
Helminth parasites	
<i>Ascaris lumbricoides</i> (roundworm)	1500
<i>Necator americanus</i> and <i>Ancylostoma duodenale</i> (hookworms)	1300
<i>Trichuris trichiura</i> (whipworm)	1100
<i>Schistosoma haematobium</i> , <i>Schistosoma japonicum</i> , and <i>Schistosoma mansoni</i>	200
<i>Strongyloides stercoralis</i> and <i>Strongyloides fülleborni</i>	200
Protozoal parasites	
<i>Giardia intestinalis</i>	200 symptomatic cases and total much higher
<i>Entamoeba histolytica</i>	400 but may be much higher
<i>Cryptosporidium</i> spp.	?

released by lymphocytes as part of the body's response to tissue damage or invasion. Additionally, however, parasitized individuals often complain of symptoms, such as nausea, abdominal pain, flatulence, and distension and discomfort, whereas the protozoal infections are associated with vomiting, diarrhea, or dysentery, all of which can be expected to reduce appetite.

Maldigestion and Malabsorption

Several GI parasites are well placed to interfere with these processes, so it is not surprising that maldigestion and/or malabsorption of fat, protein, and carbohydrate as well as many of the micronutrients have been reported during infection. Structural damage to the mucosa of the intestine, such as the flattening or thickening of villi, or villus atrophy, will reduce the absorptive surface area. Damage to the cells diminishes their absorptive properties and limits active transport processes, whereas accelerated replacement of damaged cells may result in immature mucosal cells with reduced enzymatic and transport capacity. Food that is not fully digested and absorbed in the small intestine will enter the large bowel where excessive colonic fermentation may result in diarrhea.

Nutrient Losses

Accelerated loss of nutrients from the body is probably the most important mechanism by which parasitic infections compromise the nutritional status of their host. Nutrient losses arise both directly and indirectly.

Direct losses occur during the feeding of the blood-sucking and tissue-invading parasites. Blood and tissue ingested by the worms forms part of the loss but the lesions caused by feeding and burrowing activity continue to ooze blood and tissue fluids after the parasites have moved on. Similarly, the passage of schistosome eggs through the tissues of the bladder or intestine is often accompanied by tissue damage and blood loss. Increased turnover and accelerated shedding of parasite-damaged enterocytes into the lumen of the GI tract is another mechanism of increased nutrient loss. Although some of the nutrients lost into the lumen may be reabsorbed, the process is far from complete. Vomiting or diarrhea causes loss of electrolytes and important trace elements such as zinc.

Indirect losses arise from stimulation of the host's immunological and inflammatory mechanisms that are mobilized to combat the infection and repair tissue damage. Localized inflammation at the site of the parasite activity often accompanied by lymphocytic infiltration of tissues cause further damage to the mucosa, augmenting maldigestion, malabsorption, and nutrient losses as more damaged cells are shed. Activation of the systemic inflammatory system, that is, the acute-phase response, is a general reaction of the body to pathogen invasion or tissue damage. It results in a widespread cytokine-mediated catabolic response. Growth slows or ceases, muscle tissue is broken down to provide substrates for gluconeogenesis and the repair of damaged cells, and a negative nitrogen balance ensues. Anorexia occurs and there are increased losses of amino acids, minerals, and vitamins in the urine and feces.

Competition for Nutrients

Competition for nutrients is generally unlikely owing to the considerable difference in biomass of the host and parasite. However, the tapeworm, *Diphyllobothrium latum*, does compete for vitamin B₁₂ taken in the diet. The worm concentrates large amounts of this vitamin in its own tissues, depriving the host and in some cases leading to megaloblastic anemia.

Parasite Epidemiology and Impact on Host Nutrition

Clinical Studies

Much of our knowledge of the impact of parasitism on host nutrition (Table 2) comes from hospital studies of heavily parasitized patients. Irrespective of the organism involved, nutritional status and anthropometric indices of such severely ill patients are invariably poor on admission but quickly improve following treatment. Such data must however be interpreted with caution. In developing countries, malnourished individuals admitted to hospital rarely suffer from a single parasitic infection; viral and bacterial pathogens and other parasitoses are frequently present as are frank dietary deficiencies. Patients are routinely dosed with wide-range antibiotics and anthelmintics and given high-quality rehabilitation diets; so, in general, neither the cause of their symptoms nor the basis for recovery can be established with certainty.

Helminth Parasites

Ascaris lumbricoides (Roundworm)

Approximately 73% of all infections by this worm are estimated to occur in Asia with many countries having prevalence rates greater than 50%. In some rural areas, more than 90% of children harbor the infection. It is less prevalent in Africa (approximately 12% of all cases) and in Central and Southern America (approximately 8% of all cases). It is uncommon but still present in some rural areas of Europe and southeastern parts of USA. Adult *Ascaris lumbricoides* live in the lumen of the upper part, that is, the jejunum, of the small intestine. The worms live for some 12–20 months and females grow to 20–35 cm in length and 3–6 mm in diameter. An adult female discharges 200 000–240 000 eggs per day into the lumen and these pass out of the body in the feces. Infection occurs by oral ingestion of eggs from fecally contaminated food, water, hands, kitchen utensils, or play things. Both the prevalence and intensity of infection with *A. lumbricoides* increase rapidly during early childhood and although prevalence often remains high throughout life, intensity of infection tends to peak in the 5–15 years age range.

Despite the large size of these worms, mild-to-moderate infections are generally well tolerated with few, if any, overt symptoms. Clinical studies give inconsistent results. Although anorexia, abnormal mucosal histology, decreased absorption of fat and carbohydrate, reduced lactase activity, decreased transit time, reduced nitrogen retention, reduced vitamin A absorption, and lower vitamin A status have all been reported, they are by no means present in all cases. These abnormalities are in keeping with a stimulation of the host's immune and

Table 2 Parasite interference with host nutrition

Parasite	Symptom	Nutritional effect
<i>Ascaris lumbricoides</i>	Anorexia and abdominal pain Malabsorption syndrome	Growth retardation and weight loss Reduced fat and nitrogen uptake Reduced vitamin A status
	Lactose intolerance Acute-phase response	Growth retardation and weight loss Growth retardation and weight loss
Hookworm	Anorexia and abdominal pain Diarrhea Blood loss Protein-losing enteropathy	Growth retardation and weight loss Growth retardation and weight loss Iron deficiency and anemia Hypoalbuminemia and edema
<i>Schistosoma</i> spp.	Anorexia Diarrhea Blood loss Plasma protein loss Acute-phase response	Growth retardation and weight loss Growth retardation and weight loss Iron deficiency and anemia Hypoalbuminemia Growth retardation and weight loss
<i>Trichuris trichiura</i>	Anorexia Abdominal pain and vomiting Diarrhea and dysentery	Growth retardation and weight loss Growth retardation and weight loss Loss of trace elements, e.g., zinc Growth retardation, weight loss
	Blood loss Plasma protein loss Acute-phase response	Iron deficiency and anemia Hypoalbuminemia and edema Growth retardation and weight loss
<i>Strongyloides</i> spp.	Anorexia Abdominal pain and vomiting Malabsorption syndrome Protein-losing enteropathy Acute-phase response	Growth retardation, weight loss Growth retardation, weight loss Reduced fat absorption Hypoalbuminemia, edema Growth retardation, weight loss
<i>Giardia intestinalis</i>	Anorexia Diarrhea and vomiting Malabsorption syndrome	Growth retardation, weight loss Loss of trace elements Reduced fat absorption Reduced vitamin A status
	Mucosal disruption Acute-phase response	Lowered disaccharidase activity General maldigestion Growth retardation and weight loss
<i>Cryptosporidium</i> spp.	Anorexia Abdominal pain Diarrhea and vomiting Mucosal disruption	Growth retardation and weight loss Growth retardation and weight loss Loss of trace elements Lowered disaccharidase activity General malabsorption
	Acute-phase response	Growth retardation and weight loss
<i>Entamoeba histolytica</i>	Diarrhea and dysentery	Fluid and electrolyte loss Electrolyte imbalance Loss of trace elements
	Acute-phase response	Growth retardation and weight loss

inflammatory mechanisms and it seems likely that occurrence of these symptoms depends on whether such mechanisms have been initiated. Why the immune and inflammatory response should be initiated in some cases of *A. lumbricoides* infection but not others is not known, but it may be at least partly due to genetics.

Hookworms

Although 13 different human hookworm parasites have been listed, only two species, *Necator americanus* and *Ancylostoma duodenale*, are responsible for virtually all cases of hookworm disease in humans. The two worms are similar in appearance, feeding pattern, and life history. Man is their only known host.

Necator americanus is the only species seen in North America and it predominates in central and southern America, central Africa, southern India, Indonesia, and the South Pacific. *Ancylostoma duodenale* is found in Mediterranean Europe, the Middle East, North Africa, Pakistan, Iran, and northern India. Both species occur in parts of Brazil, India, and Africa, throughout Southeast Asia, Indonesia, and the Pacific islands.

Adult worms live in the upper part of the small intestine and eggs are discharged into the lumen. Up to 10 000 (*N. americanus*) or 25 000 (*A. duodenale*) eggs per day can be produced and are passed out in the feces. Eggs hatch within 48 h and the larvae are free living for 2–3 weeks but then must reach a host or die. Adult female *A. duodenale* are 10–13 mm in

length, *N. americanus* 9–11 mm, and the males approximately 2 mm shorter. *Necator americanus* can live for up to 5 years.

Both prevalence and intensity of infection increase with age in childhood up to approximately 10–15 years, and then remain constant during adulthood. High prevalence is associated with inadequate or unhygienic disposal of feces, which contaminates the soil. Lack of footwear, a common state in developing countries, allows feet to come in contact with infective larvae.

Loss of blood, particularly of its iron content, is the most important pathological feature of hookworm infection. Iron-deficiency anemia is one of the commonest deficiency diseases in the world and there is no doubt that hookworms contribute significantly to the estimated two billion individuals who suffer from this problem. Through its feeding activity, each *N. americanus* worm causes the loss of approximately 0.03 ml of blood per day, whereas the larger *A. duodenale* accounts for approximately 0.15 ml per day. Part of this loss is blood ingested by the worm, but each time the worm moves to a new site, perhaps up to six times per day, the lesions continue to ooze blood into the lumen.

Daily blood loss from an individual passing 2000 eggs per gram of feces has been estimated at 4.3 ml (containing 2.0 mg of iron) and 8.9 ml (4.2 mg of iron) for *N. americanus* and *A. duodenale*, respectively. Although the intestine will reabsorb approximately 35% of this iron, daily losses will be 1.3 and 2.7 mg of iron, respectively. Assuming that only 10% of dietary iron is absorbed, an increased dietary intake of 13 and 27 mg, respectively, is required to make good these losses. As most diets contain only 15–20 mg of iron per day and some 10–15 mg of this is needed to cover daily metabolic requirements, intake would need to at least double to replace the loss from even this moderate hookworm load. In developing countries, this is rarely possible, so without iron supplements, iron stores are soon depleted and iron-deficiency anemia ensues. In lighter infections, subclinical iron deficiency is shown by low plasma ferritin and iron concentration, low transferrin saturation, and elevated erythrocyte protoporphyrin content.

Protein is also lost into the lumen of the small intestine during hookworm disease. Estimates of plasma loss vary considerably and values more than 100 ml (containing 6–7 g of protein) per day have been recorded, although much of this may be reabsorbed. Nevertheless, moderate-to-heavy hookworm infections are associated with hypoalbuminemia, hypoproteinemia, edema, and kwashiorkor, especially in areas where the protein content of the diet is low.

Schistosomes

The three commonest species responsible for disease are *Schistosoma haematobium*, *Schistosoma mansoni*, and *Schistosoma japonicum*, with some individuals in Africa harboring two species. Urinary schistosomiasis, found mainly in Africa and some eastern Mediterranean countries, is caused by *S. haematobium*. Infection with either *S. mansoni* (found in Africa, the Middle East, parts of South America, and the Caribbean) or *S. japonicum* (occurs in China, the Philippines, and Indonesia) results in intestinal schistosomiasis. These worms live in blood vessels: *S. haematobium* in the vesicle venules of the urinary bladder with the other two species infecting the mesenteric veins adjacent to the intestines. Adults live in male/female

pairs and damage is caused by passage of eggs through the tissues into either the bladder (*S. haematobium*) or the gut lumen. Eggs leave the body in the urine or feces. If they reach fresh water, they hatch to produce miracidia, which must find a suitable snail host. After entering the snail, the parasites multiply by asexual reproduction, eventually producing free-swimming cercariae, which are infective to man. Infection is by skin penetration during contact with fresh water containing cercaria. Egg production starts some 2–3 weeks after infection. The parasite lives for 3–8 years. The prevalence of this parasite in many developing countries is increasing as irrigation schemes allow the intermediary snail hosts to extend their range.

Iron-deficiency anemia associated with blood loss occurs in both urinary and intestinal schistosomiasis. Although blood loss can be severe in heavy *S. haematobium* infection, in a study of nonhospitalized children with low-to-moderate infection, iron losses ranged from 120 to 500 $\mu\text{g day}^{-1}$, increasing with rising egg count. This is less than losses due to hookworm, but dietary iron consumption would need to increase by approximately a third to compensate. In areas where iron status is poor, the extra burden due to *S. haematobium* will undoubtedly contribute to the onset of anemia. Intestinal schistosomiasis caused by *S. mansoni* can also result in iron deficiency, but it is generally less severe than that seen in hookworm disease. Little data are available for *S. japonicum* infection, but its effect appears to be similar to *S. mansoni*.

The poor nutritional status of infected individuals may be related to anorexia, diarrhea, and activation of the inflammatory mechanisms of the host. Blood cytokine concentrations are raised in schistosomiasis causing growth faltering and weight loss.

Trichuris trichiura (Whipworm)

This helminth is widespread throughout the tropics and subtropics. Most cases of infection (63% of the worldwide total) occur in Asia, with 11% in Africa and 14% in the Americas; however, a few cases are still seen in USA, Western Europe, and Japan.

Man is the principal host of the parasite, which lives in the large intestine. Adults are 3–5 cm in length and are whip shaped; the long thin anterior end is embedded in the mucosa, with the thicker posterior end in the lumen. Worms feed on mucosal cells but may also ingest red and white blood cells. Eggs leave the host in feces and embryonate in the soil. Infection occurs by oral ingestion of embryonated eggs on fecally contaminated food, hands, or utensils. They hatch in the small intestine and larvae develop in the villi before moving down to the large intestine. Egg production starts 30–90 days after ingestion.

In some rural areas, the prevalence can exceed 90% and although prevalence remains high throughout life, peak intensity usually occurs between the ages of 5 and 15 years.

This helminth causes loss of blood and iron from the large intestine of its host by its burrowing and feeding activities. More than 3000 worms have been found in heavy *Trichuris trichiura* infection and such individuals do have marked iron-deficiency anemia. However, in the majority, where worm counts rarely exceed 100, the infection is usually

asymptomatic. Plasma protein loss can also be substantial in heavy infections but although plasma albumin values are frequently reduced, hypoproteinemic edema is rare.

Heavy infections are characterized by persistent dysentery, abdominal pain, nausea, vomiting, and tenesmus leading to rectal prolapse. Appetite is reduced and raised plasma cytokines and acute-phase proteins indicate activation of host immune and inflammatory mechanisms. Loss of nutrients, including zinc and other trace elements, in the persistent dysentery and vomiting may further lower nutritional status.

Strongyloides stercoralis

This worm has a worldwide distribution but is found predominantly in the tropics. Prevalence rates are uncertain as detection of the larvae by direct fecal examination (the method usually employed) gives a considerable underestimate. Prevalence rates of up to 85% have been reported but are uncommon. In parts of Africa, a closely related worm, *Strongyloides fuelleborni* is often more common than *Strongyloides stercoralis*.

Adult worms are approximately 2.7 mm in length and are usually found in the duodenum and upper jejunum. Eggs are passed into the lumen but most hatch while still in the GI tract. Although most larvae pass out in the feces, some penetrate the wall of the intestine and reinfect the host, a situation known as autoinfection. Larvae passed with the feces live in the soil and grow into adults of both sexes. Eggs are laid and larvae hatch within 1–2 weeks. They metamorphose to an infective stage when they must either locate a host or die. Infection is usually by skin penetration. Because of the autoinfection process, infection with this parasite can last indefinitely and severe disease can suddenly appear many years after an individual has left an endemic area.

The impact of this worm on nutritional status has not been clearly defined. Heavily infected subjects have a severe small intestinal illness with anorexia, abdominal pain, nausea, diarrhea, and vomiting. There is some evidence for a malabsorption syndrome; steatorrhea is often present, but it is not seen in all cases. A substantial protein-losing enteropathy can occur, resulting in severe hypoalbuminemia and kwashiorkor-like edema. Protein loss arises from a combination of the burrowing activity of the worms and a local inflammatory reaction from the host. The little information on *S. fuelleborni* infection suggests that it has a similar impact on host nutrition.

Special Features of Helminth Parasites

Helminth infections all exhibit certain characteristic features by which they differ from most other infective organisms:

1. In contrast to most infective organisms, most helminths cannot reproduce within the host; each worm has to gain individual access to the host, usually by ingestion or skin penetration.
2. Intensity of infection shows an overdispersed distribution; it is usual for 20% of an infected population to harbor 80% of the parasites. Thus, a large majority of individuals will have only light infections and show few if any symptoms.
3. Some individuals appear to be predisposed to have heavy worm burdens; they quickly reacquire a heavy load after

eradication of their original infection. Household and family clustering of high parasite loads is also seen. Clearly such differences might be explained on the basis of increased exposure of individuals and family groups due to particularly unhygienic living conditions or greater occupational risk. However, increased host genetic susceptibility has recently been demonstrated to account for between 21% and 44% of the observed variance in infection intensity.

4. Infection by several different parasites at the same time (polyparasitism) is extremely common in many areas.
5. Reinfection following deworming occurs very quickly because of considerable contamination of the environment by the large numbers of eggs produced by the parasites. In a study in Myanmar, preinfection prevalence of *A. lumbricoides* was reached only 6–8 months after deworming.

Protozoal Parasites

Giardia intestinalis (= lamblia)

This organism is a common parasite of the human GI tract and is found in all parts of the world. Although its prevalence is greatest in developing countries where hygiene facilities are poor, outbreaks of giardiasis continue to occur in many developed countries. It has a simple life history. The trophozoite (the active form in the intestine) lives in the duodenum and jejunum of the host where it attaches to the enterocytes by means of a ventral disk. It reproduces rapidly by mitotic division and in heavy infections can cover large areas of the mucosa. Some trophozoites encyst; a protective wall forms around the organism, and the cysts pass out in the feces. Cysts are directly infective and after ingestion by a new host, the organisms emerge to establish a new infection. Disease can follow the ingestion of as few as 10 cysts, which, given moist conditions, are viable for several months.

In developed countries, most infections can be traced to contaminated water, but direct person-to-person transmission has been documented. In developing countries, poverty-related unsanitary conditions and inadequate disposal of feces promote orofecal spread of the parasite, but contaminated water is also likely to be important. The large number of cyst-producing individuals with asymptomatic infection constitutes a reservoir of *Giardia intestinalis*. In addition, some animals are known to harbor *Giardia* and may be a source of human giardiasis.

Infection with *G. intestinalis* can be associated with a wide range of symptoms: from mild, self-limiting watery diarrhea to persistent foul-smelling diarrhea with vomiting, abdominal pain and distension, and a severe malabsorption syndrome. However, many infected individuals (from 20% to 84% of infected cases) remain asymptomatic. It is not clear why the parasite can cause such a range of degrees of illness.

The nutritional impact varies with both the severity and duration of the symptoms. In the early stages, anorexia is of major importance, but if the disease persists, intestinal aspects compound the situation. In at least 50% of symptomatic patients, there is malabsorption of fat, carbohydrates, protein, and micronutrients (particularly vitamin A) associated with structural and functional abnormalities in the small intestine.

Damage to the mucosa can range from little to subtotal villus atrophy, but most subjects have mild villus shortening and increased crypt depth. The abnormalities are associated with a reduction in disaccharidases, notably lactase activity and in lowered intraluminal concentrations of the hydrolytic enzymes trypsin, chymotrypsin, and lipase. The small intestinal barrier function is compromised, allowing translocation of potentially antigenic macromolecules into the body with consequent stimulation of the immune and inflammatory mechanisms resulting in growth retardation. Little is known about the nutritional effects of nonsymptomatic giardiasis.

***Cryptosporidium parvum* and Other *Cryptosporidium* Species**

These organisms have only been recognized as human parasites since 1976. They have a worldwide distribution; but in developed countries, they generally cause a self-limiting disease, which occurs most commonly in child institutions and in people working with animals. However, water-borne outbreaks have occurred in which large numbers of people have become infected. Cryptosporidiosis is much more prevalent in developing countries where it is mainly a disease of children. The parasites live in the upper part of the small intestine, attached to the mucosal cells from which they feed.

Both sexual and asexual reproduction occurs in the host; and cysts are produced, most of which pass out in the feces. However, some excyst while passing through the GI tract resulting in autoinfection that can prolong the disease long after the original source of infection has been eliminated. Infection is by ingestion of cysts in fecally contaminated food, water, or utensils, or from unhygienic contact with infected persons. Continued exposure is facilitated by the many infected individuals who remain asymptomatic while passing cysts. *Cryptosporidium* spp., including *Cryptosporidium parvum*, also occur in many animals and can be transmitted to humans.

Most infected individuals remain asymptomatic, but in others, acute or chronic diarrhea associated with vomiting, abdominal pain, dehydration, and fever can occur. Immuno-compromised and previously malnourished cases tend to have more severe and prolonged disease.

The nutritional impact of the infection depends on the severity and duration of the infection but growth retardation and lowered nutritional indices occur in asymptomatic cases as well as those with symptoms. Structural damage to the mucosa of the small intestine is seen, with shortened and fused villi and lengthening of the crypts due to accelerated cell division to replace damaged cells. Surface area is greatly reduced and the immature enterocytes have lower enzymatic and transport activity than mature cells. The resulting mal-digestion, malabsorption, and stimulation of the host immune and inflammatory mechanisms are likely to account for the adverse nutritional effects. In children, growth remains poor for many months after infection has resolved. The nutritional status of asymptomatic individuals appears to be compromised by less extreme expression of these same mechanisms.

Entameba histolytica

This ameba has a very wide distribution but is most commonly found in developing countries where lack of hygienic facilities

exacerbate fecal contamination of water, food, and hands. The organism is exclusive to humans; there are no animal hosts. These parasites generally infect the large intestine where they can cause severe disease by invading mucosal tissues.

The life cycle is simple: adult amebae reproduce asexually forming substantial colonies and in some cases cause ulcerative lesions in the mucosa. Some organisms encyst and pass out with the feces. Following ingestion of the cysts by another host, the amebae emerge when the cyst reaches the large bowel. The organism can also invade other organs, notably the liver, resulting in a life-threatening illness.

Although this parasite can cause life-threatening diarrhea and dysentery in some, most infected individuals remain free of symptoms. In others, persistent diarrhea can continue for months, interspersed with periods of apparently normal bowel function. As the parasite is most commonly found in the large intestine, there is little interference with food digestion and absorption and its main effect on nutrition seems to be due to loss of trace elements and electrolytes in watery stools. In more severe cases, blood is also lost in this way but amounts are small. Infection is associated with inflammation of the large bowel (colitis) indicating that host immune and inflammatory mechanisms have been stimulated and this may account for reports of hypoalbuminemia.

Community and Intervention Studies

Iron Deficiency and Iron-Deficiency Anemia

A close relationship between the level of hookworm infection and severity of anemia has been observed in many cross-sectional field studies. Similar, although generally less severe, levels of anemia have been associated with intensity of schistosome species and *T. trichiura* disease. The cause and effect relationship suggested by this data has been confirmed by longitudinal investigations of iron status following anthelmintic administration. Community studies in Kenya, India, and Papua New Guinea have recorded substantial increases (up to 6 g l^{-1}) in hemoglobin concentration between 4 and 8 weeks after treatment for hookworm. These marked improvements were seen even when parasite loads were not completely eliminated. Effective treatment of severe *Schistosoma* and *T. trichiura* infections also results in much improved iron status.

Growth and Protein–Energy Malnutrition

Although clinical studies confirm that these parasites have the potential to interfere with growth and nutritional status, evidence that they are a major cause of the widespread stunting and protein–energy malnutrition seen in developing countries is not as conclusive as may be expected. This may be because most infected individuals in a community will have only low-to-moderate parasite loads and whether a particular disease is important in precipitating malnutrition on a community or public health scale will depend on whether or not such low-level infections impact on nutritional status. Information has come from two types of study: (1) cross-sectional surveys and (2) longitudinal, placebo-controlled intervention studies in

which nutritional improvements are sought following the use of antiparasitic drugs.

A large number of cross-sectional community studies have associated parasitic infection with growth deficits and poor anthropometric indices. Schistosomiasis has long been associated with poor growth, and an extreme condition, schistosomiasis dwarfism, in which physical and sexual development were severely retarded was reported to be quite common in China until the 1950s. Most recent studies of mild-to-moderate infection with all three schistosome species confirm an association with poor nutritional status that is more marked in girls, but the degree of impairment is variable between different regions and at best can only explain a small part of the total nutritional deficit of the subjects. Hookworm infection is similarly associated with poor appetite, slower growth, and lowered nutritional indices, all of which become more marked with increasing severity of iron-deficiency anemia. Iron supplementation of hookworm-infected children has been reported to improve appetite and growth performance as well as iron status, suggesting that the lowered nutritional status may be secondary to iron deficiency rather than a direct effect of the parasite. Growth retardation seen in moderate-to-heavy *T. trichiura* infection may be similarly explained, although heavier burdens of this worm frequently cause dysentery, which can result in loss of essential trace elements such as zinc.

The impact of the protozoal parasites *Giardia* and *Cryptosporidium* on nutritional status has been less well studied, but infection appears to be associated with persistent diarrheal disease and prolonged growth faltering even after apparent elimination of the parasites. Moreover, these parasites, unlike the helminths, are very common in children during the first 2 years of life when growth is at its greatest. Growth-retarding infections at this time of life, particularly in developing countries, appear to compromise growth throughout the whole growth period, thus the impact of these parasites on nutritional status may be far greater than currently appreciated. This is certainly an area requiring more research.

The results of these cross-sectional studies have been reinforced by longitudinal community-wide studies of nutritional improvement following reduction or eradication of parasite burden with anthelmintic drugs. The results of such studies have, however, been less than convincing. Successful treatment of heavily polyparasitized Kenyan children harboring hookworm, *A. lumbricoides* and *T. trichiura*, with albendazole resulted in improvements in weight, arm circumference, and skinfold thickness and was associated with increased appetite and fitness. Statistical analysis of this data implicated hookworm as being the most important in compromising nutritional status. Weight gain above placebo-treated counterparts averaged 1.3 kg per 6 months, and added approximately 3% points to a weight-for-age of approximately 80%. However, similar studies in many parts of the world in subjects with lower intestinal helminth burdens have reported only small improvements, whereas others found no change at all in nutritional status indices following successful deworming. Treatment of schistosomiasis in Kenyan, Brazilian, and Filipino children showed only small improvements in nutritional status, for example, in Kenya, the percent weight-for-age only increased from 72.9% to 74.9% following

eradication with praziquantel. A recent meta-analysis of these studies concluded that deworming did improve nutritional status, but that the effect was small.

Overall, both community and intervention studies do suggest that elimination of GI parasites would improve growth and anthropometric status of children in developing countries but that such improvement would be limited. This contrasts with the very substantial improvement in iron status and iron-deficiency anemia that follows effective treatment of organisms causing blood loss.

Treatment and Prognosis

Table 3 shows the drugs most commonly used in treatment of these parasitic infections. Anthelmintic drugs have improved dramatically during the last 20 years and are now highly effective; in most cases, a single course of treatment will result in parasite eradication. However, immunocompromised hosts, including malnourished children, may require more extensive courses of therapy to completely eliminate the infection. This is particularly the case in the treatment of cryptosporidiosis. Iron supplements are usually provided where blood loss has resulted in iron-deficiency anemia.

Recovery from infection is usually complete and rapid as most parasites do not cause lasting damage to their host. Schistosomiasis is the exception and can result in permanent granuloma formation in several tissues, particularly the liver and spleen, which may become life threatening.

Prevention

Although drugs are now available to eradicate infections, unless the home environment changes, most individuals will soon become reinfected. The transmission of all the parasites discussed occurs most commonly through close contact between the host and infected human feces, either orally or by skin penetration. The basic requirement for prevention is an efficient and hygienic mode of disposal of feces and improved facilities in the home, for example, clean running water, concrete floor to the home, plus a knowledge of basic hygiene.

Table 3 Drugs of choice for parasitic infections

Infection	Drug
Ascariasis	Mebendazole, albendazole, and pyrantel pamoate
Hookworm infection	Mebendazole and albendazole
Schistosomiasis	Praziquantel, metrifonate, niridazole, and oltipraz
Trichuriasis	Mebendazole and albendazole
Strongyloidiasis	Thiobendazole and ivermectin
Giardiasis	Metronidazole, tinidazole, secnidazole, furazolidone, and albendazole
Cryptosporidiosis	Nitazoxanide, spiramycin, and clindamycin
Amebiasis	Metronidazole, secnidazole, paromomycin, and nitazoxanide

Use of footwear and avoidance of contact with water likely to contain schistosome cercaria would help. For the foreseeable future, however, such control measures are quite unrealistic in many developing countries and the alternative may be the large-scale, nation-wide use of anthelmintics to regularly deworm all individuals in endemic areas. School-based regular treatment programs can be effective. Safe, effective, and relatively cheap drugs are now available and their use in this way could substantially reduce the level of helminth disease throughout the developing world. Such programs can be expected to result in a marked reduction in the prevalence and severity of iron-deficiency anemia but in most situations, to have a relatively small impact on child growth, stunting, and incidence of protein-energy malnutrition.

See also: Cytokines: Nutritional Aspects. Diarrheal Diseases. Iron: Physiology, Dietary Sources, and Requirements. Zinc: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements

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PARENTERAL NUTRITION

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Indications for Parenteral Nutrition

The use of parenteral nutrition should be entertained in those situations where feeding into the gut is contraindicated or in those conditions where oral intake or enteral feedings are not adequate or feasible. Parenteral nutrition should also be strongly considered when it is anticipated that enteral feedings cannot begin for at least 7 days. However, generally parenteral nutrition should not be initiated if it is expected to last less than 5–7 days because risk may then outweigh potential benefit of this therapy.

Enteral nutrition has many advantages over parenteral nutrition and should be considered whenever possible. Its benefits are myriad and include: lower cost, lower risk of adverse effects such as infection and hyperglycemia, and decreased length of stay for hospitalized patients. It also allows for the maintenance of gastrointestinal function and integrity. Even small quantities of enteral intake can serve to boost gut barrier function and gut-associated immune function. It has been shown in animal models that enteral feedings are associated with decreased risk of sepsis presumably through a decrease in bacterial translocation ([Table 1](#)).

[Table 1](#) lists contraindications to the use of enteral feedings. In general, the gastrointestinal tract should not be used in these conditions until the underlying problem is treated. [Table 2](#) lists the common scenarios where parenteral nutrition becomes an important means of restoring nutritional status.

Bowel Rest

When use of the gastrointestinal tract may not be prudent or suitable, parenteral nutrition can be employed. For instance, in patients with inflammatory bowel disease, parenteral nutrition may serve an invaluable role in those situations where

bowel rest may be necessary. Patients with acute exacerbations of disease characterized by refractory severe diarrhea or vomiting, or those with complications of the disease such as intestinal obstruction or fistula formation in Crohn's disease or short bowel syndrome may also benefit from parenteral nutrition. Certainly though, enteral nutrition should be utilized whenever feasible.

Enterocutaneous fistulous disease, whether it is secondary to Crohn's disease, gastrointestinal or intra-abdominal abscesses, abdominal surgery, trauma, ischemia, or tumors, may be managed with parenteral nutrition. The hope is that the bowel rest in combination with parenteral nutrition would allow for closure of the fistula and would boost the nutritional status of these patients because they are at high risk for malnutrition, dehydration, and electrolyte abnormalities.

Parenteral nutrition can also be used for management in severe cases of acute pancreatitis, when its duration is expected to be greater than 7–10 days in length. However, there have been excellent data on the use of postpyloric tube elemental low fat enteral feedings in patients with mild-to-moderate pancreatitis.

Perioperative Support in Severe Malnutrition

Malnutrition has been found to be associated with an increased risk of morbidity and mortality in the postoperative period. The Veteran's Affairs Parenteral Nutrition Cooperative Trial evaluated the benefits of preoperative parenteral nutrition in patients with varying degrees of malnutrition. Those

Table 1 Contraindications to enteral nutrition

Diffuse peritonitis
Mesenteric ischemia
Intestinal obstruction that prohibits use of the bowel
Paralytic ileus
Intractable vomiting
Intractable diarrhea

Table 2 Common diagnoses with indications for parenteral nutrition

Perioperative support in severe malnutrition
Inflammatory bowel disease and related complications
Short bowel syndrome
Severe acute pancreatitis
Mechanical intestinal obstruction or pseudo-obstruction
High output enterocutaneous fistula
Prolonged postoperative ileus
Severe malabsorption
Bone marrow transplant/peripheral stem cell transplant
Severe hyperemesis gravidarum

patients with significant benefit were the group with severe malnutrition as defined by a serum albumin $<3.0 \text{ mg dl}^{-1}$. Of note, an increased rate of infectious complications was seen in mildly-to-moderately malnourished patients receiving parenteral nutrition. If enteral feeding is feasible and tolerated, preoperative enteral nutrition has been found to be equally effective when used for 7–14 days or longer in malnourished surgical patients. In those postoperative patients who have developed a paralytic ileus from various causes, parenteral nutrition may be attempted if there is no return of gastrointestinal function for 7–10 days.

Contraindications to Parenteral Nutrition

The main contraindication to parenteral nutrition includes a functional gastrointestinal tract. Other possible instances where parenteral nutrition would not be recommended would be when a patient is do not resuscitate (DNR) status, or in those cases where patients have unstable fluid or electrolyte status or cardiopulmonary status is unstable.

Vascular Access

Parenteral nutrition can be infused either into the venous system centrally or peripherally. Central or peripheral access is not defined by the initial point of entry into the vascular system. Instead it is determined by the position of the distal catheter tip. When the tip is located outside a large-caliber central vein such as the superior vena cava or the inferior vena cava, it is considered a peripheral access device.

Lower concentration of dextrose and amino acids may be given through peripheral veins for a short duration of therapy (less than 10–14 days). Such formulas usually do not provide the patient's full nutritional needs, and may require large volumes of fluid and therefore should not be used in patients where fluid restriction is necessary (i.e., cardiac patients and renal patients). Osmolality of peripheral solutions needs to be less than 900 mOsm l^{-1} . Potential complications of peripheral parenteral nutrition include phlebitis, infiltration, or fluid-overload issues. Frequent peripheral IV site rotations are required (usually every 48–72 h).

Formulas that have higher concentrations of nutrients, thereby making them hyperosmolar, must be administered directly into the superior or inferior vena cava to allow for rapid dilution. Some of the central catheters commonly used to deliver parenteral nutrition include subclavian vein catheters, peripherally inserted central catheters, subcutaneously tunneled percutaneous catheters, or implanted subcutaneous infusion ports. These delivery systems are preferred to peripheral options because they allow for maximal amounts of calories and protein per volume to be provided.

Nutrition Components of Parenteral Nutrition

Parenteral nutrition is a compounded formulation, which includes protein as amino acids, energy in the form of dextrose and fats, as well as electrolytes, vitamins, minerals, and

trace elements. Sterile water is added to provide necessary volume to the parenteral nutrition formula. Parenteral solutions are divided into two main categories: 3-in-1 or 2-in-1 solutions. A 3-in-1 solution, referred to as Total Nutrient Admixture, is composed of amino acids, dextrose, and lipids all combined in one bag. A 2-in-1 solution contains the mixture of amino acids and dextrose in one bag and a separate intravenous fat emulsion infusion.

Ideal body weight (IBW) is calculated by the following formulas:

$$\text{Men} = 50 \text{ kg} + (2.3 \text{ kg} \times \text{each inch more than 5 ft})$$

$$\text{Women} = 45.5 \text{ kg} + (2.3 \text{ kg} \times \text{each inch more than 5 ft})$$

A common method to estimate caloric requirements per day include simple weight-based algorithms as demonstrated in [Table 3](#). Usually, 40–60% of total energy requirements are supplied in the form of dextrose whereas another 20–30% is supplied as fat calories. The remainder is then composed of protein.

Amino Acids

Amino acids yield 4 kcal g^{-1} when oxidized for energy. Amino acid solutions are available in 10% or 15% concentrations. The higher concentration formulation is useful when fluid restriction is necessary. Standard amino acid solutions are a combination of essential and nonessential amino acids. Specialty amino acid formulations are also available for specific disease states. An example of this would be a formulation that contains a higher concentration of branched-chain amino acids, which may be useful in patients with hepatic disease complicated by encephalopathy.

Ascertaining goal protein requirements is vital in terms of maintaining lean body mass and promoting positive nitrogen balance. Degree of suspected catabolism, renal function, and hepatic function all play an important role in determining this. [Table 4](#) provides a listing of protein needs estimates based on clinical condition.

Reductions in protein doses may be required in cases of hepatic encephalopathy complicating hepatic failure. Likewise, renal insufficiency may also be a situation where lower amounts of protein can be used depending on the severity of renal failure and whether dialysis is indicated. IBW should be utilized in morbidly obese individuals to estimate protein needs.

Dextrose

Carbohydrate calories come in the form of dextrose, which provides 3.4 kcal g^{-1} of energy when metabolized. Although dextrose is available commercially in concentrations ranging

Table 3 Determination of energy needs

Condition	Need (kcal kg^{-1})
Overnourished/obese	20 (upper end IBW)
Maintenance	25
Undernourished	30
Stressed/critically ill	25

Table 4 Estimation of protein needs

Condition	Need ($\text{g kg}^{-1} \text{d}^{-1}$)
Mild stress	1.0
Moderate stress	1.2–1.5
Severe stress	1.5–2
Acute renal failure (no dialysis)	0.6
Hemodialysis/CVVHD	1.1–1.5
Peritoneal dialysis	1.2–1.5
Liver failure without encephalopathy	1.2–1.5
Liver failure with encephalopathy	~6.0

from 2.5% to 70%, a concentrated 70% solution is most commonly used in compounding parenteral nutrition formulas. Higher dextrose concentrations (greater than 10%) are used exclusively in central venous administration due to the high risk of thrombophlebitis in peripheral access.

Lipid Emulsions

Parenteral nutrition formulas contain lipid emulsions as the source for fat calories and essential fatty acids. In US, the lipid formulations are mostly composed of *n*-6 long-chain fatty acids derived from vegetable oils. Intravenous fat emulsion provides 10 kcal g^{-1} of energy due to the addition of the glycerol molecule.

Lipids are contraindicated in patients with significant hypertriglyceridemia. It is recommended that intravenous fat emulsion be withheld from the parenteral nutrition regimen if serum triglyceride concentration exceeds 400 mg dl^{-1} . Fortunately, acute pancreatitis due to intravenous fat emulsion-induced hypertriglyceridemia is rare unless serum triglyceride concentrations are greater than 1000 mg dl^{-1} .

Two polyunsaturated fatty acids, linoleic and α -linolenic, cannot be synthesized by the body and are therefore considered essential in that they have to be brought into the body via diet. Thus, to prevent essential fatty acid deficiency, 1–2% of daily energy requirements should be derived from linoleic acid and approximately 0.5% of energy from linolenic acid. Clinical manifestations of essential fatty acid deficiency are important to recognize and include scaly dermatitis, dermatitis, alopecia, hepatomegaly, thrombocytopenia, fatty liver, and anemia.

Electrolytes

Electrolytes are added as salts to parenteral nutrition solutions depending on the patient's requirements for daily maintenance and to replace losses. They are available commercially as individual salts or as combination products. Sodium and potassium can be added as chloride or acetate salts, depending on acid–base needs. In general, acetate and chloride content of the solution should be adjusted carefully because they help to maintain the acid–base balance. Also, calcium and phosphorus concentrations must be watched closely to prevent precipitation. Many factors may influence the solubility of these electrolytes in the parenteral nutrition solution,

Table 5 Contents of parenteral multivitamin preparations

Vitamin component	Current FDA requirements
Vitamin A	3300 IU
Vitamin D (ergocalciferol or cholecalciferol)	200 IU
Vitamin E	10 IU
Vitamin K	150 μg
Vitamin C	200 μg
Folate	600 μg
Niacin	40 mg
Vitamin B ₂	3.6 mg
Vitamin B ₁	6 mg
Vitamin B ₆	6 mg
Vitamin B ₁₂	5 μg
Pantothenic acid	15 mg
Biotin	60 μg

Table 6 Contents of a common parenteral trace element preparation

Component	Dose
Zinc	5 mg
Copper	1 mg
Manganese	0.5 mg
Chromium	10 μg
Selenium	60 μg

including the concentration of electrolytes, the pH of the final formula, temperature, and the presence of other components.

Vitamins

Multivitamin preparations, including both water- and fat-soluble vitamins, are available for inclusion in the parenteral nutrition formulation. Available parenteral multivitamin products for adults contain 12 or 13 known vitamins (with or without vitamin K). In addition, single vitamin products are available as well for use. **Table 5** lists the composition of standard adult multivitamin products.

Iron is not usually added to parenteral nutrition solutions because it can result in destabilization of the lipid emulsion. However, addition of iron in the form of iron dextran can be administered with 2-in-1 solution. A test dose of the iron should be given separately initially to ensure that there is no adverse reaction before it is incorporated routinely into a 2-in-1 bag.

Trace Elements

Commonly used trace elements in parenteral nutrition formulas include zinc, selenium, manganese, copper, and chromium. Intravenous trace element preparations are available as single component products as well as a variety of combination products. **Table 6** provides a list of the doses of trace elements in a common combination product. The commercially available multiple trace element combinations in the US contain three- to five- times the recommended doses for manganese,

and this is important to keep in mind in those patients on long-term parenteral nutrition.

Titration of Volume

Final volume can be titrated by the addition of sterile water for injection. Concentrated substrates may be used to minimize volume in those situations where patients need to be volume-restricted. Typical parenteral nutrition volumes range from 800 to 2500 ml daily.

Parenteral Nutrition Monitoring

Careful evaluation and monitoring of patients on parenteral nutrition is essential to accomplish effective and optimal nutritional therapy while avoiding complications. Changes in patient clinical condition necessitate frequent reassessment of diagnostic testing and therapies. Once parenteral nutrition is initiated, the possibility of enteral feeding should be entertained on a regular basis. Moreover, as parenteral nutrition is transitioned to enteral intake, tolerance should be closely monitored, allowing for weaning and eventual discontinuation of parenteral nutrition.

On initiation of parenteral nutrition, the patient's ability to tolerate the therapy should be carefully assessed via laboratory, clinical, and nutritional parameters. Concurrent organ dysfunction is critical to take into account – particularly derangements in renal, hepatic, or cardiopulmonary systems. As such, initial laboratory measurements should include a complete metabolic panel, which includes liver chemistries, electrolyte levels, as well as renal profile. Electrolytes including calcium, magnesium, and phosphorus should be monitored on a daily basis until the patient is clinically stable and these levels have been within acceptable range (Table 7).

Volume status is also an important parameter to follow in these patients, and intake and output should be continuously assessed. Key aspects of the physical exam looking for evidence

of volume overload include weight gain, increasing swelling of the extremities (peripheral edema) fluid accumulation in the abdomen (ascites), and crackles in lung bases indicating pulmonary congestion. Likewise, tachycardia, low blood pressure, poor skin turgor, and dry mucous membranes may indicate insufficient volume. In general, adult patients require 30 ml kg⁻¹ body weight daily to meet volume requirements. When devising a formula for a patient, urinary losses along with other losses such as diarrhea, nasogastric suction, emesis, fistulas, and other drainage losses (i.e., biliary drains) must be taken into account as they can significantly increase the need for additional fluid.

Blood glucose levels also need to be monitored very closely – particularly during the initial days of parenteral nutrition – to detect and to prevent hyper- and hypoglycemia. Coverage with subcutaneous sliding-scale insulin is frequently utilized to manage hyperglycemia; however, separate intravenous insulin infusion or the addition of insulin as a component of the total parenteral nutrition (TPN) could also be done.

Cyclic Parenteral Nutrition

When initiating parenteral nutrition in an acutely ill patient, continuous infusion over 24 h is generally selected. Once the patient has demonstrated stability on a given formula by way of physical exam, clinical parameters such as weight, volume status, and ins/outs as well as laboratory markers such as electrolyte balance and nutritional parameters, the patient can then be cycled to have the same volume of solution run over a shorter time frame. An example would be a 12-h period of parenteral nutrition being infused, allowing for the remainder of time off parenteral nutrition. Infusion pumps can aid in programming desired volumes and administration times. Although cycling does allow the patient to have more flexibility and therefore lead to a more active lifestyle, limitations include fluid or glucose intolerance. Abrupt cessation of the infusion may be associated with a rebound hypoglycemia due

Table 7 Suggested laboratory monitoring

Parameter	Baseline	Initiation	Critically ill patients	Stable patients
CBC with differential	Yes	–	Weekly	Weekly
PT, PTT	Yes	–	Weekly	Weekly
Electrolytes (Na, K, Cl, CO ₂ , Mg, Ca, PO ₄ , BUN, Cr)	Yes	Daily × 3	Daily	1 or 2 times per week
Serum triglycerides	Yes	Day 1	Weekly	Weekly
Transferrin or prealbumin	Yes	–	Weekly	Weekly
Serum glucose	Yes	Daily × 3	Daily	1 or 2 times per week
Capillary glucose		As needed	TID until consistently <200 mg dl ⁻¹	As needed
Weight	Yes	Daily	Daily	2 or 3 times per week
Intake and output	Yes	Daily	Daily	Daily unless fluid status assessed by physical exam
ALT, AST, ALP, total bilirubin	Yes	Day 1	Weekly	Monthly
Nitrogen balance	As needed	–	As needed	As needed

Source: Reprinted from Mirtallo JM (2001) Introduction to parenteral nutrition. In: Gottschlich MM (ed.) *The Science and Practice of Nutrition Support: A Case-Based Core Curriculum*, p. 221. Dubuque, IA: Kendall Hunt Publishing Company, with permission from the American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). A.S.P.E.N. does not endorse the use of this material in any form other than its entirety.

to the presence of circulating insulin; therefore, the rate of administration is often tapered down at the end of the cycle to allow for downregulation of pancreatic release of insulin.

Complications of Parenteral Nutrition

Complications associated with parenteral nutrition can be categorized as mechanical, metabolic, or infectious. Although infectious complications are the most frequently observed problem any time intravascular access is involved, potential mechanical complications specific to central catheter placement include pneumothorax, arrhythmias, catheter occlusion, thrombosis, and breakage. Radiological confirmation of line placement is necessary before initiating parenteral nutrition therapy.

Catheter Occlusion/Thrombosis

Catheter occlusion is the most common noninfectious complication associated with long-term venous access. Symptoms of a catheter-related venous thrombosis may consist of neck vein distension, edema, tingling, or pain over the ipsilateral arm and neck, and a prominent venous pattern over the anterior chest. It may require thrombolytic treatment or line replacement.

Intraluminal clotting as a result of inadequate flushing of the catheter or blood reflux can contribute to catheter occlusion. Also parenteral formulas with inappropriate calcium/phosphate amounts favoring precipitation as well as lipid residue collecting within the catheter lumen can also create an occlusive picture. Catheter flushing protocols should therefore be followed very closely to prevent the risk of occlusion.

Kinking of the catheter tubing or angulation of the catheter can also appear to be an occlusion issue. 'Catheter pinch-off' syndrome is a common phenomenon when a subclavian catheter is compressed between the clavicle and the first rib. Findings are confirmed radiologically and management involves catheter replacement.

Infection

Infection due to catheter-related bloodstream infection, another serious complication of parenteral nutrition is unfortunately quite common and underscores the importance of careful and meticulous catheter care and sterile technique. The Center for Disease Control and Prevention recently estimated 250 000 cases of catheter-related bloodstream infections annually in the US. Moreover, there was an average of 5 cases per 1000 catheter days and mortality ranged between 12% and 25% for these infections. Infection with fungal pathogens, in particular, has an extremely high rate of mortality, ranging from 30% to 60%.

Fever and unexplained hyperglycemia can herald the presence of catheter-related infection. Usually there are no external clues of catheter infection at the insertion site. When a catheter-related infection is suspected, blood cultures should be obtained from each lumen of the catheter as well as from the peripheral blood before antibiotics are started. The most

common micro-organisms associated with these types of infections are coagulase negative staphylococci, *Staphylococcus aureus*, *Klebsiella*, *Candida*, and *Enterobacter*.

Treatment of catheter-related infection usually entails the removal of the access device as well as a course of appropriate antibiotics or antifungal therapy. In those patients on long-term home parenteral nutrition with limited venous access, catheter salvage is certainly important and can be considered with the help from an Infectious Disease Consultant. Parenteral nutrition should be withheld if there is any suspicion of infection to prevent worsening bacteremia due to infusion of hypertonic dextrose and other nutrients. A 5% dextrose solution can be given instead through a peripheral line until the infection has been adequately treated.

Metabolic

Metabolic complications are more commonly seen in patients undergoing parenteral nutrition therapy as opposed to enteral nutrition and can be seen in 5–10% of parenteral cases. Hyperglycemia is the most common complication associated with parenteral nutrition use. It is commonly related to the proportion of dextrose in the formulation, but other factors which may contribute include stress due to acute illness or sepsis (causes increased gluconeogenesis and glycogenolysis and suppressed insulin action), concomitant use of steroids, postoperative period, diabetes history, and pancreatitis. Excess sugars through parenteral nutrition can lead to hepatic steatosis, increased carbon dioxide production, and adverse outcomes. Although there is no consensus as to optimal blood glucose concentration in hospitalized acutely ill patients on parenteral nutrition, most clinicians attempt to maintain values less than 150 mg dl⁻¹.

Blood glucose levels should be monitored very closely and a sliding-scale coverage of regular insulin can be used for elevated levels. Two-thirds of the total amount of sliding-scale insulin required over 24 h can be added to the next day's parenteral nutrition formulation.

However, hypoglycemia can result from the abrupt cessation of parenteral nutrition. To decrease the risk of rebound hypoglycemia, a 1-to-2-h taper of the rate of parenteral nutrition may be required. Alternatively, if the infusion must be discontinued quickly, a 5% or 10% dextrose infusion should be utilized for at least an hour once the parenteral nutrition infusion is discontinued to avoid a possible rebound hypoglycemia.

To avert hypertriglyceridemia intravenous fatty emulsion intake should be limited to less than 30% of total calories. Hypertriglyceridemia may increase the risk of pancreatitis, particularly in those with triglyceride levels greater than 1000 mg dl⁻¹. It is generally recommended that lipid infusion be discontinued when serum triglyceride concentration exceeds 400 mg dl⁻¹.

Electrolyte disturbances also commonly occur and warrant close clinical evaluation. For example, hyponatremia can represent sodium deficiency or water excess. Electrolyte losses or shifts can be a direct result from renal or gastrointestinal losses, hormonal imbalances, medication use, or acid-base disturbances. Accumulation of electrolytes may occur with

fluid or acid–base shifts, renal insufficiency, or overzealous exogenous replacement. Generally a consistent parenteral nutrition formula is recommended, with additional electrolyte replacements provided separately from the parenteral nutrition. Lower concentrations or even elimination of selected electrolytes from parenteral nutrition are indicated in patients with renal failure.

One phenomenon seen in severely malnourished patients who are given aggressive nutritional support particularly in the early days of therapy is the refeeding syndrome. The rapid delivery of calories in the form of carbohydrates stimulates insulin secretion, which, in turn, causes an intracellular shift of phosphorus, magnesium, and potassium. This intracellular movement results in profound hypophosphatemia, hypomagnesemia, and hypokalemia.

Clinically, the patient develops symptoms of generalized fatigue, lethargy, muscle weakness, edema, cardiac arrhythmia, and hemolysis. In addition to the muscle weakness from refeeding syndrome, increased dextrose loads also increase carbon dioxide production and both of these contribute to higher predisposition for respiratory failure. Prevention of this phenomenon is achieved by identifying individuals at risk, repleting electrolytes before the initiation of parenteral nutrition, and slow advancement of parenteral nutrition with careful daily monitoring of electrolytes, including phosphorus and magnesium levels as well as weights, and fluid intake and output.

Hepatic Injury

There are three types of hepatobiliary conditions that may develop from parenteral nutrition therapy. Steatosis, which is characterized by fat accumulation in the liver, is generally a benign disorder and is seen in the setting of overfeeding. Overfeeding either combined or individual energy components (dextrose, fat, and amino acids) can promote hepatic fat deposition through insulin-triggered mechanisms of lipogenesis and inhibition of fatty acid oxidation. Most patients are asymptomatic and usually transaminases are mildly elevated. Progression to fibrosis and subsequently cirrhosis may occur rarely in long-term parenteral nutrition.

Parenteral-associated cholestasis is characterized by impaired bile secretion or biliary obstruction and presents as an elevation of alkaline phosphatase, γ -glutamyl transpeptidase, and conjugated (direct) hyperbilirubinemia. Cholestasis is universal in patients receiving parenteral nutrition for more than 6 weeks without enteral feedings. This cholestasis is a serious complication because it may progress to cirrhosis and ultimate liver failure.

Lastly, lack of enteral feeding that results in decreased cholecystokinin release, which, in turn, leads to impaired bile flow and gallbladder contractility, is what causes the development of gallstones and gallbladder sludge with subsequent cholecystitis. The duration of parenteral nutrition therapy appears to correlate with the development of biliary sludge.

When a patient on parenteral therapy develops hepatic complications, close scrutiny of the formula must be performed. A trial of decreasing calories to avoid overfeeding should be considered. A balanced carbohydrate:fat ratio needs

to be obtained with approximately 70–85% of nonprotein calories supplied as carbohydrates and 15–30% as fat. In addition, carbohydrate content should not be greater than 7 g kg day^{-1} in adults. Infusing parenteral nutrition over a cyclic period rather than as a continuous 24-h infusion may also reduce the risk of liver injury from parenteral nutrition by providing a rest period for the liver's macronutrient processing. Early transition to enteral or oral feedings should be optimized because even small amounts of intake would promote enterohepatic circulation of bile acids. Medications such as ursodiol (ursodeoxycholic acid) can be used to facilitate bile flow and improve biochemical markers and pruritus. Unfortunately there is no evidence to indicate that progression of liver disease is delayed.

Bone Disease

The prevalence of metabolic bone disease is quite high in patients on long-term parenteral nutrition and is an important issue because this contributes to significantly increased morbidity due to fracture risk. Osteoporosis was seen in 41% of patients after at least 6 months of home parenteral nutrition in one study.

Factors attributed to bone loss are multifactorial and poorly defined. Metabolic bone disease may develop due to underlying disease, inadequate intake of calcium particularly because the amount that can be included into the parenteral nutrition formulation is physically limited due to risk of precipitation and hypercalciuria. Higher protein levels in parenteral formula further enhance hypercalciuria, therefore reducing protein intake to maintenance doses is recommended. Both vitamin D deficiency and vitamin D excess can be contributing factors. Large amounts of vitamin D can cause suppression of parathyroid hormone secretion, which then directly promotes bone resorption. Calcium balance and phosphorus levels also play a critical role in the development of metabolic bone disease.

For selected long-term parenteral nutrition patients, treatment with bisphosphonates and calcium and vitamin D supplementation should be considered to prevent the development of complications. An increase in weight-bearing activity as well as avoidance of corticosteroids may also be useful in promoting improved bone health. Careful attention to magnesium, phosphorus, and calcium concentrations in the parenteral nutrition formula to ensure that there are adequate amounts and establishing a good acid–base balance are also critical to preventing metabolic bone disease. Once again, maintenance protein ($1 \text{ g kg}^{-1} \text{ day}^{-1}$ as opposed to higher levels) would reduce the rate of metabolic bone disease by decreasing renal losses of calcium.

Home Parenteral Nutrition

Home parenteral nutrition is a viable option when it has been determined that a hospitalized patient who was begun on parenteral nutrition is not able to advance to enteral or oral feedings, but the patient's medical condition is stable. The patient and/or caregiver should be taught to administer the

parenteral nutrition safely utilizing sterile technique, and they need to be actively watching for signs of infection, fluid imbalances, or other complications. Cycling of the parenteral nutrition so that it runs only at night allows the patient increased mobility and flexibility during the day. This may also help minimize hepatobiliary complications.

Conclusions

Parenteral nutrition as a way to provide essential nutrients is a potentially lifesaving therapy for individuals who are unable to utilize their gastrointestinal tract for a prolonged period of time. Although significant complications may result from this form of therapy, if patients are chosen appropriately and simple precautions such as sterile techniques and close attention to components of the parenteral formulation are taken, these patients would benefit greatly. Management of parenteral nutrition is most effective when done through a multidisciplinary approach, utilizing the expertise of physicians, pharmacists, dietitians, nurses, case managers, and social workers.

Further Reading

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PEDIATRIC FEEDING DISORDERS

Feeding Children Who Can't or Won't Eat

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Glossary

Antecedents In behavioral learning theory, antecedents are conditions present in the environment before the occurrence of a targeted behavior that influence the probability of its occurrence. Antecedent conditions cue, trigger, or set the stage for the targeted behavior to occur or not to occur.

Colonoscopy Endoscopic examination or therapy of the luminal (internal) surface of the large intestine.

Consequences In behavioral learning theory, consequences are conditions that occur following a targeted behavior that influence the probability of its reoccurrence. These can either increase or decrease the frequency, intensity, or duration of the targeted behavior.

Endoscopy Internal examination of a hollow organ of the body; conventional endoscopy refers to examination of the upper gastrointestinal tract (esophagus, stomach, duodenum).

Eosinophilic gastroenteropathy An allergic inflammatory condition of the intestinal tract, most commonly involving the esophagus.

Escape/avoidance (also known as negative reinforcement) In behavioral learning theory, escape/avoidance refers to the removal of aversive stimulation, or

removal of the individual from an aversive situation, immediately following the occurrence of the targeted behavior in order to increase its frequency, intensity, and duration.

Extinction In behavioral learning theory, extinction refers to the termination of positive reinforcement for the target behavior resulting ultimately in a decrease or cessation of the behavior.

Pediatric feeding disorders Feeding children who can't or won't eat.

Positive distraction Use of pleasurable activities to reduce anxiety by diverting an individual's attention away from an aversive event.

Positive reinforcement In behavioral learning theory, positive reinforcement refers to the application of pleasurable or rewarding stimulation immediately following the occurrence of a targeted behavior in order to increase its frequency, intensity, and duration.

Response cost In behavioral learning theory, response cost refers to the removal of pleasurable or rewarding stimulation, or the individual's access to such stimulation, immediately following the targeted behavior in order to decrease its frequency, intensity, and duration.

Introduction

Feeding is the process by which children accept and digest food in amounts adequate to meet their nutritional needs for proper growth. Though seemingly a simple instinctive act, feeding children is actually a complex process requiring a combination of successful caregiver interaction along with the child's appropriate oral-motor skills, and intact gastrointestinal motility and absorption. The term 'feeding disorder' is applied to situations where young children are unable or unwilling to eat enough to maintain their nutritional needs. The Diagnostic and Statistical Manual of Mental Disorders (DSM IV), a compendium of diagnoses and the related criteria, more specifically defines pediatric feeding disorders as "persistent failure to eat adequately as reflected in significant failure to gain weight or significant weight loss over at least one month." Feeding disorders are surprisingly common in children, and it has been reported that 25–35% of normal

children will have mild feeding disorders, and up to 75% of children with disabilities will have more severe feeding problems. Clinical manifestations include food refusal/selectivity, gagging, vomiting, swallowing difficulty, poor weight gain, or failure to thrive. Conventionally, these disorders have been grouped into medical, oral-motor, and behavioral categories with most children exhibiting overlapping problems. Therefore, it is more realistic to think of these disorders as a continuum from strictly physiologic defects to solely behavioral, with the vast majority of these disorders as a combination of many etiologies.

Certain groups may be at a higher risk for feeding difficulties. For example, children with food allergies may have accompanying gastroesophageal reflux, eosinophilic gastroenteropathy, and motility disorders that often result in food refusal. A variety of medical conditions such as cardiopulmonary, genetic, and metabolic disorders can lead to poor appetite and slow weight gain. Oral-motor and

swallowing problems are commonly seen in children with congenital and acquired neurologic conditions such as cerebral palsy, structural abnormalities, or traumatic brain injury. Premature and medically fragile infants may miss sensitive periods of oral-motor development resulting in delayed acquisition of feeding skills. This early interruption of feeding skill development and lack of experience can lead to oral aversion or more serious feeding disorders such as food refusal.

Behavioral difficulties such as food refusal or selectivity are not isolated problems. More often they develop when medical illness adversely affects feeding patterns and caregiver interactions. If a child is failing to thrive, the most immediate solution to address the lack of weight gain and growth is to start supplemental enteral feedings via a nasogastric or gastrostomy tube. However, this supplemental feeding often results in a decrease in oral intake, which may adversely impact hunger, feeding experience, and endurance.

Medical issues (i.e., GE reflux, cleft palate, etc.) that occur very early in infancy may be the initial cause for food refusal. Consequently, for the majority of children with a feeding disorder, an early food avoidance pattern is common. As a result, the parent-child interaction may be severely impacted. For example, because of severe gastroesophageal reflux, the child may learn to associate eating with pain. Consequently, when the parents attempt to feed the child, they will encounter food refusal behavior, which leads most parents to terminate the meal prematurely. At this point, the child not only has associated food with pain but also now has learned that by manifesting food refusal behavior the meal will be terminated. Thus, even when the reflux is medically managed, the child will still have the learned history of pain associated with eating, but now also has the new history of having refusal behaviors to escape the meal. Thus, an inadvertent disorder in parent-child mealtime interactions has developed as a result of medical disease.

Normal Development of Feeding and Swallowing

To understand feeding and swallowing disorders, one must recognize that there are dynamically changing developmental skills and social abilities in the growing child. Progression through the normal stages of feeding (see Table 1) requires the attainment of physical abilities such as postural stability, oral-motor coordination, and sensory awareness. In addition, factors such as emerging cognitive skills play an important role in an effective caregiver-child feeding interaction.

The Swallowing Process

Understanding the mechanisms involved in eating is useful in understanding why a child refuses to eat. The swallowing process is conventionally divided into three phases: oral, pharyngeal, and esophageal. The oral phase includes the oral preparatory phase and the oral phase of the swallow. The oral preparatory phase occurs when food is manipulated in the mouth and masticated if necessary, reducing it to a consistency ready for swallowing. This phase of swallowing

Table 1 Developmental feeding summary

<i>Age</i>	<i>Skill</i>
Birth to 4–6 months	Bottle feeding/breast-feeding Coordination of suck–swallow–breathe
4–6 months	Accepts smooth pureed food from a spoon Holds own bottle
6–9 months	Finger feeding is introduced Vertical munching of easily dissolvable solids
9–12 months	Cup and straw drinking of liquids Eats lumpy and mashed foods Finger feeds dissolvable solids Rotary jaw movements emerging
12–18 months	Self-feeding with spoon emerging Independent with finger feeding Holds a cup with two hands
18–24 months	Chewing a broad range of table foods Independent with spoon feeding
24–36 months	Circulatory jaw rotations Holds a cup with one hand Eats a wide range of solid food Independent with using a fork

includes the transit of the bolus over the tongue posteriorly until the pharyngeal swallow is triggered. In the newborn and young infant, all phases are driven reflexively by typical rooting and sucking behavior. As children age, the oral phase of sucking, chewing, and managing food comes under more voluntary control, requiring cortical integration of sensory/motor input to coordinate the complex patterns of jaw, tongue, and oral movements. The pharyngeal phase begins when swallow is triggered and the bolus is moved through the pharynx into the esophagus. During the pharyngeal phase of swallowing, the velum elevates to prevent food from entering the nasal cavity, the hyoid and larynx elevate and move anteriorly, the epiglottis tilts to prevent the material from entering the airway, the cricopharyngeal sphincter allows the material to pass from the pharynx to the esophagus, and the base of the tongue retracts to the posterior pharyngeal wall to deliver the bolus to the pharynx. The esophageal phase begins with the opening of the upper esophageal sphincter. Esophageal peristalsis then carries the bolus into the stomach. After passing the lower esophageal sphincter, food enters the stomach, beginning the gastrointestinal and absorptive phase of feeding. Food is emptied from the stomach based on the volume, nutrient composition, and caloric density of the meal. Poor coordination, timing, or motor dysfunction during any phase of swallowing may lead to aspiration.

Classification of Feeding Disorders in Children

A single underlying cause of why children refuse to eat enough to sustain normal growth is rarely evident, and therefore, this problem presents a significant diagnostic and therapeutic challenge to clinicians and parents alike. Given the complexity of this challenge, numerous attempts at classifying feeding disorders have been attempted on the basis of the apparent etiology, physical condition, or associated behaviors. Because most feeding disorders are the result of multiple factors (i.e.,

physical, motivational, skill, and parent–child relationships), a more functional classification has been developed that allows the differentiation of patient types by symptoms rather than an arbitrary disease-based diagnostic approach (see Table 2).

Children with food refusal who require any kind of enteral tube feed would be categorized as ‘food refusal-enteral tube dependent’, whereas a child who drinks the majority of his or her calorie requirement would be considered ‘food refusal-liquid dependent’. Another feeding problem category is ‘food selective-type’. In this category, children would eliminate a significant percentage of the four basic food groups. Typically, a child with this categorization would have the skill to eat but chooses to eat only one or two different foods and refuses all other foods. The child may or may not be able to sustain normal growth with this kind of diet. A child who does not eat an age-appropriate texture of food due to lack of skill or oversensitivity to a particular food texture would be classified as ‘food-selective-texture’. A classic example of texture selectivity occurs in a 5-year-old child consuming only purees when the child should have normal oral motor skills to process regular textured food. Again, the child may or may not sustain normal growth.

Assessment

An appropriate assessment of a child’s feeding disorder is the critical first step in initiating treatment. The management of complicated feeding disorders usually requires a multi-disciplinary team devoted to establishing diagnosis, assessment of the need, and developing a thorough treatment plan. This team should include a variety of pediatric specialists, including physicians (e.g., general pediatricians, developmental pediatricians, pediatric gastroenterologists, allergists, and otolaryngologists), nurse practitioners, nutritionists, occupational therapists, speech therapists, psychologists, and social workers. The assembled team must begin its approach to diagnosis and therapy with complete history taking by all interested parties. These include a careful prenatal, birth, and neonatal history. Determining the nutritional and medical status of the child must be accompanied by an appropriate psychological and developmental pediatric evaluation.

Physicians

An important goal of the physician history taking is to assess for any comorbid conditions that would require treatment before the implementation of a therapeutic treatment program

for the food refusal (see Table 3 for comorbid conditions). As part of the initial evaluation, an observation of a feeding session between the child and the primary caregiver will often offer insight into the feeding problem, especially from an oral–motor/sensory and behavioral perspective. Clinical signs of oral–motor dysfunction, length of meals, and nature of the caregiver–child interaction are all noted. Observation of the muscle tone, posture and positioning, as well as special seating systems and feeding devices are routine, as these can give insight into the child’s overall neurologic functioning. Physical examination of the child includes a general survey examination for the determination of any underlying medical disorders that may preclude safe feeding. These include evaluation of tongue and jaw movement, dentition, airway sounds, speech, and oral cavity assessment. Additionally, a complete physical examination including cardiac, pulmonary, and abdominal exams is mandatory.

Diagnostic Testing

Diagnostic evaluations may be warranted to better assess swallowing and anatomy (see Table 4). The modified barium swallow (MBS) study is the most widely used procedure to assess oral, pharyngeal, and upper esophageal phases of swallowing. Positioning, food texture, bolus size, rate, and the amount of food presented can be manipulated during the performance of the MBS study to determine the safest and most efficient method of feeding. Clinical evaluation before the MBS is essential so that the food textures, liquid consistencies, and treatment techniques can be included at the time of the study. Changes in head and neck position, such as chin tuck, should be tried before the actual study to better correlate clinical and radiologic findings. The child’s level of cooperation should also be assessed before the MBS.

Additionally, a standard upper gastrointestinal contrast series utilizing barium is required for assessment of anatomy of the gastrointestinal tract (Figure 1). The children with food allergies, repetitive vomiting, or abdominal pain will need endoscopic evaluation, many of whom will also need colonoscopy to rule out the possibility of underlying inflammatory disease such as eosinophilic gastroenteropathy. Some of the children assessed will need cranial imaging such as computed tomography or magnetic resonance imaging to look for evidence of intracranial mass lesions, hydrocephaly, or posterior fossa anomalies, such as the Chiari malformation. Fiberoptic endoscopic evaluation of swallowing (FEES) allows for direct visualization of the nasal, pharyngeal, and laryngeal structures. This procedure enables evaluation of events occurring immediately before and after the pharyngeal phase of swallow. FEES allows for visualization of any residue in the valleculae and pyriform sinuses, and at times, it may be possible to see aspirated materials below the level of the true vocal folds. This procedure, however, does not provide information on the oral phase of swallowing. FEES may also be combined with sensory testing (FEESST). Pulses of air are directed at mechanoreceptors within the larynx and reactions are observed that show potential sites of impairment. In older children, this could guide different swallowing techniques to prevent coughing, choking, or aspiration.

Table 2 Common feeding disorder symptoms

Food refusal – partial/total
Liquid dependent
Enteral tube dependent
Food selectivity
Texture
Type

Table 3 Medical conditions associated with pediatric feeding disorders

Disorders of the oral and pharyngeal phases of swallowing

Anatomic lesions

Cleft lip or palate

Pierre-Robin sequence

Choanal atresia

Laryngeal clefts

Macroglossia

CHARGE association

Acquired structural abnormalities

Dental caries

Tonsillar hypertrophy

Viral/inflammatory stomatitis

Retropharyngeal mass

Candida stomatitis

Cardiopulmonary effects

Chronic lung disease

Complex congenital heart disease

Reactive airway disease

Tachypnea

Neuromuscular disorders

Familial dysautonomia

Cerebral palsy

Pseudo-bulbar palsy

Bulbar atresia or palsy

Cranial nerve anomalies

Muscular dystrophic disorders

Arnold–Chiari malformation

Myelomeningocele

Intracranial mass lesions

Disorders of the esophageal phase of swallowing

Anatomic lesions

Esophageal atresia

Cricopharyngeal achalasia

Tracheoesophageal fistula

Esophageal mass

Esophageal stricture

Esophageal web

Esophageal rings

Vascular rings/aberrant vessels

Foreign bodies

Disorders of the lumen

Peptic esophagitis

Candida esophagitis

Viral esophagitis

'Pill' esophagitis

Inflammatory bowel disease

Behcet syndrome

Motility disorders

Achalasia

Diffuse esophageal spasm

Chronic pseudo-obstruction

Systemic lupus erythematosus

Polymyositis

Genetic disorders

Prader–Willi syndrome

Trisomy 21

Cornelia de Lange syndrome

Velo-cardio-facial syndrome

Rett syndrome

Metabolic disorders

Urea cycle abnormalities

Hereditary fructose intolerance

Table 3 Continued

Hypothyroidism

Miscellaneous

Gastroesophageal reflux

Constipation

Gas-bloat syndrome

Dumping syndrome

Food allergies

Sensory loss (visual/auditory impairment)

Table 4 Diagnostic evaluation for patients with feeding disorders

Detailed history and physical examination

Upper gastrointestinal contrast radiography

Esophogram

Small bowel follow-through

Modified barium swallow study

Gastric emptying study

pH monitoring

Esophagogastroduodenoscopy with biopsies

Antroduodenal manometry

Fiberoptic endoscopic evaluation of swallowing (FEES)

CBC

Comprehensive metabolic panel

Thyroid function

RAST analysis for food allergies

Skin test for food allergies

Plasma amino acids

Urine organic acids

Karyotype

Increasingly important is the need for allergy evaluation. Assessment of specific food-related allergies is currently difficult and often involves appropriate skin testing as well as the appropriate radioallergosorbent test (RAST) testing to look for evidence of food allergy. Reliability of these allergy testing modalities is age dependent, and therefore, clinical manifestations of allergy play a major role in the potential diagnosis of food-related allergies. Consultation with a pediatric allergist is often necessary to conduct food elimination diets and appropriate food challenges.

Oral–Motor Therapists

This term refers to the team member whose responsibilities include assessing and treating oral–motor and swallowing dysfunction and performing instrumental evaluations of swallowing when warranted. This may be either an occupational therapist or a speech/language pathologist depending on the individual's training and local facility. Occupational therapists may also evaluate fine motor, sensorimotor, and visual motor function as well as positioning and the need for adaptive equipment. Speech/language pathologists may evaluate and make recommendations for communication skills when necessary.

Nutritionists

Nutritionists dedicated to pediatric care are also essential members of the diagnostic team. The nutritionist's assessment

(Continued)

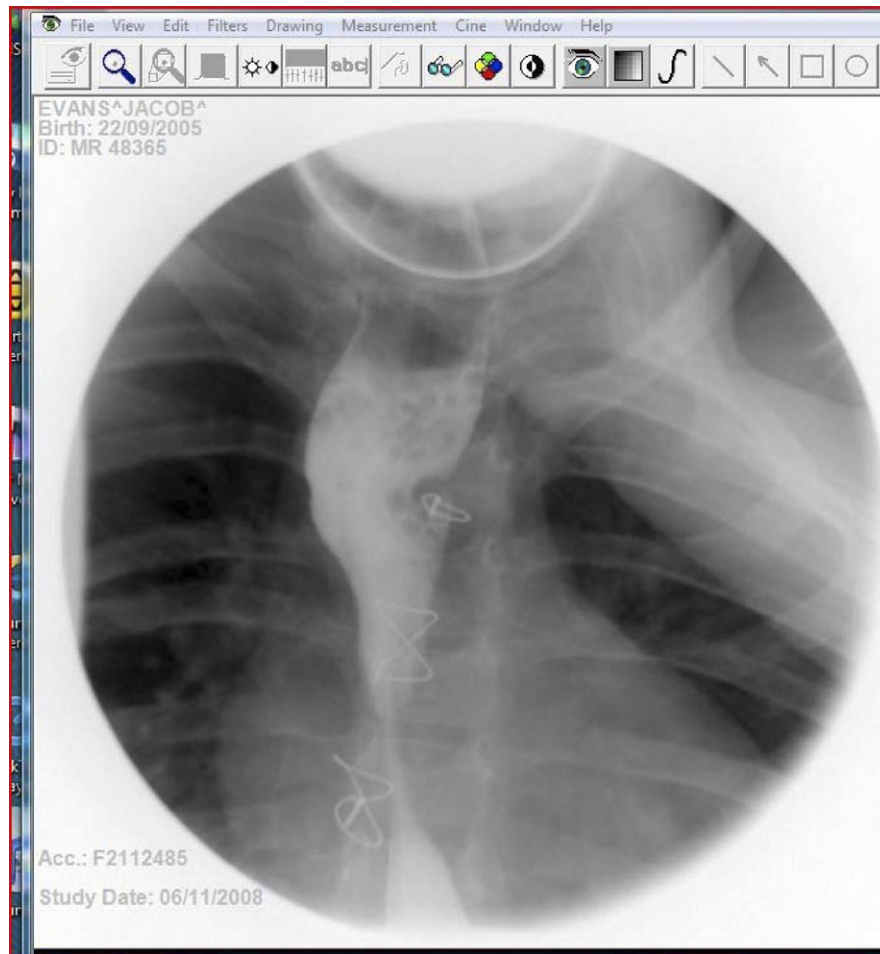


Figure 1 Abnormal esophagus secondary to esophageal stricture following repair of esophageal atresia.

of the current nutritional status, anticipated growth, and recommended energy intake (age and diagnosis appropriate) is an essential part of the diagnostic and therapeutic process.

Behavioral Psychologists

Behavioral psychologists, through detailed observations, develop an analysis of variables that may be controlling food acceptance and refusal behaviors. An integral part of the psychologist's approach is performing an in-depth assessment of the child's emotional and behavioral patterns in regard to eating. The goal of an assessment is to help the clinician identify what behaviors (e.g., spoon batting) and affective responses (e.g., anxiety) have been inadvertently learned by a child with regard to eating. Strategies are then developed to reduce mealtime anxiety, reduce or eliminate mealtime escape/avoidance behaviors, and reward eating. These behavioral strategies are then integrated with other needed interventions such as oral-motor and nutritional strategies into a comprehensive mealtime treatment plan, or 'feeding protocol.' After the child has learned new and more adaptive feeding behaviors and skills, and their medical/nutritional status has improved, the feeding protocol is taught to parents and other caregivers.

Social Workers

As the medical issues, behavioral needs, and family psychodynamics play a central role in the development of the abnormal feeding patterns, a clinical social work evaluation is necessary for assessment and treatment of underlying familial interactions and support systems. These assessments and planning help to ensure continued success once the child has returned to the home environment.

Treatment of Feeding Disorders

The goal of all therapy is directed toward allowing parents to safely feed their children in a developmentally appropriate manner. The physician in the treatment team must ensure that all appropriate diagnostic studies have been performed to determine whether an underlying medical condition has predisposed a child to developing an unusual feeding pattern. This includes appropriate utilization of consultants and diagnostic modalities (Table 4). Once these studies have been performed, the physician must coordinate all the resources and direct care so that feeding therapy may proceed with

minimal risk to the patient, keeping the child safe from aspiration and other complications.

The initial and perhaps most important part of any therapeutic approach to introducing or increasing oral food intake is to establish the safety of eating as well as the types and textures of food the child can consume most efficiently. Approaches to therapy are often described as nutritive or non-nutritive. Nonnutritive oral stimulation is performed to reduce hypersensitivity, facilitate management of secretions, establish or retrain the swallowing mechanism, maintain coordination of breathing and swallowing, and develop oral movement for sound production and communication.

Objectives for a nutritive approach include increasing oral intake, advancing food texture, transitioning to utensil use, and improvement of self-feeding. Oral-motor techniques to improve muscle strength and postural control as a foundation for feeding and swallowing are largely based on a neurodevelopmental framework. The use of adaptive seating systems is a key component to feeding a child with physical disabilities that require external devices to provide head, neck, and trunk support. Attention must be paid as to how positioning affects the feeding process, as a change in head and neck posture and oral-motor structures may affect oral-motor control.

Once airway safety, positioning, and sensitivity have been controlled, a variety of treatment approaches have been suggested for children with feeding disorders.

As noted in the preceding text, behavioral interventions for pediatric feeding disorders are the most common modality of therapy and are often included within an interdisciplinary team approach that also addresses physiology, oral-motor functioning, parent-child interactions, and community or social support.

In general, behavioral strategies are presented in a sequence beginning with the use of the most positive and least intensive strategies that have a reasonable expectation of being effective. These less intensive strategies also have the advantage of minimizing deviations from the norm in mealtimes for most families. More intensive behavioral strategies are utilized as needed. Although these more intensive strategies do require significant deviations from the norm of typical family meals, these procedures can usually be faded over time. Behavioral interventions are most typically a mixture of antecedent and consequence-based treatment packages. Some antecedent interventions would include the establishment of a systematic feeding routine (i.e., same time and place to eat), reducing or increasing the level of texture of food (i.e., puree vs. chopped fine), presenting a preferred food along with a nonpreferred food, and positive distraction to reduce mealtime-related anxiety; consequence-based treatments may include rewarding appropriate eating behavior (i.e., positive reinforcement), ignoring food refusal behavior (i.e., escape extinction), or removing positive reinforcement in response to food refusal behavior (i.e., response cost). Thus, if a child accepted a bite, he or she would be rewarded with attention or a tangible reinforcer, such as a toy or music. If the child engaged in food refusal behavior, such as batting at the spoon or turning their head away from the food, the consequence would be to withdraw attention or remove a toy and continue to present the bite to the child until it was accepted. If the child continued to refuse by expelling the food, this refusal behavior

may be responded to by representing the expelled bite of food back to the child. Some children also continue to refuse food by holding the bite of food in their mouths. This form of food refusal behavior can also be decreased or eliminated by moving or redistributing the food from between the child's cheek and teeth onto the tongue where it is more likely to be swallowed. Finally, training the parents in the use of the various feeding techniques becomes critical in maintaining long-term treatment gains. Skill-based parent training involving step-by-step criteria-based training has been shown to be superior to didactic methodology. Parent training, including instruction, discussion, handouts, role-playing, feedback, and the practice of techniques and videotape with a trained clinician can result in increased parent treatment integrity.

Conclusion

Despite the increased awareness of feeding disorders in young children, there remain many challenges in implementing the specialized treatment necessary for these children. Foremost among these challenges is the recognition by the patient's primary provider that disordered feeding is indeed compromising the child. The primary provider is often lulled into complacency by a 'normal' growth curve or assumes that feeding disorders are a normal, transient occurrence in childhood and misses the opportunity for early intervention. Children who can't or won't eat require a systematic diagnostic and therapeutic approach by a team of dedicated professionals. The goal of safe oral feeding is attainable in most children when those involved in the care of children understand the complexity of eating and the associated medical and psychological conditions that comprise a feeding disorder.

Not insignificant in the diagnosis and management of children with feeding disorders is the financial burden associated with diagnosis and therapy. However, helping these children to eat will allow independence from artificial sources of nutrition such as gastrostomy feeds and parenteral nutrition, and ultimately reduce the total cost of health care for these children.

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PHOSPHORUS

Physiology, Dietary Sources, and Requirements

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Glossary

Fibroblast growth factor 23 (FGF 23) This newly recognized hormone is synthesized by bone cells, namely osteoblasts and osteocytes, to act on the renal tubules to increase the secretion of phosphate ions into urine.

Hyperphosphatemia Elevated serum phosphate concentration may contribute to arterial calcification and chronic renal disease.

Parathyroid hormone (PTH) This hormone has a major role in the regulation of serum calcium concentration by its actions on bone and kidney.

Phosphate additives Several types of phosphate salts are used by the food industry to help maintain the physical properties of foods, including preservation. These additives are used in baked goods, meats, cola beverages, and other processed foods.

Vitamin D The hormonal form of this nutrient or skin biosynthesized molecule aids in calcium and Pi metabolism by increasing the intestinal absorption of these ions, and thereby it enhances bone formation.

Introduction

The consumption of a diet sufficient in phosphorus, in the form of phosphate salts or organophosphate molecules, is critical for the support of human metabolic functions. Too much phosphorus, in relation to too little dietary calcium, may contribute to bone loss, and too little phosphorus along with too little dietary calcium may not adequately maintain bone mass, especially in the elderly period of life. Therefore, under normal dietary conditions, dietary phosphorus is sufficient for numerous metabolic functions; it is only when too much or too little phosphorus is ingested that skeletal problems may arise. Much like calcium, elderly subjects need to consume sufficient amounts of phosphorus, like calcium, in order to maintain bone mass and density. Excess phosphorus may contribute to inappropriate elevations of parathyroid hormone (PTH) and bone loss. It is not yet clear where most elderly subjects fall along this continuum of intake patterns. This review delves into the mechanisms by which phosphate ions impact on calcium metabolism and skeletal integrity.

Calcium–Phosphate Interrelationships

Phosphorus in the form of phosphate ions is essential for numerous metabolic and structural functions. Phosphate metabolism is intricately linked to that of calcium because calcium-regulating hormones, i.e., PTH and 1,25-dihydroxy-vitamin D, also have direct or indirect effects on phosphate homeostasis by virtue of their actions on bone, the small intestine, and the kidneys. Adequate phosphorus and calcium intakes are needed not only for skeletal growth and

maintenance, but also for many cellular roles, such as energy production, i.e., adenosine triphosphate (ATP). Phosphate ions are incorporated in many organic molecules, including phospholipids, creatine phosphate, nucleotides, and nucleic acids.

Dietary Sources of Phosphorus

Phosphorus, as phosphates, is especially rich in animal products, including meats, fish, poultry, eggs, milk, cheese, and yogurt. Lesser amounts of phosphorus can also be obtained from cereal grains and many vegetables, including legumes. Because of the abundance of phosphorus in the food supply, deficiency is highly unlikely except perhaps late in life when some elderly individuals consume little food. (An extremely rare deficiency disease, hypophosphatemic rickets, occurs in infants because of inadequate phosphorus intakes.)

In the USA mean phosphorus intakes approximate 1200–1500 mg per day in adult males and 900–1200 mg per day in adult females. Daily phosphate intakes have increased as a result of phosphate additives in processed foods and cola beverages. Since no federal requirements exist to list quantities of added phosphates on labels of foods and cola beverages, the actual additional amounts consumed can only be estimated. Phosphate additives used by the food industry may be found in baked goods, meats, cheeses, and other dairy products. A conservative estimate is that most adults in the USA consume an extra 200–350 mg of phosphorus each day from these sources and cola beverages. Therefore, the total phosphorus intakes for men and women are increased accordingly. Because typical daily calcium intakes of males are 600–800 mg

Table 1 Calcium and phosphorus composition of common foods

Food category	Phosphorus mg per serving	Calcium mg per serving	Ca:P ratio (wt:wt)
<i>Milk, eggs, and dairy</i>			
Cheddar cheese, 1 oz	145	204	1.4
Mozzarella cheese – part skim, 1 oz	131	183	1.4
Vanilla ice milk, 1 cup	161	218	1.4
Low-fat yogurt, 1 cup	353	448	1.3
Skim milk, 8 oz	247	301	1.2
Skim milk – lactose reduced, 8 oz	247	302	1.2
Vanilla ice cream, 1 cup	139	169	1.2
Vanilla soft-serve ice cream, 1 cup	199	225	1.1
Egg substitute, frozen, 1/4 cup	43	44	1.1
Chocolate pudding, 5 oz	114	128	1.1
Processed American cheese, 1 oz	211	175	0.8
Lowfat cottage cheese, 1 cup	300	200	0.7
Processed cheese spread, 1 oz	257	129	0.5
Instant chocolate pudding, 5 oz	340	147	0.4
Soy milk, 8 oz	120	10	0.1

and of females are 500–650 mg, the Ca:P ratios decrease from roughly 0.5–0.6 to less than 0.5 when the additive phosphates are included. As shown later, a chronic low Ca:P dietary ratio may contribute to a modest nutritional secondary hyperparathyroidism. **Table 1** gives representative values of calcium and phosphorus in selected foods and the calculated Ca:P ratios. Only dairy foods (except eggs), a few fruits, and a few vegetables have Ca:P ratios that exceed 1.0.

Recommended intakes of phosphorus in the US have been set at 700 mg per day for men and women based on the intake required to maintain serum phosphorus in the normal range.

Intestinal Absorption of Phosphates

Because phosphate ions are readily absorbed by the small intestine, i.e., at efficiencies of 65–75% in adults and even higher in children, a prompt increase in serum inorganic phosphate (Pi) concentration follows within an hour after ingestion of a meal begins. (Calcium ions or Ca^{2+} are much more slowly absorbed.) The increased serum Pi (HPO_4^{2-}) concentration then depresses the serum ionic calcium concentration, which in turn stimulates the parathyroid glands to secrete (and synthesize) PTH. PTH acts on the kidneys to increase urinary phosphate excretion which reduces serum phosphate and ionic calcium concentrations to their normal homeostatic set-points. Recent reports suggest that an elevation of serum Pi ionic concentration directly influences PTH secretion independently of hypocalcemia. These meal-associated fluctuations in Pi and Ca^{2+} are part of normal physiological adjustments that occur typically three or more times a day. Serum phosphate concentrations also display a circadian rhythm.

Pi ions are thought to be absorbed in the small intestine primarily by transcellular mechanisms that involve cotransport with cations, especially sodium (Na^+). These mechanisms account for the rapid uptake of Pi ions in blood within an hour after ingestion of a meal. The blood concentration of Pi is less tightly regulated than the serum calcium concentration. Wider fluctuations in serum Pi concentrations reflect

Table 2 Approximate percentage (%) distributions of calcium and phosphate in blood

Serum fraction	Calcium (%)	Phosphate (%)
Ionic	50–55	55–60
Protein-bound	45–50	10–13
Complexed	0.3–0.6	30–35

both dietary intakes and also cellular releases of inorganic phosphates.

Most Pi absorption in the small intestine occurs independently of the hormonal form of vitamin D. The reported role of the of 1,25-dihydroxyvitamin D in intestinal Pi transcellular absorption is somewhat unclear because of the normally rapid influx of Pi ions after a meal, but this hormone may enhance the late or slower uptake of Pi ions. Paracellular passive absorption of Pi ions may also occur, but the evidence for this is limited.

Phosphate Homeostatic Mechanisms

The blood concentrations of Pi ions are higher early in life and then decline gradually until late life. Normal ranges for adults are from 2.7–4.5 mg dl⁻¹ (0.87–1.45 mmol l⁻¹). The percentage distribution of the blood fractions of phosphorus compared to those of calcium are given in **Table 2**. The homeostatic control of this narrow concentration range of Pi is maintained by several hormones, including PTH, 1,25(OH)₂vitamin D, calcitonin, insulin, glucagons, and others, but the control is never as rigorous as that of serum ionic calcium. In contrast to calcium balance that is primarily regulated in the small intestine by 1,25(OH)₂vitamin D, Pi balance is mainly regulated by the phosphaturic effect of PTH on the kidney, primarily the proximal convoluted tubule. In this sense, Pi regulation is less critical than that of calcium, which may result from the presence of multiple stores of this ion distributed throughout the body, i.e., bone, blood, intracellular compartments. Compared to the highly tight

regulation of serum ionic calcium, serum phosphate concentration is less tightly regulated

A major regulator of Pi is PTH which has several roles: it blocks renal tubular Pi reabsorption following glomerular filtration; it increases bone resorption of Pi (and calcium ions); and it enhances intestinal Pi absorption (and calcium absorption) *via* the vitamin D hormone, $1,25(\text{OH})_2\text{vitamin D}$. Other hormones have more modest effects on serum Pi concentration.

New understandings are emerging on the role of a new phosphatonin hormone that helps to lower the serum phosphate concentration when elevated. The actions of fibroblast growth factor 23 (FGF 23), secreted by bone cells, reduce both renal tubular reabsorption and intestinal phosphate absorption of phosphate ions which may lessen the need for PTH, the hormone generally considered most critical for the renal elimination of excessive serum phosphate ions. The roles of these two hormones in the reduction of serum phosphate need to be considered in terms of net calcium retention and the maintenance of BMD by human subjects. FGF 23 also reduces the renal production of $1,25(\text{OH})_2\text{vitamin D}$ and, therefore, it impacts intestinal calcium absorption. An elevated FGF 23 concentration has been associated with cardiovascular diseases, and if this is the case, especially in elderly subjects, then concentration of this hormone may prove to be a useful biomarker in assessing CVD and osteoporosis risk.

Functional Roles of Phosphates

Several major roles of Pi ions have been briefly noted, i.e., intracellular phosphate groups for cellular energetics and biochemical molecules as well as for the skeleton and teeth (structures). Other important functions also exist. For example, in bone tissue phosphates are critical components of hydroxyapatite crystals and they are also considered triggers for mineralization after phosphorylation of Type I collagen in forming bone. Serum phosphates, HPO_4^{2-} and H_2PO_4^- , also provide buffering capacity that helps regulate blood pH and also cellular pH.

Considerable cellular regulation occurs through the phosphorylation or dephosphorylation of Pi ions under the control of phosphatase enzymes, including protein kinases. These cell regulatory roles of Pi ions coexist with regulatory functions involving calcium ions, but Pi ions are much more widely distributed within cells and cell organelles than Ca ions.

Insulin affects Pi ions by increasing their intracellular uptake, though temporarily, for the prompt phosphorylation of glucose. Insulin may also influence the use of Pi ions when insulin-like growth factor-I (IGF-I) acts to increase tissue growth or other functions. The broad uses of Pi ions in structural components, energetics, nucleic acids, cell regulation, and buffering leaves an overall generalization that these versatile yet critical ions support life.

Phosphate in Health and Disease

Phosphate balance in adults remains zero or slightly positive, because of the effective homeostatic action of PTH on its target

organs that link the maintenance of serum concentrations of both phosphate and calcium ions. In late life, however, intestinal phosphate absorption decreases and the serum phosphate concentration declines (but remain typically within the normal range), which reflect a slightly negative balance as long as renal function remains normal. These declines, which may partly be related to lower phosphorus consumption by the elderly, may contribute to disease, especially to increased bone loss or more severe osteoporosis. Typically these changes in Pi balance are also accompanied by similar changes in calcium balance. Too little dietary phosphorus, along with too little dietary calcium, may be determinants of low bone mass and density and, hence, increased bone fragility. The usual scenario invoked to explain osteoporosis in old age, however, is that too little dietary calcium in the presence of adequate dietary phosphorus stimulates an increase in PTH secretion and, hence, bone loss (**Figure 1**).

Four human conditions that involve abnormal Pi homeostasis need explanation.

Ageing and Renal Function

The serum concentration of Pi increases with a physiological decline in renal function associated with aging and also with renal disease when glomerular filtration rate (GFR) falls below 30 ml min^{-1} . Healthy individuals excrete about 67% of their absorbed phosphate *via* the urine, and the remainder *via* the gut as endogenous secretions. As the glomerular filtration capacity of the kidneys declines, the serum Pi concentration increases and more Pi is retained by the body. PTH secretions increase but the typical serum PTH concentrations, though elevated, remain within the upper limits of the normal range, at least for a decade or so. Thereafter, however, serum Pi and PTH both continue to climb as renal function declines and increased rates of bone turnover lead to measurable bone loss. This situation is probably affecting millions in the US each year as they enter the 50s and proceed into the 60s; in the USA many of these individuals are overweight or obese and have the metabolic syndrome which may negatively impact renal function. As the syndrome worsens, many of these individuals will progress to chronic renal failure and renal secondary hyperparathyroidism (see section on Nutritional secondary hyperparathyroidism).

Nutritional Secondary Hyperparathyroidism

This mild condition has not been fully assessed in any longitudinal studies lasting as long as one year. The initiating event is a chronic low calcium and high phosphorus intake (low Ca:high P ratio) that leads to a chronic elevation of serum PTH. Elevations in PTH stimulate osteoclastic bone resorption and declines in bone mass and density. This condition has only been studied experimentally using human subjects for 28 days, but the chronic rises in PTH and vitamin D hormone suggest that even a lowering of the Ca:Pi ratio below 0.5, in this study to ~ 0.25 , resulted in adverse effects. Longer studies are needed to determine if bone losses occur under this chronic dietary regimen.

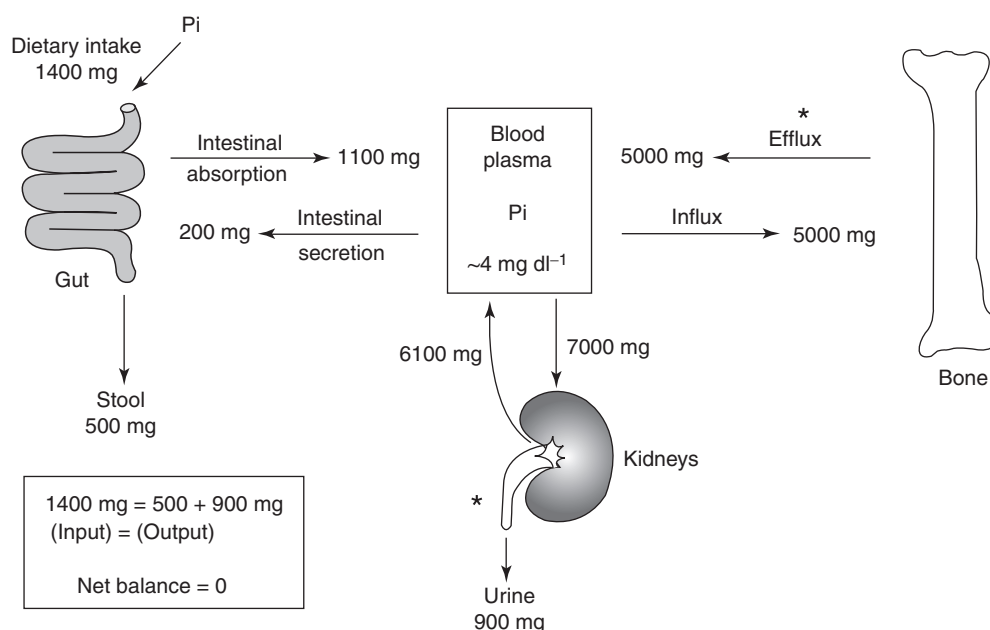


Figure 1 Phosphorus homeostasis and balance. The intestine, kidneys, and bone are organs involved in phosphate homeostasis. Fluxes of phosphate ions between blood and these organs are shown. Note the high fluxes in and out of bone each day. To convert phosphorus values from g to mmol, multiply by 32.29; from mg dl⁻¹ to mmol l⁻¹, multiply by 0.3229. * Steps enhanced by parathyroid hormone. Reproduced with permission from Anderson JJB, Sell ML, Garner SC, and Calvo MS (2001) Phosphorus. In: Bowman BA and Russell R (eds.) *Present Knowledge in Nutrition*, 8th edn., p. 282. Washington, DC: International Life Sciences Institute Press.

Renal Secondary Hyperparathyroidism

The true secondary hyperparathyroidism of chronic renal failure (CRF) has been extremely difficult to treat by clinicians because of high Pi and PTH concentrations. Traditional treatment includes the use of binders (chemical) to prevent Pi absorption from the small intestine. In recent years a calcium-sensing receptor (CaR) in the parathyroid glands has been identified and drugs are being developed that will trick the CaR into thinking that serum calcium is normal, rather than depressed, thereby reducing PTH secretion. A reduction in PTH then helps in the conservation of bone tissue, since bone loss is such a severe problem of CRF patients.

Abnormal Bone Formation in Arterial Walls

A pathologic role for phosphate ions in the initiation of bone matrix formation and subsequent mineralization associated with established atherosclerotic plaque has recently been uncovered. The arterial walls and heart valves have long been known to develop true bone at these inappropriate loci, but the mechanism remains elusive. Excessive calcium ions are also taken up to help bone generation at these abnormal sites. Inappropriate arterial bone, which occurs mainly in older adults, may increase the risk of cardiovascular diseases and death.

Conclusions

The general view of dietary phosphorus, supplied in foods as phosphates, is that too much relative to calcium skews the Ca:P

ratio to much less than 0.5. Another view, however, has been emerging that suggests that many elderly subjects, especially women, have very low phosphorus intakes in addition to low calcium intakes and that they may benefit from increased consumption of both calcium and phosphate from foods and supplements. In dietary trials designed to reduce fractures, especially nonvertebral fractures, of elderly women and men, calcium plus vitamin D have been the treatments, but at least one trial that used calcium phosphate plus vitamin D has shown significant reduction in fractures over 18 and 36 months of follow-up. Further studies are needed to target the role of phosphate ions in reducing fractures among the elderly.

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PHYSICAL ACTIVITY

Beneficial Effects

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Glossary

Energy expenditure The total energy cost of maintaining constant conditions in the body plus the energy cost of physical activities.

Exercise Physical activity that is regular, planned, and structured with the aim of improving or maintaining one or more aspects of physical fitness.

Health A state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity.

Physical activity Any bodily movement produced by skeletal muscles that results in energy expenditure.

Physical fitness A measure of the ability of the body to cope with physical activity or exercise.

Introduction

This article examines the role that physical activity plays in the regulation of energy balance, reviews physiological adaptations resulting from exercise training, and summarizes the benefits of participation in regular physical activity. Physical activity has been shown to produce beneficial effects in terms of prevention of coronary heart disease (CHD), osteoporosis and cancer, weight control, and the promotion of mental health. Recommended guidelines for the volume of physical activity required to maintain optimal health are presented. It is necessary to define key terms of reference used in the present chapter. 'Physical activity' can be defined as 'any bodily movement produced by skeletal muscles that results in energy expenditure.' 'Exercise' (often used interchangeably with 'physical activity') is defined as 'physical activity which is regular, planned, and structured with the aim of improving or maintaining one or more aspects of physical fitness.' 'Physical fitness' is 'a set of outcomes or traits relating to the ability to perform physical activity.'

Physical Activity and Energy Balance

Energy balance occurs when the total energy expenditure of an individual equals his or her total energy intake from diet. If intake exceeds expenditure, the result is an increase in the storage of energy primarily as body fat. If intake is below expenditure, body energy content or body fat decreases.

In humans, energy is expended in three ways: maintaining the physiological functions of the body at rest, often termed resting metabolic rate (RMR); ingesting food and digesting and assimilating nutrients, or the thermic effect of food (TEF);

and skeletal muscular contractions involved in spontaneous physical activity or planned exercise (see **Figure 1**). Of these components, the energy expenditure associated with physical activity is the factor that accounts for the greatest variability between individuals and is the only component that may be reasonably controlled by an individual. It may therefore represent an appropriate method for altering energy balance. In addition to its direct independent effect on daily energy expenditure, evidence suggests that physical activity may also increase RMR, TEF, and the energy expenditure caused by spontaneous physical activity.

Energy Expenditure During and After Exercise

The magnitude of energy expenditure during physical activity is dependent on several factors, including the mode, intensity, and duration of physical activity, as well as the body mass of the individual. When determining the metabolic cost of weight-bearing physical activity, energy expenditure can be expressed in relation to body size since a small person will expend less energy performing a given activity (e.g., walking up a flight of stairs) than a larger person performing the same activity. The energy cost of a given activity is often described in kilocalorie (kJ) per kilogram of body weight. The term metabolic equivalent (MET) may also be used to indicate the ratio of the rate of energy expenditure during a given activity to RMR. An example illustrates how METs are used to quantify energy expenditure during exercise. If an individual with a body mass of 70 kg expends 70 kcal (~ 300 kJ) per hour at rest (RMR), and walking at a speed of 5.6 km h^{-1} requires $280 \text{ kcal } (\sim 1200 \text{ kJ}) \text{ h}^{-1}$, the energy cost of the activity is 4 METs or four times the RMR of the individual. Because body size is a determinant of both RMR and the energy expenditure

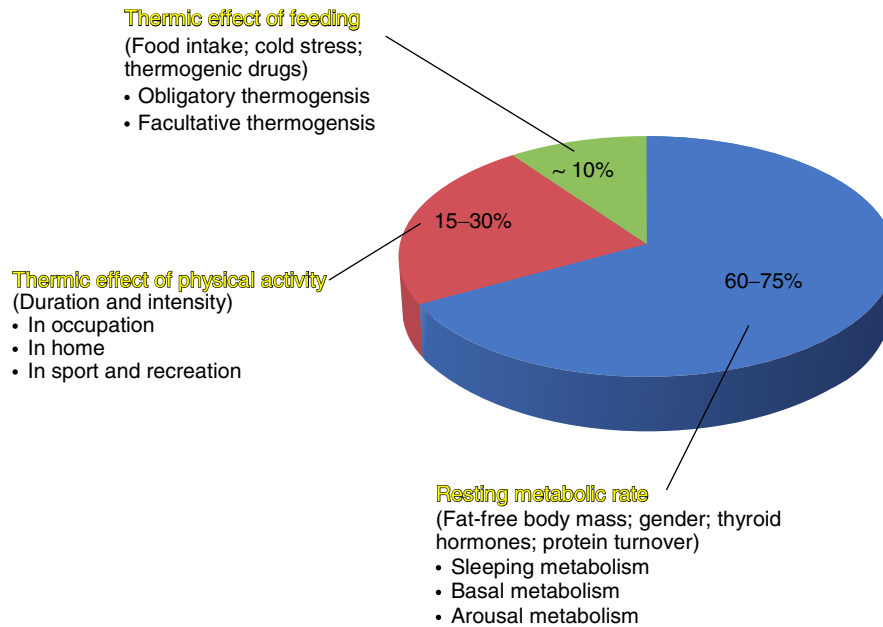


Figure 1 Components of total daily energy expenditure. Reproduced with permission from Figure 9.1, page 188 in McArdle WD, Katch FI, and Katch VL (2001) *Exercise Physiology. Energy, Nutrition and Human Performance*, 5th edn. Baltimore: Lippincott Williams & Wilkins.

Table 1 Energy costs of popular physical activities

Activity	Intensity	METs
Walking	6.4 km h ⁻¹ , very brisk pace	5
Running	10.8 km h ⁻¹	11
Cycling	20.9 km h ⁻¹	8
Swimming	Front crawl, moderate	8
Tennis	Singles	8
Aerobics	Moderate	6

Source: Adapted from Ainsworth BE, Haskell WL, Whitt MC, *et al.* (2000) Compendium of Physical Activities: An update of activity codes and MET intensities. *Medicine and Science in Sports and Exercise* 32(9): S498–S516.

during physical activity, a heavier individual will have a higher RMR but will still require four times this level of expenditure (or 4 METs) to walk at the same speed. **Table 1** indicates the energy cost in METs of many popular exercise modes.

In addition to the increased energy utilized during an exercise bout, several researchers have found that energy expenditure remains elevated for a period following physical activity. However, conclusions regarding the magnitude and duration of this postexercise elevation in energy expenditure have been equivocal. Several mechanisms underlying this increased energy expenditure during the postexercise period have been postulated, including the energy cost of replenishing fuel stores, the cost of dissipating byproducts of adenosine triphosphate (ATP) resynthesis, restoration of cellular homeostasis, and the futile cycling of energy substrates. The magnitude of this increase may be related to the intensity and duration of physical activity, with longer or more strenuous activity creating a greater perturbation to homeostasis and therefore causing greater energy expenditure in restoring the body to its preexercise condition.

Effects of Exercise Training on Resting Metabolic Rate and the Thermic Effect of Food

Although findings are far from consistent, some investigators have suggested that regular exercise causes a persistent augmentation in RMR. The mechanism for effect has yet to be confirmed, but it has been hypothesized that this increase may be due to increased muscle mass following exercise training which results in the high energy turnover in trained individuals.

Some studies have indicated that pre- or postprandial exercise may enhance the TEF. In addition to this acute effect of physical activity, regular training may alter the TEF. In males, the thermic effect of a meal is lower in highly trained compared to untrained individuals. In one study, moderate levels of fitness were associated with a greater increase in the TEF than either high or low fitness. The authors suggest that very high or very low levels of fitness may decrease the thermic effect possibly by adaptive mechanisms, such as a lower insulin or lower noradrenaline response to feeding. Studies on monozygotic twins also suggest a strong genetic factor controlling whether exercise has such an effect.

Physiological Adaptations to Exercise Training

The human body is remarkably plastic in response to the increased metabolic demands of exercise training (overload), with many adaptations occurring that enable the body to function more efficiently. The nature and magnitude of these changes are dependent on the volume (duration and frequency), intensity, and type of physical activity performed. For this reason, the physiological adaptation to training will be classified according to the nature of the exercise undertaken.

It is important to remember two principles when considering the physiological adaptations to exercise training. First, there is a degree of intraindividual variation in response to exercise training that may be attributed in part to genetic factors. Second, whereas exercise training will cause adaptation, the removal of this stimulus will result in a reversal of adaptation, or 'detraining.'

Adaptations to Submaximal/Endurance Exercise Training

Submaximal exercise generally refers to an intensity of exercise that requires less than an individual's maximal oxygen uptake. Submaximal exercise challenges the body to deliver and utilize an increased amount of oxygen in the resynthesis of ATP. With training, changes occur that increase the body's ability to utilize oxygen. For simplicity, the adaptations to submaximal exercise training have been grouped according to the site at which they occur.

Central adaptations to regular submaximal exercise include alterations in the morphology and function of the heart and circulatory systems that allow greater delivery of oxygen to the working muscle. Modest cardiac hypertrophy characterized by an increase in left ventricular volume occurs in response to training. This adaptation allows an increase in stroke volume, leading to a reduction in heart rate at rest and during submaximal workloads and an increased cardiac output during maximal workloads. An increase in total plasma volume and an increase in the total amount of hemoglobin have been observed in response to submaximal endurance training.

Peripheral adaptations to regular submaximal exercise include changes in the structure and function of skeletal muscle that enhance its ability to use oxygen to produce energy aerobically. A consequence of endurance training is an augmented blood supply to the working muscle. This is achieved by an increased capillarization in trained muscles, greater vasodilation in existing muscle capillaries, and a more effective redistribution of cardiac output to the working muscle. Increases in both the activity of aerobic enzymes and mitochondrial volume density (approximately 4–8%) within trained muscle have been noted. These are coupled with greater glycogen storage within the muscle and augmented fat mobilization allowing a higher rate of aerobic ATP resynthesis from free fatty acids and glucose.

Adaptations to High-Intensity Exercise and Strength Training

High-intensity exercise requires energy utilization rates that exceed the oxidative capabilities of the muscle. Activities such as sprinting require the anaerobic resynthesis of ATP to produce and maintain high levels of muscular force and are therefore limited in duration. Strength training also relies heavily on anaerobic energy sources and requires high force production by specific muscle groups. The main alterations that occur in response to regular high-intensity exercise or strength training are improvements in the structure and function of the neuromuscular system that allow more efficient production of the forces required for these activities and an enhanced ability to produce the energy required through anaerobic processes.

Neuromuscular adaptations to high-intensity exercise training include increased nervous system activation, more efficient neuromuscular recruitment patterns, and a decrease in inhibitory reflexes, allowing the individual to produce greater levels of force.

Regular strength training stimulates an increase in muscle size and therefore the maximum force it can exert. This hypertrophy occurs preferentially in fast twitch muscle fibers and is brought about by increased protein synthesis in response to resistance training. The degree to which muscle hypertrophy occurs is dependent on many factors, including gender and body type.

Metabolic alterations to high-intensity and strength training include improved ability of the muscle to resynthesize ATP from anaerobic sources. Intramuscular stores of the anaerobic energy intermediates, such as creatine phosphate (CP) and glycogen, increase after a period of supramaximal training. The activity of enzymes involved in anaerobic production of energy, such as creatine kinase and myokinase, is also augmented.

Benefits of Regular Physical Activity

In June 2007, the US Physical Activity Guidelines Advisory Committee was charged with reviewing existing scientific literature to identify where there was sufficient evidence to develop a comprehensive set of specific physical activity recommendations, and so updating previous national guidelines. Their report detailed the benefits of physical activity and health, and noted that improvements are attainable in cardiorespiratory health, metabolic health, energy balance, musculoskeletal health, functional health, cancer, and mental health as a result of regular physical activity.

Prevention of Coronary Heart Disease

CHD has a multifactorial etiology, and major 'biological' risk factors include elevated concentrations of blood total and low-density lipoprotein (LDL) cholesterol, reduced concentration of high-density lipoprotein (HDL) cholesterol, high blood pressure, diabetes mellitus, and obesity. In addition, 'behavioral' risk factors for CHD include cigarette smoking, a poor diet, and low levels of physical activity and physical fitness associated with the modern, predominantly sedentary way of living. Among these risk factors, a sedentary lifestyle is by far the most prevalent according to data from both the US and England (Figure 2). The World Health Organization estimates that physical inactivity is the fourth leading cause of death worldwide.

Scientific verification of a link between an inactive lifestyle and CHD has been forthcoming during the past 40 years, with the publication of more than 100 large-scale epidemiological studies investigating the relationships between physical activity and cardiovascular health. These studies, some of which are summarized in Figure 3, have produced consistently compelling evidence that regular physical activity can protect against CHD.

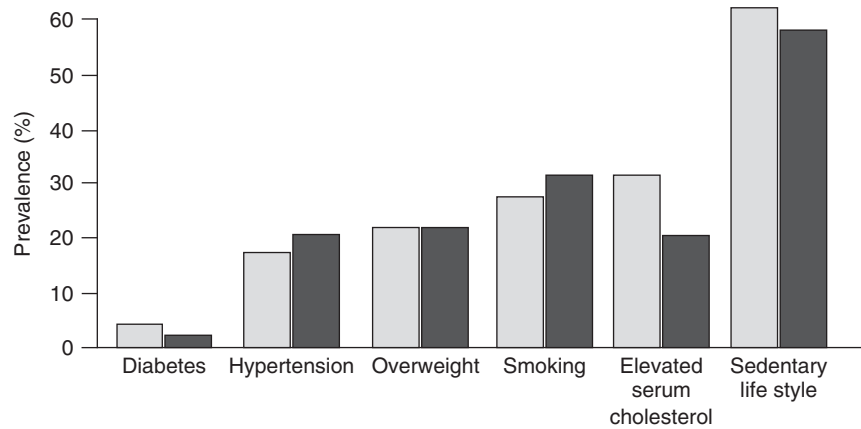


Figure 2 Estimates of the prevalence (%) of the US population with selected risk factors for coronary heart disease and the population from England. In both studies, a sedentary lifestyle was taken as 'no physical activity' or irregular physical activity (i.e., fewer than three times per week and/or less than 20 min per session). Reproduced from Killoran AJ, Fentem P, and Caspersen C (eds.) (1994) *Moving On. International Perspectives on Promoting Physical Activity*. London: Health Education Authority.

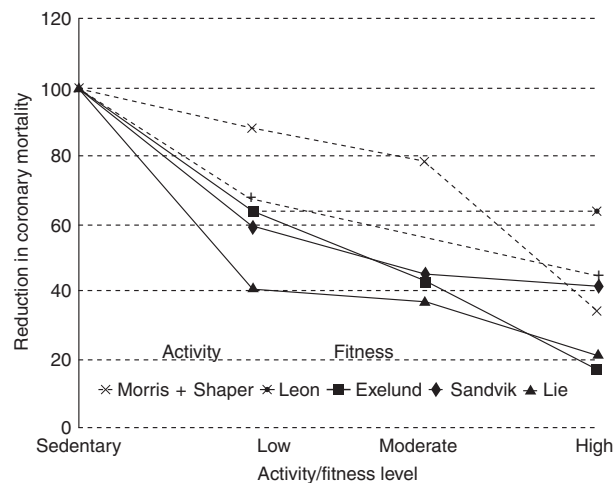


Figure 3 Summary of the results from six studies in which fitness level was determined (three studies) or activity level assessed by questionnaire (three studies) in individual populations. Follow-up was generally between 7 and 9 years except in Sandvik's study, which had a 16-year follow-up. The 'low level' group for each study represented in this figure was the activity/fitness level next to the least active/fit group. The 'high level' represents the group that was the most active/fit for the particular study. If the study participants were grouped by quintile, the 'moderate' group is the average of the third and fourth quintiles. Reproduced from Killoran AJ, Fentem P, and Caspersen C (eds.) (1994) *Moving On. International Perspectives on Promoting Physical Activity*. London: Health Education Authority.

Pooled data and meta-analyses of the 'better' studies indicate that the risk of death from CHD increases about twofold in individuals who are physically inactive compared with their more active counterparts. Relationships between aerobic fitness and CHD appear to be at least as strong. For example, in a cohort of middle-aged men longitudinally followed for an average of 6.2 years, the risk of dying was approximately double in those whose exercise capacity at baseline was < 5

METs compared with those whose capacity was > 8 METs. For both physical activity and fitness, adjustment for a wide range of other risk factors only slightly weakens these associations, suggesting independent relationships.

A common weakness of such studies is that they often rely on a single measurement of fitness or activity at baseline, with subsequent follow-up for mortality within the cohort. With such a design, it is difficult to discount the possibility that genetic or other confounding factors are influential in the observed relationship between physical activity/fitness and mortality. A further weakness in single baseline studies is that subsequent changes in activity/fitness during the follow-up are not monitored, even though they may affect the observed relationships due to the phenomenon of 'regression to the mean.'

Some prospective studies have overcome these deficiencies by examining the effects of changes in physical activity and fitness on mortality. One study reported on the relationship of changes in physical activity and other lifestyle characteristics to CHD mortality in 10 269 alumni of Harvard University. Changes in lifestyle over an 11- to 15-year period were evaluated on the basis of questionnaire information, and subsequent mortality was assessed over an 8-year period. In men who were initially sedentary but started participating in moderately vigorous sports (intensity of 4.5 METs or greater), there was a 41% reduced risk of CHD compared to those who remained sedentary. This reduction was comparable to that experienced by men who stopped smoking. The second study examined changes in physical fitness and their effects on mortality. In this study of 9777 men, two clinical examinations (including treadmill tests of aerobic fitness) were administered approximately 5 years apart, with a mean follow-up of 5.1 years after the second examination to assess mortality. Results showed that men who improved their fitness (by moving out of the least-fit quintile) reduced their age-adjusted CHD mortality by 52% compared with their peers who remained unfit. Furthermore, such changes in fitness proved to be the most effective in reducing all-cause mortality when compared with changes in other health-risk factors (Figure 4).

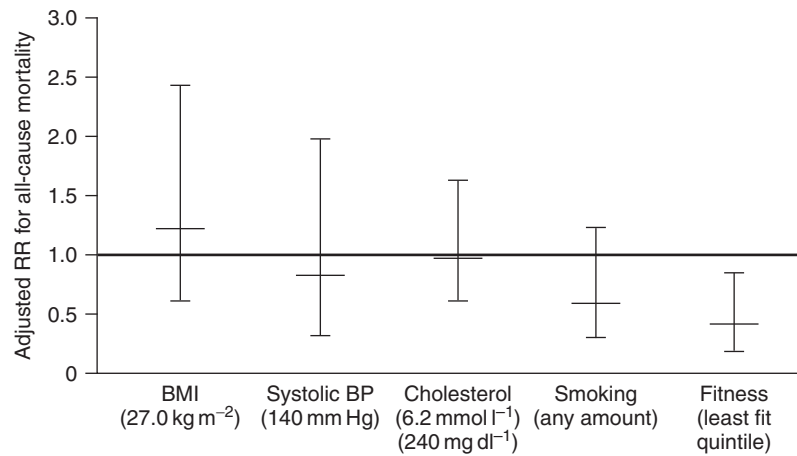


Figure 4 Relative risks (adjusted for age, family history of coronary heart disease, health status, baseline values, and changes for all variables in the figure, and interval in years between examinations) of all-cause mortality by favorable changes in risk factors between first and subsequent examinations. The analyses were for men at risk on each particular variable at the first examination. Cutoff points designating high risk are given parenthetically at the bottom of the figure. The number of men at high risk (and the number of deaths) for each characteristic were as follows: body mass index (BMI), 2691 (66); systolic blood pressure (BP), 1013 (55); cholesterol, 2212 (79); cigarette smoking, 1609 (45); and physical fitness, 1015 (56). Reproduced with permission from Blair SN, Kohl 3rd HW, Paffenbarger Jr RS, Clark DG, Cooper KH, Gibbons LW (1989) Physical fitness and all-cause mortality. A prospective study of healthy men and women. *Journal of the American Medical Association* 262: 2395–2401.

Weight Control

Obesity is defined as an excess of adipose tissue. This condition plays a central role in the development of diabetes mellitus and confers an increased risk for CHD, high blood pressure, osteoarthritis, dyslipoproteinemia, various cancers, and all-cause mortality. The prevalence of obesity has risen dramatically in recent years, concomitant with a decline in daily energy expenditure during the past two decades.

Based on the principles of energy balance, such circumstantial evidence indicates that physical inactivity may play a central role in the development of obesity in humans. Regular participation in physical activity provides benefits for weight stability, but with few data on this topic from long-term studies, the optimal amount is not known. One large-scale national study in the US evaluated the relationship of physical activity to weight gain over a 10-year follow-up of 3515 men and 5810 women. Individuals who were sedentary at both baseline and follow-up were much more likely (relative risk, 2.3 (95% confidence interval (CI), 0.9–5.8) in men and 7.1 (95% CI, 2.2–23.3) in women) to experience considerable weight gain (> 13 kg) than subjects who were active at both time-points. Evidence suggests that women who gain weight (6 kg) over a 1-year period expend on average 212 kcal/day less in light to moderate activities than those who maintain their normal body weight. With regard to weight loss, a decrease of 5% or more of body weight can be achieved with large volumes of physical activity, although a simultaneous dietary intervention is typically needed to achieve this goal.

Prevention of Osteoporosis

Osteoporosis-related fractures represent a major public health concern. Once established, osteoporosis may be irreversible, emphasizing the need for primary prevention strategies based

on minimizing bone loss and maximizing peak bone mass. Nearly half the variation in bone mineral density (BMD) may be attributable to nonhereditary factors. Performing regular weight-bearing and muscle-strengthening exercise is one of the universal recommendations to reduce the risk of falls and fractures. The 2008 Physical Activity Guidelines Advisory Committee Report noted that regular physical activity reduces the incidence of osteoporotic fractures; increases, or minimizes, the age-related decrease in BMD; and may provide protection against the development of osteoarthritis although there is limited evidence for the latter.

In addition to its osteogenic effects, regular physical activity may also promote better coordination, balance, and ambulatory muscle strength, thus minimizing the risk of falling. The reported reduced risk of fracture (relative risk, 0.41 in men and 0.76 in women) in active individuals compared to sedentary ones is likely due to these combined direct and indirect effects of physical activity.

Cancer Prevention

Estimates suggest that between 9% and 19% of cancers in Europe are attributable to lack of physical activity. Cancer is a collective term for a range of diseases in which cells of the body divide in an uncontrolled manner. Cancer is classified according to the tissue in which the diseased cells originate. There is a growing body of epidemiological evidence showing an inverse relationship between levels of lifelong physical activity and the incidence of colon cancer, endometrial cancer, and postmenopausal breast cancer. Data relating to associations between physical activity and premenopausal breast, rectal, endometrial, ovarian, prostate, and testicular cancers is weaker and less consistent. Where physical activity has been shown to have a preventive effect, it seems that 30–60 min of

daily moderate to vigorous activity across the life-course is required to reduce risk. Possible biological mechanisms for this association between physical activity and cancer risk include the modification of metabolic and sex hormones, growth factors, antitumor immune mechanisms, as well as favorable alterations in energy balance, fat distribution, antioxidant status, and intestinal food transit.

Mental Health

Physical activity has been shown to contribute to mental health. There is now strong evidence that physical activity improves mood, self-esteem, sleep quantity and quality, as well as cognitive function. In some cases these changes have been shown as acute effects – evident after just one bout of moderate to vigorous activity while in others the effect appears to be a result of more regular activity throughout the lifespan. Specifically there is evidence from population-based prospective cohort studies that regular moderate-intensity exercise reduces the risk of depression and anxiety. In addition, physical activity has been shown to have therapeutic effects in the treatment of anxiety and depression with several studies demonstrating that physical activity is as effective as psychotherapy and antidepressant drugs in the treatment of these conditions.

Although the mechanisms by which physical activity contributes to these positive effects on mental health are not yet fully elucidated, several plausible explanations have been proposed. These fall into two broad categories: physiological explanations, including changes in neurotransmitters in the brain (e.g., serotonin and opiates), increases in core temperature, improved blood flow to the brain, and reduced muscle tension; and psychological explanations including distraction from daily stressors, improved self-efficacy, and increased social interaction.

Physical Activity Prescription

For protection against CHD and other diseases associated with inactivity, physical activity needs to be habitual, predominantly aerobic in nature, and current. International recommendations are that adults should accumulate at least 150 min of moderate-intensity physical activity each week, or 75 min a week of vigorous-intensity aerobic physical activity, or an equivalent combination of moderate- and vigorous-intensity aerobic activity. Such activity may include everyday tasks such as stair climbing and walking, recreational physical activities, and more formal aerobic exercise programs and sports. Moderate-intensity activities are defined as 3.0–5.9 METs. Walking at 3.0 miles per hour requires 3.3 METs of energy expenditure. Vigorous-intensity activities are defined as ≥ 6.0 METs. Running at 10 min per mile (6.0 mph) is a 10-MET activity.

Physical activity bouts should be at least 10 min in duration and preferably spread throughout the week.

Muscle-strengthening activities on 2 or more days a week are also recommended. Additional benefits occur with more physical activity that is of greater volume, intensity, or frequency. It is also acknowledged that some activity is better than none and some various health benefits may be accrued from volumes of activity lower than current guidelines.

There is some evidence that to lose weight, or for the long-term maintenance of weight loss, approximately double the current guidelines of 150 min per week is required. It has been suggested that an increase in energy expenditure of physical activity of approximately 6300–8400 kJ per week (1500–2000 kcal/per week) is associated with improved weight maintenance.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Coronary Heart Disease: Prevention. Energy: Balance. Energy Metabolism. Obesity: Definitions, Etiology, and Assessment; Prevention. Osteoporosis: Nutritional Factors. Weight Management: Approaches

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PHYTOCHEMICALS

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Classification and Occurrence

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Abbreviations

CVD	Cardiovascular disease
eNOS	Endothelial nitric oxide synthase
NADPH oxidase	Nicotinamide adenine phosphate-oxidase

NF-κB	Nuclear factor kappa B
NO	Nitric oxide
NRF2	Nuclear factor (erythroid-derived 2)-like 2
SOD	Superoxide dismutase

Glossary

Aglycone A non-sugar compound remaining after replacement of a sugar by a hydrogen atom.
Conjugate Two or more compounds bound together.
Enzyme A protein with contains an active site which binds to a substrate to help catalyse a reaction.

Glucosidase An enzyme that cleaves the bond between glucose and another compound.

Glycoside Organic compounds formed when a monosaccharide is bound to another compound.

Introduction

There is considerable evidence to suggest that populations that consume diets rich in fruits and vegetables, whole-grain cereals, and complex carbohydrates have a reduced risk of a range of chronic diseases. This has led to the suggestion that the diversity of substances found in food, particularly plant-derived foods, may underlie the protective effects that are attributed to diets high in fruits and vegetables. Although fruits and vegetables are rich sources of micronutrients and dietary fiber, they also contain a wide variety of secondary metabolites, which provide the plant with color, flavor, UV protection, and antimicrobial and insecticide properties. Many of these substances have been attributed a wide array of biological properties but have yet to be recognized as nutrients in the conventional sense. These potentially protective plant compounds, termed phytochemicals, are receiving increasing attention and some have been termed phytonutrients, as they have been shown to exert numerous physiological functions in mammalian systems. However phytonutrients are not

currently defined as 'nutrients' as they are not essential for human/animal growth and development but may help maintain health throughout life, including the prevention of chronic disease. Many of them are ubiquitous throughout plants and as a result are present in our daily diet in varying amounts. Among the most important classes are the flavonoids, which are classified based on their chemical and structural characteristics. The present article focuses on the different classes of phytochemicals and their potential relationships to human health.

Phytochemicals: General

Phytochemicals comprise a wide group of structurally diverse plant compounds, which are predominantly associated with the cell wall and widely dispersed throughout the plant kingdom. They are secondary metabolites in plants, characterized by having at least one aromatic ring with one or more hydroxyl groups attached. The nature and distribution of these compounds can vary depending on the plant tissue in which

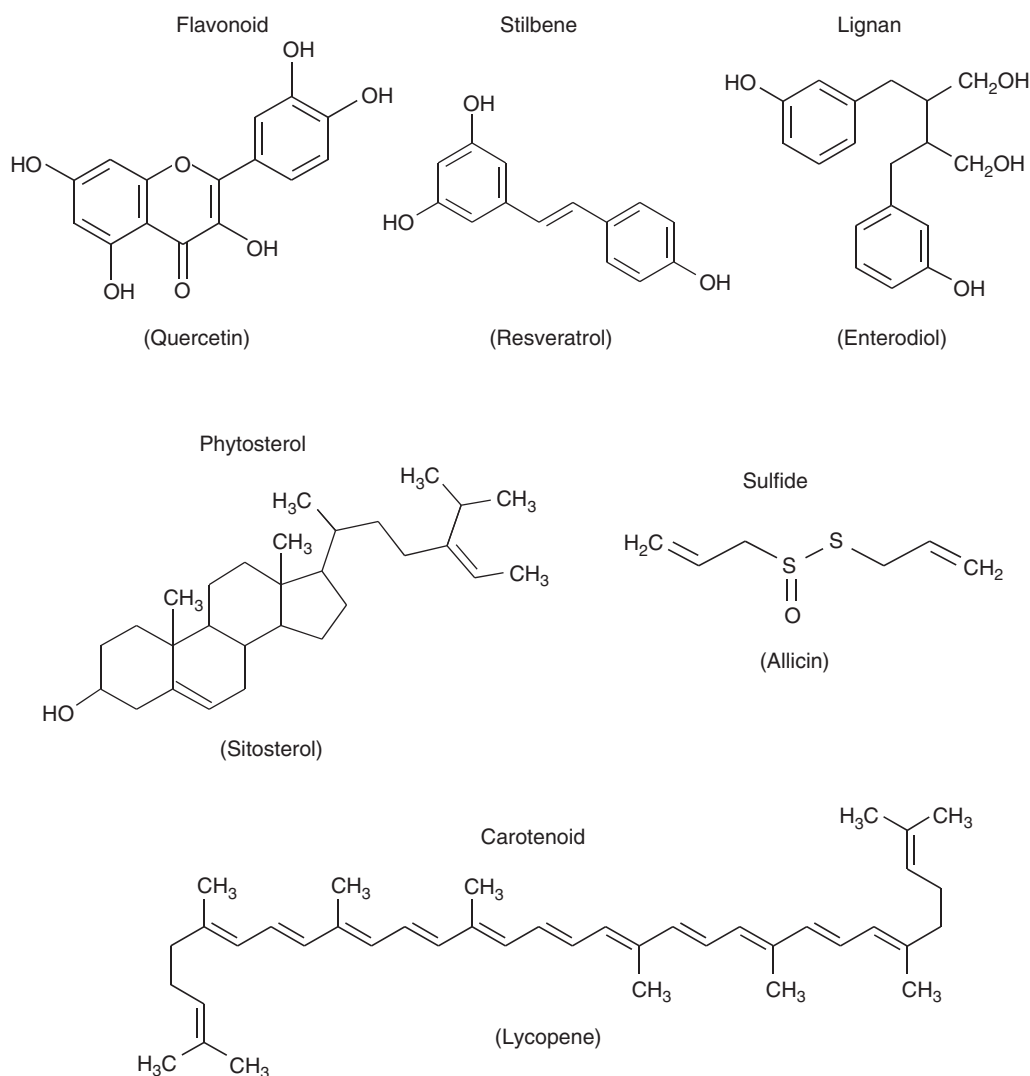


Figure 1 Chemical structure of flavonoid, stilbene, lignan, phytosterol, sulfide, and carotenoid.

they are located, but they are mainly synthesized from carbohydrates via the shikimate and phenyl-propanoid pathways. They range in chemical complexity from simple phenolic acids, such as caffeic acid, to complex high-molecular-weight compounds, such as the procyanidins/tannins, and they can be classified according to the number and arrangement of their carbon atoms. In plants, they are commonly found conjugated to sugars and organic acids and can be classified into two groups, flavonoids and nonflavonoids. Although there are many subclasses of bioactive phytochemicals, including flavonoids, stilbenes, lignans, phytosterols, sulfides, glucosinolates, and carotenoids (Figure 1), this article focuses mainly on the flavonoid subgroup, one of the most researched classes of phytochemicals to date; however, a brief introduction to the other classes is provided below:

- Stilbenes are present in a wide range of plants and one of the most studied stilbenes, *trans*-resveratrol, found in grapes and wine, is known for its effects on vascular activity and longevity.

- Lignans are phenolic compounds present in high concentrations in linseed (flaxseed) and studies suggest they have the potential to reduce the risk of cardiovascular disease (CVD) and cancer.
- Phytosterols are found in vegetable oils and are most notably known for their ability to decrease blood LDL-cholesterol levels and lower the risk of CVDs.
- Sulfides and glucosinolates are present in garlic and brassica vegetables and are most commonly investigated for their anticarcinogenic properties.
- Carotenoids are pigments (red, orange, yellow) common to many fruits and vegetables and their consumption has been linked to reduced risk of chronic degenerative diseases.

Flavonoids

Flavonoids constitute a large class of phytochemicals that are widely distributed in the plant kingdom, are present in high

concentrations in the epidermis of leaves and skin of fruits, and have important and varied roles as secondary plant metabolites. More than 8000 varieties of flavonoids have been identified, many of which are responsible for the colors of fruits and flowers. They are found in fruits, vegetables, tea, wine, dark chocolate, grains, roots, stems, leaves, and flowers and are thus regularly consumed by humans. Although it has been widely known for centuries that chemical derivatives of plant origin possess a broad spectrum of biological activities, it was first suggested that flavonoids may be important for human health in the 1930s when it was observed that a fraction from lemon juice could decrease the permeability of arteries and partially prevent symptoms in scorbutic pigs. At the time, it was suggested that these compounds should be defined as a new class of vitamins, vitamin P, and the substance responsible for these effects was identified as the flavonoid rutin. However, the data were not generally accepted and the term vitamin P was abandoned in the 1950s. There was renewed interest in flavonoids when a potentially protective role of flavonoids in relation to heart disease in humans was reported. Since then, there has been a surge of interest in the potential role of flavonoids in human health, with research suggesting antioxidant effects, hormonal actions, anti-infectious actions, anti-inflammatory and cancer-preventative effects, the ability to induce chemical defense enzymes, and actions on blood clotting and the vascular system. However, concrete clinical evidence that they positively influence human health is lacking, and potential adverse effects have also been reported for a limited number of polyphenols.

Other flavonoid groups that are thought to be less important from a nutrition perspective are the dihydroflavones, flavan-3,4-diols, coumarins, chalcones, dihydrochalcones, and aurones. The basic flavonoid skeleton can have numerous

constituents; hydroxyl groups are usually present at the 3-, 5-, and 7-positions. Sugars are very common, and the majority of flavonoids exist naturally as glycosides. The presence of both sugars and hydroxyl groups increases water solubility, but other constituents, such as methyl or isopentyl groups, increase flavonoids lipophilicity. Although many thousands of different flavonoids exist, they can be classified into a few different subclasses. The main subclasses that are important from a human health perspective are the flavones, flavonols, flavan-3-ols, flavanones, anthocyanins, and isoflavones (and their polymeric forms) (Figure 2). Extensive information on the different flavonoids present in commonly consumed plant-based foods including fruits, vegetables, and drinks is available; however, there is wide variability in the levels present in specific foods, in part due to seasonal changes and varietal differences.

Flavonols

Flavonols are arguably the most widespread of the flavonoids because they are dispersed throughout the plant kingdom. The distribution and structural variations of flavonols are extensive and have been well documented. The most common flavonols are quercetin, kaempferol, myricetin, and isorhamnetin.

Flavones

Flavones have a close structural relationship to the flavonols, but unlike flavonols they are not widely distributed in plants. Their only significant dietary occurrences are in celery, parsley, and a few other herbs, and they predominantly occur as 7-O-glycosides (e.g., luteolin and apigenin). In addition,

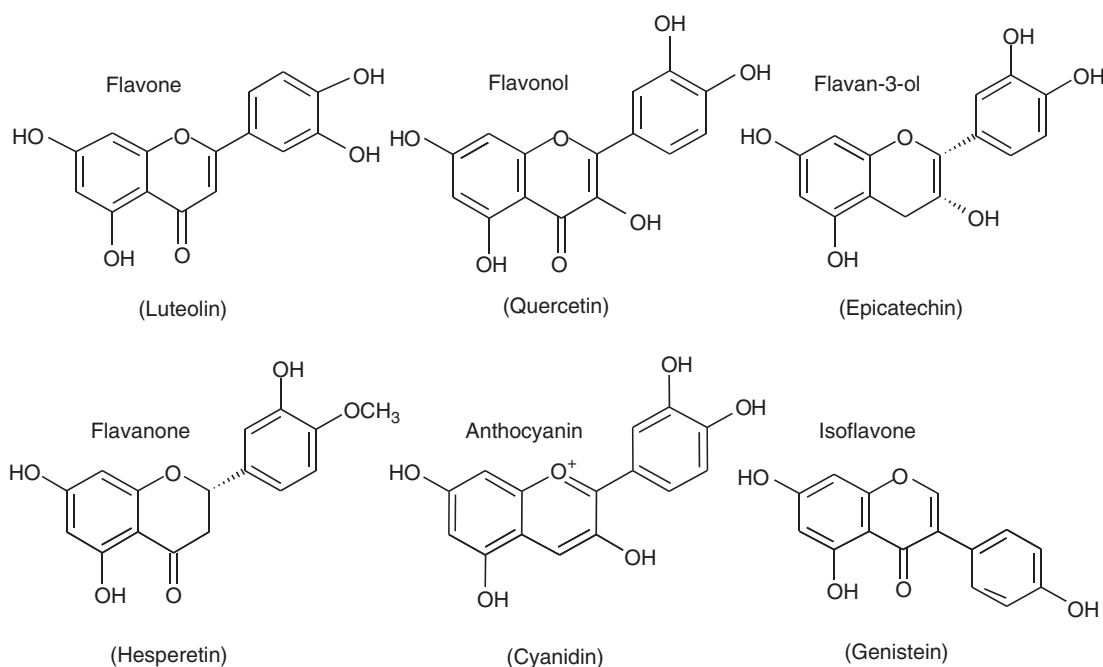


Figure 2 Structures of the major subclasses of flavonoids: flavones, flavonols, flavan-3-ols, flavanones, anthocyanins, and isoflavones.

polymethoxylated flavones have been found in citrus fruits (e.g., nobiletin and tangeretin).

Flavan-3-ols (Both Monomeric and Polymeric Forms)

Flavan-3-ols, often referred to as flavanols, are the most complex class of the flavonoids because they range in size from simple monomers (catechin and its isomer epicatechin) to the oligomeric and polymeric proanthocyanidins, which are also known as condensed tannins. Proanthocyanidins can occur as polymers of up to 50 units, and when hydroxylated they can form gallocatechins or undergo esterification to form gallic acid. Red wine contains oligomeric proanthocyanidins derived mainly from the seeds of black grapes. Green tea is also a rich source of flavan-3-ols, principally epigallocatechin, epigallocatechin gallate, and epicatechin gallate. However, during fermentation of tea leaves the levels of catechins decline and thus the main components of black tea are high-molecular-weight thearubigins, whose structures are derived from flavonoids. The catechins are widespread, but the main sources in the diet come from processed plant-foods such as tea, wine, and chocolate.

Anthocyanins

Anthocyanins are widespread in nature, predominantly in fruits, leaves, and flower tissues, in which they are responsible for the red, blue, and purple colors. They are also found in stems, seeds, and root tissue. In plants, they protect against excessive light by shading leaf mesophyll cells and scavenging radicals. Additionally, they play an important role in attracting pollinating insects. The most common anthocyanins are cyanidin, pelargonidin, delphinidin, peonidin, petunidin, and malvidin, which are present in plants as sugar conjugates.

Flavanones

The flavanones are the first flavonoid products of the flavonoid biosynthetic pathway. They are characterized by the presence of a chiral center at C2 and the absence of the C2–C3 bond. The flavanone structure is highly reactive, and they have been reported to undergo hydroxylation, glycosylation, and O-methylation reactions. Flavanones are present in high levels in citrus fruits, with the most common glycoside known as hesperidin (hesperetin-7-*o*-rutinoside), which is present in citrus peel. Interestingly, flavanone rutinosides are tasteless, whereas the flavanone neohesperidoside conjugates (e.g., neohesperidin)

from bitter orange and naringenin (naringenin-7-*o*-neohesperidoside) from grapefruit peel have an intensely bitter taste.

Isoflavones

Isoflavones are flavonoids, but they are also called phytoestrogens because of their ability to bind to estrogen receptors and exert weak oestrogenic activity. Apart from some basic structural similarities to mammalian estrogens, the key to their estrogenic effect is their unique structural orientation and the presence of the hydroxyl groups on the A and B rings. They are classified as both estrogen agonists and antagonists because they compete with estrogen for their receptors. They have also been demonstrated to exert a wide number of biological effects that are independent of their estrogen receptor activity.

Current Estimates of Intake

Diets rich in plant-derived foods can provide more than 1 g of phenolic compounds per day (which includes phenolic acids, flavonoids, and their polymers), although there are major international and inter-individual differences in exposure. There are six main diet-derived flavonoid subclasses of present interest to human health; namely, flavonols, flavones, flavan-3-ols, flavanones, anthocyanins, and isoflavones, and their principal dietary sources are shown in (Table 1).

Given the differences in dietary intake, particularly for fruits and vegetables, between populations, it is not surprising that the relationships between the predominant flavonoids and their sources will vary between populations, nor is it unexpected that there will be wide inter- and intra-individual variations in intake of the individual flavonoid subclasses. It is only recently that comprehensive databases for estimating intakes of the diverse subclasses of flavonoids have become available. Previous estimates have been based on only a few subclasses and vary considerably among studies depending on which subclasses were included and which foods were considered in the assessment of intake. Two recent studies have estimated the range of intake of the different flavonoid subclasses in the USA and Spain (Table 2). In the USA, flavan-3-ols contributed the most to total flavonoid intake, although no data were available for estimating proanthocyanidin intakes in this study. In Spain, higher intakes of flavanones and anthocyanins were observed compared to the USA. The main sources of total flavonoid intake were apples, red wine, and other fruits in Spain, whereas tea and citrus fruits and juices were the main contributors in the USA. (Table 1).

Table 1 Principal dietary sources of flavonoids

Flavonoid	Compound	Food source
Flavonol	Quercetin, kempferol, myricetin	Onion, apple, broccoli, tea, olives, kale, cranberry, lettuce, beans (green, yellow)
Flavone	Luteolin, apigenin, tangeretin	Olives, celery, parsley, tangerines
Flavan-3-ol	Catechin, epicatechin, epigallocatechin	Tea, red wine, chocolate, apple
Flavanone	Naringenin, hesperidin	Citrus fruit
Anthocyanin	Cyanidin, delphinidin, malvidin, petunidin	Grapes, cherries, berries, blood orange juice
Isoflavone	Genistein, daidzein, glycitein	Soy and soy products

Source: Reproduced from Hollman PC and Katan MB (1997) Absorption, metabolism and health effects of dietary flavonoids in man. *Biomed Pharmacother* 51(8): 305–310, and Scalbert A and Williamson G (2000) Dietary intake and bioavailability of Polyphenols. *Journal of Nutrition* 130 (8S Supplement): 2073S–2085S, with permission from JCI.

Table 2 Estimated dietary intake of flavonoid subclasses in different countries

Flavonoid subgroup	Estimated intake (mg day ⁻¹)		
	USA	Spain	Japan
Flavonol	13	19	16
Flavone	2	3	<1
Flavan-3-ol	157	33	40
Flavanone	14	79	No data
Isoflavone	<1	<1	50
Anthocyanins	3	19	No data
Proanthocyanidins	No data	189	No data

Absorption and Metabolism of Flavonoids

Many flavonoids occur as glycosides in foods, and both flavonoid structure and the type of sugar moiety determine their primary site of absorption. Some flavonoid species are absorbed in the small intestine, where the sugar moiety becomes hydrolyzed by either lactase phloridzin hydrolase, which is located at the brush border membrane or via intracellular beta-glucosidase activity. The type of glycoside present has a significant impact on the site of flavonoid absorption; glucosides are primarily absorbed from the small intestine whereas rutinosides (a disaccharide) require hydrolysis by colonic bacteria before absorption. Methylation, glucuronidation, and sulfation are also common metabolic conjugates of flavonoid metabolism. In addition, the type of flavonoid structure also impacts absorption as some forms of flavonoids, such as the isoflavones (glycosides), also require colonic hydrolysis of the sugar moiety before absorption. The absorption and metabolism of flavan-3-ols differs markedly depending on the chemical complexity of the species; for example, the location of absorption for monomeric species is primarily in the upper small intestine whereas absorption of higher-molecular-weight polymers requires prior bacterial metabolism in the large intestine before absorption of the metabolites. Absorption from the small intestine generally results in peak plasma concentrations within 1–3 h after ingestion, which is the case for most flavonoids, whereas absorption from the large intestine can take as long as 8–12 h before peak plasma concentrations of metabolites are observed.

Human and animal studies exploring the absorption and metabolism of flavonoids identify significant proportions of glucuronide, sulfate, and methylated metabolites in both the circulatory system and in tissues/organs such as the stomach, intestine, liver, brain, and eyes. Following absorption, flavonoids are readily metabolized in intestinal cells to form glucuronide and sulfate conjugates that appear in portal blood although additional conjugation (such as methylation) can occur in the liver. The conjugation of flavonoids in the small intestine and liver generally has a significant impact on polarity/water solubility and therefore rates of urinary excretion. Metabolism in other organs and tissues (such as the kidney) has also been reported. Flavonoids for the most part are processed in the body much the same as phenolic drugs, and their absorption and subsequent

metabolism can also be affected by such factors as matrix, chemical composition, relative pH, age, gender, and host genetics.

Even though some species of flavonoids are absorbed intact (i.e., primarily nonglycosylated forms), substantial amounts of lower-molecular-weight products of flavonoid degradation (spontaneous) or microbial catabolism (via colonic micro flora) are also absorbed after initial biotransformation by the colonic microflora. The colon contains numerous microorganisms, and as a result has significant capacity for catalytic and hydrolytic reactions. These colonic bacteria produce enzymes that are capable of stripping flavonoid conjugates of their sugar moieties, thus enabling free aglycones to be absorbed. The enzymes produced by colonic bacteria can also break down the flavonoids into simpler compounds, resulting in the production of a range of derivatives. The main identified products of colonic metabolism are benzoic acids, phenylacetic acids, and phenylpropionic acids. In addition various lower-molecular-weight products of ring fission can occur where all of these above listed products may be subsequently reabsorbed from the colon and enter the systemic circulation or be eliminated in feces. These products of colonic metabolism are a very active area of current flavonoid research and some of these products may be common across many species of flavonoid and in the future may prove to be useful biomarkers of flavonoid intake. The bacterial transformation of flavonoids is also an important area for future research because these metabolic reactions may result in deactivation of bioactive compounds or activation of previously inactive compounds.

Bioavailability of Flavonoids

Bioavailability in a nutritional context is a term used broadly to include a full range of digestive and metabolic factors that influence the amount and type of compound that reaches the systemic circulation. Bioavailability or pharmacokinetics of flavonoids is based on data from absorption, distribution, metabolism, and excretion (ADME) studies conducted both in humans and animals. Available data suggest that the most abundant flavonoid compounds may not necessarily lead to the highest concentrations of biologically active metabolites in target tissues nor be the most biologically active in relation to specific health outcomes. Some subclasses are rather well absorbed; for example, following ingestion, isoflavones, the flavan-3-ol epicatechin, and the flavanones can reach micromolar concentrations in plasma. On the other hand, even large oral doses of anthocyanins result in only nanomolar plasma concentrations. Absorption rates vary significantly, with anthocyanins reaching peak concentrations within 1–2 h following ingestion compared to 6–8 h for isoflavones (depending on food matrix, compositional effects, and site of intestinal absorption). Relative urinary excretion ranges from 0.3% to 43% of the ingested dose depending on the subclass; isoflavone urinary yields are high, followed by some of the flavan-3-ols, flavanones, and quercetin glucosides (flavonols) but urinary excretion rates for procyanidins, galloylated tea catechins, and anthocyanins are low. However, it is worth noting that the emphasis of previous bioavailability studies

has been on the intact parent flavonoids, and for some flavonoid subclasses it is possible that currently unidentified metabolites (arising from spontaneous breakdown or microbial catabolism) may be bioavailable and potentially responsible for significant health effects. The key role of the gut microflora in metabolism of some flavonoid compounds may explain why some exert biological effects *in vivo* even though their apparent bioavailability is low.

To date the isoflavone class has been most widely studied, and from the available evidence it is clear that in healthy adults, isoflavones are absorbed rapidly and efficiently. Following the consumption of either pure compounds, isoflavone-rich extracts or foods/beverages rich in isoflavones, the parent compounds and their metabolites can be detected in plasma and urine of human volunteers. After ingestion, isoflavones are hydrolyzed by intestinal glucosidases, which release the aglycones, daidzein, genistein, and glycitein. These may be absorbed or further metabolized to many specific metabolites including equol and *p*-ethyl phenol. Numerous studies attest to the fact that following ingestion, soy isoflavones attain maximal plasma concentrations within 4–8 h, and are then eliminated from the body through the bile and kidneys with a mean terminal elimination $t_{1/2}$ (half-life) that is approximately 8 h on average. There is evidence from several studies that high concentrations of isoflavones can be found in tissues: breast tissue of premenopausal women and in prostate glands of men. Our knowledge of the bioavailability of other flavonoid subclasses is less well-studied to date.

As with pharmacological compounds, demonstrating efficacy and understanding potential risks of flavonoids requires knowledge of their bioavailability. Further knowledge of how factors, including genetic determinants, food matrix, chemical composition, and age affect the bioavailability of flavonoids is an important area for future research.

Potential Mechanisms of Action

The effect of flavonoids on biological processes has been extensively studied, but few investigations have attempted to determine the actual flavonoid metabolites responsible for the observed effects. Much of the *in vitro* data assumes that biological activity originates from the parent/precursor flavonoids ingested without taking into consideration the biotransformation that may occur following ingestion and metabolism. It is well established that following ingestion they are transformed into a range of structurally distinct metabolic conjugates or degradation products. The majority of *in vitro* research has also been carried out with single flavonoids and few studies have investigated the relative effects of single compounds versus mixtures of compounds or the effects of factors such as matrix on absorption, metabolism, and bioactivity.

When interpreting the present mechanistic data, it is also important to note that little attention has been paid to physiologically relevant concentrations of flavonoids in *in vitro* model systems. Thus, in some instances, biological effects have been shown at concentrations that are unachievable *in vivo* following 'normal' habitual dietary consumption; therefore,

the biological relevance of these mechanisms to humans is questionable. Despite these issues, there is considerable evidence to suggest that flavonoids have beneficial biological activities.

Epidemiological and experimental evidence supports the contribution of many dietary flavonoids to improving cardiovascular health, reducing cancer, and neurodegenerative disease risk (refer to bioactivity section below), with specific indications for improvements in vascular blood flow, hypertension, and cell cycle progression. As the pathophysiological processes leading to the development of CVD and cancer are so complex, there are numerous potential mechanisms by which bioactive plant compounds present in food could act and elucidating these underlying mechanisms is a key aim for nutrition research. Given that lipid peroxidation and oxygen free radicals are thought to be involved in conditions such as atherosclerosis, cancer, neurodegenerative diseases, and various inflammatory conditions, and that the flavonoid hydroxylated benzoid ring structure lends itself to radical scavenging, the primary health focus for flavonoids was traditionally thought to be through their direct antioxidant properties. Indeed *in vitro* studies have shown that flavonoids are efficient scavengers of free radicals; however, based on the *in vivo* bioavailability of flavonoids and their blood concentrations relative to other endogenous antioxidants, their mechanisms of action are unlikely to be the result of global radical scavenging. Therefore more recent research has focused on exploring other mechanistic actions of flavonoids using more physiologically relevant concentrations. Some recent examples of these mechanistic activities include: inhibition of cyclooxygenase, which in turn reduces platelet aggregation and thrombosis; inhibition of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) and regulation of nitric oxide synthase, which are involved with inflammation and vascular function; immunomodulation, anti-inflammatory, direct effects on enzyme function and gene and protein expression, selective interactions with protein kinase and lipid kinase signalling cascades and transcription factors such as Nuclear factor (erythroid-derived 2)-like 2 (NRF2) and nuclear factor kappa beta (NF- κ B).

Potential Health Effects

There is substantial epidemiological evidence that populations that consume diets rich in plant foods have a reduced risk of CVD, various cancers, and other age-related conditions. Identification of the role of flavonoids in the primary mechanisms that may protect against cellular damage may yield clues to slowing aspects of the aging process and postpone age-related diseases.

Although historically, the biological effects of flavonoids was attributed to their antioxidant actions, through their ability to scavenge reactive oxygen and other radicals, recent evidence suggests that this classic hydrogen-donating antioxidant activity cannot account for the reported *in vivo* bioactivity of flavonoids, and recently attention has been focussed on their other biological effects, including anti-inflammatory effects and effects on cell signalling pathways.

Cardiovascular Health

Intervention and experimental studies have demonstrated roles of some flavonoids in pathways involved in CVD initiation and progression, including improvement in vascular function, hypocholesterolemic effects, reduction of foam cell formation, thrombosis and inflammation, and protection against ischemia-reperfusion injury and arrhythmia. Differential effects of different subclasses have been observed, and some subclasses have been shown to exert beneficial effects on blood pressure by increasing endothelial derived nitric oxide (NO), either via modulation of endothelial nitric oxide synthase (eNOS) activity/expression, changes in eNOS substrate availability or through the prevention of radical induced NO conversion caused by enzymes such as NADPH oxidase. Together these functions implicate the importance of specific flavonoids in a host of CVD-related conditions, including atherosclerosis, hypertension, congestive heart failure, cardiac hypertrophy, ischemic heart disease, and others.

Results from a recent meta-analysis of randomized controlled trials on flavonoids and flavonoid-rich foods provide evidence that some subclasses of flavonoids are associated with a significant reduction in blood pressure. For example, short-term interventions (1–18 week duration) with cocoa flavan-3-ols significantly reduced systolic and diastolic blood pressure. However, to date, for a number of flavonoid subclasses including anthocyanins, there are very few published studies to systematically examine their potential effects on CVD risk biomarkers, whereas for others the levels of flavonoids administered in the interventions were well beyond the range typically consumed in the diet. Further long-term trials are required to substantiate the potential cardioprotective effects of different flavonoid subclasses.

Neuroprotective Effects

Many flavonoids can cross the blood brain barrier, and therefore some flavonoids (or their metabolites) are present in the brain and have the potential to exert neuroprotective/neuroinflammatory effects. Ongoing research is therefore examining their relative effects on cognitive function and disorders such as Parkinsons disease.

In experimental studies, administration of flavonoids or flavonoid-rich foods (e.g., berries) protects dopamine neurons from oxidative damage and apoptosis and inhibits formation of α -synuclein fibrils. Other potential mechanisms for the effects of flavonoids in the brain include interactions with neuronal signalling pathways that are critical in controlling neuronal survival and differentiation and in modulating activity/expression of several oxidative-related enzymes (e.g., eNOS and superoxide dismutase (SOD)), and regulation of mitochondrial function or neuroinflammation. In a review of animal studies, oral administration of blueberry or strawberry extract consistently showed favorable neuroprotective effects including increased dopamine release, alleviating oxidative stress or suppressing neuroinflammation. However, flavonoid subclasses differ in their ability to cross the blood brain barrier and these differences depend in part on the lipophilicity and polarity of the flavonoid compound. During absorption flavonoids are extensively metabolized, with

chemical transformations resulting in O-methylation and glucuronidation during phase II metabolism, which may have a significant impact on flavonoid bioavailability to the brain. It is therefore possible that the less polar O-methylated metabolites, for example O-methylated epicatechin metabolites, which are formed in the small intestine and liver, may be more bioavailable to the brain than their parent aglycones.

Cancer

Some flavonoids have been shown to suppress proliferation and induce apoptosis in cancer cell lines; they have been shown to delete aberrant epigenetic marks, resulting in the re-expression of abnormally silenced genes, and they can inhibit angiogenesis in established tumors. In experimental animals, some flavonoids have been shown to inhibit cancer at various stages in the cancer process from initiation to metastasis. However the available evidence from epidemiological studies remains inconclusive. In a recent systematic review of green tea and cancer prevention, which included 23 cohort studies and 27 case-control studies, the data were equivocal. Results were contradictory, particularly for cancers of the digestive tract although there was some limited evidence for protection against lung cancer.

Safety

Although flavonoids may have potential health effects, the function of many of these compounds in the plant is to discourage attack by fungal parasites, herbivores, and pathogens. As a result, it is not surprising that many are toxic and mutagenic at high concentrations in cell culture systems, and excessive consumption by animals or humans may hypothetically cause adverse metabolic reactions. However, the concentrations used in cell culture experiments in general tend to far exceed the levels that are achievable *in vivo* following dietary consumption. In addition, all of these compounds have short half-lives and do not appear to accumulate in tissues thus suggesting low toxicity. For the majority of the identified phytochemicals, there are currently limited data on the 'safe level' of intake or optimal level of intake for health benefits, and it is critical that these margins be more clearly defined in future research, particularly given the growing number of flavonoid supplements in the market.

Conclusions

There is increasing evidence that flavonoids may be protective against a number of age-related disorders. Data suggest that diets high in flavonoids may not only reduce the risk of CVD and cancer but also, by protecting against cellular damage, may slow aspects of the aging process and improve quality of life by postponing age-related diseases. There is still much to be uncovered about their bioavailability, mode of action, and optimal doses or, indeed, the actual compounds responsible for their health effects (i.e., metabolites). Given that there are still significant gaps in our knowledge base of flavonoid bioactivity, there are currently no formal recommended

dietary intakes for these phytochemicals, but on the basis of the available evidence, people should consume a wide variety of foods that incorporate the various phytochemicals to maximize disease prevention. Further research is required to define optimal doses for potential health effects and to define safe levels of intakes. Many of these compounds should be viewed as pharmacologically active compounds because although they occur naturally, they still require the same levels of proof of efficacy and safety in use as synthetic pharmaceutical agents or dietary supplements.

Further Reading

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Health Effects

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Introduction

Phytochemicals are bioactive nonnutrient components of plants, commonly found in the human diet, that may have beneficial (or harmful) health effects and include flavonoids, glucosinolates, organosulfur compounds, saponins, monoterpenes, sesquiterpenes, capsaicinoids, and capsinoids. Currently, there is considerable interest in the potential health effects of dietary phytochemicals such as flavonoids, including isoflavones and other polyphenolic compounds such as resveratrol. Possible health benefits of these dietary components including protection against cardiovascular disease, cancer, osteoporosis, and cognitive-decline are evaluated. Potential mechanisms of action and possible safety concerns are also considered.

Dietary Sources

Flavonoids are a group of more than 4000 polyphenolic compounds found in many plant foods. They are present in significant quantities in a wide range of commonly consumed vegetables, fruits, herbs, grain, and beverages. Flavonoid subclasses include flavonols, for example, quercetin, flavan-3-ols (or catechins), flavones, isoflavones, for example, genistein and daidzein, flavanones and anthocyanadins. Flavonoid-rich foods include chocolate, cocoa, black tea, onions, green tea, red wine, grape juice, berries, fruit, and soy.

The isoflavones genistein, daidzen, and glycitein, are found mainly in legumes (predominantly in their glucoside forms), principally the soy bean (*Glycine max*). Soy beans are mostly consumed either as traditional soy foods such as tofu, soy milk, miso and tempeh or as soy protein products such as soy protein concentrate, isolated soy protein (used in meatless, vegetarian, and vegan food products) and soy flour.

Genistein, daidzein, and the daidzein metabolite equol (an isoflavan), produced by gut bacteria, can be considered to be phytoestrogens or plant estrogens. These are nonsteroidal polyphenolic plant compounds that are able to induce biological responses and can modulate or weakly mimic the effects of endogenous estrogens, usually by binding to estrogen receptors (ERs) (see the Section on Biological Activity).

Other phytoestrogens include prenylated flavonoids such as 8-prenylnaringenin (present in hops and beer), lignans (found in cereals, linseed also known as flaxseed, and other fruits and vegetables, and metabolized by the gut microflora to enterolactone and enterodiol), coumestans such as coumesterol (found in young sprouting legumes such as alfalfa sprouts and clover), and the stilbene resveratrol (found in grapes and red wine).

Although the main sources of dietary phytoestrogens are fruits and vegetables, they are also present in dairy products, and milk in particular has been found to contain equol and enterolactone, and in other foods of animal origin. The

highest amount of phytoestrogens in foods commonly consumed in the UK is in bread (up to 1 mg per 100 g). Isoflavones are the main type of phytoestrogens found in bread and are derived from soy, which is added to the bread dough as part of the Chorleywood Bread Process. In countries such as the US where different processes are used, the isoflavone content is considerably lower ($\sim 190 \mu\text{g}$ per 100 g for commercial white bread).

Dietary intakes of isoflavones have been estimated to be approximately $30\text{--}50 \text{ mg day}^{-1}$ for Asian populations (upper intake $\sim 100 \text{ mg day}^{-1}$: $\sim 5\%$ of population), compared with only $1\text{--}2 \text{ mg day}^{-1}$ for most Western populations. In contrast, total flavonoid and quercetin intakes in some Western populations are approximately 150 and 29 mg day^{-1} , respectively.

Metabolism and Bioavailability

The absorption, metabolism, distribution, and overall bioavailability of flavonoids are likely to be of crucial importance to their health effects. Flavonoids are substrates for phase I and phase II enzymes in the small intestine and liver, where they are deglycosylated and metabolized into glucuronides, sulfates, and *O*-methylated derivatives. In the colon, gut microflora metabolism converts flavonoids to simple phenolic acids that may be absorbed and undergo further metabolism in the liver. Many studies have been published on the absorption, metabolism and bioavailability of isoflavones in adults. After ingestion, isoflavones are hydrolyzed by gut bacterial glucosidases and by mammalian lactase phlorizin hydrolase, which release the aglycons, daidzein, genistein, and glycitein. These may be absorbed intact or further metabolized by the gut microflora to metabolites, including the conversion of daidzein to equol or *O*-desmethylangolensin. There is, however, considerable inter-individual variation in the ability to metabolize daidzein to equol. The extent of gut microflora metabolism of isoflavones in humans is variable, only approximately 25% of Western populations and 50% of Asian populations can produce equol. The bioavailability of some flavonoids is limited by the extensive biotransformation and conjugation that occurs during their absorption from the gut, in the liver, and ultimately in cells. However, there is some evidence that isoflavones may be better absorbed and more bioavailable than other subclasses of flavonoids.

Biological Activity

Flavonoids are complex molecules with multiple biological activities. Many mechanisms of action of flavonoids and related polyphenols have been suggested and these are likely to contribute to their health effects.

Isoflavones display weak estrogenic activity, and although they can compete with estradiol at the ER complex, they fail to

elicit the full response and thus can be considered to possess mixed ER agonist/antagonist properties. Furthermore, in contrast to estradiol, which has a similar binding affinity for ER α and ER β (differential tissue distributions and effects on gene expression compared with ER α), isoflavones, and metabolites, such as equol, show preferential binding for ER β . For example, genistein displays up to 20-fold greater affinity for ER β . Some of the health effects of isoflavones may be via selective activation of ER β -mediated responses and therefore isoflavones can be considered to be selective estrogen receptor modulators (SERMS) rather than simply phytoestrogens. Possible ER-independent actions of isoflavones include inhibition of enzymes such as tyrosine kinase and deoxyribonucleic acid (DNA) topoisomerase, inhibition of tumor invasiveness and anti-inflammatory and antioxidant action.

Furthermore, the biological activity of flavonoids including isoflavones is likely to involve a wide range of effects on gene expression, regulatory microRNA, and post-translational modification and modulation of cell signaling pathways. For example, flavonoid-rich berry extract (berries are rich sources of anthocyanins and quercetin) treatment has been shown to decrease expression of the dietary glucose transporters SGLT1 (apical sodium/glucose cotransporter) and GLUT2 (apical/basolateral monosaccharide transporter) and to decrease glucose uptake (in both sodium-dependent and sodium-independent pathways) in human intestinal cells. These findings may have important implications for dietary modulation by polyphenols of the postprandial glucose surge, which is associated with an increase in risk of type 2 diabetes, the metabolic syndrome, and obesity.

Although free radicals and other reactive species, such as hydrogen peroxide and peroxynitrite, can cause oxidative damage, some are also important regulatory agents in a wide range of biological phenomena. Oxidative damage may contribute to the development and pathology of age-related diseases such as cancer, neurodegenerative disease, and cardiovascular disease. The possible protective effects of dietary phenolic compounds such as flavonoids have often been attributed to antioxidant action. Although flavonoids, such as quercetin, display potent antioxidant properties *in vitro*, their ability to act as antioxidants *in vivo* is not supported by data from dietary intervention studies. However, flavonoids may exert antioxidant effects within the gastrointestinal tract because of the high concentrations present, including binding of pro-oxidant iron and perhaps inhibition of lipoxygenases and cyclooxygenases. It is of related interest that treatment of human intestinal cells with a flavonoid-rich berry extract results in a potentially beneficial modulation of expression of genes and corresponding protein abundance in the iron-uptake and copper-uptake pathways. Furthermore, if flavonoids could act as mild pro-oxidants *in vivo* (as they can *in vitro*, depending on the test system used) then they could beneficially upregulate endogenous antioxidant defenses.

In contrast, although isoflavones are relatively poor antioxidants *in vitro*, a number of dietary intervention studies have found that isoflavones can exert antioxidant effects *in vivo*, including increasing low-density lipoprotein (LDL) resistance to oxidation and lowering plasma F₂-isoprostanes (biomarker of *in vivo* lipid peroxidation). Furthermore, baseline antioxidant status of the subjects is of importance in determining

their response to isoflavones: those with impaired status showed the greatest response. These findings may reflect the ability of isoflavones to bioaccumulate in biological membranes and lipoproteins to achieve protective effects. Finally, proteomic investigations of changes in the human serum profile in response to soy isoflavone consumption suggest beneficial modulation of a number of serum proteins, including increased ceruloplasmin (antioxidant and copper regulatory properties) and decreased alpha-1-acid glycoprotein (involved in immunomodulation) levels.

Other dietary phytochemicals, such as resveratrol, also appear to interact with multiple molecular targets of diverse intracellular pathways, through numerous transcription factors and protein targets to display a wide spectrum of health effects. Potential health effects of resveratrol include anticancer (it can inhibit carcinogenesis at the stages of initiation, promotion, or progression), antidiabetic and cardioprotective actions. Resveratrol may exert antiaging activity, including a possible increase in longevity via activation of SIRT1 (an enzyme that deacetylates proteins), associated with mimicking caloric restriction and promoting life extension, although human data for these effects are still lacking.

Cardioprotection

There is evidence from epidemiological studies that consumption of dietary flavonoids such as quercetin, kaempferol, myricetin, apigenin, and luteolin found in tea, apples, onions and red wine (usually as the glycoside derivatives) may help to protect against cardiovascular disease (CVD). One of the first studies in support of this was the Zutphen Elderly Study (1993). More recently in 2008, the Kuopio Ischaemic Heart Disease Risk Factor Study found that middle-aged Finnish men (1950 men aged 42–60 years) in the highest quartile of flavonol and flavan-3-ol intake had a relative risk of 0.55 (95% CI 0.31, 0.99) and 0.59 (95% CI 0.30, 1.14) for ischemic stroke, respectively, compared with the lowest quartile. Furthermore, the relative risk for CVD in the highest quartile of flavanone and flavone intake were 0.54 (95% CI 0.32, 0.92) and 0.65 (95% CI 0.40, 1.05), respectively. These studies suggest that high intakes of certain flavonoids may be associated with decreased risk of ischemic stroke and with decreased CVD (especially coronary heart disease (CHD)) mortality.

The influence of flavonoid consumption on cardiovascular risk factors including flow-mediated dilatation (FMD; a measure of endothelial function), blood pressure (BP), and total serum cholesterol and lipoproteins has been investigated in a considerable number of dietary intervention studies, with varied results. Meta-analysis of 133 randomized controlled trials (RCTs) investigating the effects of consumption of flavonoids and flavonoid-rich foods on cardiovascular risk found that chocolate or cocoa increased FMD after acute (3.99% 95% CI 2.86, 5.12; six studies) and chronic (1.45% 95% CI 0.62, 2.28; two studies) consumption. Furthermore, chocolate and cocoa also had significant beneficial effects on systolic and diastolic BP. They decreased both systolic (−5.88 mm Hg 95% CI −9.55, −2.21; five studies) and diastolic (−3.30 mm Hg 95% CI −5.77, −0.83; four studies) BP. These effects are likely to be via an increase in circulating nitric oxide levels,

possibly via an effect of cocoa flavan-3-ols on endothelial nitric oxide synthase. Furthermore, a recent large prospective study (133 914 women and 23 043 men) found that participants in the highest quintile of anthocyanin intake (predominantly from blueberries and strawberries) had an 8% reduction in the risk of hypertension (RR 0.92, 95% CI 0.86, 0.98) compared to those in the lowest quintile. Some reduction in risk was also observed for the flavone apigenin and the flavan-3-ol catechin when the highest and lowest quintiles were compared. Selected dietary flavonoids and flavonoid-rich foods have been reported to be inversely associated with biomarkers of inflammation and endothelial dysfunction in women. Plasma levels of IL-6 were lower in the highest quintile of intake of flavones, flavanones (such as naringenin and hesperetin found in citrus fruits), and total flavonoids compared with those in the lowest quintiles by 9%, 11%, and 8%, respectively. Higher intakes of grapefruit (a rich source of the flavanone naringenin) were significantly associated with lower concentrations of plasma C-reactive protein and soluble tumor factor receptor-2. This suggests that fruits and fruit polyphenols may be beneficial in protecting against inflammation, endothelial dysfunction, and other risk factors for CVD.

Soy protein first attracted worldwide attention in 1995 (following publication of a meta-analysis) for its ability to lower cholesterol. In 1999 the U.S. Food and Drug Administration (FDA) authorized a health claim for soy protein (25 g day⁻¹ that has retained its isoflavones) and CHD, based on its cholesterol-lowering ability. Subsequently, health claims have been allowed for soy protein and cholesterol-lowering in countries around the world, including the UK (Joint Health Claims Initiative, 2002). The intrinsic effect of soy (meta-analysis of studies: 20–133 g day⁻¹ soy protein) has been shown to be a 4.3% decrease in LDL-cholesterol (LDL-C) and the extrinsic effect (displacement of food higher in saturated fat and cholesterol estimated using predictive equations for cholesterol and population survey data) a 3.6–6.0% decrease in LDL-C. The decrease in LDL-C attributable to the combined intrinsic and extrinsic effects of soy protein foods ranged from 7.9–10.3%. This indicates that soy is one of only a few food components that reduces serum cholesterol by more than 4% when added to the diet: a 4% decrease in LDL-C is associated with a 4–8% reduction in CHD risk. Furthermore, although (European Food Safety Authority (EFSA) recently rejected a health claim for the LDL-C-lowering ability of the protein component of soy, it may allow a reworded claim for soy protein containing naturally occurring isoflavones, rather than soy protein alone.

The mechanisms involved in the cholesterol-lowering ability of soy protein are not fully understood. Increasing evidence suggests that soy proteins and associated isoflavones may modulate the activities of key transcription factors leading to changes in downstream gene expression. Isoflavones are probably also acting via an ER-mediated effect leading to upregulation of the hepatic LDL receptor, which increases the removal of serum LDL-C.

Evidence from epidemiological studies, in Asian populations, suggests that the cardiovascular benefits of soy foods may extend beyond cholesterol lowering and may include improvement of endothelial function and lowering of blood

pressure. Meta-analysis of the 133 RCTs suggests that although both isolated soy protein and soy foods lowered blood pressure, soy foods were about twice as effective. This may indicate that the natural anti-hypertensive effects of soy foods are lost as a result of food processing. The beneficial effects of soy isoflavones on blood pressure are supported by the findings of two further recent meta-analyses.

Meta-analysis of 17 RCTs that investigated the effects of consumption of isoflavone-containing soy products on endothelial function found that, overall, they increased FMD by 1.15% (95% CI -0.52, 2.75) and isolated isoflavones significantly increased FMD by 1.98% (95% CI 0.07, 3.97). Furthermore, meta-analysis of nine RCTs found that isoflavone supplementation increased FMD in postmenopausal women with low (impaired) baseline FMD by 2.22% (95% CI 1.15%, 3.30%, five studies), but not in those with high (nonimpaired) baseline FMD.

Estrogen improves FMD, probably via an ER-mediated effect on nitric oxide release by endothelial cells, and isoflavones are likely to act via a similar mechanism. Additionally, isoflavones may have nongenomic ER-mediated actions on the endothelium including modulation of phosphorylation of key molecules involved in cellular signaling pathways and activation of calcium channels. Isoflavones may also improve endothelium function via non-ER-mediated effects such as anti-inflammatory and antioxidant action.

Cancer Prevention

Considerable attention has been focussed on the potential of soy isoflavones to protect against hormone-dependent cancers (breast and prostate cancer). Historically, low mortality rates due to breast and prostate cancers have been reported in Asian populations. Traditionally, the consumption of soy foods is high in Asian populations leading to the suggestion that if soy foods reduce the risk of breast and prostate cancer, it is because they contain isoflavones. It was first reported in 1991 that a reduced risk of breast cancer was observed in premenopausal Singapore Chinese women who were high-soy consumers. More recently, a meta-analysis of the eight (one cohort, seven case-control) epidemiological studies conducted in high-soy-consuming Asian populations show a significant trend of decreasing risk with increasing soy food intake. Risk was lowest (OR 0.71, 95% CI 0.60, 0.85) among those with high intake (≥ 20 mg day⁻¹) of isoflavones compared to the lowest level of soy food intake (< 5 mg isoflavones per day). In contrast, in the 11 studies conducted in low-soy consuming (highest isoflavone intake levels 0.8 mg day⁻¹ and lowest levels 0.15 mg day⁻¹) Western populations, soy intake was unrelated to breast cancer risk. Early-life exposure to isoflavones appears to be of crucial importance to their protective effects against breast cancer. Epidemiological evidence (reductions in risk ranging from 28% to 60%, four studies) is consistent with the findings of animal studies. It is likely that pubertal isoflavone exposure is protective against breast cancer by inducing changes in mammary gland morphology and signaling pathways that mimic those induced by the estrogenic environment of early first pregnancy. This may explain why, despite relatively high isoflavone exposure, the European

Prospective Investigation into Cancer and Nutrition (EPIC) found no effect on breast cancer in the vegetarian/vegan cohort of Oxford in UK, as these diets are often followed later in life.

In relation to prostate cancer, it likely appears that soy isoflavones may have a protective effect. Meta-analysis has reported a combined relative risk/OR of 0.74 for prostate cancer when comparing high with low-soy consumption. Furthermore, the combined relative risk for studies of high soy-consuming Asian populations was 0.52 compared with 0.99 for low-soy consuming Western populations. There is also some evidence that isoflavones may prevent the spread of prostate cancer. In a pilot study in prostate cancer patients, genistein exposure was found to decrease levels of matrix metalloproteinase-2 mRNA, suggesting that genistein may be able to prevent prostate tumor metastasis. This is likely to be of great importance as prostate tumors are slow growing and are often diagnosed late in life so that even modestly delaying the onset or growth of the tumor may have a big impact on prostate cancer mortality.

High intake of flavonoids has been reported to be associated with decreased risk of lung cancer in middle-aged Finnish men. Of five flavonoid subclasses (flavonols, flavanones, flavan-3-ols and anthocyanidins) flavonols and flavan-3-ols were associated with decreased risk of lung cancer. For the highest intake, the RR was 0.29 for flavonols and 0.24 for flavan-3-ols. However, no associations with prostate or colorectal cancer were found. Meta-analysis of eight prospective and four case-control studies (involving 5073 lung cancer cases and 237 981 non-cases) indicated a statistically significant association between highest flavonoid intake and reduced risk of lung cancer (RR 0.76 95% CI 0.63, 0.92). In addition, an increase in flavonoid intake of 20 mg day⁻¹ was associated with a 10% decreased risk of lung cancer (RR 0.90 95% CI 0.83, 0.97).

Cognitive Benefits

There is some less definitive evidence that consumption of flavonoid-rich foods, beverages, and dietary supplements may be beneficial to cognitive function. In addition, flavonoids may limit the neurodegeneration associated with a range of neurological disorders. One of the first dietary intervention studies to suggest cognitive protective health benefits of isoflavones in 2001 showed that consumption by young healthy male and female adults of a high-soy diet (100 mg isoflavones per day for 10 weeks) compared with a low-soy diet (0.5 mg isoflavones per day) improved cognitive function, including significantly improved short-term and long-term memory and mental flexibility. These improvements were found in both males and females. More recently, a review of 15 RCTs (predominantly interventions with isoflavone supplements in postmenopausal women) found that most studies incorporated at least one measure of executive function/working memory, with nine finding significant improvements in performance as a result of flavonoid supplementation. Isoflavones and related flavonoids are likely to mediate their beneficial effects on the brain, resulting in improved cognitive performance through processes such as improved cerebral blood flow (via increased nitric oxide release) triggering hippocampal neurogenesis and angiogenesis and scavenging

proinflammatory agents and neurotoxic species. They may also modulate cell signaling cascades in the brain, in particular, the mitogen-activated protein kinase and phosphatidylinositol 3-kinase/Akt pathways that are involved in the regulation of transcription factors related to survival and gene expression.

Bone Protective Effects

Although initial dietary intervention studies and some meta-analyses appeared to suggest beneficial effects of isoflavone on bone health, more recently a 3-year study showed that consumption of isoflavone supplements (80 or 120 mg day⁻¹) by postmenopausal women, only at the higher dose, exhibited only a modest benefit at only the femoral neck. Meta-analysis of 10 RCTs (896 women) that investigated BMD changes from baseline at the lumbar spine, total hip and femoral neck found a mean dose of 87 mg day⁻¹ of isoflavones for at least 1 year led to a small increase in spine BMD and no significant BMD changes to the hip and femoral neck. However, two recent prospective epidemiological studies that have examined the relationship between soy food intake and fracture risk in postmenopausal women both appear to show beneficial effects. In the Shanghai cohort, there were 1770 fractures among the 24 403 women over the 4.5-year follow-up and in the Singapore cohort, there were 692 hip fractures among 35 298 women over a period of 7.1 years. Although the Singapore study reported considerably lower isoflavone intakes than the Shanghai study, both studies reported a one-third reduction in risk when comparing high-soy with low-soy consumption.

The mostly contrasting results between the epidemiological studies that appear to show a bone protective effect of isoflavones and the inconsistent findings of the intervention studies may be because of differences in the exposure period because the adult Asian soy intake assessed in the epidemiological studies may reflect lifelong exposure and a 'bone healthy' lifestyle, whereas the intervention studies were of a more limited timeframe.

Menopausal Symptoms

It was suggested, in 1992, that the high consumption of soy foods by Japanese women may be responsible at least in part for the low occurrence of hot flashes reported by these women. Although more than 50 studies have evaluated the efficacy of isoflavone supplements and most support the benefits of isoflavones, the overall findings are inconsistent. In most studies the overall benefit (including placebo effect) is reduction in the severity and/frequency of flashes by approximately 50%, which appears to be the level of improvement required by women seeking an alternative to estrogen therapy. It is of considerable importance that a recent systematic review and meta-analysis has found that seven of nine of the high-genistein supplement and four of six low-genistein studies reported significant improvements and that the high-genistein isoflavone supplements were more effective. They were able to reduce frequency and severity of hot flashes by approximately 19% and 32%, respectively, beyond the placebo effect.

Health Concerns

Most of the current health concerns over phytochemicals focus on soy isoflavones and, in particular, their consumption by breast cancer patients and women at high risk of developing breast cancer, but these concerns are likely to be unfounded, as they do not appear to adversely affect markers of breast cancer risk including breast cell proliferation, breast tissue density, and circulating estrogen levels. Current advice for breast cancer patients now appears to permit soy food consumption.

There have been concerns that the estrogenic-like abilities of soy isoflavones may have feminizing effects in men, such as reducing circulating testosterone levels and sperm counts. However, the evidence, including clinical data, suggests that these concerns are unfounded, particularly as feminizing effects are not observed in response to isoflavone exposure at levels equal to or greater than typical intakes of Asian populations. Similarly, the evidence suggests that concerns relating to infant exposure to isoflavones in soy-based formulas also appear to be unfounded.

The effect of isoflavones on thyroid function is another area of concern, based primarily on *in vitro* and animal studies. Overall, the evidence indicates that isoflavones do not adversely influence thyroid function in normal iodine-replete subjects. Additionally, most soy processing reduces the levels of the goitrogens that are found in the raw soy bean, for example, a 53% loss of isoflavones in the production of soy protein isolate has been reported. However, recent findings suggest that further investigations are required in subjects with subclinical hypothyroidism.

Although, initially concerns were raised by two epidemiological studies that appeared to link soy consumption with detrimental effects on cognitive function, there were some notable limitations to these studies, and dietary intervention studies suggest that isoflavones are more likely to confer cognitive benefits, as discussed above.

Conclusions

Although, it is possible that phytochemicals such as flavonoids and especially the isoflavones, may exert beneficial health

effects including protection against cardiovascular disease, cancer, and loss of cognitive function and perhaps even increase longevity, more long-term dietary intervention studies are still required to fully determine the relevance of these dietary components to human health. In addition, further development of reliable biomarkers of polyphenol exposure is of considerable importance in identifying associations between intake and health effects.

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POTASSIUM

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The major intracellular cation in the body is potassium, which is maintained at a concentration of approximately 145 mmol l^{-1} of intracellular fluid but at much lower concentrations in the plasma and interstitial fluid ($3.8\text{--}5 \text{ mmol l}^{-1}$ of extracellular fluid). The high intracellular concentration of potassium is maintained via the activity of the Na^+/K^+ -ATPase pump. Because this enzyme is stimulated by insulin, alterations in the plasma concentration of insulin can affect cellular influx of potassium and thus plasma concentration of potassium. Relatively small changes in the concentration of extracellular potassium greatly affect the extracellular/intracellular potassium ratio and thereby affect nerve transmission, muscle contraction, and vascular tone.

In unprocessed foods, potassium occurs mainly in association with bicarbonate-generating precursors such as citrate and, to a lesser extent, with phosphate. In processed foods to which potassium is added and in supplements, the form of potassium is potassium chloride. In healthy people, a large fraction of dietary potassium is absorbed. Most potassium is excreted in urine, whereas the remainder is excreted mainly in feces, with much smaller amounts excreted in sweat. Interestingly, there are large racial differences in the percentage of dietary potassium that is excreted in the urine. Blacks excrete a lower percentage of dietary potassium than whites (e.g., 67% in blacks vs 74% in whites); this racial difference also differs by concomitant diet. Because most potassium that is filtered by the glomerulus of the kidney is reabsorbed (70–80%) in the proximal tubule, only a small amount of filtered potassium reaches the distal tubule. The majority of potassium in urine results from secretion of potassium into the cortical collecting duct, a secretion regulated by a number of factors including the hormone aldosterone. An elevated plasma concentration of potassium stimulates the adrenal cortex to release aldosterone, which in turn increases secretion of potassium in the cortical collecting duct.

Acid–Base Considerations

A diet rich in potassium from fruits and vegetables favorably affects acid–base metabolism because these foods are also rich in precursors of bicarbonate. Acting as a buffer, the bicarbonate-yielding organic anions found in fruits and vegetables neutralize noncarbonic acids generated from meats and other high-protein foods. In the setting of an inadequate intake of bicarbonate precursors, excess acid in the blood titrates bone buffer. As a result, bone becomes demineralized and calcium is released. Urinary calcium excretion increases. This state has been termed a ‘low-grade metabolic acidosis.’

Increased bone breakdown and calcium-containing kidney stones are adverse clinical consequences of excess diet-derived acids. Diets rich in potassium with its bicarbonate precursors might prevent kidney stones and bone loss. Recent studies also suggest that a diet rich in bicarbonate precursors might also retard the progression of chronic kidney disease. In processed foods to which potassium is added and in potassium supplements, the conjugate anion is typically chloride, which cannot act as a buffer.

Adverse Effects of Insufficient Potassium

Severe potassium deficiency, which most commonly results from diuretic-induced potassium losses, is characterized by a serum potassium concentration of less than 3.5 mmol l^{-1} . The adverse consequences of hypokalemia are cardiac arrhythmias, muscle weakness, and glucose intolerance. Moderate potassium deficiency, which commonly results from an inadequate dietary intake of potassium, occurs without hypokalemia and is characterized by increased blood pressure, increased salt sensitivity, an increased risk of kidney stones, and increased bone turnover. An inadequate intake of dietary potassium may also increase the risk of stroke and perhaps other cardiovascular diseases.

Kidney Stones and Bone Demineralization

Because of its effects on acid–base balance, an increased dietary potassium intake might have favorable effects on kidney stone formation. In one large observational study of women (**Figure 1**), there was a progressive inverse relationship between greater intake of potassium and incident kidney stones. At a median potassium intake of 4.7 g day^{-1} ($119 \text{ mmol day}^{-1}$), the risk of developing a kidney stone was 35% less compared to that for women with an intake of $<2.0 \text{ g day}^{-1}$ (52 mmol day^{-1}). In the one available trial, an intake of approximately $3.6\text{--}4.7 \text{ g day}^{-1}$ ($92\text{--}120 \text{ mmol day}^{-1}$) of potassium in the form of potassium citrate reduced the risk of recurrent kidney stones.

Epidemiologic studies have consistently documented that increased potassium intake is associated with greater bone mineral density. In trials, supplemental potassium bicarbonate reduced bone-turnover as manifest by less urinary calcium excretion and by biochemical evidence of greater bone formation and reduced-bone resorption. Few trials have examined the effects of potassium on bone density, and none on clinical outcomes related to osteoporosis. In the one available trial, which enrolled postmenopausal women, potassium

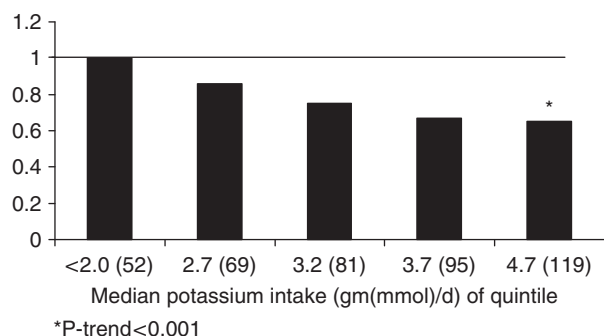


Figure 1 Relative risk of kidney stones during 12 years of follow-up by quintile of potassium intake in 91 731 women. Data from Curhan GC, Willett WC, Speizer FE, Spiegelman D, and Stampfer MJ (1997) Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk of kidney stones in women. *Annals of Internal Medicine* 126: 497–504, with permission from ACP.

citrate compared to potassium chloride significantly increased bone mineral density after 12 months of supplementation.

Elevated Blood Pressure

High levels of potassium intake are associated with reduced blood pressure. Observational data have been reasonably consistent in documenting this inverse relationship, whereas data from individual trials have been less consistent, particularly in trials of nonhypertensive individuals. Three meta-analyses of these trials have each documented a significant inverse relationship between potassium intake and blood pressure. In one meta-analysis, average net systolic/diastolic blood pressure reductions associated with a net increase in urinary potassium excretion of 2 g day^{-1} (50 mmol day^{-1}) were 4.4/2.4 mmHg. Typically, greater blood pressure reductions from potassium occur in African Americans compared to non-African Americans and in hypertensive compared to nonhypertensive individuals. Most of the trials that tested the effects of potassium on blood pressure used pill supplements, typically potassium chloride. In the few available trials, potassium chloride and potassium bicarbonate had similar effects on blood pressure.

A high potassium intake has been shown to blunt the rise in blood pressure in response to increased salt intake. The term 'salt-sensitive blood pressure' applies to those individuals or subgroups who experience the greatest reduction in blood pressure when salt intake is reduced. One metabolic study of 38 healthy, nonhypertensive men (24 African Americans and 14 non-African Americans) investigated the effect of potassium supplementation on the pressor effect of salt loading (5.7 g day^{-1} of sodium (250 mmol)). Before potassium was supplemented, 79% of the African American men and 26% of the non-African American men were termed 'salt sensitive,' as defined by a salt-induced increase in mean arterial pressure of at least 3 mmHg. There was a progressive reduction in the frequency of salt sensitivity as the dose of potassium was increased. In the African Americans with severe salt sensitivity, increasing dietary potassium to 4.7 g day^{-1} ($120 \text{ mmol day}^{-1}$)

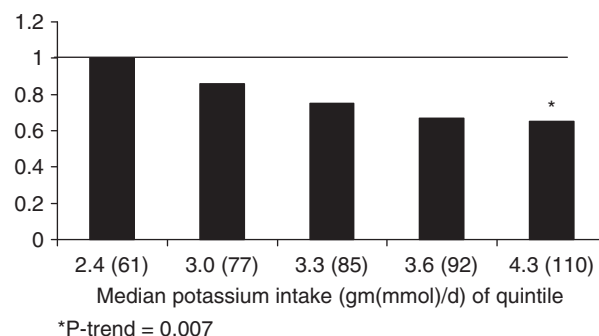


Figure 2 Relative risk of ischemic stroke by quintile of potassium intake in 43 738 men. Data from Ascherio A, Rimm EB, Hernan MA, et al. (1998) Intake of potassium, magnesium, calcium, and fiber and risk of stroke among U.S. men. *Circulation* 98: 1198–1204, with permission from LWW.

reduced the frequency of salt sensitivity to 20%, the same percentage as that observed in non-African American subjects when their potassium intake was increased to only 2.7 g day^{-1} (70 mmol day^{-1}).

Other studies indicate that potassium has greater blood pressure lowering in the context of a higher salt intake and lesser blood pressure reduction in the setting of a lower salt intake. Conversely, the blood pressure reduction from a reduced-salt intake is greatest when potassium intake is low. These data are consistent with subadditive effects of reduced-salt intake and increased potassium intake on blood pressure.

Cardiovascular Disease

The beneficial effects of potassium on blood pressure should reduce the occurrence of blood pressure-related cardiovascular disease. Potassium may also have protective effects that are independent of blood pressure reduction. This possibility has been tested in experimental studies conducted in rodents. In a series of animal models, the addition of either potassium chloride or potassium citrate markedly reduced mortality from stroke. Interestingly, these reductions occurred in the setting of stable blood pressure. Such data indicate that potassium has both blood pressure-dependent and blood pressure-independent properties that are cardioprotective.

In epidemiological studies, an inverse relationship between dietary potassium intake and subsequent stroke-associated morbidity and mortality has been noted. In a meta-analysis of 10 cohort studies, there was a significant inverse relationship between dietary potassium intake and stroke. For every 1 g day^{-1} increase in potassium intake, there was an 11% reduction in the risk of stroke. A few observational studies have also shown an inverse association between potassium intake and coronary heart disease. For example, during the course of 8 years of follow-up in 43 738 US men in the Health Professionals Follow-Up Study, there was a significant inverse relationship between baseline potassium intake and stroke after adjustment for established cardiovascular disease risk factors, including blood pressure and caloric intake (**Figure 2**). In this study, a median potassium intake of 4.3 g day^{-1}

(110 mmol day⁻¹) was associated with a 41% reduced risk of stroke in comparison to those with a median intake of 2.4 g day⁻¹ (61 mmol day⁻¹). Consistent with these studies are other observational studies that have repeatedly documented a reduced risk of stroke from an increased intake of fruits and vegetables.

In observational studies, cardiovascular events are often more strongly associated with the dietary sodium/potassium ratio, than either sodium or potassium alone. As discussed previously, such a relationship is biologically plausible. However, there is also the potential for methodological artefact, specifically, the ratio is less prone to errors from under- and overreporting in dietary assessment than absolute intake of sodium or potassium alone.

Adverse Effects of Excess Potassium Intake

In the generally healthy population with normal kidney function, a high-potassium intake from foods poses no risk because excess potassium is readily excreted in the urine. In contrast, supplemental potassium can lead to acute toxicity in healthy individuals. Also, in individuals whose urinary potassium excretion is impaired, a potassium intake less than 4.7 g day⁻¹ (120 mmol day⁻¹) is appropriate because of adverse cardiac effects (arrhythmias) from hyperkalemia. Drugs that commonly impair potassium excretion are angiotensin converting enzyme inhibitors, angiotensin receptor blockers, and potassium-sparing diuretics. Common medical conditions associated with impaired potassium excretion are diabetes, chronic renal insufficiency, end stage renal disease, severe heart failure, and adrenal insufficiency. Elderly individuals are at increased risk of hyperkalemia because they often have one or more of these conditions or take one or more of the medications that impair potassium excretion.

Recommended Potassium Intake, Current Intake, and Dietary Sources

On the basis of available data, an Institute of Medicine committee set an Adequate Intake for potassium at 4.7 g day⁻¹ (120 mmol day⁻¹) for adults. This level of dietary intake should maintain lower blood pressure levels, reduce the adverse effects of salt on blood pressure, reduce the risk of kidney stones, and possibly decrease bone loss. Current dietary intake of potassium is considerably lower than this level.

Humans evolved on a diet that was rich in potassium and bicarbonate precursors, and low in salt. However, contemporary Western-style diets have the opposite pattern – that is, relatively low content of potassium and high content of salt. Based on intake data from the National Health and Nutrition Examination Surveys (NHANESs), the percentage of men and women who consumed equal to or more than 4.7 g day⁻¹ (120 mmol day⁻¹) was low, less than 10%. Mean intake of potassium in the United States ranged from 2.8 to 3.3 g day⁻¹ (72–84 mmol day⁻¹) for adult men and 2.2–2.4 g day⁻¹ (56–61 mmol day⁻¹) for adult women.

Average potassium intake of non-African Americans exceeded that of African Americans. Because African Americans have a relatively low intake of potassium and a high prevalence of elevated blood pressure and salt sensitivity, this subgroup would especially benefit from an increased potassium intake.

Dietary intake surveys typically do not include estimates from salt substitutes and supplements. However, less than 10% of those surveyed in NHANES reported using salt substitutes or a reduced-sodium salt. Because a high dietary intake of potassium can be achieved through diet rather than pills and because potassium derived from foods also comes with bicarbonate precursors, as well as a variety of other nutrients, the preferred strategy to achieve the recommended potassium intake is to consume foods rather than supplements.

Dietary sources of potassium, as well as bicarbonate precursors, are fresh fruits, fruit juices, dried fruits, and vegetables.

Table 1 Foods rich in potassium

<i>Food</i>	<i>Portion size</i>	<i>Potassium content, g (meq)</i>
Beans		
Cooked dried beans	1/2 cup	0.4 (10.7)
Lima beans	5/8 cup	0.4 (10.8)
Fruit		
Apple	1 medium	0.1 (2.8)
Apricots	3 medium	0.3 (7.2)
Banana	6 in.	0.4 (9.5)
Cantaloupe	1/4 medium	0.3 (6.4)
Dates	10 pitted	0.6 (16.6)
Orange	1 small	0.3 (7.7)
Peach	1 medium	0.2 (5.2)
Prunes, dried	10 medium	0.7 (17.8)
Raisins	1 tablespoon	0.1 (2.0)
Watermelon	1 slice	0.6 (15.4)
Fruit juices		
Grapefruit	1 cup	0.4 (10.4)
Orange	1 cup	0.5 (12.4)
Pineapple	1 cup	0.4 (9.2)
Tomato	1 cup	0.5 (13.7)
Vegetables		
Corn	1 ear	0.2 (5.0)
Potato	–	–
White	1 boiled	0.3 (7.3)
Sweet	1 boiled	0.3 (7.7)
Tomato	1 medium	0.4 (9.4)
Squash, winter	1/2 cup boiled	0.5 (11.9)
Meats		
Hamburger	1 patty	0.4 (9.8)
Rib roast	2 slices	0.4 (11.2)
Fish (e.g., haddock)	1 medium fillet	0.3 (8.0)
Milk		
Skim milk	8 oz.	0.3 (8.5)
Whole milk	8 oz.	0.4 (9.0)

Although meat, milk, and cereal products contain potassium, their content of bicarbonate precursors does not sufficiently balance the amount of acid-forming precursors, such as sulfur amino acids, found in higher protein foods. The typical content of potassium-rich foods is displayed in [Table 1](#). Salt substitutes currently available in the marketplace range from 0.4 to 2.8 g/teaspoon (11–72 mmol/teaspoon) of potassium, all as potassium chloride.

Conclusion

Potassium is an essential nutrient that is required for normal cellular function. Although humans evolved on diets rich in potassium, contemporary diets are quite low in potassium. An increased intake of potassium from foods should prevent many of the adverse effects of inadequate potassium intake, which are higher blood pressure levels, greater salt sensitivity, increased risk of kidney stones, and possibly increased bone loss. An inadequate potassium level may also increase the risk of stroke. In view of the high prevalence of elevated blood pressure, stroke, and conditions related to bone demineralization (i.e., osteoporosis and kidney stones) in the general population, individuals should strive to increase their consumption of potassium-rich foods, particularly fruits and vegetables.

See also: Electrolytes: Acid–Base Balance. Hypertension: Dietary Factors. Nutritional Considerations for the Management of Hypertension. Osteoporosis: Nutritional Factors

Further Reading

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PREGNANCY

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Energy Requirements and Metabolic Adaptations

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The subject of energy metabolism in human pregnancy has received extensive consideration for more than 60 years, dating back to early work that assessed the contribution of fetal metabolism to the overall energy costs of pregnancy. Since then, the emphasis of much work has been on separating and quantifying the different components of gestational energy needs and on establishing appropriate recommendations for the energy requirements of pregnant women, with the intention of quantifying average amounts. Deviations from average values were mostly regarded as undesirable biological or measurement noise that needed to be overcome by studying large samples of women to get a more precise estimate of the mean values. These interindividual variations in the metabolic responses to pregnancy are increasingly recognized as biologically significant 'plasticity' that has true adaptive value in enabling women to carry a pregnancy to term under a wide range of nutritional conditions. The shorter- and longer-term consequences of such adaptations are being explored as part of fetal and infant origins of adult disease hypotheses.

Extra Energy Costs of Pregnancy

The question of how much extra dietary energy a pregnant woman needs is closely linked to the question of the amount of weight she should gain during pregnancy. This in turn is linked to her age and to her prepregnant body mass index as a proxy for energy status.

Hyttén and Leitch's theoretical estimations of the overall energy costs of human pregnancy published more than 30 years ago have subsequently been experimentally validated as reasonable average values, and they have been adopted by many national and international bodies as a partial basis for

developing recommended energy intakes in pregnancy. The costs can be divided into three main components: the energy deposited as new tissue in the conceptus, the energy deposited as fat, and the energy required to maintain this new tissue.

Tissue Deposition

Weight gain during pregnancy consists of the fetus, placenta, and amniotic fluid (the products of conception) and the extra growth of several maternal tissues. The deposition of fat in pregnancy is presumed to help meet the extra energy demands of lactation. The total energy deposited as new tissue, excluding maternal fat, averages approximately 49 MJ (11 700 kcal). If an average maternal fat gain of 2.6 kg is assumed, then the estimate of the total energy deposited as new tissue during an average pregnancy is approximately 174 MJ (41 600 kcal; **Table 1**).

Maintenance Energy Costs of Pregnancy

Because of the increase in tissue mass, the body's oxygen consumption also increases during pregnancy. Estimates suggest that the increase in oxygen consumption is equivalent to an extra 187 (45), 414 (100), 620 (148), and 951 (230) kJ day⁻¹ (kcal) at 0–10, 10–20, 20–30, and 30–40 weeks of gestation, respectively. The total maintenance cost for an average human pregnancy is approximately 150 MJ (35 800 kcal); (**Table 2**).

Theoretical Total Metabolic Costs of Pregnancy

Compared to many other mammals, humans have a relatively small and usually single infant, which develops during a long

gestation period. The energy stress to the mother is therefore low per unit time. The 49 MJ of energy deposited as the products of conception represents only 4 or 5 days of food intake for the mother. Humans also differ from most other mammals because their large fat stores can help meet some of these costs. The theoretical total metabolic costs (i.e., due to extra tissue and increased metabolism) of pregnancy are approximately 335 MJ (80 000 kcal), or 1.25 MJ day^{-1} (300 kcal). This value does not make any allowance for changes (increases or decreases) in energy expended on physical activity. It is assumed that the majority of the energy costs of human pregnancy are met by behavioral adjustments in energy metabolism rather than increased energy intake. This assumption has formed the basis for energy intake recommendations, some of which are summarized in Table 3. It should be noted that the 1985 estimates used by World Health Organization (WHO)/Food and Agriculture Organization (FAO)/United Nations University (UNU) are under revision. Future recommendations may separate the obligatory costs (e.g., by fixed increments for basal metabolic rate (BMR) and tissue deposition) and differences in physical activity (based on PAL values).

Longitudinal Studies of the Energy Costs of Pregnancy

Fat Deposition

The increase in maternal fat stores is by far the largest contributor to the energy cost of tissue deposition. It is also the most variable. Although the average increase for a well-nourished woman who has an uncomplicated pregnancy and healthy infant is approximately 3 kg, a large number of studies have reported ranges of – 2 to 8 kg and standard deviations of 2–4 kg. There is also a wide range in fat deposition between different populations, particularly when those from developed and developing countries are compared. Fat is very energy dense and therefore changes in body fat stores have a large impact on the energy costs of pregnancy. A loss of 2 kg saves approximately 78 MJ (18 600 kcal), whereas a gain of 8 kg

costs approximately 312 MJ (74 600 kcal). Women most likely to need an energy reserve to help meet the costs of lactation are often those who are least able to deposit spare energy as fat in pregnancy. Conversely, women who store large amounts of fat during pregnancy are least likely to need to use it during lactation. They are often able to increase food intake and/or decrease physical activity instead. Studies have shown that excess energy intake during pregnancy results in excess maternal weight (and fat) gain. Postpartum retention of excess fat has implications for the development of obesity and its comorbidities such as type 2 diabetes.

Basal Metabolic Rate

The cumulative increase in BMR can comprise a large part of the total energy costs of pregnancy. Although 150 MJ is a good estimate of the average energy cost of maintenance for a well-nourished woman, there is a very wide range. This has an important influence on the extra daily requirements for individual women. Studies in which BMR has been measured every 6 weeks from prepregnancy to 36 weeks of pregnancy have shown very marked differences. In some women, there is the expected response to pregnancy – an immediate and progressive increase in BMR. In other women, BMR actually

Table 2 Increases in oxygen consumption during pregnancy

	ml min^{-1}			
	10 weeks	20 weeks	30 weeks	40 weeks
Cardiac output	4.5	6.8	6.8	6.8
Respiration	0.8	1.5	2.3	3.0
Kidneys	7.0	7.0	7.0	7.0
Breasts	0.1	0.6	1.2	1.4
Uterus	0.5	1.2	2.2	3.6
Placenta	0	0.5	2.2	3.7
Fetus	0	1.1	5.5	12.4

Source: Adapted from Hytten FE (1991) Nutrition; Weight gain in pregnancy. In: Hytten F and Chamberlain G (eds.) *Clinical Physiology in Obstetrics*, 2nd edn. Oxford: Blackwell Scientific, with permission from Wiley.

Table 1 Protein and fat deposition during pregnancy for a reference woman

Site	Protein		Fat		Water (kg)	Total	
	kg	MJ (kcal)	kg	MJ (kcal)		kg	MJ (kcal)
Fetus	0.44	12.76 (3050)	0.44	20.24 (4840)	2.41	3.29	33.00 (7890)
Placenta	0.10	2.90 (690)	0.04	0.18 (43)	0.54	0.64	3.08 (740)
Amniotic fluid	0.003	0.09 (21)	0.00	0.00	0.79	0.79	0.09 (21)
Uterus	0.17	4.81 (1150)	0.04	0.18 (43)	0.80	0.97	5.00 (1200)
Breasts	0.08	2.35 (560)	0.12	0.55 (130)	0.30	0.40	2.90 (690)
Blood	0.14	3.92 (940)	0.02	0.92 (220)	1.29	1.44	4.84 (1157)
Water	0.00	0.00	0.00	0.00	1.50	1.50	0.00
Subtotal	0.93	26.83 (6400)	0.48	22.08 (5280)	7.63	9.04	48.9 (11 700)
Fat stores	0.07	1.94 (460)	2.68	123.10 (29 400)	0.60	3.35	125.04 (29 900)
Total	0.99	28.77 (6900)	3.16	145.18 (34 700)	8.24	12.38	173.94 (41 600)

Source: Adapted from Prentice AM, Spaaij CJK, Goldberg GR, et al. (1996) Energy requirements of pregnant and lactating women. *European Journal of Clinical Nutrition* 50(supplement 1): S82–S111, with permission from Nature.

Table 3 Examples of current recommendations for energy intakes during pregnancy

	Trimester(s)	Increment, MJ day ⁻¹ (kcal day ⁻¹)	Total for pregnancy, MJ (kcal)	Qualifying comments
FAO/WHO/UNU (1985)	All	1.20 (300)	336 (80 300)	For healthy women who reduce activity Energy and protein requirements are undergoing revision (interim report published 2004)
	All	0.84 (200)	235 (56 150)	
United Kingdom (1991)	Third	0.80 (190)	74 (17 000)	Underweight women and those not reducing activity may need more
United States and Canada (2002)	First	Adult EER + 0		For women aged 19–50 years
	Second	Adult EER + 160 kcal (8 kcal week ⁻¹ × 20 weeks) + 180 kcal		EERs for pregnant adolescents are based on EER for 14- to 18-year olds
	Third	Adult EER + 272 kcal (8 kcal week ⁻¹ × 34 weeks) + 180 kcal		EER is based on total energy expenditure in the nonpregnant state; increments for pregnancy are 8 kcal week ⁻¹ for total energy expenditure and 180 kcal day ⁻¹ for tissue deposition

EER, estimated energy requirement.

decreases or increases only slightly in the early stages of pregnancy and does not increase substantially until late gestation. This offsets the later increase in BMR such that there is actually a slight net saving of energy over the entire gestation period in some of these 'energy-sparing' women. The total net cost of maintenance, estimated as the cumulative area under the curve represented by the rise in a mother's BMR above the prepregnancy baseline metabolic rate, is negative or only very small. Data indicate that this between-subject variability is found in women from both well-nourished and marginally nourished populations. However, 'energy-sparing' and 'energy-profligate' responses dominate in marginally and well-nourished women, respectively. There is a more than fivefold range between the most energy-profligate and the most energy-sparing women.

In addition to the wide variability in changes in BMR between individual women, there are also wide variations between different populations. Well-nourished affluent women from developed countries tend to show an energy-profligate increase in BMR. In marginally nourished thinner women from developing countries the increase in BMR is delayed and/or preceded by a decline in early pregnancy. The total maintenance costs of pregnancy in these studies range from +210 MJ (+50 000 kcal) to -45 MJ (-11 000 kcal).

Diet-Induced Thermogenesis

A reduction in diet-induced thermogenesis (DIT) may be a mechanism by which energy is saved during pregnancy. However, when expressed as a proportion of energy intake, DIT remains essentially unaltered during pregnancy and any changes are small and unlikely to be biologically significant.

Energy Cost of Activities

Results from a number of longitudinal studies have shown that the cost of non-weight-bearing activity changes little

until very late pregnancy. From approximately 35 weeks, the gross costs (which include changes in BMR) increase by approximately 11% and net costs by approximately 6%. The gross and net costs of weight-bearing exercise (treadmill walking and standardized step testing) remain fairly constant during the first half of pregnancy and then increase progressively by approximately 15–20% at term.

Behavioral Changes in Physical Activity

It has frequently been assumed that a behavioral reduction in the energy expended on physical activity helps to counteract the increases in expenditure due to increased body weight, and in some women this leads to saving of energy that largely meets the costs of pregnancy. However, although relatively small changes in activity patterns can potentially result in significant energy savings, there is little evidence that this occurs to a large extent. A possible reason for this is that affluent women are habitually so sedentary that there is little scope for further reduction. In contrast, in developing countries habitual levels of physical activity are high and there is therefore more potential for behavioral reductions. However, many women are likely to be unable to reduce their physical activity because of the constraints imposed by a subsistence livelihood, where farm work is obligatory for survival.

This topic has been one of considerable debate in recent years, particularly because longitudinal studies that have measured total energy expenditure with doubly labeled water have shown that many women increase the energy expended on physical activity during pregnancy, and that any decreases are not sufficient to counterbalance the energy costs of pregnancy due to tissue (fat) deposition and maintenance energy metabolism. It has been recommended that the data used by the WHO should be revised to take account of changes in energy expended on physical activity and to separate these energy costs from those of maintenance and tissue deposition.

The Dietary Reference Intakes for the United States and Canada have already incorporated these changes (Table 3).

Between-Country Comparison of the Metabolic Costs of Pregnancy

The average costs across different populations result in a wide range of energy needs from -30 MJ (-7000 kcal) to 523 MJ ($125\,000$ kcal). Studies found that the average costs in the well-nourished groups were similar to the current international assumption of 336 MJ ($80\,000$ kcal). These studies have also shown that the amount of prepregnancy body fat is strongly correlated with both the maintenance costs and the total metabolic costs of pregnancy. The combined costs of maintenance, fat deposition, and conceptus across studies from different countries drawn from emerging and affluent nations show that the energy cost of fat deposition also varies according to the state of affluence and is positively correlated with variations in maintenance requirements.

This flexibility in energy metabolism acts in a protective manner, with undernourished women showing significant energy-sparing adaptive strategies that tend to normalize energy balance. Body fat content is one of the measures of fitness for reproduction; fertility is suppressed in undernourished women. However, future unfavorable conditions cannot be anticipated and pre- or early pregnant fatness may be indicative of overall nutritional status and energy balance during pregnancy.

These relationships suggested the existence of a mechanism that can monitor the mother's prepregnancy energy status and adjust the homeorhetic changes in maternal metabolism accordingly. The discovery of leptin provides a plausible mechanism by which peripheral energy status can be centrally monitored and may coordinate the metabolic responses to pregnancy. It is clear that in addition to its role in the regulation of adipose tissue, appetite, and metabolic rate; leptin plays a significant role in several components of the reproductive axis. Evidence suggests that it plays a key role in pregnancy, including the modulation of fetal growth.

Individual Variability in the Total Energy Costs of Pregnancy

Because of the marked differences between individuals in the different components of the energy costs of pregnancy (changes in BMR, body fat, and energy expended on physical activity), the total energy costs, and therefore energy requirements, are also variable. Studies of well-nourished women indicate that the total extra energy costs of pregnancy average 418 MJ ($100\,000$ kcal), considerably higher than the estimates in Table 3, and there is a large range from 34 to 1200 MJ (8000 – $287\,000$ kcal). These values are probably representative of many women in developed countries. They show that it is impossible to prescribe energy intakes for individual women because it cannot be predicted how they will respond metabolically (BMR and fat) or behaviorally (physical activity and food intake) to pregnancy.

Implications of Energy-Sparing Adaptations for Mother and Infant

Human energy metabolism is particularly adaptable during pregnancy, with early/prepregnancy body 'fatness' being a major determinant. The adaptive strategies that maintain energy balance seem to be a coordinated biological system in which energy-sensitive modulations in metabolism help to sustain human pregnancies and protect fetal growth in highly marginal environmental circumstances. However, the existence of such mechanisms should not be misinterpreted as suggesting that maintenance of optimal nutritional status in pregnant women is not a priority because the adaptive mechanisms of the women will cope. It cannot be assumed that pregnant women will have energy-sparing alterations in metabolism and/or that physical activity decreases. Any adaptations that do occur should not be overinterpreted as suggesting that this is the case. The possible long-term detrimental effects must also be considered. The biochemical and physiological processes that are downregulated in the mother causing the suppression in BMR are unknown and there may be long-term consequences to her health and that of her infant.

The associations between maintenance needs, pregnancy weight gain, and prepregnant fatness indicate that a target weight gain of 12.5 kg is associated with maintenance costs of approximately 160 MJ ($38\,000$ kcal). Although individual women or populations may have lower maintenance requirements, these may be associated with inadequate weight gain and low-birth-weight infants. A major determinant of birth weight is maternal weight gain, and the single most important determinant of infant survival is birth weight. Although birth weight is relatively well preserved at different planes of nutrition, weight alone is an inadequate measure of an infant's overall condition at birth. Even subtle nutritional influences on the fetal environment may have long-term consequences.

As mentioned previously, pregnancy weight gain is a critical component of the overall energy costs of pregnancy. The issue of whether pregnancy weight gain drives, or is driven by, the metabolic changes is interesting, but it is clear that women who consume marginal diets have small weight gains and that women from poorer countries have much lower percentage weight gains despite having lower initial body weights. Extremes of weight gains during pregnancy may have several consequences, which may or may not be mediated directly through an effect on birth weight. Other effects of weight gain may be more subtle and may be mediated through qualitative effects on fetal growth and development at different stages of intrauterine growth. There is a considerable body of evidence that suggests that many chronic adult diseases have their origins in fetal and infant nutrition, which has refocused attention on early life as a critical period in human development.

See also: Energy: Balance. Energy Expenditure: Doubly Labeled Water; Indirect Calorimetry. Energy Metabolism. Energy Requirements. Pregnancy: Nutrient Requirements; Weight Gain

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Nutrient Requirements

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Glossary

Eclampsia The final and most severe stage of preeclampsia, which often causes seizures, and can lead to maternal coma and occasionally to maternal or infant mortality.

Homocysteinemia Elevated concentrations of homocysteine in plasma, the cause of which may include a

deficiency of folate, vitamin B₁₂, riboflavin, or vitamin B₆. In pregnancy, homocysteinemia is a risk factor for poor pregnancy outcomes.

Periconceptional In the period before conception to early pregnancy.

Introduction

Providing pregnant women with their nutrient needs is a public health priority in both wealthier and poorer countries, though the local resources to attain this objective may vary widely. Inability to meet nutrient requirements during pregnancy can have serious short- and long-term effects on maternal health, the fetus, and the infant. Indeed, it is now understood that epigenetic effects of nutrient imbalances during pregnancy can affect the health of the offspring for the remainder of their life. Most of the research that provides information on nutrient requirements during pregnancy has been conducted in industrialized countries, although trials in developing countries have been important in revealing the adverse effects of maternal nutrition and the benefits of nutrient interventions. In general, even in wealthier countries there is an unacceptably high rate of pregnancy complications many of which may be prevented by improved maternal nutrition, including anemia, low birth weight, birth defects, and preeclampsia. The situation is far worse in poorer regions of the world.

Recommended Nutrient Intakes for Pregnancy

The most recent and best-described recommended intakes of nutrients during pregnancy are those of the Institute of Medicine, developed for the United States and Canada, which are the main set presented in this article (Table 1). The recommendations for the United Kingdom were published in 1992 and are discussed here when recommendations differ substantially from those of the Institute of Medicine. Many other countries have their own sets of recommendations, as do organizations such as the Food and Agriculture Organization (FAO)/World Health Organization (WHO) and the European Economic Community. However, many of these, including FAO/WHO, do not provide EARs.

The set of Dietary Reference Intake recommendations developed by the Institute of Medicine includes several values. The EAR is the intake required to meet the nutrient needs of 50% of a population group (e.g., pregnant women). It is an important value for two reasons. First, it is the value used to estimate the prevalence of inadequate intakes of a nutrient in a population group; the percentage of a group consuming less

than the EAR of a nutrient is the percentage with an inadequate intake. For energy, the Estimated Energy Requirement (EER) is equivalent to the EAR because adding a margin of safety would lead to overweight. Second, the RDA is calculated by adding two standard deviations (usually unknown but assumed to be 20%) to the EAR. The RDA should meet the requirements of 97.5% of a population group. The Tolerable Upper Level (UL) for a nutrient is the intake above which there is a risk of adverse effects.

Table 1 shows the RDAs for nonpregnant women for comparison, and the EARs, RDAs, and ULs for pregnant women.

Energy

Maternal energy requirements increase during pregnancy due to higher basal energy expenditure as well as energy deposition in maternal and fetal tissues. Basal metabolism of the mother is higher due to the increased work by the lungs and heart and because of the metabolism of the fetus and uterus. A longitudinal study by Butte *et al.* found that basal metabolic rate increased by 10.7 ± 5.4 kcal per week of gestation, mostly in the second and third trimesters. On average, the fetus requires approximately 68 kcal day⁻¹. The substantial variability in basal energy expenditure among individual women is caused mainly by differences in fat-free mass (including maternal skeletal muscle mass and fetal tissue). The cumulative increase in basal energy expenditure during pregnancy is positively correlated with maternal fatness and weight gain. Energy requirements for the thermic effect of feeding are not different from those of nonpregnant women, nor is there much change in the total energy cost of activity. Although the increasing body weight of the mother means that the energy cost of each activity is higher, the net effect is canceled out by the fact that after approximately 25 weeks of gestation women tend to become less active. The longitudinal study by Butte *et al.* suggests that energy expenditure in physical activity decreases by approximately 100–200 kcal day⁻¹ in women with a low or normal body mass index before pregnancy and by an average of more than 400 kcal day⁻¹ in those with a high body mass index (> 26 kg m⁻²).

In deriving the recommendations for the United States and Canada, the EER during pregnancy is accepted to be the sum

Table 1 Recommended Dietary Allowances (RDAs) for nonpregnant women and Estimated Average Requirements (EARs), RDAs, and Upper Limits of nutrients for pregnant women

	<i>AI/RDA,^a adult nonpregnant woman</i>	<i>EAR, pregnancy</i>	<i>AI/RDA,^a pregnancy</i>	<i>Upper Limit, pregnancy</i>
Energy (kcal)	2000–2200 ^b	+ 340 (trimester 2) + 452 (trimester 3)	–	–
Energy (MJ)	8.37–9.21 ^c	+ 1.42 (trimester 2) + 1.89 (trimester 3)	–	–
Protein (g kg ⁻¹)	0.8	0.88	+ 1.1	None
Vitamin A (μg RAE, retinol activity equivalents)	700	550	770	3000
Vitamin D	600 IU (15 μg)	400 IU (10 μg)	600 IU (15 μg)	4000 IU (100 μg)
Vitamin E (mg α-tocopherol)	15	12	15	1000
Vitamin K (μg)	90	–	90	None
Vitamin C (mg)	75	70	85	2000
Folate (μg dietary folate equivalents)	400	520	600	1000 from fortified food + supplements
Thiamin (mg)	1.1	1.2	1.4	None
Riboflavin (mg)	1.1	1.2	1.4	None
Vitamin B6 (mg)	1.3	1.6	1.9	100 as pyridoxine
Niacin (mg NE)	14	14	18	35
Vitamin B ₁₂ (μg)	2.4	2.2	2.6	None
Pantothenic acid (mg)	5	–	6	None
Biotin (μg)	30	–	30	None
Choline (mg)	425	–	450	3500
Calcium (mg)	1000	800	1000	2500
Phosphorus (mg)	700	580	700	3500
Magnesium (mg)	320	290	350	+ 350 from supplement
Iron (mg)	18	22	27	45
Zinc (mg)	8	9.5	11	40
Iodine (μg)	150	160	220	1100
Copper (μg)	900	800	1000	10 000
Selenium (μg)	55	49	60	400
Chromium (μg)	25	–	30	None
Fluoride (mg)	3	–	3	10
Manganese (mg)	1.8	–	2	11
Molybdenum (μg)	34	40	50	2000

^aValues are Recommended Dietary Intakes (RDAs) except for pantothenic acid, biotin, and choline, where value is an Adequate Intake.

^bAssuming moderately active woman. Actual requirements vary by weight and height.

^cRequirements increase throughout pregnancy and the higher end of the range is recommended during the third trimester.

Source: Adapted from Institute of Medicine, National Academies Press, for the United States and Canada (<http://www.nap.edu>).

of the Total Energy Expenditure (TEE) of the nonpregnant woman, measured using a doubly labeled water technique, plus an estimated median change in TEE of 8 kcal per week, plus 180 kcal day⁻¹ to cover energy deposited in maternal and fetal tissues. In the first trimester of pregnancy, TEE changes little and weight gain is small, so the energy requirement is increased only during the second and third trimesters. There is no RDA or UL because energy intakes greater than the EER would lead to undesirable weight gain.

The EER for pregnancy is as follows:

Trimester 1 : nonpregnant EER + 0 kcal

Trimester 2 : nonpregnant EER + 160 kcal
(based on 8 kcal per week × 20 weeks) + 180 kcal

Trimester 3 : nonpregnant EER + 272 kcal
(based on 8 kcal per week × 34 weeks) + 180 kcal

These formulae represent average requirements in trimesters 1 and 2. If a more precise estimate of requirements is needed at a specific stage of gestation, instead of the mean increment of 160 kcal in trimester 1 and 272 kcal in trimester 2, the actual weeks of gestation can be multiplied by 8 kcal per week.

The recommendation in the United Kingdom is an additional 200 kcal (0.8 MJ) per day above the prepregnant EAR but only in the last trimester. This value is based on theoretical calculation and longitudinal studies but in practice it is recognized that there are large variations in metabolic rate, fat deposition, and physical activity across women which cause large variations in energy requirements. The UK recommendation is lower than that in the United States and Canada, in part because of the observation that the actual increase in energy intake during pregnancy is usually small. The UK recommendations note that intakes may need to be increased

more for women who are underweight at conception or who continue their prepregnancy level of physical activity.

Protein

The turnover of body protein is higher after approximately 13 weeks of pregnancy, and the mother adjusts by losing less nitrogen as urea even during the first trimester. A woman who gains 12.5 kg of body weight has deposited 925 g of protein, the fetus gains 440 g, the uterus 166 g, expanded maternal blood volume contains 81 g, the placenta 100 g, and the increment in extracellular fluid, 135 g. The mother probably stores some additional protein in her body, presumably in muscle. The EAR for all age groups is $0.88 \text{ g}^{-1} \text{ kg}^{-1} \text{ day}^{-1}$ protein or 21 g of additional protein/day. The RDA is 1.1 g protein per kg per day or 25 g day^{-1} .

One-third of the 925 g total protein deposition during the 40 weeks of pregnancy occurs in the second trimester and two-thirds in the third trimester. By the end of the third trimester, the US–Canada recommendations assume that an additional consumption of 17 g protein per day is required to meet the needs for protein deposition, and as about half of this occurs during the second trimester this amounts to 8 g day^{-1} . It is also assumed that no additional protein is needed in trimester 1, but for the last two trimesters consumption of an additional 21 g day^{-1} (a total of $1.1 \text{ g kg}^{-1} \text{ day}^{-1}$) is recommended. The recommended UK protein intake is an additional 6 g day^{-1} during all three trimesters.

No UL has been set for protein, including for pregnancy, in the US–Canada recommendations due to lack of data on harmful effects. However, some earlier studies noted adverse pregnancy outcomes when high-protein supplements were given to relatively well-nourished pregnant women, so caution in this regard is certainly warranted.

Folate

Maternal folate requirements increase markedly during pregnancy due to utilization of the vitamin in cell division in the mother and fetus, single-carbon transfer reactions, and deposition in the fetus. Approximately a decade ago, research including randomized controlled trials finally proved that the risk of women giving birth to an infant with a neural tube defect (NTD) was significantly reduced if they consumed folic acid supplements before conception through approximately the first 4–6 weeks of pregnancy – during the time of neural tube closure. Some women are at greater risk of producing an infant with this birth defect, especially when their folate intake is rather low. Because such women are unaware of this risk unless they have had a previous NTD delivery, the recommendation is that all women who are capable of becoming pregnant consume at least 400 µg of folic acid daily from supplements, fortified food, or both in addition to consuming food folate from a varied diet.

In pregnancy, the recommendation is for all women to consume an additional 200 µg of synthetic folic acid (equivalent to 400 µg of dietary folate due to the higher bioavailability of the synthetic form) in addition to the RDA

for the nonpregnant woman of 400 µg day^{-1} . This amount was shown to prevent plasma homocysteine from becoming elevated during pregnancy and to maintain normal folate concentration in red blood cells. The UL of 1000 µg day^{-1} , the same as for nonpregnant women, is set to avoid potential exacerbation of vitamin B₁₂ deficiency.

In the United Kingdom, the recommendation is an additional 400 µg day^{-1} , presumably as a supplement and at least during the first 12 weeks of pregnancy, in addition to the recommendation of 200 µg day^{-1} for the nonpregnant, nonlactating woman.

In addition to its importance for lowering risk of NTDs in the periconceptional period, there is evidence that adequate folate status, which is important for maintaining normal plasma homocysteine concentrations, lowers the risk of other delivery problems and birth defects, including preeclampsia, preterm delivery, very low birth weight, club foot, and placental abruption. In the United States, Canada, and many other countries (more than 20 in Latin America alone), wheat flour is fortified with folic acid to ensure adequate folate status for pregnant women.

Other B Vitamins

Several B vitamin deficiencies cause homocysteinemia, notably folic acid, vitamin B₁₂, riboflavin, and vitamin B₆. Importantly, homocysteinemia is associated with adverse pregnancy outcomes. In a large retrospective study in Norway, for example, women in the highest 25% of plasma homocysteine concentrations had significantly more placental abruption, stillbirths, very low birth weight and preterm infants, preeclampsia, club foot, and NTDs in their offspring compared to women with values in the lowest 25%. Supplementation with folic acid up to $500\text{--}600 \text{ µg day}^{-1}$ lowers plasma homocysteine, but few studies have been done on the other B vitamins. Of these, it is most difficult for poor women to obtain their dietary vitamin B₁₂ requirement because this vitamin is found only in animal source foods, such as meat and dairy products.

The recommended intakes of most B vitamins and choline are increased above nonpregnant values as shown in **Table 1**. The increases are based on evidence for higher maternal requirements (in the case of thiamin, riboflavin, niacin, and vitamin B₆) and for fetal and placental deposition of the vitamin (thiamin, riboflavin, niacin, vitamin B₆, vitamin B₁₂, and choline). UL values, the same as for nonpregnant women, have been set for niacin when consumed as nicotinic acid in supplements based on a ‘flushing’ reaction and for choline based on cholinergic reactions and a fishy body odor.

Vitamin A

The increment in vitamin A requirements during pregnancy is based on the relatively small amount of the vitamin that is found in fetal liver at birth. The liver content is assumed to be 36 µg, mostly accumulated during the last 3 months of gestation. Using an estimated 70% absorption of the vitamin from the maternal diet, the EAR is 50 µg RAE above the 500 µg

RAE requirement for the nonpregnant woman, and the 770 μg RAE RDA is 70 μg higher.

In wealthier regions of the world, vitamin A deficiency during pregnancy is rare. Rather, there is more concern about the potentially adverse effects of consuming excessive amounts of the vitamin. Based on the potential for retinol excess to cause birth defects (malformations), especially if high doses are consumed early in pregnancy, a UL of 3000 $\mu\text{g day}^{-1}$ is set for all women who may become pregnant as well as those who are pregnant. This intake is unlikely to be achieved with natural food, although it would be possible if large amounts of liver, foods fortified with the vitamin, or supplements were consumed. One situation in which this restrictive UL becomes important is in the context of developing countries where high-dose vitamin A supplements are provided to postpartum women and their infants as part of national programs. It is accepted that it is only safe to provide these high-dose supplements to the mother during the first 6 weeks postpartum, in case she becomes pregnant again. Doing so also increases the amount of retinol secreted in breast milk, to the benefit of the infant.

In a large randomized clinical trial in Nepal, there was a 40% reduction in infection-related maternal mortality after supplementing the women with approximately their RDA as retinol per week. Supplementation with β -carotene, which is a nontoxic alternative, reduced mortality by 49%. However, when the trial was replicated in Bangladesh supplementation with vitamin A or β -carotene had no effect on all-cause maternal, fetal, or infant mortality. It did reduce gestational night blindness – which occurred in approximately 9% of these women and is an important public health issue in areas with endemic vitamin A deficiency. The explanation for differences between the study outcomes is not clear, but could be the considerably lower maternal mortality rates, more deliveries by trained birth attendants, less maternal wasting, and/or better vitamin A status in Bangladesh.

Vitamin D

In the form of 25(OH) cholecalciferol (25(OH)D), vitamin D is transferred from the mother to the fetus in relatively small amounts that do not appear to cause maternal depletion. Those women who obtain adequate exposure to ultraviolet light do not need higher amounts during pregnancy. However, if usual intake declines below ≈ 150 IU (3.8 μg) per day at high latitudes (where there is little ultraviolet radiation in the winter, such as in France), evidence of low maternal 25(OH)D and infant vitamin D depletion has been observed at delivery. Among women with more highly pigmented skin, such as African Americans, or those who are veiled, the prevalence of vitamin D deficiency near term (circulating 25(OH)D < 25 –50 nmol l^{-1}) is 30–70%, compared to 5–20% in lighter skinned populations.

The effect of maternal depletion in pregnancy on fetal bone mineralization remains controversial; the Institute of Medicine committee concluded that maternal vitamin D status does not have a major influence on calcium transfer to the fetus. Poor maternal vitamin D status in pregnancy has been implicated as a risk factor in a number of diseases of childhood and adult life, in part because risk of the diseases varies

with season. These include multiple sclerosis, schizophrenia, type I diabetes and some cancers. However, a definitive link with maternal vitamin D status has not been proven.

The IOM recommendation for both adolescent and adult women is to continue to consume the amount recommended for nonpregnant women, 15 μg (600 IU day^{-1}). The UL of 100 μg (4000 IU day^{-1}) is the same as before pregnancy, and is based on the fact that this level of intake will not cause serum 25(OH)vitamin D to exceed 125–150 nmol l^{-1} , which is at the high end of serum concentrations associated with the lowest risk of adverse events such as all-cause mortality.

In the United Kingdom, it is recommended that pregnant women take a supplement providing 10 $\mu\text{g day}^{-1}$.

Vitamin C (Ascorbic Acid)

The EAR for nonpregnant women is based on the intake that attains the maximum neutrophil concentration of ascorbic acid. Maternal plasma vitamin C concentrations decline during pregnancy, probably as a result of normal hemodilution. Oxidized ascorbic acid is transferred from the maternal circulation to the fetus, where it is retained in the reduced form. Although vitamin C deficiency in pregnancy is rare in most situations, it has been associated with increased risk of premature rupture of the membranes and infections, preterm birth, and eclampsia. However, clinical trials have not shown a benefit of higher doses of vitamin C during pregnancy. Smokers have lower levels of ascorbic acid in their serum and amniotic fluid. Based on the amount known to prevent infants from developing scurvy, the EAR is increased by 10 mg day^{-1} to 66 mg day^{-1} for 14–18 years old and to 70 mg day^{-1} for adult women, and the RDA is 80 and 85 mg day^{-1} for these groups, respectively. The recommended intake is also increased by 10 mg day^{-1} in the United Kingdom. Women who smoke more than 20 cigarettes per day and regular aspirin users may require twice as much, as may heavy users of alcohol and street drugs. The UL of 2000 mg day^{-1} is based on prevention of diarrhea and gastrointestinal disturbances that occur with high intakes.

Vitamin E

There is no increase in the recommended intake of vitamin E during pregnancy, so the RDA remains at 15 mg of α -tocopherol per day for all ages. There have been no reports of deficiency of vitamin E during pregnancy nor any evidence of benefit from maternal supplementation. The UL is 1000 $\mu\text{g day}^{-1}$ of any form of the vitamin taken as a supplement, extrapolated from data showing that high levels cause hemorrhaging in rats.

Calcium

It is clear that changes in maternal calciotropic hormones and calcium metabolism (i.e., increased intestinal absorption and reduced urinary calcium excretion) enable the fetus to be supplied with adequate amounts of this mineral, and that little change in maternal intake is needed. Nor is there a correlation

between the number of pregnancies a woman has and her risk of bone fracture. Thus, for the United States and Canada there is no increase in recommended calcium intakes for pregnancy and the RDA remains at 1300 mg day^{-1} for women aged 14–18 years and 1000 mg day^{-1} for the 18- to 51-year-old group. In the United Kingdom, the recommendation is also that no increase in intake is required during pregnancy, although the recommended level of intake for nonpregnant, nonlactating women is considerably lower at 700 mg day^{-1} .

In a series of 14 randomized, controlled calcium intervention studies in different countries, increasing calcium intake in the range of $375\text{--}2000 \text{ mg day}^{-1}$ reduced maternal blood pressure and the risk of pregnancy-induced hypertension and preeclampsia by 30–40%, with a greater effect in populations that consumed diets relatively low in calcium. The multicenter Calcium for Preeclampsia Prevention trial on 4589 pregnant women in the United States found no such benefits of a 2000 mg day^{-1} supplement, presumably because of reasonably high usual intakes of the mineral. It is possible that women at higher risk of pregnancy-induced hypertension, such as those with very low calcium intakes or adolescents, may benefit from calcium supplementation.

The UL for calcium in pregnancy is the same as that for the nonpregnant woman, 2500 mg day^{-1} . This safe level is set based on the risk of kidney stones.

Phosphorus

The efficiency of phosphorus absorption increases by 15% during pregnancy. The term infant contains approximately 17 g of phosphorus at birth, mostly in bone and water. The physiological adaptations of the mother that increase calcium retention also help to supply the fetus with more phosphorus. There is no evidence that the EAR needs to increase over that recommended for nonpregnant women, so the RDA for women aged 14–18 years is 1250 mg day^{-1} and for those aged 19–50 years it is 700 mg day^{-1} . Based on the need to avoid high serum phosphorus concentrations, and the fact that phosphorus absorption is more efficient in pregnancy, the UL is set at 3500 mg day^{-1} , slightly lower than the 4000 mg day^{-1} for nonpregnant women.

Magnesium

It is assumed that the gain in fat-free mass in pregnancy (7.5 kg) is associated with a greater deposition of magnesium. If this tissue contains 470 mg kg^{-1} , after adjustment for a bioavailability of 40%, the EAR is an increase of 35 mg day^{-1} for pregnant women of all ages, and the RDA is 10% higher than this; for women aged 14–18 years, the EAR and RDA, respectively, are 335 and 400 mg; for those aged 19–30 years, these values are 290 and 350 mg; and for those 31–50 years, they are 300 and 360 mg. In the United Kingdom, there is no recommended increment in pregnancy based on the assumption that magnesium metabolism becomes more efficient to meet fetal needs.

The UL for magnesium in pregnancy is set at 350 mg day^{-1} taken as a supplement, based on the potential for higher doses of magnesium salts to cause an osmotic diarrhea.

Iron

Incremental iron requirements for the mother and fetus are relatively well established, although how these requirements should be met is more controversial. It is generally accepted that the mother needs to absorb an additional 6 mg day^{-1} to supply the amount retained by the fetus (300 mg) and placenta (60 mg) and that used to synthesize additional maternal erythrocytes (450 mg) and replace blood loss during delivery (200 mg). Some iron is saved by the lack of menstruation in pregnancy. The fetus obtains iron from the placenta in a process that involves iron transfer from maternal transferrin to transferrin receptors on the placenta, endocytosis of holo-transferrin, and release of iron into the fetal circulation. Maternal iron absorption and transfer to the fetus increases during the second and third trimesters. This process is up-regulated if the mother is iron deficient, although in recent years it has become apparent that maternal iron deficiency does reduce the amount of fetal iron stored at birth and available to the fetus during the first months of life.

The EAR for pregnancy is set at 23 mg day^{-1} for adolescents and 22 mg day^{-1} for adult women, and the RDA is 27 mg day^{-1} for both groups. Although the requirement is mainly in the last trimester, it is important to build iron stores early and to avoid high doses later, so the higher intake recommendation is distributed throughout pregnancy. The UL is the same as that for the nonpregnant woman and is based on the need to avoid gastrointestinal distress.

It has been calculated that the maternal diet can supply enough iron to meet these increased needs during pregnancy, especially if maternal iron stores are adequate at conception. For this reason, the United Kingdom does not recommend that iron intake be increased during pregnancy, except when there is evidence of iron deficiency anemia. Iron deficiency anemia is a relatively common occurrence during pregnancy, especially if maternal iron status is poor at conception, and her diet is low in absorbable iron including heme iron from meat, fish, and poultry. The WHO estimates that approximately 35–75% of women in developing countries develop iron deficiency anemia during pregnancy. In the United States, the Centers for Disease Control and Prevention reports that in 2010 anemia affected 48% in the third trimester of pregnancy and 28% postpartum. Accepted cut points for adequate hemoglobin concentration are 110 g l^{-1} in trimesters 1 and 3 and 105 g l^{-1} in trimester 2 due to midpregnancy hemodilution.

In most countries, iron supplements are recommended routinely for all pregnant women. Benefits clearly include reduction of anemia risk, improved maternal and iron status that can persist through the early postpartum period, and possibly some protection against low birth weight. The amount recommended has been reduced from former levels of 60–120 mg to 30 mg for nonanemic women and 60 mg for anemic women. The WHO recommends 60 mg day^{-1} plus $400 \mu\text{g}$ folic acid, starting as soon as pregnancy is confirmed, but recognizes that 30 mg day^{-1} may be as effective as 60 mg day^{-1} . The folic acid recommendation was originally set based on older studies showing development of folate deficiency anemia in women. Although the risk of this anemia is probably low on a global scale, folic acid supplementation is recognized to have other potential benefits.

Some countries still recommend iron supplementation only when pregnant women become anemic. There has also been considerable controversy concerning the best time to start supplementation.

Zinc

The estimated additional zinc required for pregnancy is approximately 100 mg, equivalent to 5–7% of the mother's body zinc, part of which is obtained through more efficient intestinal zinc absorption. Approximately half of this is deposited in the fetus. The EAR for pregnant women is based on an additional requirement of 2.7 mg day^{-1} during the last 10 weeks of gestation. The UL is based on evidence of impaired copper status at high intakes, as for nonpregnant women. No increment is recommended for pregnancy in the UK report, based on the assumption that needs can be met through adjustments in maternal zinc metabolism.

Zinc plays critical roles in cell division, hormone metabolism, protein and carbohydrate metabolism, and immunocompetence. Because zinc deficiency in pregnant animals causes birth defects and fetal growth retardation, there has been considerable effort to determine the effects of human zinc status on pregnancy outcome, especially in developing countries, where zinc intakes are often inadequate. In an analysis of 12 randomized, controlled intervention trials, only two (one in India and one in the United States) found that zinc supplementation increased birth weight and reduced preterm delivery risk, whereas six found no effect. In the United States study, a positive effect was found in low-income, obese African American women with below-average plasma zinc concentrations. Trials in Peru and Bangladesh showed no such benefits. In general, however, meeting recommended zinc intakes is more difficult but more critical for women whose diets are low in animal source foods and higher in fiber. High intakes (supplements) of iron and calcium may also impair zinc absorption and therefore increase requirements.

Iodine

In the many countries with endemic iodine deficiency, which include parts of the United States, Canada, and substantial areas of Europe and many other industrialized and developing countries, there is clear potential for the harmful effects of this deficiency to emerge during pregnancy. The most damaging effect of iodine deficiency is on the brain of the fetus because iodine is required for thyroid hormone, which in turn affects myelination and function of the developing central nervous system. The clinical expression of severe maternal iodine deficiency during pregnancy is cretinism, including severe mental retardation, deaf mutism, short stature, and spasticity. Injections of iodized oil before mid-pregnancy have markedly reduced cretinism and neonatal mortality in areas of severe iodine deficiency. In most countries, Universal Salt Iodization has reduced the prevalence of cretinism substantially, but milder indications of maternal

deficiency persist even in Western Europe, including countries such as Belgium.

The EAR for pregnancy is set at $160 \text{ } \mu\text{g day}^{-1}$ and the RDA at $220 \text{ } \mu\text{g day}^{-1}$ for the United States and Canada based on the amount needed to prevent increased thyroid size in previously deficient women. The UL is $1100 \text{ } \mu\text{g day}^{-1}$, the same as for nonpregnant, nonlactating women, and it is based on the need to avoid elevated thyroid-stimulating hormone concentrations. In 2008 the WHO increased its recommendation by $50 \text{ } \mu\text{g day}^{-1}$ to $250 \text{ } \mu\text{g day}^{-1}$, and established $500 \text{ } \mu\text{g day}^{-1}$ as the intake above which no additional health benefit could be expected.

Trace Elements: Copper, Selenium, Chromium, Fluoride, Manganese, and Molybdenum

Copper is required for the function of many enzymes, primarily oxidases. In pregnancy, an increased intake of this mineral is recommended to cover deposition of approximately 18 mg day^{-1} , most of which is in fetal liver. The UL ($10\,000 \text{ } \mu\text{g day}^{-1}$) is the same as for nonpregnant women, based on the need to prevent the liver damage that occurs with high intakes.

Recommended intakes of selenium for adults are based on the criterion of maximizing plasma glutathione peroxidase activity. Based on an estimated selenium content of the fetus of $1000 \text{ } \mu\text{g}$, across pregnancy this would require that an additional $4 \text{ } \mu\text{g day}^{-1}$ be consumed. The EAR is therefore increased from 45 to $49 \text{ } \mu\text{g day}^{-1}$ and the RDA from 55 to $60 \text{ } \mu\text{g day}^{-1}$. The UL is determined on the basis of hair loss and brittle nails, which occur at higher levels of intake, and is the same as that set for nonpregnant women. An intake of 60 mg day^{-1} is also recommended throughout pregnancy in the United Kingdom, which is the same as the prepregnancy value for that population.

Chromium is required for normal insulin metabolism. There are no data from which to derive a recommendation for pregnancy, so an increase of $5 \text{ } \mu\text{g day}^{-1}$ is recommended (as an AI) based on the additional weight and tissue chromium gained in pregnancy. No UL was set due to lack of documented adverse effects in humans.

For fluoride, there is no evidence that increasing the AI in pregnancy above that for the nonpregnant woman will benefit fetal tooth or bone content or afford protection against later tooth decay in the child. The UL is set at 10 mg day^{-1} to avoid fluorosis (discoloration of tooth enamel, joint pain, and skeletal abnormalities).

Manganese is required for bone formation and the normal metabolism of amino acids, lipids, and carbohydrates. The AI for pregnancy, estimated from the manganese content of maternal weight gain, is 2 mg day^{-1} . The UL is based on avoidance of elevated blood manganese and neurotoxicity, and it is not increased for pregnancy.

Recommended molybdenum intakes, based on the mineral's role as a cofactor for several enzymes, increase by 16 mg day^{-1} in pregnancy to cover the increment in fetal and maternal weight. The UL is derived from adverse reproductive effects seen in animals.

Water and Electrolytes

The US–Canada recommended intake of water for pregnant women is based on median intake from a large national survey in the United States. The AI of 3 L day^{−1} is anticipated to come from foods (0.7 l) and beverages (2.3 l). No UL was set because individuals stop drinking once their intake is adequate.

The AI for sodium in pregnancy is 1500 mg day^{−1} based on an intake level to cover daily losses, provide adequate intakes of other nutrients, and maintain normal function. The UL of 2300 mg day^{−1} is based on the adverse effects of higher intakes on blood pressure in susceptible members of the population.

The AI for potassium in pregnancy (4.7 g day^{−1}) is set at a level that will lower blood pressure, reduce the extent of salt sensitivity, and minimize the risk of kidney stones. There is no evidence that adverse effects of potassium are seen with high intakes from food and no UL was set, but potassium supplements can cause high blood potassium in some chronic diseases, such as renal disease and type 1 diabetes.

Summary

In the US–Canada recommendations, the recommended intakes are increased for most, but not all, nutrients during pregnancy. However, the recommendations are often based on less than ideal experimental data, in part due to the difficulty of conducting experiments on pregnant women.

For most nutrients, it is likely that some population groups may have higher requirements than those recommended in **Table 1**, notably women bearing more than one fetus or adolescents (see the Institute of Medicine volumes for specific recommendations for this age group). To meet the recommended nutrient increases, dietary quality often needs to be improved during pregnancy. It is often advised that pregnant women should also take iron supplements and/or a multiple vitamin–mineral supplement. The specific benefits of supplementation in pregnancy, optimal timing, and optimal doses are still somewhat controversial and the subject of ongoing research. Currently, some countries recommend routine supplementation for all pregnant women, whereas others recommend supplementation only when there is

evidence of anemia, other nutritional deficiencies, a poor diet, or other problems such as drug or alcohol abuse.

See also: Ascorbic Acid (Vitamin C): Physiology, Dietary Sources, and Requirements. Calcium. Choline and Phosphatidylcholine. Chromium. Copper. Folic Acid. Iodine: Physiology, Dietary Sources, and Requirements. Iron: Physiology, Dietary Sources, and Requirements. Magnesium. Manganese. Phosphorus: Physiology, Dietary Sources, and Requirements. Potassium. Pregnancy: Energy Requirements and Metabolic Adaptations; Safe Diets. Protein: Requirements and Role in Diet. Sodium: Physiology. Vitamin A: Physiology, Dietary Sources, and Requirements. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements. Vitamin E: Metabolism and Requirements. Zinc: Physiology, Dietary Sources, and Requirements

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Placental Regulation of Nutrient Delivery to the Fetus

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Glossary

Endothelium Thin layer of cells lining the blood vessels.

Imprinting Parent of origin specific marking or expression of genes.

In utero In the uterus.

Intrauterine growth restriction (IUGR) Pathological deviation from normal growth.

Trophoblast Placental cells formed from the outer part of the blastocyst.

Vesicle Spherical preparation of membrane bilayer used to study transport.

Villi Hair- or finger-like projection from a cell which increases the surface area.

Fetal Nutrient Requirements

Prenatal development can usefully be divided into two periods; the embryonic period, which covers the first eight weeks of life and the fetal period, which lasts from the 9th week of gestation until term. During the latter period the fetus is entirely dependent on the placenta for its supply of nutrients (Figure 1). The fetus has an absolute requirement for the same essential nutrients as the adult but the adequacy of supply is particularly critical during *in utero* life when all the structures of the body are being established. In addition, because of the particularly high demand for some strictly nonessential nutrients these may be considered as 'conditionally essential' if the rate of utilization exceeds the fetal capacity for *de novo* synthesis.

The placenta has to maintain the supply of all nutrients at a rate adequate to allow fetal growth to proceed along its optimum trajectory. It also has to provide an appropriate mix of

nutrients to meet the needs of the fetus at the different stages of pregnancy. For example, in the first two-thirds of pregnancy the fetus deposits mainly protein whereas in late gestation fat takes over as the dominant form of deposition (Figure 2).

The availability of individual nutrients to the fetus depends not only on the maternal dietary intake but also on the function of the placenta and the many physiological and biochemical adaptations, which occur during pregnancy (Figure 2). An understanding of placental function and its interaction with diet is essential to the setting of appropriate dietary guidelines for pregnancy.

The Human Placenta

The human placenta is a hemochorial, villous type where the maternal blood enters the intervillous space *via* the spiral arteries and flows directly around the terminal villi of the fetal circulation without any intervening maternal vessel wall. The surface area available for exchange gradually increases throughout pregnancy, reaching approximately 10–15 m² in the last trimester (Figure 2). The nature of the exchangeable surface of the placenta also changes throughout gestation. Mature intermediate villi appear toward the end of the second trimester and the terminal villi – the main site of feto-maternal exchange – develop a few weeks later. The rate of fetal blood delivery to the placenta (umbilical flow) also increases with gestational age. It is approximately linearly related to fetal weight, and hence the fetal nutrient requirement, throughout gestation (Figure 2).

The human placenta typically weighs approximately half a kilogram at term. However, its physical bulk belies the delicate nature of the separation between the maternal and fetal circulations, which consists of only two cell layers; the syncytiotrophoblast and the capillary endothelium. The endothelium allows the passage of nutrients through pores within the interendothelial cleft and therefore is not a significant barrier to nutrient exchange between the maternal and fetal circulations. The effective barrier between the circulations is a thin trophoblastic sheet in the form of a syncytium (a tissue in which the cytoplasm of constituent cells is

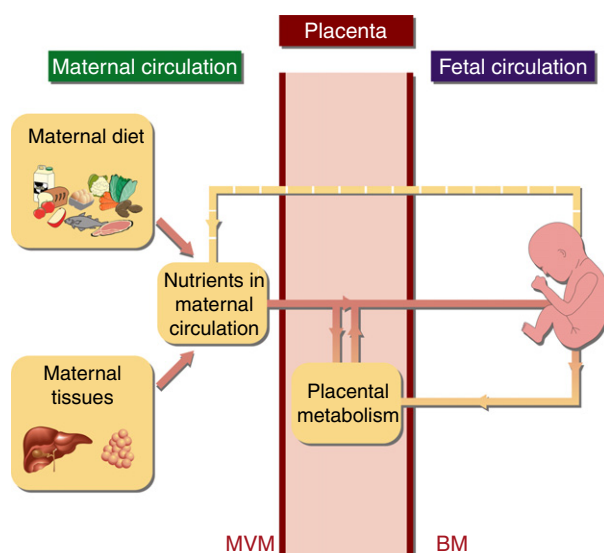


Figure 1 Nutrient exchanges between the maternal circulation, placenta, and fetus.

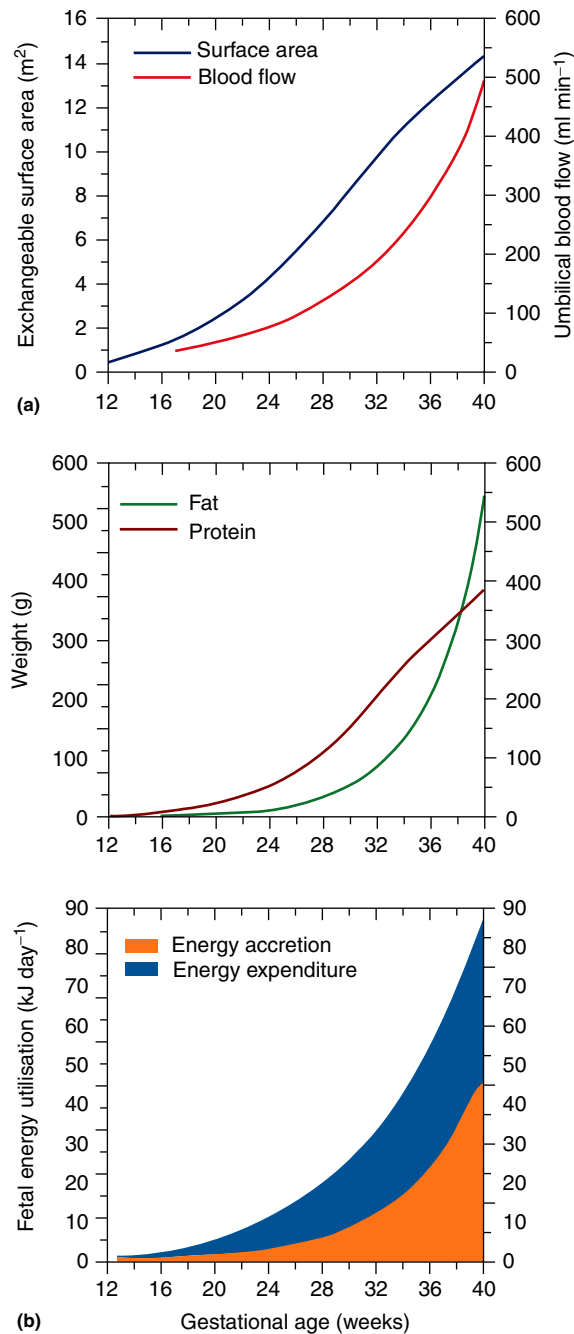


Figure 2 Changes with gestational age in placental exchangeable surface area and umbilical blood flow (a), accretion of fat and protein in the fetus (b), and the components of fetal energy requirements.

continuous), known as the syncytiotrophoblast. Between 10 weeks and term the thickness of the villous trophoblast falls from approximately 10 μm to 4 μm . Over the same period the overall materno–fetal diffusion distance drops from 40 μm to 5 μm . Any substance crossing between the maternal and fetal circulation has to pass through this barrier, which consists of two membranes; the microvillus membrane (MVM) facing the maternal blood and basal membrane (BM) facing the fetal blood. The surface area of the maternal facing MVM is approximately five- to six-times that of the fetal facing BM. There

are other cell types and structures within the placenta, such as maternal myometrium and decidua, connective tissue, Hofbauer cells, and persisting cytotrophoblast cells, which contribute to the metabolic activity and nutrient requirements of the placenta but which are not thought to be significant barriers to transport.

Methods Used to Study Placental Function

Direct measurement of placental nutrient transport function in human pregnancy is practically and ethically extremely difficult to achieve and the available techniques require a tradeoff between physiological relevance and the quality of the information derived. Stable isotope labeled amino acids and fatty acids have been administered to the mother and their appearance measured in the cord blood. Such studies are clearly physiologically relevant but their interpretation is severely constrained by the number of sequential cord blood samples which can be taken. Placental function is often inferred by measurements of concentration differences in the maternal and fetal circulations. Measurements of arterio–venous differences across the umbilical cord can be made at cesarean section but, given the significant changes in placental function during development, their relevance to the younger placenta is not clear. Cord blood nutrient levels can be measured at earlier stages of development using the invasive method of cordocentesis but this procedure carries risks and may only be justified when carried out opportunistically as part of a clinical test, and this in turn may bias the population sample toward those with fetal or placental pathology. An important limitation to the interpretation of placental function from such ‘snapshot’ measurements is that the cord blood nutrient status is the net result of both placental delivery and fetal utilization. A number of *in vitro* approaches to the study of placental function are also available. The dually perfused placenta has the advantage that it retains the cellular structure and metabolic activity of the syncytiotrophoblast and the placental vascular structure. This preparation also allows the nutrient composition of the maternal and fetal circulation to be controlled and transfer rates to be measured dynamically using isotopic tracers. However, the placenta tends to be very mature, the efficiency of perfusion cannot be assumed to exactly mimic the *in vivo* situation. Also, care has to be taken to ensure that the composition of the maternal and fetal perfusates are made up in such a way that they are relevant to the form in which nutrients are actually transported in the maternal and fetal circulations. Vesicles formed from the syncytiotrophoblast are particularly well suited to the detailed study of nutrient transport mechanisms under highly controlled conditions. Finally, there is the identification and characterization of individual transport proteins.

The Mechanisms of Placental Nutrient Transport

The transport of individual nutrients across the placenta generally depends on the same principles and the presence of the same or similar transport systems to those in the tissues and organs of the adult although there are some additional

factors specific to the placenta (Figure 3). In particular, unlike most tissues in the adult where either uptake or export dominate at any given time, the syncytiotrophoblast whose primary function is transport has to do both simultaneously.

The placental transport systems for the macronutrients (carbohydrate, fat, and protein) have been extensively studied. Glucose transport within the placenta appears to be mediated exclusively by the GLUT1 transporter, which has been located on both the MVM and BM. GLUT3 and GLUT4 are also present in the placenta but not in the syncytiotrophoblast itself. They are located on the vascular endothelium and the intra-villous stromal cells, respectively. The syncytiotrophoblast also contains a wide range of amino acid transporters; system A, ASC, Asc, B⁰, B⁰⁺, L, N, Gly, y⁺, y⁺L, and X_{AG} and β . A number of fatty acid binding proteins are also found in the placenta. Of these proteins FAT/CD36 and FATP have been located on both the MVM and BM but there is also a placenta specific protein (p-FABPpm), which has been located exclusively on the MVM. This p-FABPpm is similar in size (~40 kDa) to the ubiquitous FABPpm found in most mammalian cells but it has a different amino acid composition.

The driving force, which results in the net transfer of nutrients to the fetus is different for different nutrients and this is reflected in their transplacental gradients (Figure 4). Where the nutrient concentrations are lower in the cord than maternal blood this has been cited as a reason to supplement the mother but in many cases it is precisely this gradient which drives placental nutrient transfer. Glucose is thought to flow down a concentration gradient from the mother to the fetus and this process of 'facilitated diffusion' is mediated by GLUT1. Unlike glucose the concentration of most amino acids

in the fetal circulation is greater than that in the maternal circulation suggesting some form of active transport. For many amino acids the concentration is even higher within the placenta than the fetal circulation and the key gradient generating step for amino acids is the active transport across the MVM. The amino acids can then diffuse down a concentration gradient into the fetal circulation, and to some extent back to the mother. The concentration of water-soluble vitamins and lactate in the fetal circulation also exceeds that in the maternal circulation.

Like glucose, the fats and fat-soluble vitamins also flow down a concentration gradient from the mother to the fetus mediated by the various fatty acid transport proteins. However, unlike glucose or the amino acids, fat-soluble compounds can also cross the syncytiotrophoblast, and all other membranes for that matter, by simple diffusion and partition without the intervention of a carrier protein. The role of the fatty acid binding proteins appears to be to improve the efficiency of this process. The key factor in understanding the driving force for the placental transfer of fat-soluble nutrients is that these compounds are only sparingly soluble in water (13 μ M for C18:0 at 37 °C) and have to be transported in the plasma in hydrophobic binding sites on carrier proteins. The partition of fats between the maternal and fetal circulations is largely determined by the relative abundance of available hydrophobic binding sites within those compartments. Because only NEFA is thought to cross membranes, it is the NEFA concentration gradient which is most relevant to the transplacental flow of fatty acids. The concentration of NEFA in the maternal plasma at term is approximately three-times that in the fetal circulation but the concentration of its primary carrier protein, albumin, is actually 10–20% higher in the fetal circulation. This results in a ratio of NEFA to albumin on the fetal side of the placenta of around a quarter of that on the maternal side at term. The fat-soluble vitamins (A, E, and D) are also present in the fetal circulation in lower concentrations than in the maternal circulation. These materno–fetal concentration differences for the macronutrients develop gradually throughout gestation.

It is less easy to generalize about the transplacental gradient for minerals as some are at a lower concentration in the fetal circulation (Se, Cu, Ba) some are higher (Ca, Zn, Be, Rb) and some are about the same (Co, Mg, Mo, Sn, Bi, Cd, Cs, La, Li, Pb). Iron is particularly important during pregnancy and its concentration in the fetal venous blood leaving the placenta is almost three-times that of the maternal serum. Iron is transported in the serum on the transport protein transferrin and, like the fats and fat-soluble vitamins, its rate of transfer may be influenced by the availability of free binding sites.

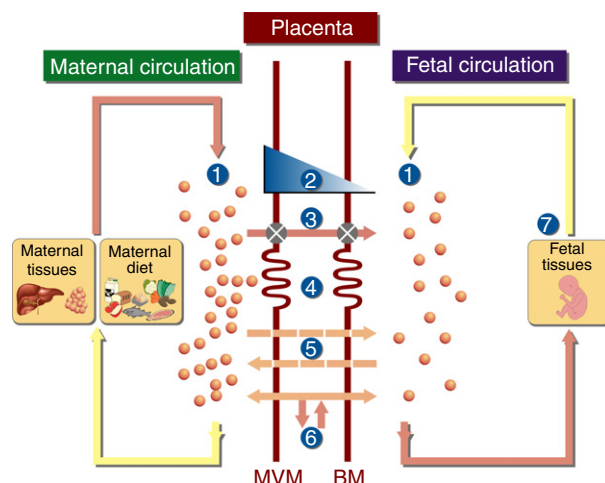


Figure 3 Factors affecting nutrient transfer across the syncytiotrophoblast. These include: (1) maternal and fetal blood flow, (2) the concentration gradient across the syncytiotrophoblast for nutrients and, where relevant, their transporters in the maternal and fetal circulations, (3) the concentration of transport proteins to facilitate or actively transport nutrients, (4) the exchangeable surface area, (5) the rate of diffusion of some nutrients across membranes without the intervention of transport proteins, (6) metabolism (utilization and *de novo* synthesis) within the placenta, and (7) the rate of nutrient utilization by the fetal tissues.

Epigenetics and the Placenta

There is growing interest in the role of epigenetics, and imprinting in particular, in controlling placental function and nutrient delivery to the fetus. The vast majority of human autosomal genes are thought to be equally expressed from the two parental alleles. In the imprinted genes the expression pattern is different for the maternally and paternally derived alleles, with information on the parental origin of each allele

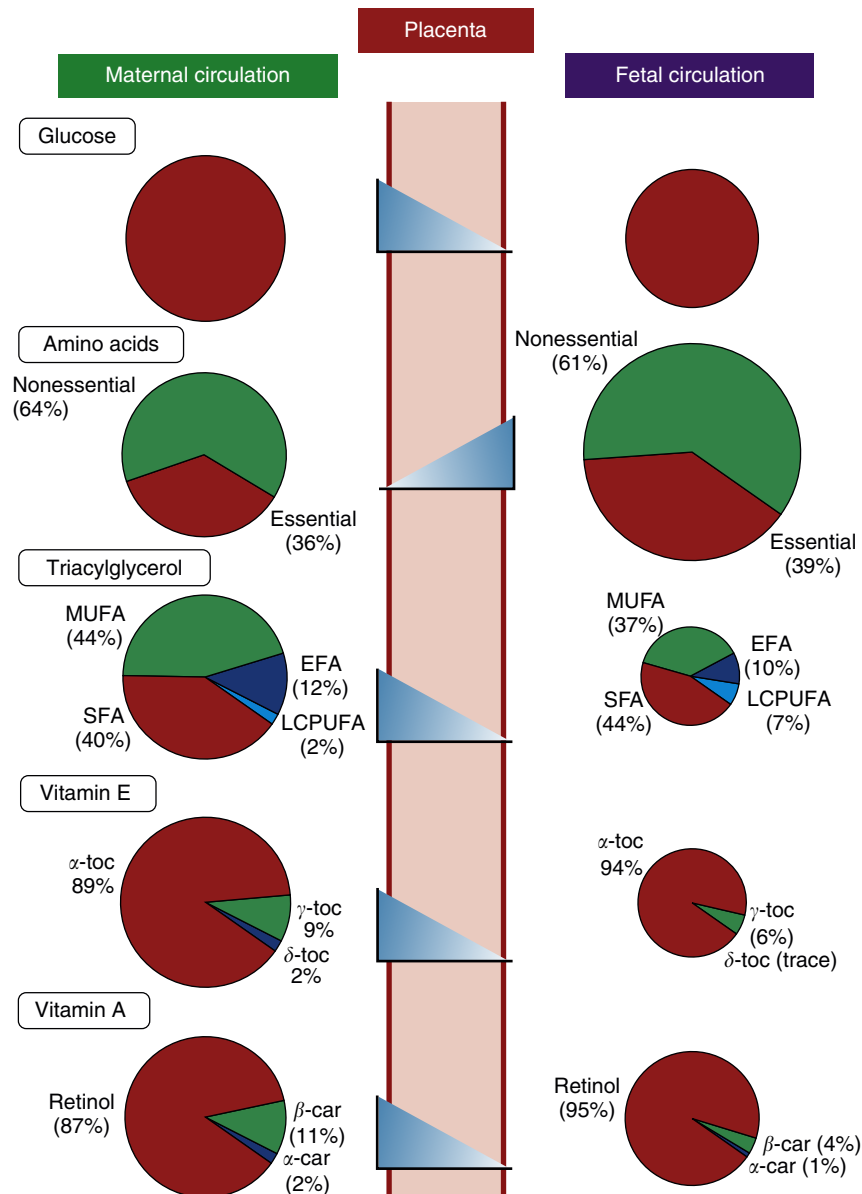


Figure 4 The relative concentration of nutrients in the maternal and fetal circulations. The concentration differences for each nutrient class are represented by the area of the circle in the fetal circulation relative to the maternal circulation. Apart from glucose the relative concentrations of individual nutrients within the nutrient groups are shown as segments of the circle. For triglyceride the fractions are saturated (SFA) monounsaturated (MUFA), essential (EFA) and long chain polyunsaturated fatty acids (LCPUFA). For vitamin E the fractions are α -tocopherol (α -toc), γ -tocopherol (γ -toc) and δ -tocopherol (δ -toc). For vitamin A the fractions are β -carotene, (β -car), and α -carotene (α -car).

being retained in the conceptus through epigenetic mechanisms. Epigenetics encompasses a collection of mechanisms that control gene function and chromatin structure without altering the nucleotide sequence of DNA and the most commonly studied is DNA methylation. Imprinted genes make up only approximately 1% of all human genes. Their main functions include control of placental function, fetal growth, and brain development but the way in which the imprint is acquired and propagated is not fully understood. Much of what is known about imprinting and developmental epigenetics comes from studies in mice, though data from human reproduction are increasingly available. Although there are

important differences between species, particularly in the timing of epigenetic events, a number of themes appear to be universal. Following demethylation and genome wide *de novo* methylation at the embryonic pregastrulation stage, striking differences in the methylation status of embryonic cell lineages within the early embryo are apparent. The trophoblast lineages, which give rise to the placenta, typically achieve a much lower level of methylation than the heavily methylated somatic cells, which give rise to the fetal tissues. Such differences, and the parent of origin specificity of the imprinted genes, have given rise to the main hypothesis that seeks to explain the phenomenon of imprinting in placental

mammals. The 'conflict theory' of imprinting is based on the premise that the function of the imprinted genes is to control resource allocation to the fetus. The logic being that the mother is more likely to pass on her genes if she spreads her body resources over a number of pregnancies whereas the father's genes are more likely to be successful in evolutionary terms if they maximize resource allocation to his offspring, even at the mother's expense. Paternally expressed imprinted genes are generally thought to enhance fetal growth by promoting resource allocation to the conceptus whereas maternally expressed imprinted genes are thought to protect the maternal resources by suppressing fetal growth. Experimental data to support this model has been obtained in mice, but it has yet to be confirmed in detail for humans.

Placental Selectivity

One of the key functions of placental nutrient transport is to maintain the most appropriate balance of nutrients in the fetal circulation and the balance of nutrients transferred by the placenta may be as important as the overall transfer capacity in influencing the pattern of fetal growth. Nutrients such as the fatty acids and amino acids occur in many forms yet they are translocated across membranes by a relatively small number of transporter molecules (**Figure 3**). This nutritional 'bottle-neck' results in competition for transfer and the possibility of placental selectivity. An example of the resulting change in nutrient quality can be seen in the increase in the relative proportion of the essential to nonessential amino acids in the fetal circulation compared to the maternal circulation (**Figure 4**). The same is true of the fat-soluble vitamins where the relative concentration of the most biologically active form is increased in the fetal circulation. In the case of the fatty acids it is the long chain polyunsaturated fatty acids (LCPUFAs) such as arachidonic acid [20:4 n-6; AA] and docosahexaenoic acid [22:6 n-3; DHA], which perform most of the essential functions in the fetus. Although the overall concentration of the lipid classes are greatly reduced in the fetal circulation the critical LCPUFA make up a greater proportion of total fatty acid in the fetal circulation. In the case of the fatty acids the placenta has multiple mechanisms including preferential binding of LCPUFA by p-FABPpm, selective uptake by the syncytiotrophoblast, intracellular metabolic channeling of individual fatty acids, and selective export to the fetal circulation, which allow it to preferentially deliver DHA and AA to the fetal circulation.

Placental Metabolic Activity

Although the barrier between the maternal and fetal circulation is effectively only one cell thick, the placenta is a substantial organ, made up of many cell types. It is extremely active metabolically and has its own requirement for nutrients and this is consistent with the observations that the surface area of the maternal facing membrane (MVM) is approximately five-times greater than that of the fetal facing membrane (BM), that the concentration of expression of GLUT1 is greater on the MVM, that the MVM contains additional fatty

acid binding proteins, which are not present on the BM, and that the amino acid transporters act to produce the maximum amino acid gradient across the MVM. The metabolic transformations within the placenta are intimately linked to fetal metabolism and represent another way in which the placenta can regulate nutrient transport availability within the fetal circulation.

In late pregnancy the overall contribution of fat to whole body oxidation is reduced and this is thought to result from the preferential utilization of carbohydrate and amino acids such as glutamate as an energy source in the feto-placental unit and the sparing of fatty acids to maximize fetal accretion of the critical LCPUFA in particular. The inter-relationships between the placenta and fetus are particularly complex for the amino acids. The placenta is a net user of serine, glutamate, leucine, isoleucine, and valine and there is significant interconversion of alanine, pyruvate, and lactate between the placenta and fetal tissues. The concentration of lactate in the fetal circulation is considerably greater than that in the maternal circulation and a considerable proportion of the glucose taken up by the placenta is converted into lactate before export into the fetal circulation for use by the fetus. The placenta takes up serine from both the maternal and fetal circulation, converting this into glycine and exporting it into the fetal circulation for oxidation by the fetal liver and there is significant cycling of glutamate and glutamine between the placenta and fetal liver. This partition of the various segments of metabolic pathways between the placenta and fetal tissues is a general phenomenon and in many respects the feto-placental unit can be considered as a metabolic whole with the placenta acting as an extra fetal organ in addition to its role as a simple nutrient transporter. Metabolic activity in the feto-placental unit is also responsive to nutrient supply and fetal demand. For example, AA is an important precursor of the prostacyclins, prostaglandins, thromboxanes, and leukotrienes, which play key roles in pregnancy. When the maternal circulation of AA is low there is net uptake by the placenta of AA from the fetal circulation, presumably to maintain placental synthesis of these important compounds.

Placental Buffering of Maternal Dietary Intake

In cases where the increased demand for nutrients during pregnancy is not met by the diet alone the shortfall may be made up from the maternal stores and the placenta may play a role in orchestrating some of the maternal nutritional adaptations in pregnancy (**Figure 3**). For example, placentally derived leptin is a potent stimulator of lipolysis and there is evidence that the rate of export into the maternal circulation is controlled to allow the placenta to modulate its own substrate supply in response to the fetal demand for fats. The various homeostatic mechanisms within the placenta and their interaction with maternal physiological adaptations during pregnancy act to ensure a constant supply of substrate to the fetus, free of large diurnal fluctuations corresponding to the timing of maternal meals, and to protect the fetus against a transiently poor intake during critical periods of fetal growth. These adaptations help the mother to meet the full fetal

requirement for nutrients such as LCPUFA and iron while consuming apparently poor diets.

Placental Insufficiency and Fetal Growth

Low birth weight is a significant public health problem in developing countries where maternal nutrition may be marginal or poor. Low birth weight resulting from poor nutrition is also a concern in industrialized societies but maternal deficiency here is relatively rare and potentially a more important public health issue relating to nutrition in pregnancy is the apparent epidemiological association between birth weight and adult disease susceptibility (cardiovascular disease, diabetes, and hypertension). The highest risk is associated with the lowest birth weight but, because of the nature of the normal distribution, in terms of the numbers potentially affected in adult life it is the small variations in the normal birth weight range, which have the largest public health implications. A causal connection between birth weight and adult disease has been proposed in the 'fetal origins' hypothesis which is that fetal undernutrition in middle to late gestation, leads to disproportionate fetal growth and programs later disease susceptibility. The close association between birth weight and placental weight has led to speculation that the placenta may limit fetal growth within the normal weight range. However, the available evidence suggests that the capacity of the normal human placenta to transport macronutrients exceeds the fetal requirement and that a considerable

proportion of transport function would have to be lost before it became limiting for fetal growth.

Intrauterine growth restriction (IUGR) resulting from utero-placental insufficiency is a serious pathology, which is associated with a greatly increased risk of adverse outcomes including perinatal mortality and morbidity, impaired mental, visual and aural development, autism, and cerebral palsy and strongly associated with serious adverse maternal outcomes especially pre-eclampsia (Figure 5). IUGR is often detected indirectly by measuring abnormal umbilical artery flow velocity waveforms and abnormal fetal heart rate. The abnormal waveforms are thought to result from increased vascular resistance associated with abnormal arteriolar tree and villi branching and a reduction in the villous capillary tree. Pregnancies in which these abnormalities are observed are also associated with fetal hypoxia and reduced concentrations of glucose and amino acids in the fetal circulation and reduced activity of the system A amino acid transporter within the placenta. However, *in vitro* studies have shown that the hypoglycemia observed in some IUGR fetuses is not caused by a decreased glucose transport capacity within the placenta (expression and activity of GLUT1) and IUGR fetuses are actually hypertriglyceridemic compared to their appropriately grown counterparts. The fetal blood concentrations of the trace elements are also either normal or elevated in IUGR. Thus although it is possible that the placenta from IUGR fetuses may limit the supply of amino acids there is no evidence that placental delivery is the first limiting factor in the supply of glucose, lipids, or trace elements. IUGR is a

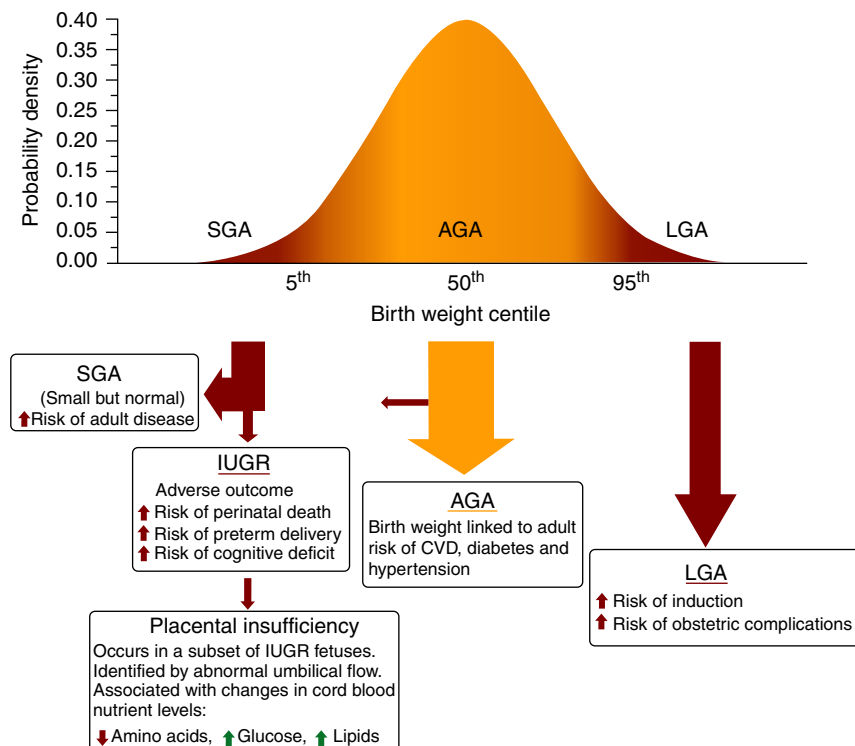


Figure 5 The normal distribution of birth weights and relative risks associated with babies who are small for gestational age (SGA), appropriate for gestational age (AGA), large for gestational age (LGA) and those subjected to intrauterine growth retardation (IUGR) and the relationship to placental insufficiency.

complicated syndrome in which almost all aspects of placental and fetal metabolism are altered and many researchers have emphasized the primary importance of the fetal hypoxia and its effects on fetal metabolism rather than a simple limitation of placental nutrient transfer capacity.

There is considerable uncertainty about the magnitude of the problem of IUGR. The lowest 5–10% of weight for gestational age babies may be referred to as small for gestational age (SGA) but babies in this range need not be growth retarded. They may be naturally small and have no increased risk of adverse outcome. Conversely, a baby born within the apparently normal birth weight range could have suffered growth retardation *in utero* if its genetic potential was for a higher birth weight (Figure 5). The true incidence of IUGR resulting from utero-placental insufficiency is therefore unknown but if it is defined in relation to umbilical flow or fetal heart rate abnormalities then it is only a fraction of even those in the lowest 5% of weight for gestational age that are affected by utero-placental insufficiency. At the other end of the spectrum babies who are large for gestational age (LGA) are at higher risk of adverse obstetric outcomes and early developmental problems but there is no evidence that LGA or macrosomic babies are produced as a result of a primary alteration in the placenta.

The Role of the Fetus

The nutrient composition of the human diet varies enormously between populations yet the healthy human newborn is essentially the same the world over. The available evidence points to extensive homeostatic mechanisms at work within the placenta to ameliorate some of the variations in the quality of the maternal diet by regulating the mix of nutrients to the developing fetus. However, these mechanisms can only operate on the nutrients already available in the maternal circulation. The maternal diet and maternal circulating concentrations of many nutrients are major determinants of the concentrations in the fetal circulation and the fetus clearly has the ability to cope with relatively large variations in nutrient availability in the cord blood. The fetus also plays an active role in regulating placental nutrient transfer. The rate of placental nutrient transport is directly influenced by the

transplacental concentration gradient, which is in turn largely determined by the rate of uptake by the fetal tissues. Another major determinant of placental nutrient transfer is the umbilical blood flow, which is approximately linearly related to the fetal weight, and hence the fetal nutrient requirement, throughout gestation. Finally, the most intimate connection between the fetus and the placenta is the way in which different parts of metabolic pathways and cycles are distributed between the placenta and fetal tissues, mainly the fetal liver. Thus although the placenta has to provide the correct mix of nutrients in sufficient quantities to support fetal growth and development throughout pregnancy it is the fetus itself, which ultimately regulates many key aspects of placental nutrient transfer function.

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Pre-eclampsia and Diet

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Glossary

Abruptio placentae Abnormal separation of the placental lining from the uterus occurring after 20 weeks of gestation and prior to birth.

Confidence interval A particular kind of interval estimate of a population parameter used to indicate the reliability of an estimate.

Eclampsia Acute and life-threatening complication of pregnancy characterized by the appearance of

tonic-clonic seizures in a patient who had developed pre-eclampsia.

Ischemia Restriction in blood supply, generally due to factors in the blood vessels, with resultant damage or dysfunction of tissue.

Relative risk A ratio of the probability of the event occurring in the exposed group versus a non-exposed group.

Introduction

Hypertensive disorders during pregnancy are one of the main causes of maternal death worldwide, most of these deaths being attributed to eclampsia. Eclampsia is the occurrence of fits in a pre-eclamptic woman that cannot be attributed to other causes (such as epilepsy). Hypertensive disorders occur in 6–8% of all pregnancies contributing significantly to stillbirths and neonatal mortality and morbidity, particularly intrauterine growth restriction, low birth weight, and preterm delivery. Pregnant women with hypertension, either newly diagnosed or pre-existing, are prone to the development of potentially lethal complications, notably abruptio placentae, disseminated intravascular coagulation, cerebral hemorrhage, pulmonary edema, hepatic failure, and acute renal failure.

The most important consideration in the classification of the disease is differentiating hypertensive disorders that antedate pregnancy from those that are pregnancy specific, of which the more ominous are pre-eclampsia and eclampsia. Pre-eclampsia is a syndrome of reduced organ perfusion secondary to vasospasm and activation of the coagulation cascade occurring only during the second half of pregnancy, labor and delivery, or the puerperium. Although our understanding of this syndrome has increased, the criteria used to identify the disorder remains a subject of confusion and controversy. In chronic hypertension, elevated blood pressure is the cardinal pathophysiologic feature, whereas in pre-eclampsia, increased blood pressure is important primarily as a sign of the underlying disorder. As might be expected, the impact of the two conditions on mother and fetus is different, as is their management.

Classification

There is controversy about the definition of hypertensive disorders during pregnancy, and several classifications have been suggested. The USA National High Blood Pressure Education

Program Working Group on High Blood Pressure in Pregnancy classifies the hypertensive disorders during pregnancy as: (a) chronic hypertension defined as hypertension observable before pregnancy, or diagnosed before the 20th week of gestation; (b) pre-eclampsia, which is a pregnancy-specific syndrome occurring usually after 20 weeks of gestation, determined by hypertension with proteinuria; (c) pre-eclampsia superimposed on chronic hypertension; and (d) pregnancy-induced hypertension or gestational hypertension, which is transient hypertension detected for the first time after mid-pregnancy if pre-eclampsia is not present at the time of delivery and blood pressure returns to normal by 12 weeks postpartum (a retrospective diagnosis). The system suggested by the International Society for the Study of Hypertension in Pregnancy (ISSHP) defines hypertension as a diastolic blood pressure of 90 mmHg or above on two consecutive occasions at least 4 h apart, or a single diastolic blood pressure of 110 mmHg or more. The definition of pre-eclampsia has the same criteria for high blood pressure, but with the addition of significant proteinuria, usually at least 300 mg per 24 h or 1+ on dipsticks.

Pathophysiology of Pre-eclampsia

Pre-eclampsia is characterized by vasospasm, activation of the coagulation system, and perturbations in many humoral and autacoid systems related to volume and blood pressure control. The pathologic changes in this disorder are primarily ischemic in nature and affect placenta, kidney, liver, and brain. Of importance, and distinguishing pre-eclampsia from chronic or gestational hypertension, is that pre-eclampsia is more than hypertension; it is a systemic syndrome, and several of its 'nonhypertensive' complications can be life-threatening even when blood pressure elevations are quite mild.

The cause of pre-eclampsia is not known. Many consider the placenta as the pathogenic focus for all manifestations of pre-eclampsia because the delivery of both the baby and the placenta is the only definitive cure of this disease. There is no disease without the placenta. Failure of the spiral arteries to

remodel is postulated as the morphologic basis for decreased placental perfusion in pre-eclampsia, which may ultimately lead to early placental hypoxia. Research on how alterations in the immune response at the maternal interface might lead to pre-eclampsia addresses the link between placenta and maternal disease. A nonclassical human leukocyte antigen (HLA), HLA G, is expressed in normal placental tissue and may play a role in modulating the maternal immune response to the immunologically foreign placenta. Placental tissue from pre-eclamptic pregnancies may express less or different HLA G proteins, resulting in a breakdown of maternal tolerance to the placenta. Finally, there are increased levels of inflammatory cytokines in the placenta and maternal circulation, as well as evidence of increased 'natural killer' cells and neutrophil activation in pre-eclampsia.

The mechanisms underlying vasoconstriction and altered vascular reactivity remain obscure. Research has focused on changes in the ratio of vasodilative and vasoconstrictive prostanoids, since prostacyclin may be suppressed and thromboxane may be raised. The vasoconstrictive potential of pressor substances (e.g., angiotensin II and endothelin) is magnified in pre-eclampsia as a consequence of a decreased activity of nitric oxide (NO) synthesis and decreased production of NO-dependent or NO-independent endothelium relaxing factor (EDRF). It has also been hypothesized that placental ischemia early in gestation upregulates the soluble Fmslike tyrosine kinase-1 (SFlt-1) and endoglin (sEng), anti-angiogenic proteins that enter the maternal circulation and inactivate circulating vascular endothelial and placental growth factors (VEGF and PlGF), which results in endothelial dysfunction and leads to the maternal clinical phenotype of hypertension and proteinuria, as well as the kidney lesion characteristic of the disease.

The Possible Role of Nutrition in the Pathophysiology of Pre-eclampsia

Calcium

There is considerable evidence linking calcium intake and hypertension during pregnancy from observational and experimental studies. However, there is still no satisfactory explanation for the mechanisms involved in the calcium-mediated effect on blood pressure reduction. It has been postulated that parathyroid hormone could be involved in this relationship. Demonstrated alterations in extracellular calcium homeostasis in pre-eclampsia include hypocalciuria and decreased serum levels of calcitriol. Increases in intracellular free calcium concentration in circulating cells are hypothesized to result from fluctuation in hormones or vasoactive substances that cause similar alteration in vascular smooth muscle. Pregnancy is a state of high calcium requirements as a result of fetal demands whereas maternal adaptive mechanisms are partially inhibited. These phenomena lead to the hyper-parathyroid state of pregnancy. An increase of parathyroid hormone serum levels would involve an increase of free intracellular calcium. Then, the concentration of intracellular free calcium in vascular smooth muscle cells determines the degree of tension, and is the trigger for muscular

contraction. So the vasoconstrictive effect, with a rise in blood pressure, results from an increase in vascular smooth muscle tension.

Antioxidant Agents

Antioxidants are important in maintaining cellular integrity by inhibiting peroxidation reactions and thus protecting enzymes, proteins, and cells from destruction by peroxides. Antioxidant defense mechanisms include cellular and extracellular enzymes and free-radical scavengers, including vitamins C and E, carotenoids, glutathione, serum albumin, and metabolites such as bilirubin and uric acid. Vitamins C and E are antioxidants derived from the diet. Vitamin C is central for the neutralization of both water-soluble and lipid-soluble free radicals; as a water-soluble molecule its ability to neutralize free radicals in the aqueous compartment is clear. The lipid-soluble vitamin E acts *in vivo* to prevent the formation of lipid peroxides and thus protect cell membranes.

Other Nutrients

Nutritional factors other than antioxidants can also contribute to oxidative stress. Hyper-homocysteinemia can occur as a result of dietary deficiencies, and is said to be altered, at least in part, by the genesis of oxidative stress. Vitamins B₆ and B₁₂ and folic acid are involved at different steps in the metabolic pathway for removing or recycling homocysteine to methionine. Dietary deficiencies of any of these micronutrients can increase circulating homocysteine. Pre-eclampsia is characterized by increased triglycerides that favor the formation of small, low-density lipoproteins (LDLs). This lipoprotein variant has increased access to the subendothelial space where it is sequestered from blood-borne antioxidants. The relevant role of triglycerides in the genesis of pre-eclampsia is indicated by the fact that they are increased long before clinically evident diseases. Similarly, free fatty acids are increased in pre-eclampsia and this increment can be observed months before the diagnosis. Recent studies indicate that this effect may be secondary to altered copper binding by albumin to which large amounts of free fatty acids are bound. Unbound copper is a potent stimulator of free radical formation. Ordinarily this effect of copper is prevented by protein binding (quantitatively, primarily to albumin). However, with fatty acid binding, albumin binds copper differently. In this configuration, copper bound to albumin maintains its ability to participate in redox reactions. Thus, it appears that increased free fatty acids can also contribute to oxidative stress.

All of these nutritional alterations may be amenable to dietary modification raising the possibility of nutritional prophylaxis.

Nutritional Interventions and Hypertensive Disorders of Pregnancy

Prevention

The ability to prevent hypertensive disorders of pregnancy is limited by lack of knowledge of its underlying etiology.

Prevention is currently focused on identifying women at higher risk of developing pregnancy-induced hypertension or pre-eclampsia during pregnancy, followed by close clinical and laboratory monitoring to recognize the clinical symptoms of the disease in its early stages for more intensive monitoring or delivery. Although these measures do not prevent the disease, they may be helpful for preventing some adverse maternal and fetal sequelae.

As part of many other nonpharmacological interventions, some dietary interventions have been proposed to prevent the development of pregnancy-induced hypertension and pre-eclampsia. We will describe here the evidence from randomised controlled trials on such interventions.

Nutritional Advice in Pregnancy

The effects of advising pregnant women to increase their energy and protein intakes on the outcome of pregnancy, and maternal and fetal/infant morbidity and mortality was evaluated on a Cochrane systematic review and appears to be effective in increasing pregnant women's energy and protein intake, but the implications for fetal, infant, or maternal health cannot be judged from the available evidence. Pre-eclampsia prevention was assessed only in one small trial involving 136 women with no beneficial effects.

Balanced protein/energy supplements for pregnant women on gestational weight gain and pregnancy outcomes were also evaluated. Pre-eclampsia prevention was assessed in three trials involving 516 women, with no significant beneficial effects. However, these trials had methodological flaws, so the results should be interpreted cautiously. In another pre-specified subgroup, only one trial involving 782 women evaluated pre-eclampsia prevention when isocaloric balanced protein/energy supplements were given to underweight pregnant women, with no effect.

Energy/protein Restriction for Obese Pregnant Women

Excessive weight gain during pregnancy has long been recognized as a risk factor for edema and impending pre-eclampsia. Epidemiological studies suggested that high maternal weight was positively associated with the risk of pre-eclampsia.

Energy/protein restriction for high weight-for-height or weight gain during pregnancy was another subgroup assessed in this systematic review. There was no reduction in the risk of pre-eclampsia (three trials, 334 women), and of pregnancy-induced hypertension (four trials, 434 women). The limited evidence available suggests that protein/energy restriction of pregnant women who are overweight or exhibit high weight gain is unlikely to be beneficial and may be harmful to the developing fetus. Although weight reduction may be helpful in reducing or preventing high blood pressure in nonpregnant women, there is no effect on preventing pre-eclampsia, even in obese women. Clinicians frequently ask pregnant women to restrict their food intake in an attempt to prevent pre-eclampsia, despite the absence of evidence that such advice is beneficial.

Salt Restriction

Even in the early phase of pregnancy, marked hemodynamic changes occur including a fall in vascular resistance and blood pressure and a rise in cardiac output. To compensate for the

increased intravascular capacity the kidney retains more sodium and water. Apparently, the set point of sodium homeostasis shifts to a higher level at the expense of an expansion of extracellular volume. In nonpregnant individuals, a strong positive association of sodium intake with blood pressure has been established, but the relationship between sodium intake and blood pressure in human pregnancy remains obscure to date. For decades a low-salt diet has often been recommended as treatment for edema, in the hope that restricting salt intake would treat, and also prevent pre-eclampsia. The concerns about the effect of a low-sodium diet during pregnancy on maternal nutritional status led researchers to investigate if such changes could alter other nutrient intake. It was shown that the reduction in sodium intake also caused a significant reduction in the intake of energy, protein, carbohydrates, fat, calcium, zinc, magnesium, iron, and cholesterol. Even though the majority of clinicians no longer advise women to alter their salt intake during pregnancy, this is still current practice in many countries worldwide.

A Cochrane systematic review evaluates the effect of the advice about low dietary salt intake during pregnancy. The review includes two trials with data reported for 603 healthy nulliparous women. Women with established pre-eclampsia were not enrolled, so this review provides no information about restricting salt intake for treatment of pre-eclampsia. No effect was found in preventing pre-eclampsia or pregnancy-induced hypertension (one trial, 242 women). Women's preferences were not reported, but the authors presumed that a low-salt diet was not very palatable and was therefore difficult to follow.

Calcium Supplementation

In a recently updated Cochrane systematic review of calcium supplementation during pregnancy, authors prespecified comparison groups taking into account the women's risk of hypertensive disorders of pregnancy (low versus increased), and the women's baseline dietary calcium intake (low: $<900 \text{ mg day}^{-1}$ versus adequate: $\geq 900 \text{ mg day}^{-1}$).

High blood pressure with or without proteinuria was evaluated in 12 trials involving 15,470 women. Overall, there was less high blood pressure with calcium supplementation (relative risk (RR) 0.65; 95% confidence interval (CI) 0.53–0.81), but there was a variation in the magnitude of the effect across the subgroups. The effect was considerably greater in women at high risk of developing hypertension than in those at low risk. Taking into account the women's calcium intake, the effect was also greater in those with low baseline dietary calcium than in those with adequate calcium intake.

There was a reduction in the risk of pre-eclampsia when evaluated from 13 trials involving 15,730 women (RR 0.45; 95% CI 0.31–0.65). When predefined subgroups were considered, again a significant reduction was shown in women with low baseline dietary calcium intake, but not in those with adequate calcium intake. Pre-eclampsia was considerably reduced in women at high risk of hypertension, and less consistently in those at low risk. Calcium supplementation also appears to reduce the risk of preterm birth and to reduce the rare occurrence of serious morbidity related to pre-eclampsia.

The results from the trial conducted by the National Institutes of Health (NIH), which studied low-risk women with adequate baseline calcium diet, and in whom all women in both groups received low-dose calcium supplementation as part of their antenatal care, showed no significant effect on hypertension and pre-eclampsia. Based on this, authorities from developed countries where adequate dietary calcium intake is common, discourage the use of routine calcium supplementation during pregnancy. Evidence from this review, however, support the view that calcium might benefit women at high risk of gestational hypertension and women with low dietary intake, and current guidelines suggest supplementation with calcium in these groups.

Iron and Folate Supplementation

Numerous trials involving various populations of pregnant women have evaluated the effects of iron and folate supplementation on several outcomes, some of them including hypertensive disorders of pregnancy. A Cochrane systematic review of iron and folate supplementation during pregnancy showed no effect on the occurrence of pre-eclampsia when daily iron supplementation was compared with no treatment (two trials, 774 women). The same findings were observed in the subgroup of daily iron + folate supplementation versus no intervention (one trial, 48 women). Although iron and folate are not effective in preventing hypertensive disorders during pregnancy, they should be prescribed for other established beneficial effects on pregnancy such as prevention of maternal anemia at term.

Magnesium Supplementation

Magnesium is one of the essential minerals needed by humans in relatively large amounts. Magnesium works with many enzymes regulating body temperature and synthesizing proteins as well as maintaining electrical potentials in nerves and muscle membranes. Magnesium occurs widely in many foods; dairy products, breads and cereals, vegetables, and meats are all good sources. It is therefore not surprising that frank clinical magnesium deficiency has never been reported to occur in healthy individuals who eat standard diets. However, dietary intake studies during pregnancy consistently demonstrate that many women, especially those from disadvantaged backgrounds, have intakes of magnesium below recommended levels. Observational studies based on medical records reported that magnesium supplementation during pregnancy was associated with a reduced risk of fetal growth retardation and pre-eclampsia and that magnesium intake was associated with increased birth weight. Stimulated by these studies, randomized clinical trials have been undertaken to evaluate the potential benefits of magnesium supplementation on pregnancy and neonatal outcomes.

A Cochrane systematic review of these trials shows no apparent effect of magnesium treatment on either maternal systolic or diastolic blood pressure (three trials, 1432 women) or in pre-eclampsia (two trials, 474 women). However, these results may have been confounded by the fact that in the largest trial all women (both magnesium supplemented and placebo groups) received a multivitamin and mineral preparation containing low doses of magnesium. Several of the trials also have poor methodological quality, especially related

to concealment of allocation, which could give biased results. Authors conclude that dietary magnesium supplementation of pregnant women cannot be recommended for routine clinical practice.

Fish Oil Supplementation

Studies of nonpregnant subjects suggest that fish oil, rich in long-chain n-3 fatty acids, has a moderate effect on blood pressure in normotensive as well as hypertensive individuals. A meta-analysis of controlled clinical trials of the effect of fish oil on blood pressure has demonstrated a significant reduction in systolic and diastolic blood pressure in untreated hypertensive nonpregnant individuals, but found no significant effect on normotensives. Fish oil has been shown to modify prostaglandin metabolism, and its effect on blood pressure has often been assumed to be due to such interference. Epidemiological studies suggested that marine diets could have a preventive effect on early delivery and hypertensive disorders of pregnancy.

Marine oil, and other prostaglandin precursors, supplementation during pregnancy was evaluated in a systematic review of six trials (2783 women) updated in 2008, showing no effect on high blood pressure (five trials, 1831 women; RR: 1.09, 95% CI 0.90 to 1.33) or in the incidence of pre-eclampsia (RR 0.86, 95% CI 0.59 to 1.27; four trials, 1683 women). Based on current evidence, fish oil supplementation is not recommended during pregnancy for the prevention of pre-eclampsia.

Zinc Supplementation

Zinc is proposed as playing an important role in many biological functions, including protein synthesis and nucleic acid metabolism. There is controversy in the literature in demonstrating the relationship between low serum zinc levels and abnormalities of pregnancy outcomes such as pregnancy-induced hypertension, prolonged labor, postpartum hemorrhage, preterm or post-term pregnancies, small-for-gestational age babies, or poor perinatal outcomes. Although severe zinc deficiency is now considered rare, mild to moderate deficiency may be relatively common in some disadvantaged populations throughout the world.

The role of routine zinc supplementation during pregnancy on outcomes for both mother and newborn was assessed in a Cochrane systematic review. There is no detectable effect on pregnancy hypertension or pre-eclampsia (seven trials, 2975 women, RR 0.83, 95% CI 0.64 to 1.08), although a reduction is possible in populations with low baseline zinc status or deficient nutrition (five trials, 2434 women, RR 0.65, 95% CI 0.42–0.98). However, it appears to be inconsistent among trials regarding the effects from other pregnancy outcomes. This may be related to variable population characteristics of women recruited in the various trials, as some included normal pregnant women with no systemic illness, other studies specifically selected women at high risk of low-zinc status, and in one study, participants were selected on the basis of proven low plasma zinc levels. There is at present no conclusive evidence of overall benefit from routine as opposed to selective zinc supplementation in pregnancy-induced hypertension or pre-eclampsia.

Vitamin (A, E, and C) Supplementation

An oxidant/antioxidant imbalance has been suggested among the possible pathogenic factors involved in pre-eclampsia. As vitamin E is one of the most important antioxidants, its levels and their relation with circulating levels of lipid peroxides in pre-eclamptic women has been intensively studied in recent years. As with other antioxidants, several studies found decreased vitamin E levels in serum from women with gestational hypertension and pre-eclampsia compared with controls. Increased ascorbate radical formation and ascorbate depletion were also found in plasma from women with pre-eclampsia. A promising randomized controlled trial involving 283 women at very high risk of developing pre-eclampsia published in 1999 found a significant reduction in the risk of developing pre-eclampsia in the group supplemented with vitamin C (1000 mg day⁻¹) and E (400 IU day⁻¹) compared to controls. The preventative potential of vitamins C and E was then evaluated in several large double-blind randomized trials in North America, the UK, Australia, Venezuela, South Africa and Tanzania. A Cochrane systematic review of these trials (6212 women) was published in 2008. Studies evaluated vitamin C and E, vitamin C alone, vitamin C and E plus fish oil and aspirin, multivitamins containing vitamin C and E, lycopene, red palm oil, and the antioxidant mineral selenium. There was no clear difference in the risk of pre-eclampsia between antioxidant supplemented and control groups (nine trials, 5446 women, RR 0.88, 95% CI 0.75–1.02). This result was not significantly different in the sensitivity analysis

restricted to the quality of the studies, or the participant's baseline risk. There was also no clear difference between the groups for women allocated vitamin C and E alone (RR 0.92, 95% CI 0.68 to 1.25; four trials, 4655 women). Two trials (2495 women) of vitamins C and E reported on severe pre-eclampsia, showed no effect. Results from more than 16 000 women randomised in five large multicentre, randomised controlled trials conducted in USA, Canada, the UK, Brazil, India, Peru, South Africa, and Vietnam were published later on, all with similar conclusions. Current evidence does not support routine antioxidant supplementation during pregnancy to reduce the risk of pre-eclampsia.

The role of vitamin A in pregnancy-induced hypertension and pre-eclampsia is another subject of controversy. It was proposed as a chain-breaking antioxidant in the free radical cascade. Some studies found significantly reduced serum vitamin A levels in pre-eclamptic and eclamptic women when compared to levels in healthy women in the third trimester. No trials have been published to date to assess the effect of vitamin A supplementation on pregnancy-induced hypertension or pre-eclampsia. Although a large trial of low-dose supplementation with vitamin A or beta-carotene conducted in Nepal showed a reduction in maternal mortality related to pregnancy in supplemented women, these findings could not be confirmed in a recently updated Cochrane systematic review. Differences in causes of death, including pre-eclampsia and eclampsia, could not be reliably distinguished between supplemented and placebo groups. Use of vitamin A

Table 1 Effectiveness of nutritional interventions in hypertension during pregnancy and pre-eclampsia

Intervention	Hypertension during pregnancy		Pre-eclampsia	
	Practice	Research	Practice	Research
Nutritional advice	No evidence	–	No effect; RR = 0.89 (0.42–1.88)	–
Balanced protein (<25%)/energy	No evidence	–	No effect; RR = 1.20 (0.77–1.89)	–
Iso-caloric balanced protein (<25% of total energy)	No evidence	–	No effect; RR = 1.00 (0.57–1.75)	–
Energy/protein restriction for high PI or high weight gain	No effect; RR = 0.94 (0.72–1.22)	–	No effect; RR = 1.07 (0.57–2.02)	–
Salt restriction	No effect; RR = 0.97 (0.49–1.94)	–	No effect; RR = 1.11 (0.46–2.66)	–
Calcium	Possibly beneficial for women at high risk (RR = 0.47 (0.22–0.97)) and with low baseline intake (RR = 0.44 (0.28–0.70))	–	Possibly beneficial for women at high risk (RR = 0.22 (0.12–0.42)) and with low baseline intake (RR = 0.36 (0.20–0.65))	–
Iron and folate	No effect; RR = 1.15 (0.41–3.18)	–	No effect; for iron alone RR = 2.58 (0.81–8.22); for iron and folate RR = 3 (0.13–70.16)	–
Folate	No evidence	Needed	No evidence	Needed
Magnesium	No evidence	Needed	No effect; RR = 0.87 (0.57–1.32)	Needed
Fish oil	No effect; RR = 1.09 (0.90–1.33)	–	No effect; RR = 0.86 (0.59–1.27)	–
Zinc ^(a)	No effect; RR = 0.83 (0.64–1.08)	Needed	–	Needed
Antioxidants	No effect; RR = 0.89 (0.62–1.26)	–	No effect; RR = 0.73 (0.51–1.06)	–

^(a) reported as pregnancy hypertension or preeclampsia.

supplements for the prophylaxis and management of pregnancy-induced hypertension and pre-eclampsia needs to be evaluated further before being recommended.

Treatment

The objectives of treatment for established pre-eclampsia or pregnancy-induced hypertension are to prevent eclampsia as well as other severe maternal complications. Close maternal evaluation is aimed at observing progression of the condition, both to prevent maternal complications and to determine whether fetal well-being can be assessed. As this disorder is often completely reversible and usually begins to abate with delivery, an imbalance between the mother's condition and the risk for fetus survival without significant neonatal complications *in utero* or in the nursery must be continuously evaluated. Even though the only definitive treatment of pre-eclampsia is delivery, some nonpharmacological approaches have been proposed as part of an overall strategy of management of the disease to achieve these goals.

Unfortunately, there is no information from randomized controlled trials related to dietary approaches to the management of the disease in its mild to moderate stage, at which point conservative management is generally decided.

Conclusions

In short, based on the available data from systematic reviews (see **Table 1**) we can conclude that there is some evidence that calcium supplementation in populations with low calcium intake and at risk of developing pregnancy-induced hypertension could be beneficial. Data on antioxidants (particularly vitamins E and C) confirm their inefficacy in preventing pre-eclampsia and reveal some concerns about their safety. Although pregnant women living in developing countries could be exposed to several other nutrient deficiencies, a lack of evidence precludes recommending other nutrient supplementation as part of their routine antenatal care in order to prevent the occurrence of pregnancy-induced hypertension or pre-eclampsia.

See also: Ascorbic Acid (Vitamin C): Deficiency States; Physiology, Dietary Sources, and Requirements. Calcium. Copper. Cytokines: Nutritional Aspects. Folic Acid. Hypertension: Dietary Factors. Lipoproteins. Magnesium. Obesity: Definition, Etiology, and Assessment. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases. Salt: Epidemiology. Sodium: Physiology. Supplementation: Dietary Supplements. Vitamin A: Deficiency and Interventions. Vitamin E: Metabolism and Requirements; Physiology and Health Effects

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Prevention of Neural Tube Defects

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Glossary

Anencephaly Failure of the section of the neural tube to close in the area of the brain, causing the absence of a large part of the brain and skull. Anencephaly is uniformly fatal.

Craniorachischisis A severe form of neural tube defect where the area in which the neural tube fails to close extends from the head down into the spine.

Encephalocele A form of neural tube defect where there is failure of ossification of a portion of the skull. A sac like protrusion occurs which may contain only meninges (encephalocele) or brain tissue and meninges (meningoencephalocele).

Iniencephaly A severe form of neural tube defect in which the head is flexed in an upward gaze and both anencephaly and spinal defects are common.

Meningocele A failure of the neural tube to close in the area of the spine causing a defect in the vertebral arches through which cerebrospinal fluid filled meninges but not neural tissue protrude.

Meningomyelocele Failure of the neural tube to close in the area of the spine causing a defect in the vertebral arches through which cerebrospinal fluid filled meninges and neural tissue protrude.

Epidemiology

Neural tube defects (NTDs) are the most common anomalies of the central nervous system. The neural tube closes to form the brain and spinal cord within the first 28 days after conception. The type of lesion depends on the portion of the neural tube that fails to close. Failure of the cranial portion to close causes anencephaly, a uniformly fatal defect. Failure of the more caudal neural tube to close causes meningocele or meningocele (spina bifida). The severity of the defect depends on the location; the higher the defect, the more severe the disability. Less common forms include encephalocele, iniencephaly, and craniorachischisis. Combinations of these lesions may also be present in severely affected cases. Most children with NTDs who survive have serious disabilities.

The birth prevalence of NTDs ranges from approximately six to 60 cases per 10 000 births. In the past 40 years the prevalence has dropped significantly in developed countries, particularly in regions that traditionally had high rates. The reason for this decline before the introduction of folic acid fortification of foods is not well understood, but is probably due in part to prenatal diagnosis and termination and possibly to improved diets. Because the neural tube closes very early in pregnancy, before many women are aware that they are pregnant, NTDs have been difficult to study and to prevent. In fact prevention essentially requires that interventions be initiated before conception.

Genetic and Environmental Factors

Both genetic and environmental factors are involved in the etiology of NTDs. Evidence for a genetic component includes racial differences in prevalence, recurrence risks approximately

10-times the general occurrence risk, higher rates in females and well recognized mendelian syndromes that include NTDs. Over 240 laboratory mouse strains exhibiting NTDs have been generated by single or multiple gene manipulations, further substantiating a strong genetic component. Nevertheless, the majority of NTDs are isolated, consistent with a low penetrance etiology exhibiting unclear and probably variable genetic and environmental influences.

Marked variations in prevalence over time, between areas and across social class provide evidence of the importance of environmental factors. Most notably, evidence for nutritional factors has accumulated since the 1960s. The observation that women of low socio-economic status were at increased risk for having children with NTDs stimulated Smithells and coworkers to examine diet as a risk factor and to measure vitamin levels in pregnant women. His finding that women carrying affected fetuses had significantly lower red cell folates stimulated many case-control studies and later clinical trials. These intervention and case control studies generally showed that periconceptional use of vitamin supplements containing folic acid reduced NTD rates by 35–71%. Intervention studies are shown in [Table 1](#).

Folate/Folic Acid

Evidence from Intervention Studies

The protective effect of folic acid was established by two randomized controlled trials. The Medical Research Council trial (1991) randomized women with a history of an affected fetus to folic acid, other vitamins, both, or neither. The recurrence rate in the two groups that received folic acid was significantly lower than the rate in the groups that did not receive folic acid, (odds ratio 0.28; confidence interval 0.12–0.71) suggesting that

Table 1 Intervention studies of periconceptional folic acid supplementation and NTD risk

Study	Location	Design	Daily dose folic acid (mg)	Outcome: No. of NTDs	Relative risk	Comments
UK Medical Research Council (1991)	International	Randomized controlled trial	4.0	6/593 supplemented 21/602 not supplemented ^a	0.29	Significant ^b
Laurence <i>et al.</i> (1981)	Wales	Randomized controlled trial	4.0	2/60 supplemented 4/51 not supplemented	0.42	Not significant Small numbers
Kirke <i>et al.</i> (1992)	Ireland	Randomized controlled trial	0.36	0/172 supplemented 1/89 not supplemented	0.00	Not significant Small numbers
Czeizel and Dudas (1992)	Hungary	Randomized controlled trial	0.8	0/2104 supplemented 6/2052 not supplemented	0.00	Significant ^b
Indian Council of Medical Research (2000)	India	Randomized controlled trial	4.0	4/137 supplemented 10/142 not supplemented	0.41	Not significant Small numbers
Smithells <i>et al.</i> (1983)	UK	Nonrandomized controlled trial	0.36	3/454 supplemented 24/519 not supplemented	0.14	Significant ^b
Vergel <i>et al.</i> (1990)	Cuba	Nonrandomized controlled trial	5.0	0/81 supplemented 4/114 not supplemented	0.00	Not Significant Small numbers
Berry <i>et al.</i> (1999)	China	Nonrandomized controlled trial	0.4	Northern region 13/13 012 supplemented 16/3 318 not supplemented	0.21	Significant ^b
				Southern region 34/58 638 supplemented 28/28 265 not supplemented	0.59	Significant ^b

^aSix NTD pregnancies in 593 women supplemented with folic acid and 21 NTD pregnancies in 602 women not supplemented with folic acid.

^bStatistically significant difference in NTD rate between supplemented and nonsupplemented groups.

Table 2 Distribution of case and control mothers and risk of NTDs by early pregnancy red cell folate concentration

Red cell folate ($\mu\text{g l}^{-1}$)	No. of cases (%)	No. of controls (%)	Risk of NTD per 1000 births	95% confidence interval
0–149	11 (13.1)	10 (3.8)	6.6	3.3–11.7
150–199	13 (15.5)	24 (9.0)	3.2	1.7–5.5
200–299	29 (34.5)	75 (28.2)	2.3	1.6–3.3
300–399	29 (23.8)	77 (29.0)	1.6	1.0–2.4
≥ 400	11 (13.1)	80 (30.0)	0.8	0.4–1.5
Total	84 (100.0)	266 (100.0)	1.9	1.5–2.3

Source: Reproduced with permission from Daly LE, Kirke PN, Molloy A, Weir DG, and Scott JM (1995) Folate levels and neural tube defects – Implications for prevention. *Journal of the American Medical Association* 274: 1698–1702. Copyright © 1995, American Medical Association.

72% of recurrences could be prevented by folic acid. It is important to note that 28% of NTD recurrences were not prevented by the 4-mg daily folic acid dose used in the trial. The multivitamin combination without folic acid had no protective effect. Vitamin B₁₂ was not included in the multivitamin tablets but vitamins B₂ (riboflavin) and B₆ (pyridoxal phosphate), other vitamins that have links with folate metabolism, were included. The Hungarian trial on NTD occurrence, published one year later, found no NTD occurrences in the treatment group who were given 800 $\mu\text{g d}^{-1}$ folic acid plus other vitamins including 4 μg vitamin B₁₂. The placebo arm received a multimineral combination without folic acid or vitamin B₁₂.

Evidence from Maternal Blood Folate Status

Observational studies generally demonstrate that women who have had pregnancies affected by NTD tend to have lower blood folate concentrations (both in plasma and red cells) than nonaffected mothers. Daly and coworkers showed that the risk for having an affected child was strongly related to the mother's red cell folate level. Those with red cell folates levels below 150 ng ml^{-1} (340 nmol l^{-1}), had more than eight times the risk of those whose red cell folates were over 399 ng ml^{-1} (906 nmol l^{-1}) (Table 2). Notably, the data showed that risk remained elevated in women whose red cell folate levels were well above the deficiency cut off (150 ng ml^{-1}).

Vitamin B₁₂

The role of vitamin B₁₂ in NTDs is of particular interest because of the close metabolic relationship between this nutrient and folate. There are no trials demonstrating that vitamin B₁₂ can prevent NTDs. However, many studies that examined maternal and amniotic fluid vitamin B₁₂ status both during and after an NTD affected pregnancy suggest that low maternal vitamin B₁₂ status is an independent risk factor for having an NTD affected pregnancy. Differences have been observed in maternal serum total circulating vitamin B₁₂ and in serum holotranscobalamin (holoTC), which is the fraction of B₁₂ in the circulation that is destined for tissue uptake. Lower amniotic fluid B₁₂ concentrations and lower B₁₂ binding capacity have also been reported in NTD affected pregnancies. Several of the largest studies indicate that there is an approximately three-fold increase in risk for mothers in the lowest quartile or quintile of blood B₁₂ concentration compared to the highest. All studies with more than 50 case mothers are summarized in **Table 3**. The negative reports were either from studies with low numbers (less than 50 case mothers) and were therefore probably underpowered, or were from an area of low indigenous NTD risk (e.g., Finland). It has been considered that low levels of vitamin B₁₂ may reflect low levels of folate, however, analysis of both vitamins in several studies suggest that they are independent risk factors and one study found the highest risk in women who were in the lowest quartile for both folate and vitamin B₁₂.

Other Causes of NTDs

Nutritional Factors

Vitamin C levels were found to be significantly lower in the Smithells *et al.* study reported above, but this observation has not been followed up. Zinc deficiency has also been reported to be a risk factor, but the data are inconsistent. Several studies have implicated low maternal choline status as a risk factor for NTDs. Choline has close metabolic interactions with folate; supplementation with one nutrient may ameliorate deficiency in the other. The possible role of riboflavin in people homozygous for the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T genetic polymorphism is discussed later. It is possible that the observed protection by folic acid in some instances occurs through indirectly surmounting a deficiency or impairment caused by one of these other nutrients. One mutant animal strain that has served as a model of NTDs for decades is resistant to folic acid but responsive to maternal periconceptional supplementation with the B vitamin inositol. There have been calls for supplementation trials with inositol in women who are not protected from NTD pregnancies by folic acid.

Obesity

There are other potentially modifiable risk factors for NTDs. Women who are obese (defined as prepregnancy body weight of more than 80 kg or body mass index (BMI) greater than 29 kg m⁻²) are more likely to have infants with NTDs (and other malformations such as congenital heart defects) than

women of normal BMI. Several recent meta-analyses reported a consistent trend toward higher risk of having an NTD affected pregnancy with increasing obesity such that a three-fold higher risk prevailed in severely obese women compared with women of normal weight. Although the overall risk of an NTD pregnancy for obese women may be small, the increasing prevalence of obesity in Western populations may make obesity an important population health burden for NTDs.

Diabetes Mellitus

Diabetes mellitus is a well known consequence of obesity and maternal pregestational diabetes poses increased risk for many congenital defects, including NTDs. Although the teratogenic mechanisms in diabetes are poorly understood, good glucose control in diabetic women can reduce the risks of having pregnancies affected by NTDs and other birth defects.

Other Risk Factors

Antifolate drugs (including carbamazepine, phenobarbital, phenytoin, primidone, sulfasalazine, triamterene, and trimethoprim) have been associated with increased risk for NTDs. The antiepileptic drug valproic acid is a well established risk factor. Hyperthermia has also been associated with increased risk.

Mechanisms

The possible mechanisms underlying the involvement of folate/folic acid in the etiology and prevention of NTDs are examined in this section.

Functions of Folate and Vitamin B₁₂ and NTD Etiology

Folate acts as an intermediary in the transfer of one-carbon groups for two important processes in metabolism, namely the provision of methyl groups for methylation reactions and the *de novo* synthesis of purines and thymidylate (dTMP) for DNA and RNA (**Figure 1**). The vitamin B₁₂ dependent enzyme methionine synthase is central to both the methylation and DNA synthesis aspects of one-carbon metabolism. Through this enzyme, vitamin B₁₂ and folate control the intracellular flux of one-carbon units between these two major metabolic cycles. Folate enters the cell as 5-methyltetrahydrofolate (5-methylTHF) and must release its methyl group through the methionine synthase reaction in order to be retained in the cell. As free tetrahydrofolate (THF), it can then be polyglutamated and can accept one-carbon units from serine, formate, and other sources for use in nucleotide synthesis or regeneration of 5-methylTHF. The released methyl group from 5-methylTHF is used *via* methionine synthase, to methylate homocysteine, thereby producing methionine, which is then converted to S-adenosylmethionine (SAM) for methylation of proteins, lipids, DNA, and many other cellular components. Deficiency, impaired function or limited cellular availability of folate or vitamin B₁₂ leads to an increase in intracellular

Table 3 Large case/control studies (> 50 case mothers) assessing measures of blood vitamin B₁₂ status in mothers of NTD affected children

Study	Country	Sample	Time of sampling	Cases /controls	Cases/controls pmol l ^{-1a}	OR highest to lowest quantile ^{b,c,d}	95% CI	Significant (yes/no)/ P value (if given)
Suarez (2003)	Texas-Mexico border (1995–2000)	Serum B12	Postpartum	225/378	317/367 (median)	3.0 ^c	1.4–6.3	Yes; <i>p</i> = .001
Molloy (2009) (3 cohorts) ^g	(1) Ireland (1983–1984)	Serum B12	15 weeks Median	95/265	155/179 (median)	3.14 ^{b,e}	1.46–6.72	Yes; <i>p</i> = .003
	(2) Ireland (1986–1990)	Plasma B12	15 weeks Median	76/222	180/221 (median)	2.45 ^{b,e}	1.12–5.32	Yes; <i>p</i> = .024
	(3) Ireland (1986–1990)	Plasma B12	15 weeks ^f Median	107/414	199/232 (median)	2.75 ^{b,e}	1.43–5.28	Yes; <i>p</i> = .003
Ray (2007)	Canada (1993–2004)	Serum holoTC	15–20 weeks	89/422	68/81 (geometric mean)	2.9 ^{b,e}	1.2–6.9	Yes;
Zhang (2008)	China (Shanxi) (2004–2005)	Serum B12	20 weeks median	84/110	73/91 (geometric mean)	4.96 ^d	1.94–12.7	Yes; <i>p</i> < .01
Mills (1992)	Finland (1983–89)	Serum B12	6–16 weeks	78/150	356/384 (mean)			No
Christensen (1999)	Canada (pre-1998)		Non pregnant	59/88	298/350 (mean)			Borderline; <i>p</i> = .05

^aFor comparison across studies all vitamin B₁₂ concentrations are presented as pmol l⁻¹. In studies that reported data as ng ml⁻¹, mean or median values were converted to pmol l⁻¹ using a multiplication factor of 0.738.

^bQuartile.

^cQuintile.

^dComparison of B₁₂ above and below 55 pmol l⁻¹.

^eAdjusted for maternal folate.

^fCases were mothers with a history of NTD pregnancy but currently undergoing an unaffected pregnancy.

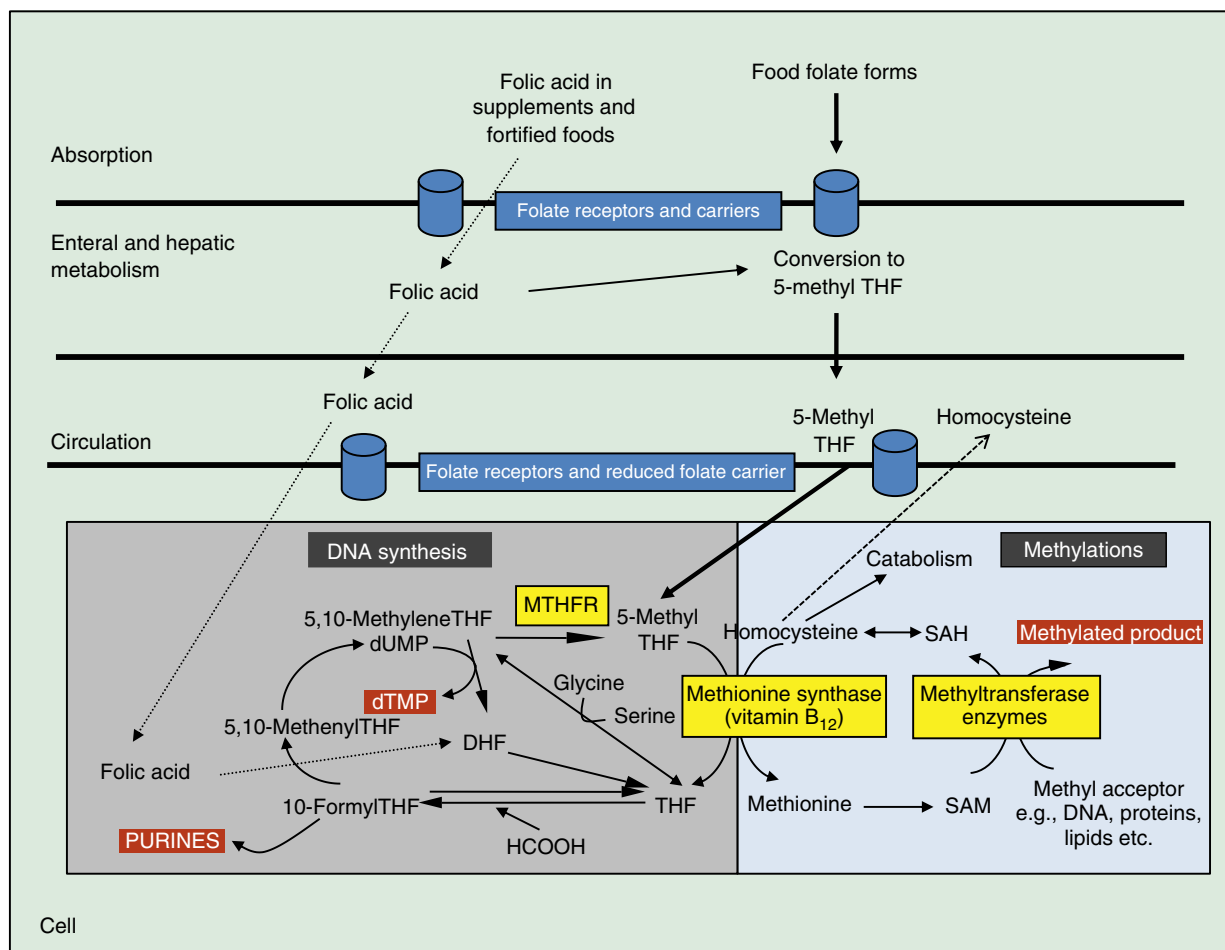


Figure 1 Intracellular pathways of folate and homocysteine metabolism and their relation to vitamin B₁₂. Folate cofactors are used in the transfer of one-carbon groups across two distinct metabolic cycles involving (a) the *de novo* synthesis of purines and thymidylate (dTMP) for DNA and (b) the provision of methyl groups for methylation reactions. Folate interacts with vitamin B₁₂ at the methionine synthase reaction, through which the THF cofactor pool provides methyl groups for biological methylation reactions *via* the conversion of homocysteine to methionine. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methyleneTHF to 5-methylTHF, thereby committing one-carbon units to methylation reactions and away from DNA synthesis. In addition to enzymes involved in folate pathways, the reduced folate carrier, folate receptors, the proton coupled folate transporter and mitochondrial folate enzymes play key roles in maintaining one-carbon homeostasis within the cell. Vitamin B₁₂ binding proteins and receptors also have important effects on one-carbon metabolism through their role in maintaining optimal cellular B₁₂ levels.

homocysteine that culminates in higher plasma concentrations of this metabolite.

Neural tube closure is a highly complex developmental process, involving multiple cycles of cell proliferation and apoptosis; the details of which are not well understood. There are many ways in which abnormal one-carbon metabolism could result in abnormal closure of the neural tube. Inadequate production of DNA for the embryo, lack of methyl groups for methylation reactions that regulate cell signaling, gene expression and activation or repression of apoptotic pathways are obvious candidates. Several studies found higher total homocysteine (tHcy) in maternal plasma or in amniotic fluid during NTD pregnancy, suggesting that these women were less able to metabolize homocysteine. These biochemical studies point to a subtle alteration in folate homeostasis in families with NTDs and suggest that folic acid supplementation works by overcoming a metabolic block in folate related

processes due to specific genetic variants within folate pathways. This hypothesis would explain the finding that both environment and genes contribute to NTD risk and has prompted an intensive investigation of genes encoding folate-related proteins as risk factors.

Folate Related Genetic Risk Factors

The MTHFR enzyme catalyzes the essentially irreversible conversion of 5,10-methyleneTHF to 5-methylTHF, providing methyl groups for SAM mediated methylation reactions. The common 677C→T variant in the gene for this enzyme is associated with reduced enzyme function, resulting in lower blood folate concentrations and higher tHcy levels, and an increased requirement for folate. Riboflavin is also important because the variant enzyme binds its cofactor flavin adenine dinucleotide (FAD) with lower affinity than the wild-type variant and can be

stabilized by addition of the cofactor FAD or by addition of folate. In individuals homozygous for the TT variant, poor folate status causes an altered distribution of intracellular folate cofactors and reduced global methylation of DNA.

MTHFR 677C→T has been strongly associated with NTDs. A meta-analysis concluded that homozygosity for the variant in the child or mother doubles the risk of NTD. Frequency of the TT genotype ranges from approximately 0.20–0.36 in Mexican and Southern European populations to 0.12 in Northern Europeans and less than 0.01 among African groups. Studies have also shown evidence of a strong gene–nutrient interaction; low maternal blood folate levels increase the risk associated with the variant allele. This variant, along with variations in folate and riboflavin intake in different countries, may explain observations of a clear association in some populations but not others. Homozygosity for the MTHFR 677C→T variant may account for approximately 13% of NTDs in some populations.

The discovery of the MTHFR association stimulated the search for other folate-related enzyme gene variants that might be related to NTDs. **Table 4** gives details of NTD association studies involving high priority SNPs in a number of primary candidate genes. Perhaps not surprisingly, results from candidate gene studies to date have been disappointing and inconsistent, with positive outcomes usually being weak and rarely replicated in other studies. There are many reasons for this, such as the underlying low penetrance of the genetic effects, differences in population genetic and environmental susceptibility, inadequate sample size, and often poorly matched controls in study design. One important limitation is the lack of knowledge on what genes should be included as

candidates. Because the biological mechanisms leading to NTDs are so poorly understood, it is highly likely that alteration in the function of a gene with no apparent link to folate pathways could play a role in NTDs. A further problem is that nearly a third of all human genes have no known function, yet most of these un-annotated genes are conserved through evolution, indicating that nature has assigned them important functions. Consequently, despite nearly two decades of research no genes have emerged as clearly underlying the biological mechanism leading to an NTD occurrence during a woman's pregnancy. An alternative approach of carrying out a genome-wide association study (GWAS) is now being discussed by NTD researchers. The GWAS design has the advantage of being able to screen every region in the genome for association with NTDs, and can pick up variations that are clustered within case families compared with control groups.

Primary Prevention

There was general agreement that the folic acid doses in both the MRC and Hungarian trials were too high to recommend for use in low risk women. The evidence of benefit from 400 µg folic acid per day, used by most women in the case–control studies, led to the recommendations that were set by public health authorities in most countries (see section on Minimum dose). However, campaigns to encourage women to take folic acid supplements have been generally unsuccessful, with studies reporting between 33 and 49% of women taking folic acid before pregnancy. Unplanned pregnancy is the greatest logistical obstacle to the success of these

Table 4 NTD association studies involving high priority polymorphisms in a number of folate and vitamin B₁₂ candidate genes

<i>Gene</i>	<i>Enzyme</i>	<i>Association with NTDs</i>
MTHFR 677C→T	5,10-Methylene tetrahydrofolate reductase A222V	Significant risk factor for NTDs in some but not all populations. Important cause of low folate status.
MTHFR 1298A→C	As above E429A	No clear risk associations that are independent of 677C→T.
MTR 2756A→G	Methionine Synthase D919G	May interact with other genes as a maternal risk factor but no clear independent risk.
MTRR 66A→G	Methionine synthase reductase I22M	Inconclusive maternal risk associations and possible interactive effects with low B12 or other genes.
MTHFD1 1958G→A	Trifunctional C1 Synthase R653Q	Maternal risk factor for neural tube defects in some populations.
SHMT1 1420C→T	Serine hydroxymethyltransferase L474F	No reported risk association with NTD.
RFC-1 80G→A	Reduced folate carrier H27N	Inconclusive maternal risk associations. Possible interaction with maternal nutrient intake.
FR α , FR β , FR γ Several SNPs	Folate receptors	No reported risk associations with NTD or biochemical changes in humans.
GCPII 1561C→T	Folyl- γ -glutamate carboxypeptidase H475Y	Inconclusive maternal risk associations.
TSER (Promoter enhancer region)	Thymidylate Synthase (28 bp double or triple repeat)	Inconclusive maternal risk associations.
CBS 844ins68	Cystathionine β synthase	No reported risk association with NTD.
TCII 776C→G	Transcobalamin II R259P	Inconclusive maternal risk associations. Several studies found changes in maternal serum TC II concentrations or in amniotic fluid during NTD affected pregnancies.
TCBIR Several SNPs	Transcobalamin II receptor	Several rare polymorphisms conferred highly significant risk in one large population study.
DHFR Intron 1	Dihydrofolate reductase (19 bp deletion)	Conflicting reports of risk association and protection.
BHMT 742G→A	Betaine-homocysteine methyltransferase R239Q	Inconclusive maternal risk associations.

supplementation strategies. Because of the large number of women who were not protected, food fortification was instituted as a means of passively exposing all women capable of becoming pregnant to increased amounts of folic acid.

Minimum Dose

There has been much debate on the minimum effective dose to prevent NTDs. One dose response study used the achievement of a red cell folate concentration greater than $400 \mu\text{g l}^{-1}$ as a target outcome and estimated that delivery of $200 \mu\text{g d}^{-1}$ would provide substantial protection and $100 \mu\text{g d}^{-1}$ would also be protective if delivered over a longer period. The minimum duration of supplementation necessary for prevention is not known. Although most national health authorities recommend that women take extra folic acid for at least four weeks before conception and until week 12 of pregnancy, supplementation for a shorter duration before closure of the neural tube may also be effective, but more evidence is needed.

Fortification

Fortification of enriched cereal grains with $140 \mu\text{g}$ of folic acid per 100 g of grain became mandatory in the USA in 1998. The target was to increase folate intake in women by an average of $100 \mu\text{g d}^{-1}$ while avoiding over-exposure to folic acid in high consumers of enriched cereal grain products. A similar program was adopted in Canada. Although the exact amount of folic acid being provided to women of childbearing age in these countries is difficult to quantify, it is reasonable to assume that most women are receiving

roughly $100\text{--}200 \mu\text{g d}^{-1}$. In the ensuing 10 years, mandatory fortification was adopted in many countries world-wide, using different enrichment vehicles and different folic acid doses. Several European countries have set mandatory fortification policies in place but have not yet implemented them. Voluntary fortification of food products with folic acid is also allowed in many countries world-wide. These programs have been effective in improving folate status generally but are not targeted to any particular sector of the population.

Effects on NTD Prevalence

The effect of mandatory fortification programs on NTD rates has been striking, especially in Canada. It is worth noting that data from Canada are generally more accurate than data from the US because of the limited information on prenatally diagnosed cases in the US. **Figure 2** shows data from countries that have implemented fortification and have recorded the change in NTD rates. These data contrast with countries in Europe where there has been no mandatory fortification program and no significant change in NTD rates over the past decade. Both intervention study data and data from pre- and postfortification programs indicate that not all NTDs are folate preventable. The greatest drop in prevalence has been apparent in countries or states with the highest indigenous NTD rate and the lowest rates achieved in most countries to date is five to six per 10 000 births. A study from the National Birth Defects Prevention Study in the US showed that 10 years after fortification was initiated, women who took folic acid supplements did not have lower risk of having NTD affected children than women who did not. Their findings suggest that

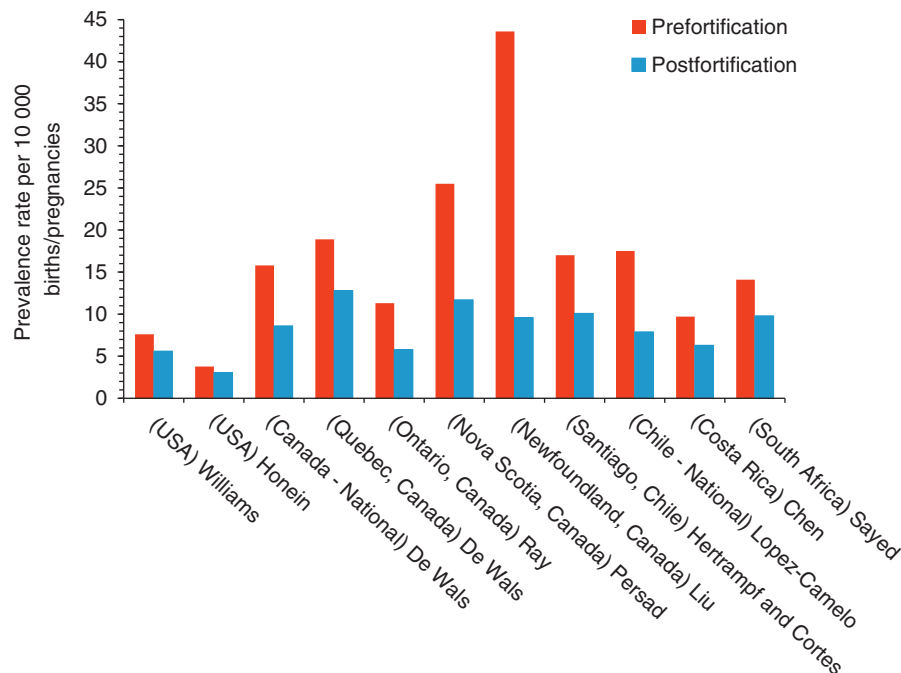


Figure 2 NTD prevalence rates pre- and post fortification of foods with folic acid in 11 areas where mandatory fortification has been implemented.

in the US and Canada fortification may be preventing nearly all folate-related NTDs.

Effects on Folate Status

Data from the US National Health and Nutritional Examination Survey (NHANES) and from retrospective longitudinal studies in Canada demonstrate increases in both short term and long term blood markers of folate status (serum and red cell folate, respectively). During the first two years of fortification, there was approximately a tripling of serum folate and an increase between 40–60% in red cell folate. Since that time, the levels have stabilized at the higher concentrations, with minor fluctuations. The prevalence of low serum and red cell folate status in US women of child-bearing age dropped from 21% and 30%, respectively to 0.8% and 2.8%, respectively. Fortification has produced similar results worldwide. Several European countries with liberal voluntary fortification policies have also seen substantial increases in the population folate status. In general, although fortification programs have been successful in increasing the folate status of the target population, greater increases in blood folate status have been seen in children and older persons than in women of child-bearing age.

Effects on Other Health Outcomes

There is some evidence that folic acid can prevent other major birth defects. The Hungarian intervention trial found a 47% reduction in birth defects other than NTDs. Several studies reported decreasing trends in the prevalence of congenital heart defects and other rarer defects since fortification began in the US and Canada and after implementation of folic acid intervention studies in China, but an association with increased folic acid intake is not established. There have also been reports of a decreased prevalence in several childhood cancers although the data are inconsistent. There is no convincing evidence of an effect on the prevalence of orofacial clefts.

Numerous studies have shown that elevated plasma tHcy is independently associated with risk of vascular disease. Because increased folate/folic acid intake can lower plasma tHcy, one hypothesis was that folic acid food fortification would have beneficial effects on risk of cardiovascular disease. This is no longer considered likely because clinical trials showed no beneficial effect of lowering plasma tHcy among patients with existing cardiovascular disease. Some reports indicate that a reduction in the incidence of stroke may have occurred but more evidence is needed. It should be noted that the trials did not address the question of primary prevention of vascular disease resulting from long-term better folate status in the population. There is a similar lack of clarity regarding the effect of folic acid fortification on population cancer rates.

Safety

The main concern with folic acid fortification was that some sectors of the population, particularly the elderly, would be exposed to very high intakes because of concomitant

fortification and supplement use. Intake of folic acid doses above 200 µg can cause unmetabolized folic acid to appear in the bloodstream. Vitamin B₁₂ is not required for folic acid uptake by cells, therefore, folic acid might initiate DNA synthesis in a vitamin B₁₂-deficient person, thereby preventing the development of anemia and potentially delaying diagnosis whereas the neurologic damage produced by B₁₂ deficiency progressed and became irreversible. Although such an effect has not been demonstrated to date, there are concerns about the increasing number of elderly people who have very high blood folate concentrations together with low vitamin B₁₂ concentrations. The physiological impact of such extreme imbalance of nutrient status, if any, is unclear, but is a topic of considerable current research interest. Adding vitamin B₁₂ as well as folic acid to fortified food has been suggested as a solution, but more evidence on efficacy, dosage, and feasibility is required. Another fear is the possibility that, because of its role in DNA synthesis, high folic acid status will help to advance established cancers and promote malignant transformation of premalignant lesions. Several other possible adverse effects of high folic acid intake are currently under scrutiny, including reports of decreased natural killer cell cytotoxicity, increased twinning rates, interference with the efficacy of anticonvulsant and antifolate drugs, and increased childhood asthma and autism rates. None of these reports have been substantiated but caution and vigilance remains an important public health position in relation to the food fortification strategy.

Recommendations

Internationally established recommendations distinguish between occurrent (first-time) and recurrent NTDs. Women with a previously affected pregnancy are advised to take 4.0 mg of folic acid daily from at least four weeks before conception until the end of the third month of pregnancy. The 4.0 mg dose should be taken under the supervision of a doctor because giving high doses of folic acid can complicate the diagnosis of vitamin B₁₂ deficiency. Epileptic women on anticonvulsant therapy require individual counseling before starting folic acid. For the prevention of occurrence of NTDs, most public health authorities recommend that all women capable of becoming pregnant consume 400 µg of folic acid per day and that total folic acid consumption should not be more than 1.0 mg d⁻¹ to avoid the possible risks of high intakes. The only practical ways of obtaining the recommended extra 400 µg folic acid daily are by consuming folic acid supplements or fortified foods. Despite the availability of fortified foods, women should be advised to take supplements to ensure adequate intake. If multivitamins are used to provide folic acid, care should be taken not to exceed safe levels of other components, particularly vitamins A and D. In the future it may be possible to demonstrate that fortified food alone is providing sufficient folic acid to prevent all folate-related NTDs, at least where fortification is mandatory. At present, however, such definitive evidence is lacking. Therefore, all women at risk should consume supplements; this is the surest method for preventing NTDs.

See also: Bioavailability. Folic Acid. Homocysteine. Nutrient–Gene Interactions: Health Implications. Obesity: Complications; Definition, Etiology, and Assessment. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements

Further Reading

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Safe Diets

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Glossary

Congenital Acquired during development in the uterus and not through heredity.

Fetal alcohol spectrum disorders (FASD) Describes the range of effects that can occur in an individual whose mother had a high alcohol intake during pregnancy. These effects may include physical, mental, behavioral, and/or learning disabilities with possible lifelong implications.

Fetoplacental unit The fetus and the placenta as a single physiological unit.

Hydrocephalus An abnormal increase in the amount of cerebrospinal fluid within the cranial cavity that is

accompanied by expansion of the cerebral ventricles, enlargement of the skull and especially the forehead, and atrophy of the brain.

Intracranial Affecting or involving intracranial structures.

Intrauterine Situated or occurring in the uterus.

Isoretinoin An oral retinoid (derivative of vitamin A).

Neonate An infant less than a month old.

Retinochoroiditis Inflammation of the retina and choroid.

Teratogenic Of, relating to, or causing malformations of an embryo or a fetus.

Introduction

A balanced diet that contains adequate amounts of all the nutrients needed by a mother and her growing fetus is essential for a healthy pregnancy. Pregnant women also need to be advised about how to reduce their risk of exposure to substances that may be toxic to the fetus during development (teratogenic) and therefore associated with the production of physical defects in the developing embryo (e.g., alcohol and excess vitamin A), as well as other dietary and lifestyle behaviors that could optimize maternal health and reduce the risk of health problems in their children. The aim of this article is to describe evidence relating to food safety issues during pregnancy, including potential risks to the fetus as a result of prenatal exposure to food pathogens or toxic food components (e.g., heavy metals and dioxins) and the potentially harmful effects of high doses of alcohol, caffeine, and vitamin A.

Food-Borne Infections during Pregnancy

For many years it has been recognized that food-borne antenatal infections may cause death or serious fetal damage. Women may be more susceptible to the effects of infection during pregnancy because of immunological changes leading to suppression of the immune system (most commonly cell-mediated immunity), probably as a result of increases in pregnancy-associated sex steroids, such as oestradiol or progesterone. Among the most common causes of diarrhea during pregnancy are several food- or water-borne pathogens (bacteria, protozoa, or viruses), including salmonella species, *Helicobacter pylori*, *Shigella*, *Escherichia coli*, and *Cryptosporidium*. Hepatitis A is also a food- or water-borne pathogen of concern, particularly in countries where sanitation is poor. In pregnant women, severe vomiting and diarrhea may negatively affect the

availability of important nutrients to the growing fetus. For example, impairment of the supply of folate (or the synthetic form, folic acid) during a critical stage of development could increase the risk of associated neural tube defects, such as spina bifida.

Although rare, infection with *Listeria* or *Toxoplasma* during pregnancy is of particular concern because even in a mild form these infections can prove fatal. Listeriosis caused by the consumption of food containing the bacterium *Listeria monocytogenes* leads to flu-like symptoms, such as fever, muscle aches, and sometimes nausea or diarrhea. If the infection spreads to the nervous system, it may also cause headaches, stiff neck, confusion, loss of balance, or convulsions. The bacterium has been found in a variety of raw foods, including unpasteurized (raw) milk and cheeses, uncooked meats, and vegetables, and in processed foods that become contaminated after processing, such as soft cheeses, pâtés, cold cuts of meat and smoked fish. According to the Centers for Disease Control and Prevention, pregnant women in the US are approximately 20 times more likely than other healthy adults to get listeriosis and approximately one-third of listeriosis cases occur during pregnancy. In the UK, the Health Protection Agency Centre for Infections reported approximately 30% of human cases to be pregnancy-associated (both mother and neonate affected) in England and Wales between 1983 and 2009. The fetus and newborn are at greatest risk of this infection and its consequences can be severe, leading to miscarriage, stillbirth, and premature delivery or to meningitis in the newborn infant. When infection occurs during pregnancy, antibiotics given promptly to the pregnant woman can often prevent infection of the fetus or newborn, and infants developing the infection can also be treated in the same way.

Toxoplasma gondii is a parasite that can be transmitted to the fetus *in utero* through transplacental transmission, causing stillbirth, miscarriage, or mental retardation (in

immunocompetent people infection is asymptomatic or mild). The parasite has been found in raw, inadequately cooked or cured meat, cat feces, and unwashed raw fruit and vegetables. It has also occasionally been reported in unpasteurized goats' milk. In the UK, toxoplasmosis occurs in approximately 2.5–5.5 in 1000 pregnant women (1750–2850 cases per year), generally causing flu-like symptoms, swollen lymph glands, or muscle aches and pains that last for a few days to several weeks. If a pregnant woman contracts the infection, there is an approximately 30–40% chance of fetal infection (congenital toxoplasmosis). The incidence and risk of placental transmission is dependent on the trimester during which maternal infection is contracted. The risk of transmission in the first trimester is estimated to be 10–15% and the outcome if contracted at this stage can be severe or life-threatening to the fetus. Conversely, the risk rises to 70–80% in the third trimester but the clinical outcome is usually less severe, or may be asymptomatic. Congenital toxoplasmosis can cause hearing loss, hydrocephalus, eye and brain damage, epilepsy, growth retardation, intrauterine death, and other problems. Congenital toxoplasmosis affected 0.34 per 10 000 births in England and Wales in 2002–2004. Studies over the past 15 years have estimated between 1 and 10 per 10 000 births elsewhere in Europe, and similar rates for the US. The most common symptoms observed in newborns are retinochoroiditis (inflammation of the retina and choroid) and/or intracranial abnormalities (with or without developmental delay). In Europe, 1% or 2% of infected infants develop learning difficulties or die and 4–27% develop permanent loss of vision. Mothers can be tested to determine if they have developed an antibody to the infection. Fetal testing may include ultrasound and testing of amniotic fluid or cord blood. When toxoplasmosis is diagnosed during pregnancy, antibiotic treatment can often help reduce the severity of symptoms in the newborn.

The risk of food poisoning can be minimized by ensuring adequate attention to good hygienic practice when preparing, cooking, and storing foods (Table 1). Pregnant women should therefore be advised of the need for a high regard for food hygiene and personal cleanliness during this vulnerable time. In addition, there are a few foods that may pose a particular (although small) risk, which should be avoided during pregnancy where possible (Table 2).

Alcohol

Excessive Alcohol Consumption during Pregnancy

Chronic alcohol abuse may result in a wide spectrum of secondary disturbances of the absorption and utilization of many nutrients, including glucose, amino acids, fat, sodium, and some vitamins (especially thiamin, vitamin B₁₂, and folate). The inhibition of folate absorption by alcohol is of particular concern because of the risk of neural tube defects associated with an inadequate supply of this vitamin to the fetus before conception and during the first trimester of pregnancy. Alcohol may also directly impair the placental transfer of nutrients essential for growth (e.g., amino acids), which at critical phases of fetal organogenesis could compound any direct fetotoxic effects of ethanol or acetaldehyde.

Table 1 General guidelines on good hygienic practices in the home

The risk of food poisoning can be minimized by adopting the following practices:

Cleanliness in the kitchen

- Keeping all work surfaces scrupulously clean
- Washing cooking utensils after coming into contact with raw meat, poultry or eggs to prevent cross contamination
- Using separate chopping boards for foods that are to be cooked (e.g., raw meat)
- Keeping kitchen cloths clean; rinsing crockery in hot water, leaving it to dry, then wiping it with a clean tea towel
- Using kitchen towels to mop up spills, rather than a dishcloth
- Ensuring waste bins are covered and away from food and keeping pets away from the kitchen.

Hygienic food handling

- Washing all equipment and work surfaces before and after touching raw food
- Washing all foods to be eaten raw thoroughly
- Cooking meat thoroughly to an internal temperature of at least 70 °C
- Keeping raw and cooked foods separated during preparation and storage
- Cooling cooked foods as quickly as possible if they are to be stored in a fridge or freezer
- Covering foods and not leaving them standing around in the kitchen
- Storing food at the correct temperature (less than 4 °C in the fridge or less than –18 °C in the freezer)
- Storing raw meat, well covered, at the bottom of the fridge
- Storing eggs in a fridge, if possible
- Never overloading the fridge as this can reduce the circulation of cool air
- Keeping foods for as short a time as possible (especially meat and fish) and following storage instructions (i.e., not using beyond the 'use by' or 'best before' date)
- Thawing frozen meat thoroughly before cooking
- Avoiding reheating and food more than once
- Reheating foods thoroughly (if this is done in a microwave, the standing times recommended by the manufacturer should be observed to ensure that food attains an even temperature before it is eaten).

Personal hygiene

- Washing hands thoroughly before preparing food, after visiting the toilet and after emptying the rubbish bin
- Never licking fingers or utensils and put them back into food
- Washing hands after blowing or touching the nose whilst handling food
- Keeping nails clean and hair out of food
- Wearing a clean apron
- Not handling food during periods of illness, e.g., heavy cold, sickness or diarrhea
- Covering all cuts, spots and pimples, particularly on the hands, with a waterproof dressing and replacing it often
- Wearing rubber gloves when emptying cat litter trays.

Both alcohol and its primary metabolite, acetaldehyde, are teratogenic. Excessive alcohol consumption (> 80 g of ethanol or 10 units per day) during pregnancy can result in a child being born with a specific combination of physical and mental disabilities known as fetal alcohol syndrome (FAS). Such fetuses usually survive until birth but are growth retarded and display a characteristic range of clinical features, principally

Table 2 Foods to avoid during pregnancy

<i>Foods to avoid</i>	<i>Reason</i>
Some types of cheese ^a : <ul style="list-style-type: none"> ● Mould-ripened cheeses, e.g., Camembert, Brie ● Some goats' cheeses ● Soft blue cheeses, e.g., Stilton All types of pâté (including vegetable)	To minimize risk of listeriosis
Unwashed fruit and vegetables Raw or undercooked meat Cured meats, e.g., Parma ham and salami Unpasteurized goats' milk or goats' cheese	To minimize risk of toxoplasmosis
Raw or partially cooked eggs or foods made from them, e.g., homemade mayonnaise, soft- whip ice cream, cake mix, mousses, and hollandaise sauce (eggs should be cooked until both the white and yolk are solid). Raw or undercooked meat, poultry, shellfish (e.g., oysters) and fish (e.g., smoked salmon, trout, sushi) Undercooked ready meals and ready-to-eat poultry (unless they have been reheated to a very high temperature). Unpasteurized milk and milk products Untreated water	To minimize risk of food poisoning from Salmonella and Campylobacter
Liver products and supplements containing vitamin A or fish liver oils	To avoid excess vitamin A intake
Some types of fish: shark, swordfish, king mackerel, tilefish, and marlin	To avoid high intakes of mercury and other contaminants
Limit intake of tuna, no more than two tuna steaks a week (approximately 140 g cooked or 170 g raw each) or four medium-size cans of tuna a week (approximately 140 g when drained) No more than two portions of oily fish per week, e.g., fresh tuna (not canned tuna, see above), salmon, mackerel, sardines and trout.	

^aFoods containing these cheeses that have been properly cooked will be safe to eat.

craniofacial abnormalities and neurological damage (Table 3). However, FAS is not the only outcome of prenatal alcohol exposure, and it has been suggested that it can present as a spectrum of disorders. Fetal alcohol spectrum disorder (FASD) is an umbrella term used to describe a range of effects that can occur due to the presence of alcohol during the prenatal stage. FASD is characterized by the presence of some of the criteria for FAS and is associated with lesser degrees of harm from maternal alcohol consumption.

The extent of the damage from alcohol varies depending on the stage of development at which high doses are encountered. The fetus is most vulnerable to organ damage from the time the umbilical cord begins to function (5 weeks) to the completion of organ development (11 weeks). Inhibition of growth and neurobehavioral development occurs in the second and third trimester. Although the facial features of FAS become more subtle with age, growth deficits and central nervous system impairment may be permanent. The reported worldwide incidence of FAS is 0.97 in 1000 births; however, the incidence of FASD remains unclear largely due to the absence of robust and routine data collection. Nevertheless, it is clear that FASD is more common in some populations than others. For example, in Australian aboriginal populations the incidence of FASD is estimated at 5 per 1000 births, whereas in the Western Cape Province of South Africa the incidence of FASD exceeds 60 cases per 1000 births.

FAS is only seen in infants born to women who are excessive drinkers, but it is not an inevitable result of heavy drinking in

Table 3 Symptoms of fetal alcohol syndrome (FAS)^a

The diagnosis of FAS requires signs in all of the three following categories:
● Prenatal and postnatal growth retardation Intrauterine growth retardation, including smaller than normal head circumference, continued growth below the 10th centile and failure to thrive.
● Central nervous system involvement Neurological abnormalities, developmental delay, intellectual impairment, brain malformation, hearing and visual disabilities.
● Physical anomalies Characteristic facial deformity including short upturned nose, receding forehead and chin, smaller than normal eye apertures, absent philtrum and asymmetrical ears.

^aThe diagnosis of FAS requires signs in all three of the categories.

pregnancy, and even children born to mothers who are active alcoholics may not show it. This differing susceptibility of fetuses to the syndrome is thought to reflect the interplay of genetic factors, social deprivation, nutritional deficiencies, and tobacco and other drug abuse, along with alcohol consumption.

Binge Drinking and Social Alcohol Consumption during Pregnancy

Binge drinking is generally defined for women, as the consumption of four or more drinks in approximately 2 h

Table 4 Definition of a unit of alcohol

1 unit of alcohol approximately equals 8 g of absolute alcohol, which is equivalent to:
½ pint of ordinary strength beer, lager, or cider
¼ pint of strong beer or lager
1 small glass wine
1 single measure of spirits
1 small glass sherry

(Centers for Disease Control and Prevention) or at least 6 units of alcohol (Table 4) per occasion (Strategy Unit, London) in the US and in the UK, respectively, and may be particularly harmful because it exposes the fetus to high blood alcohol concentrations over relatively short periods of time and may be associated with repeated withdrawal episodes. Animal studies have demonstrated binge-like exposure to alcohol to be as teratogenic as long-term exposure throughout gestation, even if the overall alcohol amount consumed by binge drinking is less than intake during more continuous drinking patterns. Human studies have found associations with binge drinking and neurodevelopment outcomes, such as an increase in 'disinhibited behavior,' a reduction in verbal IQ, an increase in delinquent behavior and learning problems. However, findings for other health outcomes have been inconsistent, possibly because of the notorious problems of recording binge drinking during pregnancy.

The question of whether moderate or occasional alcohol consumption is safe during pregnancy has been widely debated. Currently, there is little evidence that modest drinking (< 10 units per week) has any harmful effects. In a systematic review of the evidence, reported in the National Institute of Health and Clinical Excellence (NICE) 2008 guidelines "Antenatal care: routine care for the healthy pregnant woman," the highest risk suggested was a three- to fourfold increase in the risk of miscarriage in women who consume approximately 10 units of alcohol per week compared to abstainers. For other health outcomes, the majority of the studies did not report a statistically significantly increased risk with low-to-moderate alcohol intake, but the evidence was not entirely consistent. Many studies are confounded by factors such as cigarette smoking, social class, drug abuse, very high levels of caffeine intake, and different cross country categorization of 'light,' 'moderate,' and 'heavy' drinking. Although there is general agreement that women should not drink alcohol excessively during pregnancy, a consensus opinion of a safe threshold level of alcohol consumption has not been established at any stage of pregnancy, and advice differs among countries.

Despite the lack of evidence of detrimental effects on any outcome at low-to-moderate maternal alcohol consumption, many professional bodies err on the side of caution. The Royal College of Obstetricians and Gynaecologists and NICE suggest that the only way to minimize any harmful effects to the fetus from alcohol is to not drink at all during pregnancy. The UK Department of Health advises that pregnant women or those planning to conceive to avoid alcohol completely and if they do choose to drink they should not consume more than 1 or 2 units once or twice a week (Table 4), and should avoid intoxication. Advice in North America (US and Canada) is that women should not consume alcohol at all during pregnancy,

and there are warnings on products and advertisements. Anecdotal, many pregnant women develop a spontaneous aversion to the taste and/or smell of alcoholic beverages and so may limit their intakes anyway.

Vitamin A

During the period of early development, the supply of preformed vitamin A (retinol) must be carefully managed to ensure that the developing fetus is exposed to neither too little nor too much of the nutrient because either condition can have teratogenic consequences. Adequate vitamin A is required for normal embryonic development, and an insufficient supply during pregnancy can result in malformations in the offspring as well as increased mortality and morbidity during early childhood from infectious diseases, such as diarrhea, measles, and respiratory infections.

Excess vitamin A intake has also been associated with teratogenicity in animals and may represent a risk in humans, particularly within the first trimester of pregnancy. Characteristic features include severe motor deficit and malformations of the heart, thymus, face, jaw, ears, palate, and brain. Although adverse effects from dietary sources are very rare, events have occurred with the ingestion of high-dose supplements and with isotretinoin treatment for severe acne; this medication is now only permitted under strong supervision in women of reproductive age. Therefore, as presented in the SACN report 'The influence of maternal, fetal and child nutrition on the development of chronic disease in later life,' pregnant women are advised to avoid taking preformed vitamin A (retinol) supplements and to also avoid consuming liver or liver products. This advice is based on the risk of teratogenesis associated with retinol consumption at doses higher than 3000 µg retinol equivalents (RE) per day.

In Western countries, where vitamin A deficiency is rare, women who are or might become pregnant are advised against taking vitamin A supplements (including cod liver oil), except on the advice of a doctor or antenatal clinic, and not to consume liver or liver products. In developing countries with a high prevalence of vitamin A deficiency, vitamin A supplementation programs have resulted in decreased pregnancy-related mortality and lower rates of childhood morbidity and mortality, with benefits clearly outweighing any potential risks. The initiation of such programs in any population should be carefully examined in each case according to the risk-benefit ratio, with the final decision taking into account the vitamin A status of the population, the availability of vitamin A-rich foods, and whether supplementation can be supervised. The World Health Organization recommends that high-dose vitamin A supplements for women be restricted to the first 6 weeks postpartum, before they are likely to become pregnant again.

Fish and Pregnancy

Fish is a good source of protein, vitamins, and minerals. In particular, oil-rich fish (e.g., mackerel, salmon, kippers, herrings, trout, sardines, and fresh tuna) contain the long-chain

n-3 fatty acids eicosapentenoic acid (EPA) and docosahexenoic acid (DHA), which may confer many health benefits to the developing fetus. For example, DHA is required for nerve and retinal development, and eating oily fish has been found to have a slight beneficial effect on birth weight and length of gestation. In the US and Canada, the position of the American Dietetic Association (ADA) and Dietitians of Canada, is that adults, including pregnant and lactating women, should consume a combined intake of 500 mg day⁻¹ of DHA and EPA. In the UK, 450 mg day⁻¹ of DHA and EPA combined is recommended for adults and pregnant women, the equivalent of consuming two servings (one serving=140 g) of fish a week, one of which is oil-rich. However, consumption of oil-rich fish has been positively associated with intakes of certain contaminants, namely mercury, dioxins, and polychlorinated biphenyls (PCBs), and concern has been expressed about the consequences of prenatal exposure to these toxic chemicals on the risk of brain and nervous system abnormalities. As a result, pregnant women in the UK are advised not to consume more than two portions of oily fish a week.

Mercury

Mercury is a metal that is present in the environment from natural and man-made sources (e.g., coalburning or other industrial pollution). It is converted primarily by micro-organisms to a more toxic form, methylmercury, which is bioaccumulated in the aquatic food chain, reaching its highest levels in large, longer living predatory fish. Among humans, the sole source of exposure to methylmercury is the consumption of fish and sea mammals.

Methylmercury is neurotoxic and accumulates in the brain and central nervous system. It inhibits the division and migration of neuronal cells and disrupts the cytoarchitecture of the developing brain. Although a mother may show no signs of neurotoxicity, the developing fetus may be damaged following exposure to methylmercury. The concentration of methylmercury in fetal brain has been shown to be 5–7 times higher than that in maternal blood, and it has been estimated that the fetus is 5–10 times more sensitive to methylmercury exposure than an adult, although the reason for this is unknown.

Disasters in Minamata, Japan, in the 1950s and in Iraq in 1971–72 demonstrated that acute prenatal exposure may result in severe mental retardation, cerebral palsy, blindness, and deafness. However, whether exposure to lower chronic doses, which may occur if pregnant women consume large amounts of fish, can also lead to adverse neuro-developmental consequences is less certain. Large, long-term prospective epidemiological studies of high fish-eating populations have not found a consistent pattern of association between exposure and neuropsychological outcomes. Although subtle neuropsychological changes were reported in a study of children in the Faroe Islands study, where exposure was mainly from whale consumption, a similar study in the Seychelles found no adverse effects from fish consumption alone.

The Joint FAO/WHO Committee on Food Additives revised its safety guideline for weekly intake of methylmercury, known

Table 5 Concentrations of methylmercury (mg kg⁻¹) in surveyed fish in the UK

<i>Fish</i>	<i>Methylmercury mg kg⁻¹</i>
Shark	1.52
Swordfish	1.35
Marlin	1.09
Fresh Tuna	0.40
Canned Tuna	0.19
Herring	0.09
Pink Shrimps	0.09
Cod	0.07
Plaice	0.06
Mackerel	0.05
Haddock	0.04
Scallops	0.01

as the provisional tolerable weekly intake (PTWI), to 1.6 mg kg⁻¹ body weight per week. The UK government's independent expert Committee on Toxicity of Chemicals in Food, Consumer Products, and the Environment (COT) has applied a lower PTWI limit of 0.7 mg kg⁻¹ body weight per week to women who are pregnant or those intending to become pregnant and to mothers who are breast-feeding.

Any public health recommendations to pregnant women regarding fish consumption must recognize the important role that it plays as part of a healthy, balanced diet. Most fish contain trace amounts of methylmercury, but high concentrations of the metal have only been found in large, predatory fish, such as shark, marlin, and swordfish (Table 5). If a pregnant or breast-feeding mother were to consume one portion of these predatory fish, she would exceed the lower PTWI set by COT and the EPA by 400%. Therefore, as a precaution, pregnant women, breast-feeding mothers, and those who intend to become pregnant within the next 12 months are advised to avoid consumption of these types of fish (in the US, this also includes king mackerel and tilefish). Some samples of tuna have also been found to have higher levels than other species. In the UK, pregnant women (and those who may become pregnant) are advised to restrict their weekly intake to two 140 g portions of fresh tuna or four 140 g portions of canned tuna.

Dioxins and Polychlorinated Biphenyls (PCBs)

Fish can also contain other organic pollutants, such as PCBs and dioxins. Whereas mercury accumulates in the muscles of larger predatory fish, PCBs and dioxins are found in the fatty tissues of fish. Most human exposure to PCBs and dioxins comes from dietary sources because they accumulate in the lipid fractions of meat, fish, milk and milk products, eggs, grains, and oils.

PCBs and dioxins have been linked with increased rates of some cancers in studies of individuals exposed to high amounts through either vocational exposure or accidental environmental contamination. Prenatal exposure to large amounts of these pollutants (e.g., through contaminated fish) has been associated with neurobehavioral alterations in newborn children. Some studies have also suggested that exposure

to smaller quantities of PCBs and dioxins in utero may lead to more subtle cognitive and motor developmental delays, although a favorable home environment appears to counteract any effect. However, the difficulty of separating the effects of PCBs and dioxins from potentially confounding factors (e.g., exposure to other contaminants, breast-feeding, smoking, and maternal education) makes it difficult to reach firm conclusions. Further research is also needed to ascertain whether any cognitive changes are temporary or persist into later life.

The potency of dioxins is expressed as toxic equivalents (TEQs), which have been internationally accepted. In the UK, the tolerable daily intake (TDI) recommended by COT is 2 pg TEQ kg⁻¹ body weight, which is in line with recommendations of other international and European expert committees. In common with the US and the European Union, approximately one-third of the UK population may exceed the TDI in their daily diet. The TEQ, therefore, provides a target to reduce dioxins and PCBs in the environment internationally. Since the 1960s, following the prohibition of many dioxins and PCBs by governments, concentrations have been declining in breast milk, which is commonly used to determine exposure. For example, between 1982 and 1997, consumption of dioxins and PCBs in the UK decreased by 75% and between 1997 and 2001 fell by a further 50%, largely due to strict controls concerning production, use, and disposal of PCBs and dioxins: it is anticipated that intakes will continue to decrease.

Caffeine

Caffeine is a methylated xanthine that acts as a mild central nervous system stimulant. It is the most widely consumed xenobiotic in pregnancy and is found in a number of foods and beverages (Table 6). The main sources are coffee, tea, cocoa, chocolate, and soft drinks, as well as prescription and nonprescription medicines, such as diet pills, headache treatments, and cold and flu medicines. Tea and cocoa also contain significant quantities of theophylline and theobromine, which are caffeine derivatives that have not been as widely researched.

Once caffeine and its derivatives are consumed, they are absorbed into the blood and body tissues and can cross the placenta to the fetus. Cytochrome P450 1A2 (CYP1A2), the principal enzyme involved in caffeine metabolism in the liver, is absent in the placenta and the fetus, therefore the exposure of the fetoplacental unit to caffeine depends on maternal

caffeine metabolism, which is influenced by genetic and environmental factors. Typically the body metabolizes caffeine more slowly during pregnancy, especially in the last few months; the half-life of caffeine increases from approximately 5–18 h during the second and third trimesters. Blood caffeine concentrations are therefore raised during pregnancy with no change in intake. In contrast, smoking is known to increase caffeine metabolism appreciably.

Although very high doses of caffeine are teratogenic in animals, no link between consumption during pregnancy and birth defects has been demonstrated in humans. However, high maternal caffeine intakes (>500 mg day⁻¹) have been associated with increased fetal heart rate and newborn cardiac arrhythmias. Maternal caffeine intake has been reported to be associated with low birth weight, but the safe threshold level remains unknown. A number of studies have found an association with caffeine intakes greater than 300 mg day⁻¹ and fetal growth restriction and some have also demonstrated increased risks at intakes as little as 141 mg day⁻¹. In 2001, the Committee on Toxicology of Chemicals in Food, UK, conducted a thorough review of the literature and concluded that although caffeine consumption of more 300 mg day⁻¹ might be associated with spontaneous miscarriage and low birth weight, the evidence was inconclusive.

The lack of consistency between studies, particularly in relation to the dose at which an effect is reported, has made it very difficult to identify a threshold level of caffeine intake that presented an increased risk during pregnancy. However, in 2008 a large prospective observational study was conducted to reduce the uncertainties of previous evidence and provide a more robust basis on which to advise pregnant women on caffeine consumption. Results of the study, published in the British Medical Journal, linked maternal caffeine intake with increased risk of fetal growth restriction and concluded that sensible advice would be to reduce caffeine intake before conception and throughout pregnancy. Based on this evidence, guidance has changed in the UK on the consumption of caffeine for women trying to conceive and pregnant women, to limit their daily intake to 200 mg day⁻¹ (previous recommendation was a maximum daily intake of 300 mg day⁻¹). This is the equivalent of approximately two mugs of coffee a day (Table 7).

The revised level in the UK of 200 mg day⁻¹ is endorsed by the March of Dimes in the USA, however, the ADA, in their 2008 Position Paper, suggest 300 mg day⁻¹ as a safe upper limit and this is in line with the advice given by the EU Scientific Committee on Foodstuffs. In practice, many pregnant women reduce their coffee intake as a result of a spontaneous aversion to the taste and smell, particularly in early pregnancy. For example, in the UK average daily caffeine consumption decreased from 238 to 139 mg day⁻¹ during the first trimester,

Table 6 The caffeine content of beverages and foods

1 cup (190 ml) of instant coffee: ~75 mg
1 cup (190 ml) of brewed coffee (filter or percolated): ~100–115 mg
1 cup (190 ml) of decaffeinated coffee (brewed or instant): ~4 mg
1 cup (190 ml) of tea: ~50 mg
1 cup (200 ml – using manufacturers' instructions) of drinking chocolate: 1.1–8.2 mg
250 ml serving of energy drinks (containing either caffeine or guarana): 28–87 mg
330 ml serving of cola (regular and diet) – 11–70 mg
50 g bar of chocolate – 5.5–50 mg

Table 7 200 mg of caffeine is roughly equivalent to

3 average cups or 2 average size mugs of instant coffee
2 average cups of brewed coffee
4 average cups of tea or 2 mugs of tea
5 cans of regular cola drinks (e.g., 40 mg each)
2 cans of 'energy' drinks (e.g., 80 mg each)
200 g (4 standard 50 g bars) of milk chocolate (e.g., 50 mg each)

and then increased to an average of 153 mg day^{-1} by the third trimester of pregnancy, so most pregnant women are unlikely to be affected by the change in advice.

Avoiding Foods to Prevent Allergy

Food allergy has been estimated to affect approximately 3–7% of infants and young children in Western Europe, although the majority of children outgrow food allergies by the time they start school. Prevalence is assumed to be increasing in line with other forms of atopic disease, although evidence to support this is limited. Some food allergies (e.g., peanut allergies) can persist into adulthood and in severe cases can be life threatening. Most confirmed food allergies are associated with a relatively limited range of foods, including cows' milk, eggs, tree nuts, peanuts, soybeans, wheat, fish, and shellfish.

The development of food allergy depends on several factors, including genetic factors and early exposure to allergenic proteins in the diet, food protein uptake and handling, and the development of tolerance. However, it remains uncertain whether sensitization occurs *in utero* and, if so, whether this occurrence is restricted to specific stages of gestation.

There is little evidence to support any benefit of avoiding specific foods during pregnancy to reduce the risk of allergic disease in a genetically susceptible child. Indeed, such a strategy may be counterproductive because it has been suggested that exposure to foreign proteins that cross the placenta is important to establish a normal immune response that enables the infant to develop normal tolerance to the many foreign proteins in the environment. Because restrictive diets may limit the supply of essential nutrients, these should only be practiced under medical supervision. Inappropriate and unnecessary exclusion of foods could prevent both mother and infant from obtaining the nutrients they need, resulting in significantly reduced weight gain and a tendency toward lower birth weights.

Owing to the severity of reactions experienced from peanut allergy, some countries have issued specific advice around intake during pregnancy. In the UK, COT had previously issued cautionary advice that women may wish to avoid peanuts during pregnancy and breast-feeding and not introduce peanuts into their child's diet before three years of age, if their child has a family history of allergy. However, in 2009 the Government revised its advice based on a systematic review which indicated that there is no clear evidence that eating or not eating peanuts (or foods containing peanuts) during

Table 8 A summary of advice regarding dietary habits and foods safe during pregnancy

- Pregnant women should pay careful attention to food and personal hygiene so as not to expose themselves to any risk of food poisoning, which is not only highly unpleasant but also potentially very dangerous to the unborn child in some cases (e.g., with listeriosis and toxoplasmosis).
- Foods that have been linked with the bacteria *Listeria monocytogenes* should be avoided. These include pâtés and mould-ripened, soft cheeses (e.g., brie and camembert). Preprepared foods should be heated until they are piping hot and fruit and vegetables washed well, especially if they are to be eaten raw.
- To reduce the risk of toxoplasmosis, pregnant women should avoid eating raw or uncooked meat, unpasteurized goats' milk or goats' cheese, or unwashed fruit and vegetables. After handling raw meat, chopping boards, utensils and hands should be washed thoroughly. When gardening or emptying cat litter trays, rubber gloves should always be worn.
- Undercooked foods (e.g., meat, poultry, eggs), foods containing raw egg (e.g., mayonnaise, soft whip ice cream) and raw fish (e.g., sushi, smoked salmon) should also be avoided.
- Drinking alcohol heavily throughout pregnancy ($>80 \text{ g}$ or 10 units per day) is linked with fetal alcohol syndrome. Modest drinking (<10 units per week) does not appear to have harmful effects but most professional bodies err on the side of caution and recommend that pregnant women abstain from drinking alcohol or limit their consumption to no more than 1–2 units per day.
- Supplements containing high doses of preformed vitamin A and foods containing large amounts of this vitamin (liver and liver products) are best avoided in countries where intake from a well-balanced diet should be sufficient. In areas of endemic vitamin A deficiency, supplementation can reduce pregnancy-related mortality and reduce rates of childhood morbidity and mortality. However, most experts agree that preformed vitamin A supplements in doses of more than 3000 RE should not be taken by women who may become pregnant. Beta-carotene is safe for pregnant women.
- Although it is not presently clear if intakes of mercury and other contaminants such as PCBs and dioxins at levels that can be obtained from eating fish can influence children's neurological development, government organizations in a number of countries recommend that pregnant women avoid species of fish that have been found to contain high levels of these substances. This includes shark, marlin, swordfish, tilefish and king mackerel. Some countries have also recommended limiting tuna intake (e.g., in the UK pregnant women are encouraged to consume no more than two tuna steaks or four medium-size cans of tuna per week).
- Consumption of caffeinated beverages (e.g., coffee, tea and colas) has been associated with miscarriage and low birth weight, although many studies are confounded by high alcohol intakes, smoking, and drug and other substance abuse. In the UK, the recommendation is that pregnant women limit their caffeine intake to 200 mg day^{-1} which is endorsed by the March of Dimes in the US, however, the ADA and EU Scientific Committee on Foodstuffs suggest a safe upper limit of 300 mg day^{-1} .
- Avoiding specific foods during pregnancy is unlikely to reduce the risk of allergic disease in a susceptible child. In the UK, the Government recommends if women would like to eat peanuts or foods containing peanuts during pregnancy, they can choose to do so as part of a healthy, balanced diet. The March of Dimes in the US now advises that women who are not allergic to peanuts can safely eat peanuts during pregnancy.
- Additives permitted for use in foods undergo stringent safety tests over a long period of time before being approved and are safe for consumption during pregnancy by all but a small proportion of women who experience rare reactions to specific additives.
- Many pregnant women who would not consider taking over-the-counter medications often view herbal products as a safe and natural alternative. However, very few randomized, clinical trials have examined the safety and efficacy of alternative therapies during pregnancy. Pregnant women should be advised to seek advice from a doctor or pharmacist before taking any medication, including herbal supplements.

pregnancy, breast-feeding or early childhood has any effect on the chances of a child developing a peanut allergy. Pregnant women in the UK are now advised that they can choose to eat peanuts irrespective of whether their child has a family history of allergies. Similarly in the US, the American Academy of Pediatrics previously suggested that pregnant women avoid peanuts, until research from the Avon Longitudinal Study of Parents and Children (ALSPAC) reported no association between maternal consumption of peanuts during pregnancy and childhood peanut allergy. The March of Dimes in the US now advises that women who are not allergic to peanuts can safely eat peanuts during pregnancy.

Food Additives and Herbal Supplements

Pregnant women often express concern about food additives. However, all additives have to be approved as safe for almost all but a small proportion of the population who may experience rare reactions to them before they can be used in foods. The presence of an 'E' number demonstrates that it has passed safety tests and been approved for use by the European Community. In the UK, COT sets an Acceptable Daily Intake for each additive, which is the amount that can be consumed daily with no risk to health. This may limit the amount of an additive used or restrict its use to certain food products. Even when an additive has been approved, new research is constantly reviewed and approval for any additive will be withdrawn if doubt is raised about its safety.

A number of herbal supplements and preparations may be used during pregnancy, most commonly to relieve gastrointestinal symptoms. Although the use of many herbal remedies is safe during pregnancy, this cannot be assumed simply because a product is described as 'natural.' Many plants, trees, fungi, and algae can be poisonous to humans, and several pharmaceuticals have been developed or derived from these sources because of the powerful compounds they contain. Very few randomized, clinical trials have examined the safety and efficacy of alternative therapies during pregnancy, and women should be warned to use any medicine, including herbal remedies, with care during pregnancy and with the advice of a doctor or pharmacist.

Summary

In addition to the consumption of a healthy, balanced diet, there are food safety precautions that need to be followed to ensure a safe pregnancy. A summary of the evidence and current advice described in this article is given in **Table 8**. However, it is important to highlight that recommendations during pregnancy across the globe, continue to change from time to time as new evidence emerges and guidelines are published. A recent virtual issue report by *Maternal & Child Nutrition* and *Nutrition Bulletin* has been published which pulls together articles describing these recent changes in nutritional recommendations which are also summarized in the above article.

See also: Fertility. Fish and seafood: Nutritional Value. Folic Acid. Food Safety: Bacterial Contamination; Heavy Metals; Other Contaminants. Pregnancy: Energy Requirements and Metabolic Adaptations; Nutrient Requirements; Placental Regulation of Nutrient Delivery to the Fetus; Pre-eclampsia and Diet; Prevention of Neural Tube Defects; Weight Gain. Vitamin A: Deficiency and Interventions

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Weight Gain

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Glossary

Body Mass Index Weight (in kg) divided by height (in meters squared). Used as an indicator of undernutrition or overweight.

Gestational diabetes mellitus Abnormal glucose metabolism accompanied by major alterations in the metabolism of fat and protein. Diagnosed by high blood glucose and/or urinary glucose. This is the most common medical disorder in pregnancy.

High birth weight (macrosomia) >4500 g.

Low birth weight <2500 g.

Preeclampsia A condition which can appear suddenly in late pregnancy, with symptoms of high blood pressure, edema, urinary protein, severe headache, and vision problems. This may turn into eclampsia (convulsions or coma) if not treated.

Preterm delivery <37 weeks from conception. A normal duration of gestation is 40 weeks.

Pregnancy Weight Gain Recommendations

In 1970, the US National Academy of Sciences published guidelines for weight gain during pregnancy in the report “Maternal Nutrition and the Course of Pregnancy.” The recommended pregnancy gain was 24 lb (10.9 kg), with a range of 10–25 lb (9.1–11.4 kg). The report advised health care providers and pregnant women not to restrict weight gain – a practice that had been fairly widespread during the previous decade in order to reduce the perceived increased risk of labor complications, preeclampsia, and excess weight retention postpartum. In fact, many obstetricians had been recommending gains of only 15–20 lb (6.8–9.1 kg).

Even with the more generous recommendations set in 1970, by the 1980s it had become clear that average gain of women in the United States far exceeded these guidelines. An analysis of data from the National Natality Survey in 1980 showed the average pregnancy weight gain to be 29 lb (13.2 kg), and by the time of the National Maternal Infant Health Survey in 1988 the average had increased to 32 lb (14.5 kg). The range of gain among women was very wide, from no gain to more than 75 lb (34.1 kg).

Based on this realization, in 1990 the weight gain recommendations were revised completely by a committee established by the Institute of Medicine (IOM) of the National Academy of Sciences. Existing data from a national survey were analyzed to determine the weight gain that was compatible with a normal pregnancy outcome. The latter was defined as the infant being born full term and of normal birth weight, and the absence of pregnancy or delivery complications. It became apparent from these analyses that maternal weight-for-height at conception, expressed as body mass index (BMI) (weight in kilograms divided by height in meters squared), was an important predictor of actual weight gain. Thin women (with a low BMI) gained more weight than fatter women. Different weight gain recommendations were therefore developed for women entering pregnancy with different BMIs.

The 1990 weight gain recommendations were revised again in 2009, the justification being that women are now becoming pregnant at an older age and heavier weight, and are more likely to have multiple pregnancies and to gain excessive amounts of weight during pregnancy. The increasing prevalence of maternal overweight and obesity at conception is a global phenomenon, and associated with greater risk of preeclampsia, gestational diabetes, cesarean delivery, problems with breast-feeding, and subsequent overweight in the child. As in 1990, the known relationship between pregnancy weight gain and BMI formed the basis of the recommendations, but the BMI categories were changed to those used by the World Health Organization. The recommended gains in **Table 1** are consistent with the lowest risk of: cesarean delivery, giving birth to a premature infant, excessive weight retention after delivery, low or high birth weight, and subsequent childhood obesity in each BMI category.

For thinner women (BMI <18.5), recommended gains are 12.5–18 kg (28–40 lb) or 0.51 kg (1 lb) per week; for women with a normal BMI (18.5–24.9), gain should be 11.5–16 kg (25–35 lb) or 0.42 kg (1 lb) per week; for overweight women (BMI >25–29.9), gain should be at least 7 kg (15 lb) and not more than 11.5 kg (25 lb) or 0.28 kg (0.6 lb) per week; and obese women (BMI ≥30) should gain the least – 5–9 kg (11–20 lb) or 0.22 kg (0.5 lb) per week. New weight gain charts were constructed that show the recommended gains over the course of pregnancy for each BMI group (**Figure 1**), enabling the adequacy of weight gain to be tracked for individual women. To use the chart, women’s height and weight should be measured as near to the time of conception as possible (because pregnancy causes a temporary reduction in height) and used to obtain their BMI from a table. Maternal recall of prepregnancy weight and height can be used as a practical alternative but may be substantially less reliable. The US recommendations are deemed to be appropriate for women in developed countries worldwide.

Table 1 Recommendations for pregnancy weight gain by BMI at conception

BMI category	Recommended weight gain range, kg (lb)	Rate of weight gain in second and third trimester range, kg (lb)/week
Low (BMI < 18.5)	12.5–18 (28–40)	0.44–0.58 (1–1.3)
Normal (BMI 18.5–24.9)	11.5–16 (25–35)	0.35–0.50 (0.8–1)
Overweight (BMI > 25.0–29.9)	7–11.5 (15–25)	0.23–0.33 (0.5–0.7)
Obese (BMI ≥ 30.0)	5–9 (11–20)	0.17–0.27 (0.4–0.6)

Source: Modified from Institute of Medicine (2009) Weight gain during pregnancy: Reexamining the guidelines. In: Rasmussen KL and Yaktine AL (eds.) *Food and Nutrition Board and Board on Children, Youth, and Families*. Washington, DC: National Academies Press.

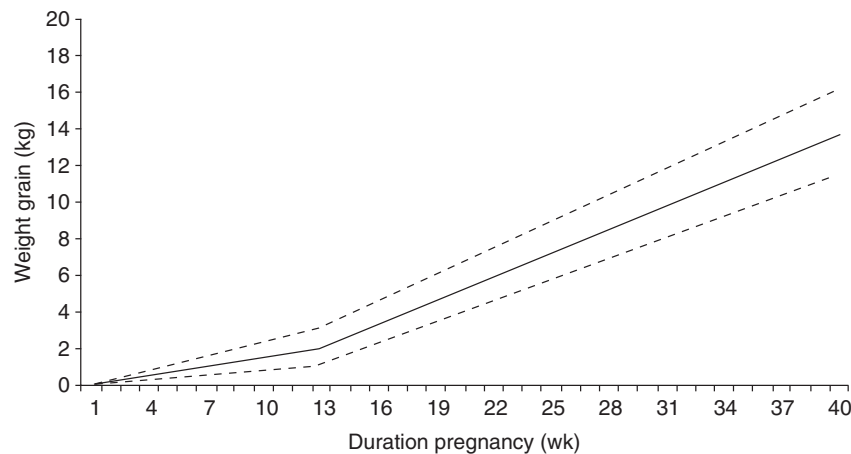


Figure 1 Recommended weight gain by week of pregnancy for women with a normal BMI at conception. Similar charts are available for underweight and obese women. Reproduced with permission from the Institute of Medicine (2009) Weight gain during pregnancy: Reexamining the guidelines. In: Rasmussen KL and Yaktine AL (eds.) *Food and Nutrition Board and Board on Children, Youth, and Families*. Washington, DC: National Academies Press.

Excessive Weight Gain

Most of the literature on pregnancy weight gain and maternal outcomes is observational so it usually cannot be confirmed that poor outcomes are caused by, rather than associated with, high weight gain. However, in general it appears that the evidence is inconclusive concerning whether excess weight gain leads to diabetes or hypertension, but reveals more strongly that it leads to increased risk of cesarean delivery and failure to initiate breast-feeding.

Pattern of Weight Gain

Relatively little (1–2.5 kg) of the total weight gain during pregnancy occurs during the first trimester; gain in the second trimester is highest, followed by a slightly lower gain in the third. Nevertheless, it is important to pay attention to the quality of pregnant women's diets during the first trimester and to ensure that they do not restrict their intake during this time, when there is the strongest risk of nutrition-related birth defects and spontaneous abortions. In some studies, an association has been noted between low weight gain in the first trimester and increased risk of spontaneous preterm delivery.

Variability in Weight Gain

The BMI-specific target ranges for pregnancy weight gain are relatively narrow, but a very wide range of gain actually occurs. In a California study, for example, only 50% of the mothers who had an uncomplicated pregnancy with a normal birth-weight infant gained the recommended range of weight, with the remainder gaining more or less. Because a substantial amount of the variation in weight gain is due to physiological variability and prepregnancy BMI, deviation from the recommended range may not necessarily be cause for concern. However, it is especially important to assess the dietary patterns and other behaviors of women whose weight gain is unexpectedly high or low.

Maternal Weight Gain and Birth Weight

Inadequate maternal weight gain is associated with poor fetal growth even when the contribution of fetal weight and factors such as length of gestation are taken into consideration. Birth weight is an important determinant of child health and survival; low-birth-weight (<2.5 kg) infants are 40 times more likely to die in the neonatal period. Low weight-for-length at birth may be a risk factor for chronic disease in later life. It has been estimated that in women with a normal prepregnancy

BMI, each kilogram of total pregnancy weight gain has an average effect on birth weight of 20 g. In California, women with pregnancy weight gains below recommendations had a 78% higher risk of the infant being born small, whereas women who gained in excess of recommendations were twice as likely to give birth to a large infant.

As noted previously, maternal BMI at conception is strongly inversely related to expected pregnancy weight gain. Nevertheless, heavier women still tend to deliver heavier infants and thinner women tend to have smaller infants. In thinner women, birth weight is more strongly related to pregnancy weight gain, so the greatest risk of low birth weight is for thin women with a low pregnancy weight gain. It is crucial that thin women gain adequate amounts of weight. These associations are not explained by other risk factors associated with thinness, such as smoking.

Changes in Body Composition and Maternal Energy Status

It used to be assumed that maternal energy intake during pregnancy was the main determinant of the amount of weight gained. Although our knowledge of this relationship is still inadequate, newer information indicates that other maternal factors, and especially body composition, are more important predictors.

The weight gained during pregnancy can be roughly divided into the weight of the fetus, placenta, and amniotic fluid (a total of approximately 5 kg), maternal gain in the uterus, breasts, blood, and fluid (approximately 4 kg), and maternal fat. The latter component is the most variable, accounting for approximately 70% of the variability in pregnancy weight gain. Although average fat gain in different studies is approximately 2–5 kg, values for individual women range from a loss of several kilograms to a gain of approximately 12 kg. Even in a group of women with normal BMIs at conception, the range of fat gain was 0.5–9.5 kg. Fatter women at conception gained less fat during pregnancy, as would be expected from their lower weight gains. The greater fat gain of thinner women is a potential energy store for the fetus and would afford some protection against maternal malnutrition in late pregnancy – a situation that is not uncommon in some economically disadvantaged countries.

Maternal BMI at conception influences not only the amount of maternal weight and fat gained during pregnancy but also changes in maternal basal metabolic rate (BMR). In studies of well-nourished pregnant women, BMR has been reported to increase by approximately 20–30%. However, for undernourished women the increment in BMR may be only 20% of that seen in those who are well nourished. In contrast, in a group of well-nourished Californian women weighing 55–116 kg, the BMR of those with higher BMIs was almost twice that of the thinnest women in the group.

Overall, it is clear that heavier women gain less weight and fat during pregnancy and have a larger increase in BMR. It has not been determined how these changes translate into energy requirements for women in the different BMI groups used to predict weight gains. Therefore, the values for energy requirements, which vary by trimester, are used for all pregnant women regardless of their BMI at conception.

Weight Gain for Special Population Groups

Adolescents, Short Women, and Ethnic Groups

Well-nourished adolescents tend to gain at least as much, if not more, weight than adult women. The relationship between BMI, pregnancy weight gain, and birth weight is probably no different in this group, there is insufficient evidence for creating different weight gain guidelines for young adolescents. To ensure adequate nutrition for those who are still growing special attention should be given to ensuring that the quality of their diets is good. The effects of this recommendation on weight retention postpartum have not been evaluated adequately.

Women who are less than 157 cm tall tend to give birth to infants who are large relative to maternal pelvic size, with a subsequently slightly greater risk of a more difficult delivery. However, pregnancy weight gain recommendations do not differ by maternal height.

There is insufficient evidence to recommend different pregnancy weight gains for ethnic groups. However, Black women in the United States tend to gain less weight in pregnancy and to produce lower birth-weight infants. The reasons for this are not known, but it could not be explained by differences in gestational age or other factors. Adequate weight gain in this group is known to be especially important for the prevention of fetal growth retardation. In one study, 18% of nonobese black women who gained less than the IOM recommendations gave birth to low-birth-weight infants compared to 10% whose gain was in the ideal range and 4% who gained more than the recommendations. In obese black women, the low birth weight prevalence was approximately six times higher than that for those who gained less than the recommendations.

Most surveys indicate that Hispanics seem to gain approximately the same amount of weight as Anglo women. In the 1980 National Natality Survey, Hispanic and non-Hispanic white women gained a similar amount of pregnancy weight, but the risk of low birth weight was twice as high in Hispanics. Surveillance of a predominantly Hispanic population indicated that half of the underweight women and one-third of the normal weight women gained the recommended amount of weight, whereas more than half and three-fourths of overweight and obese women, respectively, had excessive gains. Inadequate weight gain during the third trimester was predictive of preterm birth. Underweight Hispanic women had nearly twice the risk of premature delivery.

The maternal weight gain recommendations have been evaluated in a group of Chinese women with good pregnancy outcomes (N=504) to assess the need for an ethnic-specific recommendation for this group. The BMI categories were used at different levels. Women with weight gain in the lowest quartile had twice the risk of having a low-birth-weight infant, and those with excessive weight gain were in greater need of assisted delivery (either vaginal or cesarean delivery).

Substance Abusers

Cigarette smokers tend to gain less weight during pregnancy and to produce smaller infants. This effect is not explained by

a lower food intake. Alcohol and drug use have similar effects. Simply gaining more weight during pregnancy will not compensate for the adverse effects of these practices on fetal outcome or pregnancy complications.

Multiple Births

Relatively few data are available from national surveys on which to base weight gain recommendations for women with twins. Recommendations by the recent IOM Committee are: normal weight at conception, 37–54 lb; overweight, 31–50 lb; and obese, 25–42 lb. There is insufficient information to make recommendations for underweight women.

Obese and Overweight Women

Obesity during pregnancy is associated with higher morbidity for both the mother and the child. Higher prepregnancy weights have been shown to increase the risk of late (>28 weeks of gestation) fetal deaths. In addition, the prevalence of gestational hypertension increases threefold and there is a three to four times greater risk of gestational diabetes in obese pregnant women. However, in prolonged fasting, i.e., 16–18 h, there is more risk of blood ketones being elevated in pregnant women that may increase risk of poor intellectual development of their offspring, thus prolonged fasting and weight loss in pregnancy is not recommended.

Exercising Women

Women who are physically fit at conception are able to continue to exercise during pregnancy without harm to themselves or the fetus, as long as the activity is not too strenuous or prolonged. In several studies exercising women gained 2 or 3 kg less than those who were more sedentary.

Pregnancy Weight Gain and Postpartum Risk of Obesity

On average, well-nourished women retain relatively little weight a year after delivery (approximately 0.5–1.5 kg). Delivery is followed by a rapid loss of weight in the subsequent 2 weeks due to fluid loss then a slower rate of loss for the next 6 months, so a complete return to preconception weight should not be expected in less time than this. In general, weight still retained at 1 year postpartum is unlikely to be lost without lowering intake and/or increasing physical activity. If weight retention is substantial, it can add to the risk of obesity in the longer term, and obesity is a major public health concern in many countries.

The relatively low average weight retention postpartum obscures the fact that many women do retain an excessive amount of weight. Those who retain most are likely to have gained large amounts of weight during pregnancy. At 10–18 months postpartum, weight retention was 2.5 kg for women who gained more than the IOM recommendation compared to 0.7 kg for white women, and 3.2 kg for black women who gained the advised amount. These large racial differences in

weight retention have not been explained and certainly may be a risk factor for the higher prevalence of later obesity in this group.

Most women breast-feed their infants exclusively or partially for a relatively short time. There is little difference in weight loss between women who breast-feed and those who do not for periods up to 6 months postpartum. This is presumably due to the greater appetite and energy intake of women who are breast-feeding and perhaps to dieting on the part of nonbreast-feeders. One study of women who breast-fed until 12 months postpartum did report 2 kg more weight loss compared to women who stopped breast-feeding before 3 months. Even more weight was lost by those who breast-fed more often and gave longer feeds.

Women with a high BMI at conception tend to either lose or gain more weight postpartum than those with a normal BMI; approximately one-third end up weighing less than at conception, and one-third weigh substantially more. The reasons for the highly variable weight retention in this group are not known.

Although inadequate intake of nutrients during lactation can lead to maternal nutrient depletion and lower breast milk content of some nutrients and especially vitamins, breast-feeding women who choose to lose weight can do so by exercising and/or reasonable restriction of energy intake. Exercising by jogging, biking, and aerobics for 45 min, four or five times per week for 12 weeks did not affect well-nourished mothers' ability to lactate or influence their milk composition. However, it is possible that severe energy deficit in lactation, especially of thinner women, will reduce breast milk volume.

Impact of Supplementation

Numerous investigators have explored the benefits of energy and/or protein supplementation for pregnancy weight gain and other outcomes. However, relatively few trials have randomly assigned these supplements and used control diets. A statistical analysis was conducted of the 10 such studies that met this criterion in 1995. Most, but not all, of these studies were performed in developing countries. A 5-year controlled trial in The Gambia provided daily prenatal dietary supplements (two biscuits) that contained 4250 kJ (1000 kcal) energy and 22 g protein. This supplement increased pregnancy weight gain and birth weight during the hungry and harvest seasons. There was a significant but very small increase in head circumference and a significant reduction in perinatal mortality.

Supplementation of undernourished women in the third trimester is most likely to increase birth weight. In the Dutch famine during World War II, low food intakes during the third trimester rapidly reduced birth weight and length, and head circumference. These adverse outcomes did not occur a few months after the food supply improved. Low intakes in the second trimester had less effect, although insufficiency in the first trimester had no effect. An increase in low birth weight prevalence was also observed in The Gambia when third-trimester gestation overlapped with the hungry season. Nonetheless, nutrition interventions initiated earlier and continued throughout pregnancy will have the strongest effect

on birth weight. There are enduring advantages to continued supplementation postpartum (during lactation) and into the ensuing pregnancy. A longitudinal study in Guatemala reported a significant increase (approximately 350 g) in birth weight in the second pregnancy when the mother was supplemented during the previous pregnancy and throughout subsequent lactation and the second pregnancy, compared to those who were not supplemented during the prior pregnancy. Overall, it is appropriate for supplementation of undernourished women to begin as early in the pregnancy as possible so that both mother and fetus receive the maximum benefits for optimal health and development.

See also: Adolescents: Requirements for Growth and Optimal Health. Breast Feeding. Lactation: Dietary Requirements; Physiology. Obesity: Complications. Pregnancy: Energy Requirements and Metabolic Adaptations; Nutrient Requirements; Pre-eclampsia and Diet; Prevention of Neural Tube Defects; Safe Diet

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PROSTAGLANDINS AND LEUKOTRIENES

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Introduction

Prostaglandins (PGs) and leukotrienes (LTs) belong to a large, heterogeneous group of lipid mediators, collectively named eicosanoids, that exhibit a diverse array of physiological activities (Figure 1). Eicosanoids are synthesized by oxygenation and remodeling of their precursor 20-carbon polyunsaturated fatty acids (PUFAs), namely arachidonic acid (AA; 20:4, *n*-6). Although pivotally involved in many homeostatic processes, eicosanoids are also implicated in the pathophysiology of many chronic disorders (Figure 1). Since the discovery in the mid-1930s of prostaglandins as a component of human semen that potently induced uterine contractility, the field of eicosanoid biology has expanded to include the PGs, LTs, thromboxanes (TXs), hydroxyeicosatetraenoic acids (HETEs), lipoxins (LXs, including epi-LXs), isoprostanes, and the cyclopentanone PGs (Figure 2). Except for the latter two classes, which are generated by nonenzymatic oxidation, synthesis of these mediators is tightly regulated by a number of enzymes. Eicosanoids generally act as paracrine or autocrine agents, in that they exert their biological effects locally, either on the cell from which they were synthesized or on neighboring cells. This article will focus primarily on the synthesis and physiological roles of the PGs and LTs and the regulation of their synthesis by dietary fatty acids.

Synthesis

Following an appropriate physiological or pathological stimulus, AA is released from cell membrane phospholipids by

one of the many forms of phospholipase A₂ (PLA₂), which is generally regarded as the rate-limiting step in eicosanoid synthesis (Figure 2). There are two major biosynthetic pathways: the cyclooxygenase (COX/PGH synthase) pathway, which synthesizes the PGs and TXs; and the 5-lipoxygenase (5-LO) pathway, which synthesizes the LTs, HETEs, and LXs. The predominant cellular origins of PG and LT synthesis, their receptors, and their major physiological activities are summarized in Table 1.

Major Biosynthetic Pathways

Cyclooxygenase

COX catalyzes two enzymatic activities, namely, the conversion of AA to the hydroperoxy endoperoxide PGG₂, followed by its subsequent reduction to the labile product PGH₂. PGH₂ is the common substrate for a number of different cell-specific synthases, which convert PGH₂ to the individual PGs or TX, including PGE₂, PGI₂ (prostacyclin), PGD₂, PGF_{2α}, and TXA₂ (Figure 3). In the early 1990s, two isoforms of COX named COX-1 and COX-2 were identified and this led to renewed interest in the field of PG biology. Both isoforms catalyze the same reactions but are produced by different genes, and although they share only approximately 61% sequence identity, the three-dimensional crystal structures are virtually identical. After the discovery of the two isoforms, it quickly became apparent that their roles in many physiological processes were distinctive and that their expression and tissue profiles were differentially regulated. In general terms, COX-1 is constitutively expressed in most tissues and cell types and is responsible for the synthesis of PGs required for the maintenance of normal physiology in the noninflamed state. Although COX-2 is generally undetectable in most tissues, its expression can be rapidly induced by a variety of inflammatory stimuli, such as bacterial lipopolysaccharides (LPS), cytokines, and growth factors. It is this isoform that synthesizes most PGs during inflammation and carcinogenesis. However, this division of the biological roles of COX-1 (physiological PGs) and COX-2 (inflammatory PGs) is an oversimplification of the biological reality. More recent studies have shown that COX-1 can be induced or upregulated under certain conditions and that COX-2 is constitutively expressed in the brain and kidney. Thus, both COX-1 and COX-2 are involved in physiological as well as pathological responses.

The COX pathway is of high clinical importance because it is the major pharmacological target of nonsteroidal

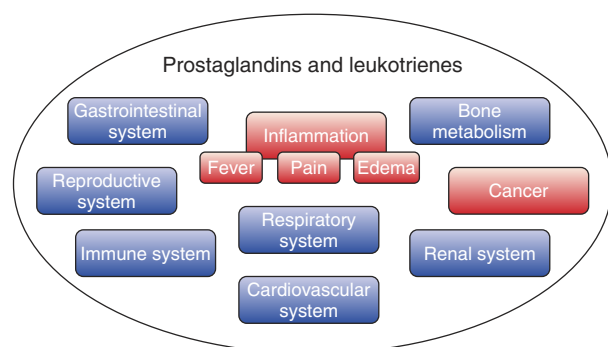


Figure 1 The diverse activities of PGs and LTs are reflected by their involvement in both normal homeostasis (blue) and pathophysiology (red).

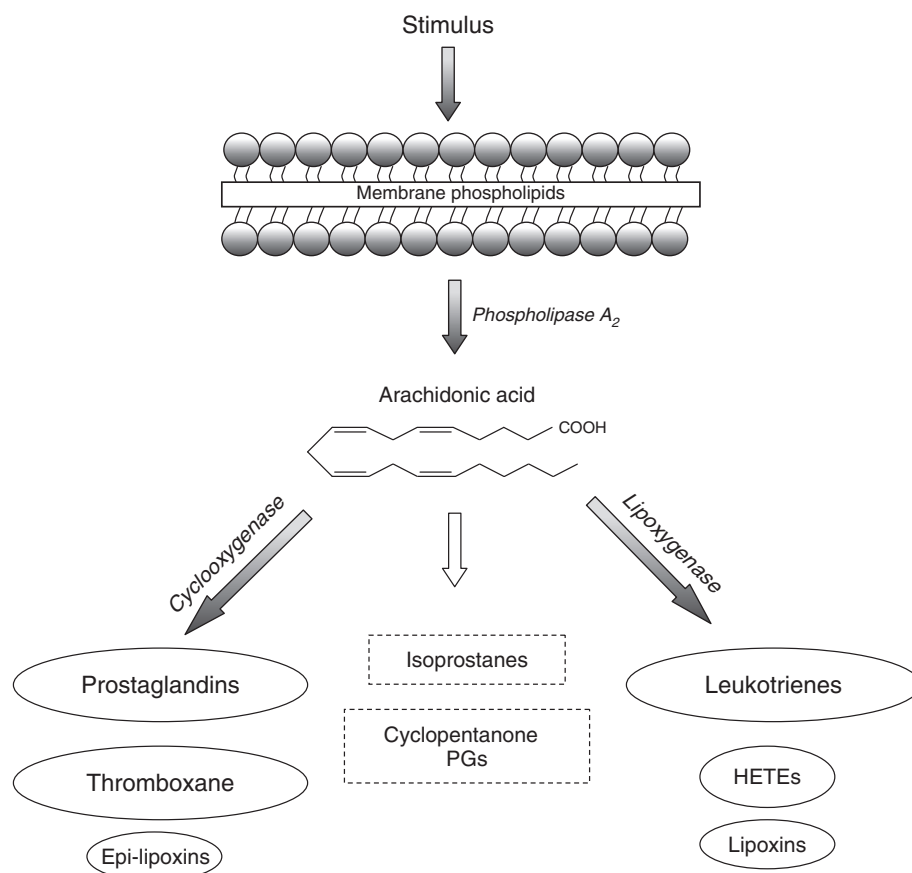


Figure 2 Metabolism of arachidonic acid to either the PGs and TX via the COX pathway or LTs (and HETEs, lipoxins) via the lipoxygenase pathway. The isoprostanes and cyclopentanone PGs are generated by nonenzymatic oxidation (dashed boxes). The epi-lipoxins are formed via interactions with COX and aspirin.

anti-inflammatory drugs (NSAIDs) and aspirin. Inhibition of PG synthesis is considered the primary mechanism responsible for both the therapeutic (anti-inflammatory and analgesic) and the toxic effects of NSAIDs. The clinically significant side effects of NSAIDs include renal impairment, dyspepsia, and upper GI bleeding, the latter being particularly associated with inhibition of COX-1. By comparison, the anti-inflammatory and analgesic effects are associated with COX-2 inhibition. These observations provided the rationale for fast-track development of selective COX-2 inhibitors, which were promoted under the premise that they would have similar anti-inflammatory efficacy to conventional NSAIDs but would have significantly fewer GI side effects. Highly selective COX-2 inhibitors have been relatively successful with regard to their reduced GI toxicity. However, based on the role of COX-2-derived PGs in normal physiology, there exists the potential for other side effects such as increased cardiovascular events in at-risk patients and aggravated renal impairment in patients with reduced renal function.

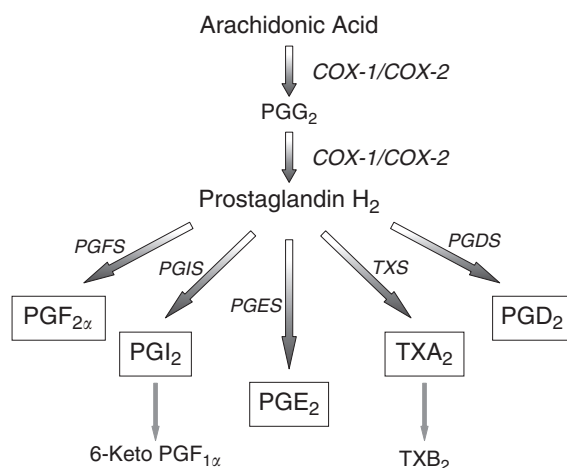
Lipoxygenase

An alternative pathway available for the metabolism of AA is the 5-LO pathway, which gives rise to the LTs that contain a

conjugated triene structure. The 5-LO enzyme catalyzes the addition of oxygen at the fifth carbon of AA to produce 5-hydroperoxyeicosatetraenoic acid (5-HPETE), as well as the subsequent conversion of 5-HPETE to LTA₄. The 5-LO is dependent on adenosine triphosphate and Ca²⁺ for activation, following which it translocates from the cytosol to the cell membrane in association with a transmembrane protein termed 5-LO activating protein. This translocation step facilitates substrate presentation because most AA is found in the cell membrane. LTA₄ may undergo one of two enzymatic reactions depending on the cell type. In the first, glutathione S-transferase (LTC synthase) catalyzes the addition of glutathione to the sixth position of LTA₄ to produce the first of three cysteinyl LTs (CysLTs), LTC₄. LTC₄ is then exported to the extracellular space through a specific transmembrane transporter. In the extracellular space, subsequent peptide cleavage yields LTD₄ and then LTE₄. This represents metabolism from one active mediator to another and not a catabolic inactivation process. Collectively, these three CysLTs (historically known as the slow-reacting substance of anaphylaxis) contribute to the bronchoconstricting activity generated during anaphylaxis and they play a key role in asthma and allergic reactions. In the second reaction, LTA₄ hydrolase converts LTA₄ to the dihydroxy fatty acid LTB₄. Once formed, LTB₄ is actively exported from the cells where it acts as a potent chemoattractant and triggers

Table 1 Predominant cellular origins and physiological activities of PGs and LTs

Eicosanoid/receptor	Major cell origins	Physiological activities
PGE ₂	EP ₁ –EP ₄	Most cell types
		Potent vasodilator Stimulates bone and cartilage resorption Increases microvascular permeability Mediator of febrile responses Hyperalgesic
PGI ₂	IP	Endothelial cells
		Potent vasodilator Inhibits platelet aggregation
TXA ₂	TP _α , TP _β	Platelets Monocytes
		Potent vasoconstrictor and inducer of platelet aggregation
PGD ₂	DP ₁ , DP ₂	Mast cells
		Vasodilator Inhibits platelet aggregation
PGF _{2α}	FP	Monocytes Macrophages Uterine cells Epithelial cells
		Potent vasoconstrictor and bronchoconstrictor Myometrial and smooth muscle cell contraction
LTB ₄	BLT ₁ , BLT ₂	Neutrophils Monocytes Macrophages Eosinophils Mast cells
		Potent neutrophil chemotactic and chemokinetic agent Induces leukocyte adhesion Induces release of reactive oxygen species and hydrolytic enzymes by neutrophils
LTC ₄ LTD ₄ LTE ₄	CysLT ₁ , CysLT ₂	Eosinophils Mast cells Macrophages Smooth muscle cells
		Potent bronchoconstrictor Promotes vasoconstriction

**Figure 3** Metabolism of AA to PG, PGF_{2α}, PGI₂ (prostacyclin), PGE₂, TXA₂, and PGD₂ by the COX pathway. PGI₂ and TXA₂ have very short half-lives (30 s) and are converted to the stable but inactive 6-keto PGF_{1α} and TXB₂, respectively.

adherence and aggregation of leukocytes. In addition, LTB₄ modulates immune responses and host defense against infections. Release of LTB₄ may contribute to the pathology of many inflammatory disorders, including asthma, arthritis, and inflammatory bowel disease (Figure 4).

PG and LT Receptors

The biological actions of both PGs and LTs are mediated through G-protein-coupled cell surface receptors, which are

coupled to specific signal transduction pathways. Eight subtypes of PG receptors encoded by separate genes are characterized and include the PGE receptors (EP1, EP2, EP3, and EP4), the TX receptor (TP), the PGI receptor (IP), the PGF receptor (FP), and the PGD receptor (DP). The tissue distributions of these receptors is linked to specific functional roles and can be grouped into three categories based on their signal transduction and activities:

1. Relaxant receptors (EP2, EP4, IP, and DP): mediate an increase in cyclic adenosine monophosphate (cAMP) and smooth muscle relaxation.
2. Contractile receptors (EP1, TP, and FP): mediate an increase in intracellular calcium and smooth muscle cell contraction.
3. Inhibitory receptors (EP3): mediate inhibition of cAMP and smooth muscle cell relaxation.

Two receptors for LTB₄, termed BLT1 and BLT2, have been identified at the molecular level and these differ in their affinity and specificity for LTB₄. BLT1 is a high-affinity receptor specific for LTB₄, and BLT2 is a low-affinity receptor to which other eicosanoids can also bind. The major activities of LTB₄ appear to be mediated via BLT1. The precise role of BLT2 remains to be identified.

Two subtypes of the receptor for the CysLTs, termed CysLT₁ and CysLT₂, were postulated based on pharmacological studies. This classification has recently been confirmed by molecular identification. To date, several high-affinity CysLT₁ receptor antagonists have been developed, which have been shown to be clinically efficacious in chronic asthma. By contrast, no high-affinity selective CysLT₂ antagonists have yet been described.

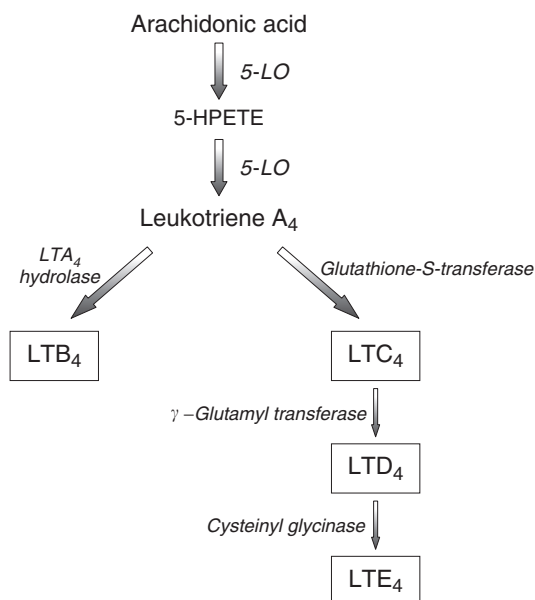


Figure 4 Metabolism of AA to LTB₄ and the cysteinyl LTs, LTC₄, LTD₄, and LTE₄ by the 5-lipoxygenase (5-LO) pathway.

The development of specific agonists and antagonists for each of the PG and LT receptors, in addition to receptor knockout animal models, have aided in the characterization and understanding of the roles of these mediators in both normal physiology and disease states.

Physiological Activities of PGs and LTs

Bone Metabolism

Bone remodeling, the continuous process of bone resorption by osteoclasts and bone formation by osteoblasts, is mediated by a number of factors, one of which is PGE₂. However, the role of PGs in bone metabolism is somewhat contradictory, in that PGE₂ can have both anabolic and catabolic effects. For example, PGs can stimulate the *in vitro* differentiation of precursors of both osteoclasts (responsible for bone resorption) and osteoblasts (bone growth). Both bone resorption and formation by PGE₂ is mediated by the EP4 receptor. It has been suggested that the opposing actions of PGE₂ may serve to maintain a coordinated regulation of bone resorption and formation, and may be dose related with stimulation of bone formation at low concentrations and inhibition at high concentrations.

Cancer

The involvement of PGs, in particular those arising from COX-2, in the causation and prevention of cancer has been identified recently. Considerable evidence has come from epidemiological studies indicating that chronic use of aspirin or other NSAIDs can significantly decrease the risk of developing certain cancers (e.g., colorectal cancer). Additionally, aspirin and NSAIDs can lower the mortality rates and induce

tumor regression in colorectal cancer and other forms of cancer. Angiogenesis, the development of new blood vessels, which is an essential step in tumor growth, is associated with the upregulation of COX-2 and presumably PG synthesis. COX-2 is upregulated in a variety of premalignant and malignant states and there is evidence that selective COX-2 inhibitors can inhibit the early development of malignant tumor growth, cause premalignant tumors to regress, and lead to the death of established cancer cells.

Cardiovascular System

The PGs and TX have a central role in the regulation of platelet aggregation and vascular tone, and as such are particularly important regulators of the cardiovascular system. TXA₂ is a potent vasoconstrictor and inducer of platelet aggregation, whereas PGI₂ dilates blood vessels and prevents platelet aggregation. Under normal conditions, a dynamic balance based on the opposing actions between TXA₂ and PGI₂ produced by platelets and vascular endothelial cells, respectively, maintains cardiovascular homeostasis and prevents thrombotic events (Figure 5). Altered metabolism of these mediators has been reported in association with atherosclerosis, in which there is a shift in the TXA₂/PGI₂ balance due to both increased TXA₂ and decreased PGI₂ synthesis. The consequence is a proatherogenic state with increased platelet adhesion and aggregation at sites of endothelial injury. This may lead to subsequent thrombus formation and vessel occlusion.

Platelets only express COX-1, and the cardioprotective effect of low-dose aspirin is attributed to its ability to irreversibly inhibit platelet COX-1 and hence TXA₂ synthesis. In the vasculature, it appears that endothelial cell COX-2, possibly upregulated by shear blood flow, is primarily responsible for the synthesis of PGI₂. Traditional NSAIDs inhibit both COX-1 and COX-2, thereby inhibiting platelet-derived TXA₂ and endothelium-derived PGI₂. The balance may therefore be maintained but in a less stable state. In contrast, the selective COX-2 inhibitors reduce PGI₂ synthesis but importantly have no effect on platelet COX-1 activity, potentially altering the balance of TXA₂/PGI₂ to a prothrombotic state that may explain the potential thrombotic side effects associated with use of these drugs. Low-dose aspirin inhibits platelet COX-1, which cannot be regenerated by these anuclear cells thereby shifting the balance between TXA₂ (principally platelet COX-1 derived) and PGI₂ toward a less coagulable state.

LTB₄ can also be implicated in cardiovascular events by virtue of its potent chemotactic effects and ability to induce leukocyte adhesion to vascular endothelial cells at the sites of injury.

GI System

Of the COX isotypes, only COX-1 is constitutively expressed throughout the GI system, where the main PGs produced are PGE₂ and PGI₂. Both have important cytoprotective effects on the GI mucosa, including reducing gastric acid secretion from stomach parietal cells, increasing mucosal blood flow, and stimulating the release of protective mucus. As stated previously, the upper GI toxicity commonly associated with



Figure 5 A dynamic balance between TXA_2 production by platelets and PGI_2 by vascular endothelial cells maintains cardiovascular homeostasis.

classical NSAIDs is thought to arise from the nonselective inhibition of COX-1 activity in the stomach. Clinical trials with highly selective COX-2 inhibitors have demonstrated clinically meaningful reductions in the incidence of serious upper GI events in comparison with conventional NSAIDs.

COX-2 is expressed in peptic ulcers and the inhibition of COX-2 has been associated with delayed ulcer healing.

Immune System

Within the immune system, PGE_2 regulates a wide range of functions, particularly in the cell populations central to the cell-mediated immune response, namely T cells and macrophages. In these immune-modulating cells, the actions of PGE_2 are generally immunosuppressive. For example, PGE_2 inhibits antigen-induced T-cell proliferation and activation, cytokine production, cytokine receptor expression and macrophage proliferation, and class II major histocompatibility complex expression. Additionally, PGE_2 can regulate the overall characteristic of an immune response by its ability to promote a Th2-type response, which is characterized by immunoglobulin (Ig) class switching to IgG1 and IgE and increased production of interleukins (IL), such as IL-4, IL-5, and IL-10. The ability of PGE_2 to inhibit many of the responses initiated by T-cell activation supports a central role for PGE_2 within the immune response. Moreover, PGE_2 has inhibitory and protective functions in autoimmune disease. Administration of PGE_2 or its analogs can ameliorate the manifestations of autoimmunity and reduce immune-mediated organ injury and can also delay or prevent allograft rejection.

Inflammation

Inflammation is a complex of sequential and partly recursive cellular and biochemical changes in tissues in response to injury or infection. It is a normal homeostatic process that protects the host against the effects of everyday and incidental trauma and invasive microorganisms. However, when this process becomes dysregulated, unwanted inflammation and tissue destruction arises. Acute inflammation is characterized by hyperemia, pain, edema, and leukocyte infiltration. PGs are involved in these processes, as further illustrated by the analgesic effects and reduction in inflammatory swelling of NSAIDs. PGE_2 has been regarded as the principal PG mediator of pain and edema, but both PGI_2 and PGD_2 can exert similar

effects. These PGs exert their hyperalgesic effects by increasing the sensitivity of pain receptors to peripheral inflammation. PGE_2 dilates vessels and along with LTB_4 , it leads to tissue swelling with both edema and leukocyte infiltration.

Although levels of PGs and LTs are generally low in uninfamed tissues, their synthesis is increased substantially during an inflammatory response. As immune cells infiltrate the tissues, further increases in levels of PGs and LTs are observed. Induction of PLA_2 and COX-2 by inflammatory stimuli accounts for the high levels of PGs found at sites of inflammation. Cellular infiltration mediated by LTB_4 is contributed by the chemotactic effects on leukocytes and altered adhesion molecule expression on endothelial cells.

Resolvins are a group of inflammatory agonists involved in resolution of the inflammatory response and are synthesized from *n*-3 PUFA. Two molecular species of resolvins, D and E, have been identified, acting on specific receptors such as BLT1 and GPR32. By antagonizing cytokine responses and gradually resolving the inflammatory response, these compounds help protect tissues from its damaging effects.

Fever

Fever, a common symptom of many diseases, is elicited by exogenous pyrogens (such as bacterial LPS) or an inflammatory insult, which results in the production of cytokines such as IL- 1β that act as endogenous pyrogens. These cytokines then stimulate the neural pathways that increase body temperature. PGE_2 is an important contributor to the febrile response and NSAIDs are used to treat pyresis. In COX-2-deficient mice, the febrile response to LPS is ameliorated, suggesting an important role for COX-2-derived PGs in fever production. Furthermore, mice deficient in the EP3 receptor fail to respond to either endogenous or exogenous pyrogens.

Respiratory System

The bronchoconstrictor activity of the CysLTs underlies their pathogenic role in asthma. Other relevant biological actions of CysLTs include: (1) increased microvascular permeability, which leads to airway edema and (2) mucus hypersecretion. Both the 5-LO inhibitors and CysLT receptor antagonists have been used to treat asthma.

PGs, in particular PGD_2 , are involved in many processes within the lung, including regulation of pulmonary vascular tone, maintenance of lung surfactant, regulation of capillary and alveolar permeability, and control of bronchial mucous secretion.

Renal System

Maintenance of normal kidney functions is dependent on PGE_2 , which regulates vascular tone, blood flow, sodium and water homeostasis, and renin secretion. PGE_2 can reduce sodium and water reabsorption and mediate the release of renin, which in turn can act to regulate blood pressure. Under conditions of increased sodium reabsorption, PGE_2 can act as a counter-regulatory factor. PGI_2 is involved in potassium secretion by stimulating the renin-angiotensin system. Both

isoforms are constitutively expressed in the kidney with quite selective and distinct localization. For example, COX-2 is highly expressed in the *macula densa*, which plays an important role in the coordinated regulation of glomerular filtration, proximal tubule function, and renin production, processes that are responsible for sodium and water homeostasis. In those with poor renal function, reversible renal failure has been associated with NSAIDs and highly selective CXO-2 inhibitors. This presumably reflects a crucial compensatory role for PGs in the compromised failing kidney.

Reproduction

PGs play important regulatory roles in the reproductive processes of ovulation, implantation, and parturition. Just before ovulation, there is an increase in PGE₂ synthesis by the pre-ovulatory follicle in response to an increase in luteinizing hormone (LH). Induction of COX-2 (by LH) is necessary for this increase in PGE₂ synthesis and for the successful rupture of the follicle. After fertilization, PGs (PGE₂ and PGI₂) play a role in the successful implantation of the embryo and, again, PGE₂ production appears to be COX-2 dependent. The importance of COX-2 in the reproductive process is further emphasized by studies in which COX-2-knockout mice (but not COX-1 knockout mice) have impaired fertility based on multiple reproductive failures, at the level of both ovulation and implantation, which can be restored by exogenous administration of PGE₂. Both PGE₂ and PGF_{2α} have potent uterotonic activities and are involved in uterine contraction during the initiation of labor and parturition. Since the 1960s, administration of PGF_{2α} and PG analogs has been used extensively to induce labor. In addition, at the time of parturition, there is an increase in the uterine expression of EP1, EP3, and FP receptors, which act to potentiate smooth muscle cell contraction. In the neonate, COX-2-derived PGE₂ is required for closure of the ductus arteriosus through an action by EP4 receptors on smooth muscle cells.

Regulation of PG and LT Synthesis by Dietary Fatty Acids

The diverse physiologic and pathologic functions mediated by eicosanoids highlight the importance of their fatty acid precursors in the diet. Unlike cellular proteins that are genetically predetermined, the PUFA composition of cell membranes is dynamic and is pivotally dependent on dietary intake. The typical Western diet is high in the *n*-6 family of PUFA (up to 25-fold more *n*-6 fats than *n*-3 fats are consumed). This predominance of *n*-6 fat is due to the abundance of the 'parent' 18-carbon PUFA linoleic acid (LA; 18:2, *n*-6) in the diet, which is present in high concentrations in corn, soy, safflower, and sunflower oils. Once ingested, LA can be converted to AA by a series of elongation and desaturation enzymes (Figure 6). Hence, AA is the predominant PUFA of membrane phospholipids and substrate for eicosanoid biosynthesis in the Western context.

The enzymes involved in the metabolism of the 20-carbon PUFAs to PGs and LTs can use either *n*-9 (eicosatrienoic acid (EtrA); 20:3), *n*-6 (AA; 20:4), or *n*-3 (eicosapentaenoic acid (EPA); 20:5) PUFAs as the substrate (Figure 7). When *n*-3

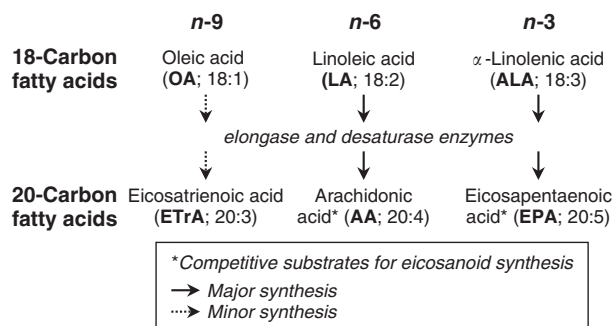


Figure 6 Dietary fatty acids and their metabolism after ingestion via the desaturase/elongase pathways.

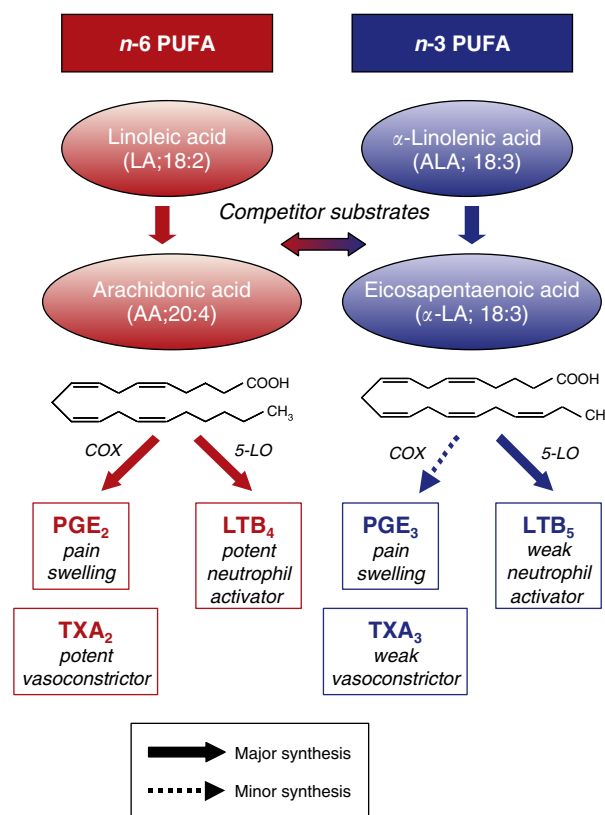


Figure 7 A comparison of the physiological activities between the *n*-6- and *n*-3-derived PGs and LTs.

PUFAs are included in the diet, EPA, the *n*-3 homolog of AA, competes with AA for incorporation into the cellular phospholipids. An increase in the concentration of EPA in cell membranes displaces AA, which will result in reduced substrate for the synthesis of the *n*-6 eicosanoids. EPA can also compete with AA as the substrate for either COX or 5-LO enzymes. This results in inhibition of the synthesis of *n*-6-derived PGs and LTs and the formation of the *n*-3 PGs and LTs. The *n*-3-derived PGs and LTs are similar in structure but can be considerably different in their biological activity. On balance, the *n*-3 PGs and LTs are less thrombotic and less inflammatory than the homologous *n*-6-derived mediators (Figure 6).

Although the *n*-9 fatty acid oleic acid (OA; 18:1, *n*-9) is consumed in substantial amounts in the diet, the elongase and desaturase enzymes that catalyze the conversion of OA to ETrA (20:3, *n*-9) preferentially metabolize the *n*-3 and *n*-6 20-carbon PUFAs, α -LA, and LA, respectively. Metabolism of OA to ETrA is only quantitatively significant in essential fatty acid deficiency, which is very rare due to the abundance of essential fatty acids available in the diet and the small amounts required to avoid deficiency. Furthermore, ETrA can be metabolized by 5-LO but not COX because it lacks the *n*-6 bond necessary for PG and TX formation.

Although Western diets are rich in *n*-6 and relatively poor in *n*-3 fats, there are populations in which *n*-6 fats are the less dominant source in diets and more *n*-3 fats are consumed in total and relative terms (e.g., Greenland Eskimo, Japanese, and Mediterranean diets). In the extreme case of the Eskimos eating their aboriginal diet, which is based almost entirely on marine foods, there is a striking reduction in thrombotic vascular events and inflammatory diseases. The cardiovascular benefit has also been associated with traditional Japanese and Mediterranean diets. These benefits may be, in part, ascribed to a more favorable balance of *n*-6- and *n*-3-derived eicosanoids, although a myocardial membrane stabilizing effect of *n*-3 fats, independent of PG and LT synthesis, is also important.

See also: Cytokines: Nutritional Aspects. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids; Health Effects of Saturated Fatty Acids. Pregnancy: Nutrient Requirements

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PROTEIN DEFICIENCY

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Glossary

Deamination Removal of an amino group from a molecule.

Enteropathy A disease of the intestinal tract.

Gluconeogenesis A process by which glucose is made, primarily in the liver, from noncarbohydrate sources.

Immunostimulation Stimulation of an immune response, e.g., by use of BCG vaccine.

Transamination Transfer of an amino group from one chemical compound to another.

Introduction

Protein is one of the three ‘macronutrients’ (protein, carbohydrates, and fats) that our bodies need in balanced amounts. Almost every function of our body is dependent on proteins. The building blocks of proteins are 20 amino acids, only 11 of which can be synthesized in the human body from other amino acids. The other nine are considered essential amino acids and have to be found in our food. Without adequate amounts of each of these essential building blocks, the human body experiences malnutrition and begins to shut down – or, in extreme cases of starvation, to consume itself for the protein needed for necessary enzyme and hormone functions (e.g., in kwashiorkor and marasmus, two diseases caused by protein starvation). The term protein deficiency can be defined as a state of relative or absolute deficiency of body proteins or one or more of the essential amino acids. Thus, the term protein deficiency can also be considered synonymous with negative nitrogen balance. The deficiency can result from a protein-deficient diet or other diseases and, in general, can also result from a global deficit of food. It may also occur despite adequate protein intake if the protein is of poor quality (i.e., the content of one or more amino acids is inadequate and thus becomes the limiting factor in protein utilization). Although protein-energy malnutrition is the more common form of protein deficiency, in general the features are comparable to those seen with kwashiorkor. Dietary protein contributes all of the amino acids and fixed nitrogen necessary for the biosynthesis of tissue proteins and nonprotein nitrogenous compounds such as purines and pyrimidines.

Dietary amino acids are required for the synthesis of new tissue constituents at all ages, particularly during growth. Amino acids consumed in excess of these needs are not stored but are degraded, the nitrogen being excreted and the carbon skeleton recycled. Each day more amino acids are degraded and resynthesized in the body than are ordinarily consumed in the diet.

All of the 20 fundamental amino acids must be present for protein synthesis to occur. The remarkable range of functions mediated by proteins is a function of the diversity and versatility of these 20 distinct building blocks of proteins. Nine of these amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine) are not synthesized in the human body and are therefore ‘essential’ for well-being. In addition, arginine is essential in infancy, and in the preterm infant there is a transient need for dietary tyrosine and cysteine as well. The nutritional quality of dietary protein is influenced by its essential amino acid content. This implies that the protein intake contains sufficient ‘nonessential’ amino acids to minimize metabolic diversion of essential amino acids to cover nonspecific nitrogen requirements.

An adequate protein intake contains all of the essential amino acids in sufficient quantities to satisfy maintenance needs and to provide a surplus sufficient for the processes of normal growth and development. Serum concentrations of albumin and total protein serve as clinical indicators of the sufficiency of dietary protein intake in the absence of systemic disease. Dietary proteins can be divided into two types of proteins, i.e., complete proteins and incomplete proteins. Complete proteins consist of all the essential amino acids. Animal products (milk, meat, fish, and eggs) are a good source of complete proteins. Some plant proteins such as soybeans are also complete proteins. Incomplete proteins may have lower (limiting) quantities of one or more essential amino acids. Most plant food proteins, particularly cereal proteins, are incomplete proteins.

Although the importance of hormonal regulation of protein metabolism is well recognized, there is increasing evidence that dietary protein may play a regulatory role by modulating the hormonal milieu leading to tissue accretion. Studies in recovering malnourished children and in normal children have shown a significant increase in circulating insulin-like growth factor I associated with higher protein

intakes. Protein amino acid composition, however, appears to be of less relevance for these effects, which were observed with both animal and vegetable protein sources. Thus, it appears that dietary protein quantity is the major factor for this observed response. The amino acid composition of dietary proteins has a direct effect on growth by determining the supply of amino acids at the cellular level. Protein synthesis requires the presence of each component amino acid at the time of chain elongation. Thus, a dietary protein intake deficient in one or more essential amino acids will not be able to sustain protein synthesis. Many vegetable proteins have one or more limiting amino acids, e.g., with levels below, or close to the minimum requirement pattern, and certainly lower than those in high-quality reference proteins such as egg or milk.

It is important to maintain a balanced intake of amino acids in the diet, and to understand the relationships between different groups of amino acids and other nutrients such as vitamins. For example, when the most limiting amino acid in a diet generally poor in protein is increased, a deficiency of the next most limiting amino acid may be precipitated. Excessive intakes of certain amino acids, which may or may not be limiting, when added to a diet that is marginal in certain of the B vitamins, may result in an increased severity of the vitamin deficiency. In other cases, an excess of an amino acid may reduce the utilization of another amino acid that is provided in normally adequate amounts to such an extent that a deficiency occurs.

Protein Turnover and Regulation

The term protein turnover reflects the balance of protein degradation and resynthesis. More synthesis than breakdown indicates an anabolic state that builds lean tissues, more breakdown than synthesis indicates a catabolic state that burns lean tissues. From a quantitative standpoint, by far the greatest influence on amino acid turnover and metabolism is this turnover cycle in which proteins are continuously degraded and resynthesized. Coregulation of the anabolic and catabolic arms of the cycle is crucial to maintaining cellular viability, to regulation of growth and cellular protein mass, and to control of enzyme levels. At least 20% of basal energy expenditure is used in maintaining whole-body protein synthesis. Body protein mass and rates of protein gain or loss in a cell are entirely dependent on the balance of these mechanistically distinct processes, i.e., the relative rates, of protein synthesis and degradation. Although both processes are influenced by protein and energy nutritional status and by the same hormones (e.g., insulin, growth factors, growth hormone, and glucocorticoids), direction and magnitude of a response of either process are not easily predicted.

Nutritional status, especially amino acid intake, and the response of protein turnover to endocrinological changes interact in a complex way. As a result of these complexities it has proved difficult to identify a common response even when the same outcome variable (e.g., increased protein deposition) is achieved. For example, stimulation of proliferative growth involves a simultaneous increase in protein synthesis and decrease in protein degradation, whereas hypertrophic growth (e.g., of a muscle in response to increased workload) involves

simultaneous increases in both protein synthesis and degradation. Similarly, increases in whole-body protein retention brought about by either increased intake of energy, limiting amino acid, or insulin infusion appear to involve primary changes in whole-body protein degradation. However, separate evidence implies that changes associated with total protein intake or following growth hormone administration involve primarily protein synthesis. Furthermore, the magnitude of changes in whole-body protein turnover, even in response to a common nutritional manipulation, can depend on the prior nutritional status of the individual.

Developmental factors influence regulation of protein turnover as it relates to protein deposition. Protein synthesis appears to be of particular importance to nutritional regulation of growth of immature tissues during childhood, but the response of protein synthesis to protein intake becomes progressively smaller as subjects approach adulthood. In adults, protein degradation seems to be the critical factor regulating protein balance in the short term.

General Nutritional Factors Regulating Amino Acid Catabolism

All tissues have some capability of synthesizing the non-essential amino acids and other derivatives that contain nitrogen. However, liver is the major site of nitrogen metabolism in the body. In times when there is surplus of dietary proteins, the potentially toxic nitrogen of amino acids are eliminated via transaminations, deamination, and urea formation; the carbon skeletons are generally conserved as carbohydrate, via gluconeogenesis, or as fatty acid via fatty acid synthesis pathways. Essential amino acid catabolism is primarily influenced by the following nutritional factors:

1. The degree to which total nitrogen intake approximates total nitrogen needs of the individual. This factor affects amino acid catabolism in general and is reflected in adaptations in urea synthesis.
2. The degree to which the pattern of amino acids in dietary protein matches the amino acid needed by the body. This is reflected directly in the efficiency with which a given dietary protein is utilized in productive processes (e.g., growth, lactation) and is the principal factor underlying differences in biological value of dietary proteins. This factor determines the regulation of the catabolism of individual indispensable amino acids independently of the total. This is the premise underlying recent nutrition interventions with specific high-quality protein intake in infected malnourished children, as a means of preventing amino acid diversion to acute phase protein synthesis.
3. The balance between essential and nonessential amino acids (see [Table 1](#) for list of essential and nonessential amino acids). In adults, dietary indispensable amino acids represent approximately 27% of the total minimum amino acid needs for maintenance; in children, the need for protein deposition is also required for growth, although the proportion of protein (amino acids) required for growth is a significant proportion only at very early ages, and falls to approximately 10% in middle school children. The rest of the minimum amino acid requirement consists

Table 1 Essential and nonessential amino acids

<i>Nonessential amino acids</i>	<i>Essential amino acids</i>
Alanine	Arginine ^a
Asparagine	Histidine
Aspartate	Isoleucine
Cysteine	Leucine
Glutamate	Lysine
Glutamine	Methionine ^a
Glycine	Phenylalanine ^a
Proline	Threonine
Serine	Tryptophan
Tyrosine	Valine

^aThe amino acids arginine, methionine, and phenylalanine are considered essential for reasons not only related to lack of synthesis. Arginine is synthesized by mammalian cells but at a rate that is insufficient to meet the growth needs of the body and the majority that is synthesized is cleaved to form urea. Methionine is required in large amounts to produce cysteine if the latter amino acid is not adequately supplied in the diet. Similarly, phenylalanine is needed in large amounts to form tyrosine if the latter is not adequately supplied in the diet.

of dispensable amino acids. Although nonessential amino acids do not have to be supplied in the diet, there is still a metabolic need for these nutrients, and if the diet fails to provide them, these amino acids must be synthesized by the body. An imbalance between dietary essential and nonessential amino acids intake leads to catabolism of essential amino acids to supply nitrogen for nonessential amino acid synthesis.

4. The degree to which energy intake matches energy needs. Amino acid catabolism is also part of the body's energy supply in order to maintain ATP synthesis. Variations in nonprotein energy intake can have rapid and marked effects on overall amino acid catabolism.

Relationship Between Protein Intake and Protein Need

Amino acid catabolism changes rapidly after protein intake. Even in the fed state, amino acid catabolism changes within hours in response to a change in overall level of dietary protein. An important factor in the immediate response to protein intake is the concentration of amino acids. The quantitative relationship between circulating amino acid concentrations and their rate of catabolism is not uniform, either between individuals or between diets. A persistently high or low intake of protein leads to an overall increase or decrease in rate of amino acid catabolism that is partially independent of circulating amino acid concentrations.

Both short- and long-term changes in protein intake alter the levels of insulin, glucagon, and glucocorticoids, all of which are capable of altering the function of amino acid catabolic enzymes. Glucagon, for example, both activates and induces a wide range of amino acid catabolic enzymes. The positive relationship between glucocorticoid level and hepatic amino acid catabolism has been known for many years. There is now additional evidence for direct regulation of catabolic enzyme synthesis by amino acids that is independent of hormonal effects. For example, the initial enzyme of the urea cycle, carbamoyl-phosphate synthase, is immediately responsive to changes in ammonia production via activity of glutaminase, which is in turn activated by ammonia.

Adaptation to Low Protein Intakes

Nitrogen Balance

Nitrogen equilibrium is a state in which, for given intake of nitrogen, an equivalent amount of nitrogen is lost from the body via urine, feces, skin, sweat, etc. In general, when protein intake is low, dietary protein is used more efficiently, urea nitrogen excretion is reduced, and amino acid synthesis pathways are stimulated. The liver plays an important role in this adaptive process because it is the only organ that can transform the nitrogen from amino acids into urea. The metabolic activity of the gastrointestinal tract is important in this adaptive process. Normally, one-third of the urea produced is passed into the bowel and can be hydrolyzed by the microflora. As a process of adaptation to reduced protein intake, the body retains a greater proportion of urea. Similarly, the intravascular circulating albumin mass is maintained by reduced breakdown and a shift of albumin from the extravascular to the intravascular compartment.

Factors Affecting Adaptation

Among the factors that can affect the adaptation to low protein intake are infections, diarrheal disease, and injuries. In infections, protein from muscle and skin is needed for the immune response and synthesis of acute phase proteins. This immunostimulation can lead to an overall negative nitrogen balance. **Table 2** indicates the relative contribution of various amino acids to the production of acute phase proteins.

In other disorders such as injuries or burns, there may be more severe direct losses of nitrogen and altered adaptation. Energy balance is critical for nitrogen balance because of its nitrogen-sparing effect. Thus, if protein deficiency is accompanied by energy deficiency, the adaptation to a low protein intake cannot be achieved completely.

The process of adaptation is clearly dependent on prior nutritional status and overall protein deficits or reserves. It is estimated that the body of a human adult (65 kg) contains 12 kg of protein, approximately 50% of which is found in muscles. The well-fed human adult can lose approximately 3 kg of protein without disturbances to his or her health. The amount of body protein depends on, among other things, the dietary protein and carbohydrate intake; if carbohydrate is lacking, the amino acids are utilized for gluconeogenesis.

Protein reserves are not comparable to special fat depots, and not all body proteins can serve as protein reserve. Reserves are primarily organs that contain labile body protein such as liver, plasma (with protein such as albumin and enzymes), and the gastrointestinal tract. Although the protein turnover rate in muscle is very slow, this tissue is a very important protein reserve owing to its large mass. In general, however, during protein deficiency the labile body proteins are metabolized first, sparing the reserves. However, when deficiency is long term, all organs are affected to various extents. **Table 3** indicates the rates of loss of protein from various organs and tissues in rats on a protein-deficient diet. **Table 4** shows some different amino acid responses to a range of stresses.

Table 2 Estimated amino acid requirements for synthesis of some acute phase proteins (grams of amino acid per kilogram of protein)

Amino acid	C-reactive protein	Fibrinogen	α -Acid glycoprotein	α -Antitrypsin	Serum amyloid A	Haptoglobulin
Valine	77	48	46	59	18	84
Leucine	91	62	101	124	29	82
Isoleucine	54	32	48	49	29	47
Threonine	58	60	74	66	30	54
Tryptophan	42	35	30	11	45	32
Phenylalanine	105	46	64	83	103	30
Serine	84	91	31	49	47	40
Arginine	36	84	52	23	116	28
Alanine	31	29	36	43	106	54
Lysine	71	77	75	92	33	92
Histidine	16	27	17	37	35	38
Cysteine	13	15	18	6	0	24
Tyrosine	50	56	74	27	67	70
Methionine	16	32	11	28	16	22
Proline	44	48	34	41	34	44
Glycine	46	59	19	33	61	44

Source: Data from Reeds PJ, Fjeld CR, and Jahoor F (1994) Do the differences between the amino acid composition of acute-phase and muscle proteins have a bearing on nitrogen loss in traumatic states? *Journal of Nutrition* 124: 1754S–1764S, with permission from ASN.

Table 3 Relative losses of protein in different organs and tissues from rats over 7 days

Organs or tissues	Loss (percentage of primary content)
Liver	40
Prostate gland	29
Seminal vesicle	29
Gastrointestinal tract	28
Kidney	20
Blood plasma	20
Heart	18
Muscle, skin, skeleton	8
Brain	5
Eyes	0
Testicle	0
Adrenal gland	0

Source: Reproduced from Kraut H (ed.) (1981) *Der Nahrungshedarf des Menschen. I- Stoffwechsel Ernährung und Nahrungshedarf. Energiebedarf, Proteinbedarf*, pp. 140–153. Darmstadt: Steinkopff Verlag, with permission from Springer.

Causes of Protein and Amino Acid Deficiency

Causes

Although the main cause of protein deficiency is a protein-deficient diet, the disorder can commonly occur in a variety of pathologic states. In particular, the disorder can be seen in the general context of starvation (although the deficits may be both protein and energy) or in disorders where there are specific protein losses from the body as in nephrotic syndrome or after burns.

Secondary protein deficiencies can be ascribed to six causes:

1. Irregular food habits and starvation states; this may be seen in both developed and developing countries in a variety of pathological states.

Table 4 Plasma amino acid response to different disease states and different intakes

	Starvation	Protein-free diet	Infection	Malnutrition
Valine	↑	↓	↓	↓
Leucine	↑	↓	↓	↓
Isoleucine	↑	↓	↓	↓
Phenylalanine			↑	↓
Alanine	↓	↑		
Glycine	↑	↑	↓	↓

2. Inability to digest and absorb the protein that is consumed; this occurs in patients with chronic gastrointestinal disorders such as celiac disease, persistent diarrhea, or protein-losing enteropathy.
3. A disturbed protein metabolism, which may exist in patients with cirrhosis of the liver, but also in patients with hormonal disorders or in some cases of diabetes.
4. A continuous loss of protein; this predominates in patients with diseases such as chronic renal disease, bleeding, or exudative gastroenteropathy. High losses of albumin into the urine are indicators of the nephrotic syndrome.
5. Increased protein turnover, which is characteristic in cases of systemic infection or fever. In many instances this may be subclinical and associated with protein diversion due to immunostimulation.
6. Enhanced catabolism of protein, with increased nitrogen losses, seen in patients with severe injuries, especially burns, or in postoperative stress.

Principles of Treatment of Protein Deficiency

The dietary treatment of protein deficiency depends on the cause of the deficiency and must depend on a sound

understanding of the underlying disorder. In most instances, isolated protein deficiency due to deficient intake is rare and most deficits include both macronutrients and micronutrients. In this situation, isolated repletion of protein or amino acids alone is inadequate and may even cause harm. This is well illustrated by the great 'protein fiasco' of the past when attempts to provide high protein supplements to malnourished children were found to be both inadequate and deleterious. Similarly, high protein supplements in pregnancy have been shown to actually increase rates of adverse pregnancy outcomes. Thus, the mainstay of treatment in such states of global deficiency includes balanced energy-protein and micronutrient supplementation.

In other instances, protein supplementation is critical. For example, in children with nephrotic syndrome, the daily intake of protein should be increased to 3–4 g kg⁻¹ day⁻¹ so that hepatic synthesis of albumin can compensate in part for the urinary losses. In other acute circumstances, infusion of albumin can be used to acutely correct deficits and circulatory abnormalities. However, in states of metabolic adaptation, care should be used in increasing protein intakes. For example, in cases of cirrhosis, the protein intake should be restricted to 20 g day⁻¹ to reduce the risk of precipitating hepatic encephalopathy.

See also: Amino Acids: Chemistry and Classification; Metabolism; Specific Functions. Protein Digestion and Bioavailability.
Protein: Quality and Sources; Requirements and Role in Diet; Synthesis and Turnover

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PROTEIN DIGESTION AND BIOAVAILABILITY

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Glossary

Dipeptide A dipeptide is a molecule consisting of two amino acids joined by a single peptide bond.

Hydrolysis Decomposition of a chemical compound by reaction with water, such as the dissociation of a dissolved salt or the catalytic conversion of starch to glucose.

Oligopeptide An oligopeptide (oligo=few) consists of between two and 20 amino acids (includes dipeptides, tripeptides, tetrapeptides, pentapeptides, etc.).

Peptidase An enzyme that catalyzes the hydrolysis of peptides into amino acids.

Polypeptide A peptide, such as a small protein, containing many molecules of amino acids, typically between 10 and 100.

Proenzyme A compound that is an inactive precursor of enzymes and requires some change (such as the hydrolysis of a fragment that masks an active enzyme) to become active.

Proteolysis The hydrolysis of proteins into peptides and amino acids by cleavage of their peptide bonds.

Tripeptide A tripeptide is a molecule consisting of three amino acids joined by peptide bonds.

Introduction

Proteins are the principal nitrogenous constituents of the protoplasm of all animal and plant tissue, and it is estimated that almost half of the dry weight of animal cells is composed of proteins. Proteins are crucial for the synthesis of body tissues and regulatory proteins, and it is also recognized that approximately 90% of all cellular proteins are present as enzymes.

The basic structural units of proteins are the amino acids, which are characterized by the presence of an amino NH_3 component and an acid or carboxyl group. Nitrogen thus comprises approximately 16% of all proteins by weight. Most naturally occurring amino acids are of the L configuration. These amino acids are in turn linked together by peptide bonds. Units of two or three amino acids are called dipeptides or tripeptides, respectively, whereas by convention any protein structure of less than 100 amino acid residues is called a polypeptide. The primary structure of a protein refers to the chains of amino acids constituting it, whereas the secondary structure is formed by the linkages between close amino acids by hydroxyl or sulfide bonds. More complex proteins have a tertiary structure due to the amino acids being held together by strong interatomic forces. The quaternary structure of a protein refers to the manner of association or binding between different units.

Dietary proteins are the major sources of protein intake and constitute on average approximately 10–20% of daily energy intake. In addition, they are the main sources for the essential amino acids, which cannot be synthesized by humans. Despite wide variations in dietary composition, the average daily protein intakes in different populations of the world range from 50 to 70 g day⁻¹, although it must be recognized that the intake

may be much lower in deprived populations, in both qualitative and quantitative terms. Almost half of the total protein entering the gastrointestinal tract daily is derived from endogenous sources, mainly intestinal secretions and cellular desquamation. Salivary, gastric, biliary, pancreatic, and intestinal secretions contribute approximately 20–30 g day⁻¹, whereas desquamated villus epithelial cells contribute an additional 30 g, and a relatively smaller amount (2 g) is derived from plasma proteins leaking into the lumen.

An intricate and coordinated system of digestion ensures that under normal conditions, approximately 95% of ingested protein is digested and absorbed.

Digestion

The purpose of digestion is to hydrolyze proteins to small peptides and amino acids so that these can be absorbed. The daily protein load requiring digestion within the gastrointestinal tract includes both exogenous protein derived from the food consumed and that from endogenous intestinal enzymes and cellular debris. The latter may constitute approximately 40% of the total gastrointestinal protein load, approximately 160–170 g daily. The digestion of proteins in the gastrointestinal tract involves a coordinated series of events at different levels, with sequential digestion by proteolytic enzymes to a form that can be absorbed into the bloodstream. **Figure 1** is a sequential representation of the various sites of protein digestion and absorption in the gastrointestinal tract. The main gastric and pancreatic proteolytic enzymes and their physiological functions are summarized in **Table 1**.

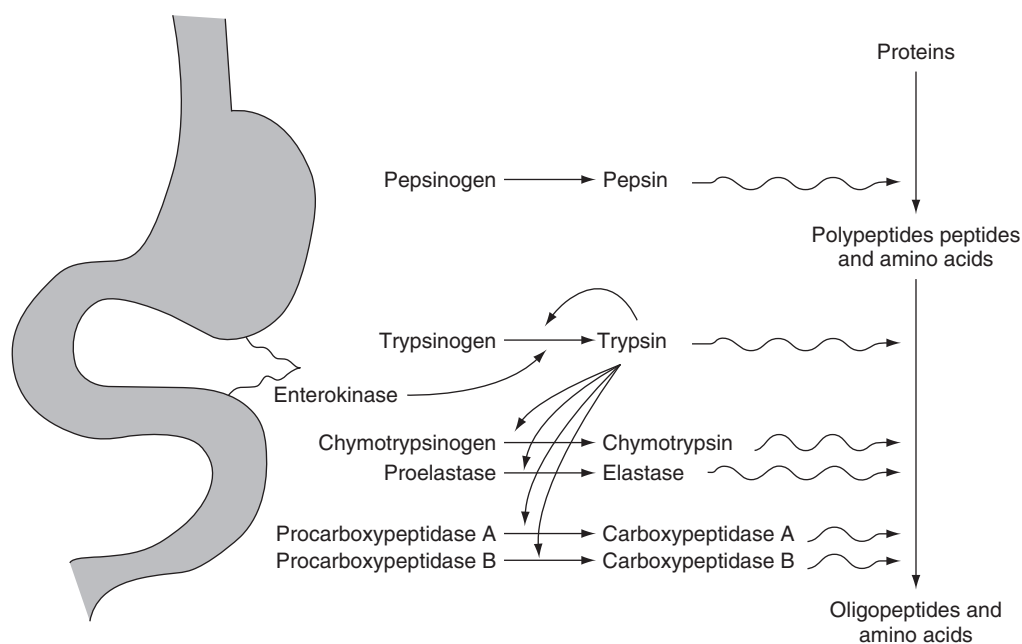


Figure 1 Cascade of protein hydrolysis in the gastrointestinal tract.

Table 1 Proteolytic enzyme activity in the gastrointestinal tract

Enzyme	Precursor	Products	Catalyst	Substrate	Action
<i>Stomach</i> Pepsins	Pepsinogens	Polypeptides of diverse sizes and some amino acids	Acid pH	Protein	Hydrolyze bonds between aromatic amino acids (e.g., phenylalanine or amino acid)
<i>Pancreatic proteases</i> Trypsin	Trypsinogen	Oligopeptides	Enterokinase	Proteins	Cleaves internal bonds at lysine or arginine amino acids; cleaves other pancreatic proenzymes
Chymotrypsin	Chymotrypsinogen	Oligopeptides	Trypsin Trypsin	Polypeptides Protein	Cleaves bonds of aromatic or neutral amino acids
Elastase	Proelastase	Oligopeptides	Trypsin	Polypeptides Elastin	Cleaves bonds of aliphatic amino acids (e.g., alanine, glycine, and serine)
Carboxypeptidase A	Procarboxypeptidase A	Aromatic amino acids and peptides	Trypsin	Other proteins Polypeptides at the free C-terminal end of the chain	Cleaves aromatic amino acids from C-terminal end of protein and peptides
Carboxypeptidase B	Procarboxypeptidase B	Arginine, lysine, and peptides	Trypsin	Polypeptides at the free C-terminal end of the chain	Cleaves arginine or lysine from C-terminal end of protein and peptides

Stomach Peptic Activity

Digestion of proteins begins in the stomach by the actions of pepsins, which are secreted as the precursor from pepsinogen by the gastric mucosa main cells. The release of pepsinogen is effected by gastrin, histamine, and cholinergic stimulation and

sinogens are converted to the active form pepsin by the loss of a small basic peptide. Pepsins are most active at a pH of approximately 2 and totally inactive at a pH above approximately 5. So, for pepsin to affect any digestive action on protein, the stomach juices must be acidic. Pepsins have a broad proteolytic specificity, splitting peptide bonds mostly

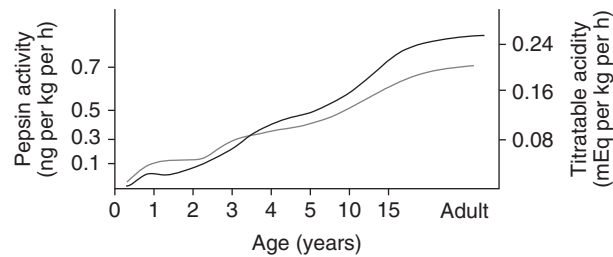


Figure 2 Postnatal development of gastric acid secretion and titratable acidity. Modified from Koldovsky (1987) Digestion and absorption of carbohydrates, proteins and fat in infants and children. In: Walker WA and Watkins JB (eds.) *Nutrition in Pediatrics*, Boston: Little Brown, with permission from Little Brown and Company.

involving phenylalanine, tyrosine, and leucine. The level of peptic activity and acid production is lower in premature infants and increases in relation to gestational age; pepsin activity increases approximately twofold between infancy and adulthood (Figure 2). Immunohistochemistry indicates two distinct forms of pepsinogen: Pepsinogen I is only found in acid-secreting regions of the stomach, whereas pepsinogen II is also found in the mucous cells of the oxyntic and pyloric regions of the stomach as well as in the duodenal Brunner's glands. Although these two forms of pepsinogen have slightly different pH optima, their substrate specificity is very similar and both are rapidly inactivated by the alkaline pH beyond the pylorus.

A gelatinase liquefying gelatin is also found in the stomach. There is controversy regarding the presence of rennin (a peptidyl peptide hydrolase) in the stomach of young infants; however, the mild clotting activity in human infants is fairly rapid.

The completeness of gastric protein digestion is dependent on several factors, including the rate of gastric emptying, the pH of intragastric contents, and the type of protein ingested. Given the significant buffering capacity of food, it is unlikely that gastric proteolysis plays a major role in protein digestion. This is also verified by the fact that neither patients with achlorhydria nor those recovering from major gastric surgery appear to have a major problem with protein digestion.

Pancreatic Proteases

The pancreatic proteases are secreted as proenzymes and are activated in the lumen. The enteropeptidase (also called enterokinase) released from the brush border membrane removes a hexapeptide from the N-terminal end of trypsinogen, converting it to the active form trypsin. Trypsin, in turn, activates the other protease proenzymes and also autocatalytically promotes further activation of trypsinogen. The pancreatic proteases include the endopeptidases trypsin, chymotrypsin, and elastase, primarily splitting peptide bonds located within the protein molecules resulting in the production of short-chain polypeptides. These are further hydrolyzed by the exopeptidases carboxypeptidase A and B, acting on aromatic/aliphatic C terminals or basic C terminals, respectively, to remove single amino acids. The end product of this coordinated intraluminal digestion by these endopeptidases and exopeptidases is a mixture of neutral and basic amino acids (30%) with

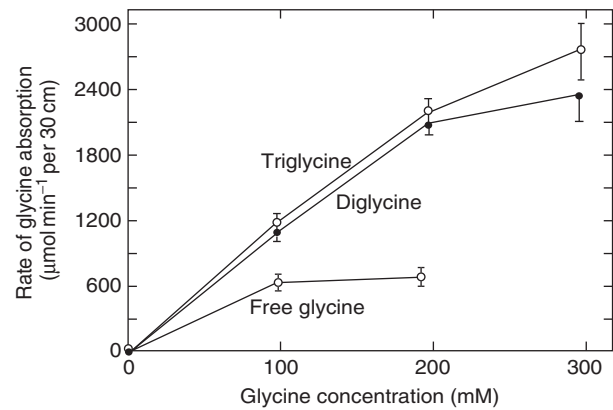


Figure 3 Rates of glycine absorption (mean \pm SEM) from perfusion solutions containing equivalent amounts of glycine in free or peptide form. Reproduced from Adibi SA, Morse EL, Masilamani SS, and Amiu P (1975) Evidence from two different modes of tripeptide appearance in human intestine: Uptake by peptide carrier systems and hydrolysis by peptide hydrolases. *Journal of Clinical Investigation* 56: 1355–1363, with permission from American Society for Clinical Investigation.

peptide chains varying in length from two to six amino acids (70%). The presence of excess amino acids in the lumen can further limit peptide hydrolysis (product inhibition).

The activity of enterokinase is noticeable after 26 weeks of gestation and its activity at term is approximately 10% of that of adults. Although pancreatic trypsin levels are substantial in both preterm and term infants, the secretory response to secretin and pancreozymin stimulation is somewhat blunted at birth compared with that at 2 years of age. However, such comparatively lower levels of protease activity in newborn infants do not appear to limit protein digestion significantly.

Brush Border Membrane and Cytoplasmic Peptidases

An important step in the final hydrolysis of peptides is their proteolysis to amino acids, either at the level of the intestinal brush border or within the cytoplasm of the intestinal mucosa. An important physiological observation is that protein absorption can occur both as amino acids and as peptides; indeed, absorption as peptides is considered a more efficient way of amino acid absorption compared with that of single amino acids (Figure 3). Even when a di- or tripeptide is subject to rapid hydrolysis by brush border peptidases, 30–50% of it is directly absorbed unconverted. The recognition that peptides are the main physiological routes of entry of amino acids into the enterocytes is a point of fundamental importance in the formulation of special protein hydrolysates and enteral feeds.

A range of peptidases are present at the level of the brush border membrane or cytoplasm with the capability of hydrolyzing oligopeptides of up to eight amino acid residues (Table 2). These oligopeptidases are synthesized in the rough endoplasmic reticulum of enterocytes and, after transfer through the Golgi apparatus, are transported to the brush border and extruded by exocytosis. There is little posttranslational processing of these peptidases, and they are attached to the brush border membrane by short anchoring pieces.

Table 2 Peptidases present at the brush border membrane and cytoplasm of villous epithelial cells

Peptidase	Action	Products
<i>Brush border membrane peptidase</i>		
Aminooligopeptidases (at least two types)	Cleave amino acids from C-terminal of 3–8 amino acid peptides	Amino acids dipeptides
Aminopeptidase A	Cleaves dipeptides with acidic amino acids at N-terminal	Amino acids
Aminopeptidase I	Cleaves dipeptides containing methionine	Amino acids
Aminopeptidase III	Cleaves glycine-containing dipeptides	Amino acids
Dipeptidyl aminopeptidase IV	Cleaves proline-containing peptides with free C-terminal	Peptides and amino acids
Carboxypeptidase P	Cleaves proline-containing peptides with free C-terminal	Peptides and amino acids
Angiotensin I converting enzyme (ACE) γ -glutamyl transpeptidase	Cleaves γ -glutamyl bonds and transfers	γ -Glut amino and/or peptide
Endopeptidases (two, including PABA peptidase)		
Folate conjugase	Cleaves pteroyl polyglutamates	Monoglutamate
<i>Cytoplasmic peptidases</i>		
Endopeptidases (several, including Gly–Leu dipeptidase)	Cleaves most dipeptides	Amino acids
Aminotripeptidase	Cleaves tripeptides	Amino acids
Proline depeptidase	Cleaves X-Pro bonds in proline-containing depeptides	Proline and amino acids

PABA, *para*-aminobenzoic acid.

The brush border peptidases differ in several ways from the cytoplasmic peptidases; the bulk of the hydrolysis of tetrapeptides and longer peptides occurs at the brush border, whereas the converse is true for dipeptidase activity, which is primarily within the cytoplasm. Most oligopeptidases are aminopeptidases, acting at the N-terminal amino acid. The brush border proteolysis rate is most rapid for tripeptides and least rapid for dipeptides, whereas the rates of hydrolysis of tetrapeptides and pentapeptides are somewhat intermediate. The brush border peptidases are capable of hydrolyzing all peptide bonds except those with proline at the C-terminal.

In general, the cytoplasmic peptidases are more heat labile than brush border peptidases. Of the cytoplasmic peptidases, the most abundant is a dipeptidase that cleaves neutral dipeptides, whereas the aminotripeptidase has a high specificity toward tripeptides with N-terminal amino acids or those containing proline terminally.

Very little is known about the developmental aspects of brush border and cytoplasmic proteases. However, the activity of many of these proteases is discernible by 10–16 weeks of gestation and progressively increases during development. In contrast, γ -glutamyl transpeptidase activity decreases with increasing gestational age, but the significance of this transition is unknown.

Colonic Digestion

Although colonic digestion and fermentation is an important mechanism for energy production in plant-eating animals, its role in human nutrition is of minor importance. Colonic fermentation may lead to the production of short-chain fatty acids from undigested starch, nonstarch polysaccharides, or proteins reaching the colon, providing approximately 5–10% of daily energy requirements from this source. The contributory role of colonic protein digestion may become important for people with reduced small intestinal function such as short bowel syndrome.

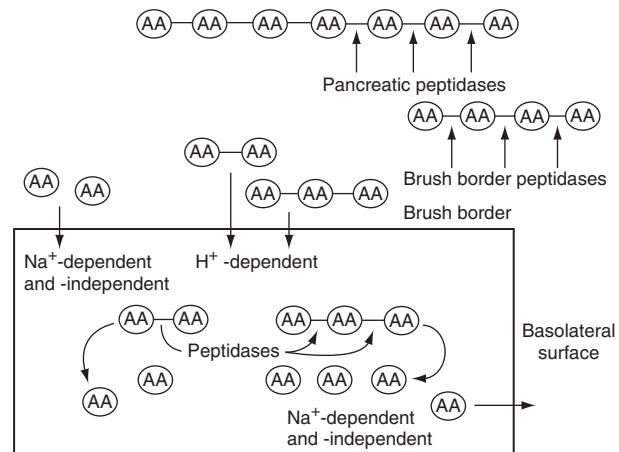


Figure 4 Small intestinal protein digestion and absorption. Adapted from Shulman RJ (1996) Intraluminal digestion and absorption in the small intestine. In: Gluckman PD and Hayman MA (eds.) *Pediatrics and Perinatology: The Scientific Basis*, 2nd edn., pp. 634–637. London: Arnold.

Absorption

As already indicated, although the final end product of protein digestion is amino acids, small peptides are the dominant form of entry of amino acids into enterocytes, where they are further hydrolyzed into amino acids and absorbed into the bloodstream (Figure 4). Thus, the vast majority of products of protein digestion that reach the bloodstream are single amino acids. Amino acid transport systems develop *in utero* by the end of the first trimester, whereas peptide transport systems can be demonstrated by the beginning of the second trimester.

It is recognized that the intestinal permeability of the preterm and newborn infant may be high, allowing the entry of small amounts of undigested proteins. The maternal antibodies from colostrum can enter the newborn's bloodstream relatively unaltered by a process of endocytosis and

subsequent exocytosis. Although the intestinal permeability decreases with age, adults can still absorb larger proteins in abnormal circumstances. However, the predominant form of absorption and presentation of large foreign proteins is through the specialized microfold or M cells overlying the lymphoid Peyer's patches. This mode of absorption of intact proteins or polypeptides, however, is nutritionally insignificant.

Peptide Absorption

Di- and tripeptides can cross the brush border membrane by a main peptide transport system with broad specificity. This carrier protein can transport dibasic as well as diacid peptides and peptides consisting of up to three amino acid residues. However, there is some stereospecificity for this transporter because the longer the length of the amino acid side chain on the peptides, the easier the absorption. The transporter system also has greater affinity for dipeptides than tripeptides, and the acidic and basic amino acid residues in dipeptides lower the affinity for the transport system compared with neutral amino acids. In general, the absorption of L-isomers of amino acids in dipeptides is preferred over the D forms. The peptide transport system is coupled to the proton pump system rather than the sodium gradient. The oligopeptide transporter (Pept-1) in the brush border membrane is the major mechanism for protein absorption in the human intestine and is primarily responsible for the transport of di- and tri-peptides. Several factors may determine the levels of Pept-1, such as insulin, which may stimulate membrane insertion of the oligopeptide transporter from a preformed cytoplasmic pool, and cholera toxin, which decreases the activity of Pept-1 through an increase in the intracellular concentration of cyclic AMP.

Once in the absorbing cell, the di- and tripeptides are further hydrolyzed to the constituent amino acids by the cytoplasmic peptidases before absorption. The only small peptides that are known to enter the portal blood directly are those from gelatin that contain proline and hydroxyproline, and those from certain meats containing carnosine and anserine. However, their relative proportion in comparison to amino acids is inconsequential.

Amino Acid Absorption

Although some diffusion of amino acids does occur, they are mostly absorbed by active transport. Unlike peptides, which are absorbed equally well in both proximal and distal small intestine, amino acids are absorbed more rapidly in the duodenum and jejunum. Also in contrast to the parsimonious peptide transport system, there are multiple transport mechanisms for various amino acids at both the luminal end and the basolateral membrane of the enterocyte (Table 3). At the luminal end, the transporters are mostly located at the villous enterocytes. The villous enterocytes utilize approximately 10% of the absorbed amino acids for their own protein production, whereas the crypt cells derive their amino acid supply from the portal circulation. Of the various amino acids, glutamine appears to have a major role in the nutrition and regeneration of enterocytes, and it is now recognized that in the human

Table 3 Major amino acid transport systems in the intestinal epithelial cells

Transport system	Substrates	Sodium gradient-dependent
<i>Brush border membrane</i>		
B	Dipolar α amino acids	+
B ^{0,+}	Dipolar α amino acids	+
	Basic amino acids	
	Cystine	
B ^{0,+}	Dipolar α amino acids	—
	Basic amino acids	
	Cystine	
Y ⁺	Basic amino acids (e.g., lysine)	—
	Cysteine	
IMINO	Imino acids (e.g., proline)	+
	β -Alanine	
X _{GA} [—]	Acidic amino acids (e.g., glutamate, aspartate)	+
β	β -Amino acids (e.g., alanine)	+
<i>Basolateral membrane</i>		
L	Broad selectivity	—
A	Broad selectivity	—
ASC	Neutral amino acids (e.g., alanine, serine)	+
	Cysteine	
N	Glutamine, histidine, asparagines	+

Source: Modified from Shulman RJ (1996) Intraluminal digestion and absorption in the small intestine. In: Gluckman PD and Heyman MA (eds.) *Pediatrics and Perinatology: The Scientific Basis*, 2nd edn., pp. 634–637. London: Arnold.

intestine the predominant mechanism for assimilation of glutamine dipeptides is absorption as intact dipeptide rather than hydrolysis.

There are at least five different sodium-dependent transport systems for amino acid uptake. The sodium-dependent transport is facilitated by energy derived from Na⁺/K⁺-exchanging ATPase at the basolateral membrane. Most energy-dependent transporters are coupled either to cotransport of Na⁺ or Cl[—] or to the countertransport of K⁺. An additional system of sodium-independent facilitated diffusion also exists and is predominantly geared toward basic and dipolar α amino acids. These passive transporters are either facilitated transporters or channels.

Digestibility

The digestibility of a protein is a measure of the amount of protein available from it for absorption after digestion; this is usually obtained from estimates of dietary nitrogen and fecal and urinary nitrogen. Digestibility is different from the other measures of protein quality, such as the amino acid or chemical scores and biological value, which, respectively, represent the essential and nonessential amino acid composition of the protein and the proportion of available nitrogen retained for growth or maintenance. Thus, a protein-based diet of high amino acid or chemical score may be poorly

digested and of limited nutritional value. The digestibility of a protein is also dependent on the physical shape of the protein and the relative ease with which peptide bonds can be hydrolyzed. Fibrous proteins with long polypeptide chains, such as collagen, keratin, and elastin, are relatively insoluble. In contrast, globular proteins, which are coiled and tightly packed, are comparatively soluble and thereby more digestible. Such proteins are insulin enzymes, hemoglobin, and albumin.

The apparent protein digestibility is a measure of the amount of protein intake (%) available for absorption and is usually calculated by estimation of fecal nitrogen and corresponding dietary intake:

$$\text{Apparent digestibility} = \frac{(\text{dietary nitrogen} - \text{fecal nitrogen})}{\text{dietary nitrogen}} \times 100$$

However, since not all fecal nitrogen is of dietary origin and some is derived from obligatory endogenous intestinal losses, a more appropriate measure is that of 'true protein digestibility'. This is derived as

$$\text{True protein digestibility} = \frac{[\text{dietary nitrogen} - (\text{fecal nitrogen} - \text{obligatory fecal nitrogen})]}{\text{dietary nitrogen}} \times 100$$

The obligatory fecal intestinal protein losses have been variably estimated to range from 20 mg kg⁻¹ day⁻¹ in young infant and preschool children to approximately 12 mg kg⁻¹ day⁻¹ in adults. These losses may result in some difficulty in interpreting digestibility findings. The estimated value of true digestibility of food and feed proteins is dependent on the excretion of metabolic fecal nitrogen (MFN). Results of many studies show that a high-fiber content of the diet increases MFN excretion and lowers the true digestibility of the diet protein. The exact estimation of MFN is only possible with isotopic methods. Experimental studies indicate that for human subjects the fecal digestibility values are significantly higher than the ileal values for Arg, Asp, Gly, Phe, Pro, Ser, Thr, and Trp, with the exception of fecal digestibility of Met, which is significantly lower than the ileal value.

Table 4 gives the true digestibility values for several common foods and diets. In general, milk and eggs have the highest true digestibility values of approximately 97%, followed by meats, fish, and poultry. Plants and legumes have comparatively lower protein digestibility values, ranging from 75% to 85%. Thus, in mixed diets, increasing the relative amounts of animal proteins compared with plant-based proteins results in increased protein digestibility of the diet. However, some fibrous animal proteins, such as keratin and collagen, are relatively indigestible. A useful approximation is to assume a protein digestibility of 75–80% for diets based on whole grain cereals and vegetables, 95% for diets based on refined cereals and animal proteins, and 85–90% for mixed diets. In general, the lower the true digestibility of a protein, the greater the amount required to achieve nitrogen equilibrium.

In addition to the differences in the nature of proteins highlighted previously, several other factors affect protein digestibility of a diet, including the presence of additional

Table 4 Illustrative values of protein digestibility in humans

Protein sources	True digestibility (%)	Digestibility relative to reference protein (%)
Eggs	97	100
Milk and cheese	95	100
Meat and fish	94	100
Maize	85	89
Oatmeal	86	90
Whole wheat	86	91
Refined wheat	96	101
Polished rice	88	93
Soy flour	86	91
Soybean isolate	94	99
Millet	79	83
Peanut butter	95	100
Beans	78	82
Chinese mixed diet	94	99
Brazilian mixed diet	78	82
Guatemalan mixed diet	79	92
Indian rice and milk diet	87	92
Mixed American diet	96	101

Source: Modified from Torun B (1985) Proteins: Chemistry, metabolism, and nutritional requirements. In: Brunser O, Carraza F, Gracey M, Nichols B, and Senterre J (eds.) *Clinical Nutrition of the Young Child*, pp. 99–119. New York: Raven Press.

dietary factors such as trypsin inhibitors. The latter may be present in certain foods, such as navy beans and soybeans, and can be largely inactivated by heating, thus improving protein digestibility. Although moderate heating can promote digestibility by promoting breakdown of peptide cross-linkages and inactivation of protease inhibitors in natural food, strong heating, especially in the presence of a carbohydrate or oxidized lipids, may make the protein resistant to enzymatic hydrolysis. The Maillard or 'browning reaction' occurs after high, usually prolonged heating of a protein in the presence of a reducing sugar such as lactose or glucose, resulting in cross-linkages of the sugar with the free side chain of the lysine residues. This may make up to 30% of the lysine biologically unavailable. These changes are of particular importance in situations of marginally sufficient protein intake, in which cooking procedures may further aggravate protein malnutrition. The effect of heat treatment on the protein digestibility of a formulation was highlighted by a study using an elegant suckling rat model to investigate the digestibility of different infant milk formulations. The data indicate that proteins from ultraheat-treated milk formulate were most rapidly digested (84%), resulting in an amino acid profile closest to that of breast-milk-fed pups, whereas the digestibility from powdered formulations (77–82%) and soy milk-based formulas was slower. The slowest digestion of protein was found in sterilized milk formula (72–74%), where the canned formulation was exposed to high temperatures for extended time periods.

The Maillard reaction is highly influenced by the pH of foodstuffs or other agents. The reduction of pH that may be performed by increasing fermentation in the baking industry lessens the decomposition of lysine and tryptophan in proteins. Fermentation is widely used as a strategy to increase the

digestibility of starch and improve the organoleptic properties of weaning foods and cereal-based preparations in developing countries. However, although the impact of fermentation on starch digestion is well established, the impact on protein digestibility is variable. Although some studies have suggested an impact on protein quality and digestibility of legumes and finger millet-based foods, other studies suggest that fermentation only modifies the gastric emptying rate and does not significantly affect the level of diet hydrolysis, the endogenous nitrogen stimulation, or the digestibility rate.

Despite several limitations, the digestion and absorption of ingested proteins is remarkably complete, with only a small fraction (3–5%) of ingested protein nitrogen escaping hydrolysis and excreted in the stools. In the context of infant nutrition, although breast milk is well digested, some proteins, such as secretory IgA, lactoferrin, and α_1 -antitrypsin, escape digestion.

The protein digestibility-corrected amino acid score (PDCAAS) has been adopted by FAO/WHO as the preferred method for the measurement of the protein value in human nutrition. The PDCAAS is the product of the fecal digestibility of the protein and the chemical score of the limiting amino acid. The method is based on comparison of the concentration of the first limiting essential amino acid in the test protein with the concentration of that amino acid in a reference (scoring) pattern. This scoring pattern is derived from the essential amino acid requirements of the preschool-aged child. The chemical score obtained in this way is corrected for fecal digestibility of the test protein. PDCAAS values higher than 100% are not accepted as such but are truncated to 100%. Although the principle of the PDCAAS method has been widely accepted, critical questions have been raised in the scientific community about the basis of correction for fecal instead of ileal digestibility, and the truncation of PDCAAS values to 100%. At the time of the adoption of the PDCAAS method, there were no acceptable experimental data on the amino acid requirement of the preschool child, and a factorial approach to determining these requirements was used, based on recent data defining the growth and protein accretion in these children. However, there is still a need for experimental validation of the scoring pattern. Also, the scoring pattern does not include conditionally indispensable amino acids.

These amino acids also contribute to the nutrition value of a protein. There is strong evidence that ileal, and not fecal, digestibility is the correct parameter for correction of the amino acid score. The use of fecal digestibility overestimates the nutritional value of a protein because amino acid nitrogen entering the colon is lost for protein synthesis in the body and is, at least in part, excreted in urine as ammonia. The truncation of PDCAAS values to 100% can be defended only for the limited number of situations in which the protein is to be used as the sole source of protein in the diet. For evaluation of the nutritional significance of proteins as part of mixed diets, the truncated value should not be used. In these cases, a more detailed evaluation of the contribution of the protein to the amino acid composition of the mixed diet is required. From such an evaluation, it appears that milk proteins are superior to plant proteins in cereal-based diets. Other studies have assessed the validity of the PDCAAS method in predicting the quality of protein products compared with the commonly used protein quality methods, protein efficiency ratio and net

protein ratio. These data demonstrate that the PDCAAS method is inappropriate for predicting the protein quality of protein sources that may contain naturally occurring growth-depressing factors or antinutritional factors formed during alkaline and/or heat processing.

See also: Amino Acids: Chemistry and Classification; Metabolism. Protein: Quality and Sources; Requirements and Role in Diet; Synthesis and Turnover. Protein Deficiency

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PROTEIN

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Glossary

Amino acid score (or 'chemical score') Value of the limiting amino acid with the lowest score in a protein (i.e., the 'most limiting amino acid'). A protein is assigned a percentage score of 100 (or a fractional score of 1.00) when none of its EAAs are limiting.

Amino acid scoring pattern Amino acid composition of a hypothetical reference protein that contains all EAAs in the amounts necessary to satisfy requirements.

Amino acid scoring procedure Calculation of the proportion of each EAA in a protein or diet relative to the scoring pattern (Table 1). It can be expressed as percentage or as a fractional value.

Essential amino acids (EAAs) Also called 'indispensable amino acids'. Amino acids that the diet must provide because humans cannot synthesize them from other

components at a rate commensurate with normal bodily needs.

Limiting amino acids EAAs in food proteins that are present in lower proportions than in the reference protein (i.e., with fractional value <1.00, relative to the reference protein, Table 1).

Nitrogen balance (NB) The average amount of nitrogen that is retained or lost from the body. It is calculated from measurements of dietary, urinary, and fecal nitrogen and estimates of integumental (sweat, skin, nails, and hair) nitrogen losses (Table 1).

Protein (or nitrogen) digestibility The proportion of dietary nitrogen that is absorbed. 'True' protein digestibility is calculated correcting for endogenous or obligatory fecal nitrogen losses (i.e., nitrogen in epithelial cells, gastrointestinal secretions, and intestinal flora, Table 1).

Table 1 Calculation of operational definitions^a

Definition	Calculation
Apparent digestibility	$\frac{I_N - F_N}{I_N}$
True digestibility	$\frac{I_N - (F_N - F_E)}{I_N}$
Nitrogen balance	$I_N - U_N - F_N - \text{Integ}_N$
Amino acid score	$\frac{\text{mg of EAA in 1 g of food protein}}{\text{mg of EAA in 1 g of reference protein (or EAA scoring pattern)}}$
Limiting amino acid	EAA with a score <1.00 (or <100%)

^aDigestibility and amino acid scores can be expressed as fractional values (≤ 1.00) or multiplied by 100 and expressed as percentages.

F_E , endogenous fecal nitrogen; F_N , total fecal nitrogen; I_N , nitrogen intake; Integ_N , integumental nitrogen; U_N , total urinary nitrogen.

The amino acid composition of food proteins and the efficiency with which they are digested to allow amino acid absorption determine their capacity to provide nitrogen and essential amino acids (EAAs) for human growth and functions. This capacity, known as protein quality, influences dietary requirements: the lower the quality, the higher the required dietary protein intake. The nutritive value of food proteins is also influenced by the density of protein as well as the concentration and bioavailability of its amino acids. Some forms of food storage and processing can affect the latter.

This article examines the ways of assessing the protein quality of foods and diets and the quality inherent to various protein sources.

Assessment of Protein Quality

Metabolic Studies

The most accurate assessment of protein quality of foods for humans is through clinical or metabolic studies that measure nitrogen balance. A fixed amount of protein is fed to a group of individuals until a steady state is reached. At that point, excreta are collected and analyzed for their nitrogen content, and integumental nitrogen losses are generally estimated at approximately $5 \text{ mg N kg}^{-1} \text{ day}^{-1}$ to calculate NB as follows:

$$\text{NB} = I_N - U_N - F_N - \text{Integ}_N \text{ (see abbreviations in Table 1).}$$

Measurements are repeated with different amounts of food protein and the relationship between nitrogen intake and nitrogen balance is evaluated (Figure 1). The slope of the line before nitrogen balance reaches a plateau and the amount of dietary protein needed to attain zero nitrogen balance are indicators of protein quality: the steeper the slope and the lower the amount of dietary protein to achieve balance, the higher the quality of the protein being tested.

Influence of Energy Intake on Nitrogen Balance

When food energy intake is insufficient to satisfy energy needs, amino acid oxidation increases in an effort by the human body to satisfy energy requirements. This raises urinary nitrogen excretion and reduces nitrogen balance. However, increased energy intake may reduce amino acid oxidation and urinary nitrogen excretion, thereby improving N balance until it reaches a plateau. This response, known as the protein-sparing effect of dietary energy, can be attenuated if the quantity or quality of food protein intake is inadequate. It has been postulated that the protein-sparing effect of dietary carbohydrates is mediated by increased insulin secretion, which inhibits proteolysis, hepatic gluconeogenesis, and renal ammoniogenesis. The protein-sparing effect of dietary fat may be due to a reduction of amino acid oxidation through an effect of free-fatty acid oxidation in the liver, whereby the increase in NADH/NAD inhibits branched-chained keto-acid dehydrogenase. For these reasons nitrogen balance must not be used to estimate protein quality when the amount of dietary energy is such that it produces weight loss or gain in an otherwise well-nourished individual.

Because of their high cost and experimental complexity, metabolic studies are done mainly to evaluate new,

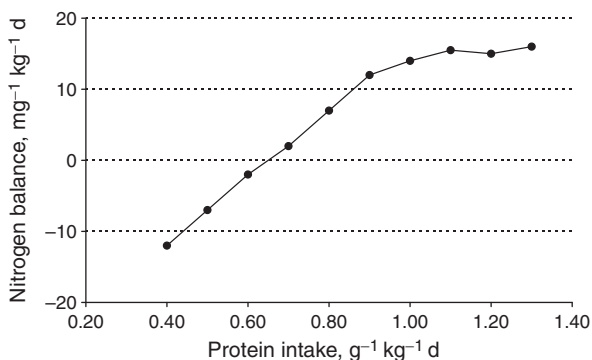


Figure 1 Relation of N balance to protein intake.

nonconventional protein sources and novel food processes that may affect protein quality. Other methods that can predict protein quality for humans rapidly and at low cost are used to evaluate diets and conventional foods routinely.

Assays in Laboratory Animals

Biological assays in laboratory animals have been used to assess food protein quality, based either on a protein's ability to support growth in young rats (protein efficiency ratio (PER)) or on nitrogen retention (net protein utilization (NPU)). However, these assays underestimate the quality of some vegetable and animal proteins for humans. For example, the proteins of pulses and milk casein have a lower quality for rats than for humans because rats have a higher requirement of sulfur-containing amino acids. Thus, application of rat assay results to human nutrition can result in important quantitative errors. The discrepancy usually has economic rather than public health implications because rat assays generally err by underestimating protein quality for humans, but the value of certain animal proteins can be overestimated because of higher efficiency of utilization by the rat. Nevertheless, the PER remains a useful model to validate theoretical models based on the amino acid composition of the protein in question.

Amino Acid Score Adjusted for Digestibility

The concept of assessing protein quality on the basis of a protein's constituent amino acids was introduced in the late 1940s. It was later suggested that the calculations be corrected by the protein's digestibility. The validity of this approach and its correlation with results of metabolic and clinical studies were initially limited by lack of accurate procedures to measure tryptophan and sulfur amino acids, insufficient information on digestibility of proteins from various sources, and uncertainty about human amino acid requirements to prepare an adequate scoring pattern. Significant scientific and technological advancements now allow the use of an amino acid scoring procedure adjusted for digestibility as a good and practical predictor of protein quality for humans.

This method is recommended by expert committees of the World Health Organization (WHO), United Nations Food and Agriculture Organization (FAO), United Nations University (UNU), and the Codex Committee on Vegetable Proteins (CCVP), as well as by regulatory agencies of several countries, for routine evaluation of protein quality for humans. The elements required for its application are knowledge about the amino acid composition and digestibility of the food protein(s) under evaluation and a scoring pattern based on human amino acid requirements.

Amino Acid Analysis of Food Proteins

Modern methods that involve acid or alkaline hydrolysis of the protein followed by separation and quantification of the released amino acids by ion exchange, gas-liquid or high-performance liquid chromatography, and other chemical and microbiological methods for specific amino acids, such as

lysine, methionine, cysteine, and tryptophan, provide data with a repeatability within laboratory of approximately 5% and a reproducibility between laboratories of approximately 10%. Although several national and international food composition tables include amino acid contents of foods, it is preferable to use analytical results from a reliable laboratory owing to technical shortcomings in the preparation of some tables and to the considerable variability between the reported values, especially for tryptophan, cysteine, and methionine.

Amino acid data are usually calculated as milligrams amino acid per gram of protein. If they are reported as milligrams amino acid per gram of nitrogen, they can be converted to the protein equivalents multiplying by specific protein factors that range from 5.7 (17.5% nitrogen) to 6.4 (15.6% nitrogen) for the major protein sources in the diet. The factor used for a mixture of protein sources is 6.25, corresponding to a nitrogen content of 16%.

To calculate the amino acid content of a combination of food proteins, as in a processed food based on several protein sources or in a mixed diet, a weighted mean of the published or analytical results of each component should be used, as illustrated in Table 2.

Amino Acid Scoring Pattern

For infants younger than 0.5 year, the scoring pattern should be based on the amino acid composition of breast milk, even if some EAAs in human milk exceed minimum requirements for infants of this age. For example, infants consuming cow's milk proteins, which have less sulfur-containing amino acids than human milk, show adequate growth and nitrogen balance. Thus, although the use of a scoring pattern based on human milk composition may somewhat underestimate the protein quality of some foods for infants, there is consensus to accept errors on the side of safety for this highly vulnerable age group.

International expert committees (WHO/FAO/UNU 2007) have now agreed that the scoring pattern should be based on the best available estimate of daily EAA and protein requirements for each age group. For preschool children as well as older children, the EAA and protein requirements were determined by a factorial method that took maintenance and growth requirements into account, as well as the EAA

composition of the maintenance requirement and of tissue deposition in children of different ages. This was because published nitrogen balance studies to determine the EAA requirement in older children and adults have experimental flaws. In adults, EAA requirements have now largely been determined by amino acid oxidation techniques, which are more accurate, and coupled with the adult protein requirement, allow for an adult scoring pattern to be established. For a universal scoring pattern, because proteins with amounts of EAAs that satisfy the needs of young children will probably be adequate for older children and adults, the scoring pattern for preschool children is currently used for all after 0.5 years of age.

Table 3 shows the internationally accepted patterns for amino acid scoring applicable to infants and to persons after 0.5 years of age; the composition of high-quality animal foods is shown for comparison. The content of each EAA in a food

Table 2 Calculating the lysine (lys) content of a rice, lentil, and chicken mixture

<i>1. Protein source in 100 g of the cooked mixture</i>			
10 g dry polished rice			
10 g dry lentil			
20 g raw white chicken meat			
<i>2. Chemical composition</i>			
	<i>Rice</i>	<i>Lentil</i>	<i>Chicken</i>
Protein (g per 100 g food)	7.0	23.7	19.2
Lysine (mg per 100 g food)	255	1739	1590
<i>3. Lysine content of the mixture (mg per g protein)</i>			
<i>Food</i>	<i>mg lys per g protein</i>	<i>g component per 100 g mixture</i>	
Rice	(255/7.0)	×	10
Lentil	(1739/23.7)	×	10
Chicken	(1590/19.2)	×	20
Weighted mean = (364 + 734 + 1656)/(10 + 10 + 20) = 60 mg lys per g protein			

Table 3 Amino acid scoring patterns for infants under 0.5 year, preschool children, and adults (mg amino acid per g protein)^a

<i>Amino Acid</i>	<i>Infant 0.5 year</i>	<i>Preschool children 0.5–2 year</i>	<i>Adult</i>	<i>Egg, cow's milk, and beef protein</i>
Histidine	20	(18) ^b	(15) ^b	22–34
Isoleucine	32	31	30	47–54
Leucine	66	63	59	81–95
Lysine	57	52	45	70–89
Methionine + cysteine	28	26	22	33 ^c –57
Phenylalanine + tyrosine	52	46	38	80–102
Threonine	31	27	23	44–47
Tryptophan	8.5	7.4	6	12 ^c –17
Valine	43	42	39	50–66

^aComposition of animal proteins shown for comparison.

^bEssentiality of histidine not clearly determined.

^cCow's milk proteins have less sulfur-containing amino acids and tryptophan than human milk.

protein is evaluated relative to the age-specific scoring pattern, to determine the protein's amino acid score and to identify the limiting amino acids as shown in **Table 1**. All EAAs present in proportions that exceed requirements are assigned a fractional score of 1.00 (or a percentage score of 100%), even if mathematical calculation gives a higher value. The EAA with the lowest value (i.e., the most limiting amino acid) determines the protein's amino acid score.

The only EAAs that are likely to limit the protein quality of mixed diets for humans are lysine, the sulfur-containing amino acids (methionine and cysteine), threonine, and tryptophan. Consequently, when information on all EAAs is not available, protein quality can be estimated on the basis of its score for these four amino acids.

Correction for Protein Digestibility

A protein may have a good amino acid composition relative to the scoring pattern, but if it is not fully digested and its constituent amino acids are not absorbed, its capacity to provide nitrogen and EAAs for human function will diminish. Not all food proteins are digested, absorbed, and utilized to the same extent because of inherent differences in their source (e.g., inside vegetable cells with indigestible membranes), their physicochemical nature (e.g., protein configuration and amino acid binding), the presence of food constituents that modify digestion (e.g., dietary fiber, tannins, and other polyphenols), the presence of antiphenological factors that interfere with protein breakdown (e.g., trypsin inhibitors and lectins), and processing conditions that alter the nature or release of amino acids (e.g., Maillard reaction and formation of polyamino acids and methylmercaptan). Consequently, amino acid scores as predictors of protein quality must be adjusted for protein digestibility and amino acid availability.

The standard for obtaining digestibility data is through metabolic studies in humans, in which the nitrogen excreted in the feces is subtracted from the amount ingested with the diet and expressed as a percentage of intakes. This apparent digestibility value must be corrected for the amount of fecal nitrogen excreted when a person is consuming a protein-free diet to calculate 'true' digestibility (**Table 1**). Ethical constraints and practical complexities do not permit the determination of obligatory fecal nitrogen losses on a protein-free diet in all age and physiological groups. It is recommended that existing published values for daily obligatory fecal losses in preschool children (approximately 20 mg N kg⁻¹) and adults (approximately 14 mg N kg⁻¹) be used to correct apparent protein digestibility values. In the absence of human data, the FAO/WHO 1991 Expert Committee

recommended the standardized rat fecal-balance method as the most suitable practical method for predicting protein digestibility. However, fecal digestibility is unlikely to be a true measure of amino acid digestibility. Digestibility measurements at the ileal level may provide a better measure of amino acid digestibility, however this may pose significant challenges for many researchers.

Protein digestibility values of specific foods and well-defined diets may be taken from reliable published data. **Table 4** shows some examples. When such data are not available for a mixed diet, a weighted average can be calculated from the true digestibilities of its constituent protein sources, as illustrated in **Table 5**. For new or novel products or processes, digestibility must be determined, preferably in humans. When cost and practicality do not permit metabolic studies in humans to be performed, standardized fecal-balance methods in rats have been used. These methods have given true protein digestibility values of 93–100% for animal foods or food products (casein, beef salami, skim milk, tuna, and chicken sausage) and soya protein isolate; 86–92% for beef stew, chick peas, rolled oats, and whole-wheat cereal; and 70–85% for lentils and different types of beans. These value ranges are similar to those from human studies. Nevertheless, rat data must be used with caution for foods and diets that are known or suspected of being handled differently by the human and rat intestines.

Table 4 True protein digestibility of selected foods and diets

	True protein digestibility (%)
Egg white	97
Whole egg, milk, beef, poultry, fish	95
Wheat, refined flour	95
Soya protein isolate	94
Polished rice	88
Soya flour	86
Wheat, whole	86
Maize products	85
Rice, whole	84
Beans	69
<i>Mixed diets</i>	
USA	96
China	94
Colombia, high-income	93
Philippines, urban	88
Chile, middle class	82
Mexico, rural	80
Guatemala, rural	79
Brazil, rural	78
India, vegetarian	78

Table 5 Calculation of true digestibility of a mixed diet of rice, beans, wheat, and egg

Diet	True protein digestibility (%)	Proportion of total protein (g per 100 g protein in whole diet)
Polished rice	88	40
Black beans	69	35
Whole wheat	86	15
Whole egg	95	10
Estimated digestibility of whole diet	$(0.88 \times 40) + (0.69 \times 35) + (0.86 \times 15) + (0.95 \times 10) = 82\%$	

Table 6 Calculation of amino acids scores of single protein sources corrected for digestibility and in relation to the protein quality to cow's milk

<i>Food</i>	<i>Most limiting amino acid</i>	<i>Noncorrected amino acid score</i>	<i>True protein digestibility</i>	<i>Corrected amino acid score</i>	<i>Protein quality relative to milk</i>
Cow's milk	None	$>100 \rightarrow 100\%$	$\times 95\%$	$= 95\%$	–
Polished rice	Lysine 36 mg per g protein	$(36/58) \times 100 = 62\%$	$\times 88\%$	$= 55\%$	$(55/95) \times 100 = 58\%$
Egg white	None	$>100 \rightarrow 100\%$	$\times 97\%$	$= 97\%$	$(97/95) \times 100 = 102\%$

In vitro procedures have also been developed using combinations of trypsin, chymotrypsin, peptidase, and bacterial protease. Further research is needed to validate their use as predictors of protein digestibility in humans.

Calculations and Examples

The EAA composition and protein digestibility of the food or mixed diet being tested are determined. Then the percentage or fractional value of the most limiting EAA (noncorrected amino acid score) is multiplied by the percentage or fractional value of 'true' protein digestibility to obtain the corrected score, which is equivalent to protein quality. This value can be used as such or it can be expressed in relation to the corrected amino acid score of a reference protein or food, usually casein or an animal food (milk, egg, or beef).

Proteins that have no limiting amino acids are assigned an amino acid score of 100% (or 1.00) that must be only corrected for digestibility. Similarly, if the clinical or experimental assessment of 'true' protein digestibility gives a value greater than 100% (generally due to experimental variability), a digestibility correction factor of 100% (or 1.00) is applied to the amino acid score. **Table 6** shows examples of calculations for a single food as protein source. The same procedure can be used for food mixtures using a weighted average procedure based on the protein content, amino acid composition, and digestibility of the individual components. **Table 7** shows an example of those calculations. For simplicity, the example uses only the four EAAs that are most often limiting.

Protein Concentration

Protein concentration or density (i.e., the amount of protein per unit of food) is another factor of a food's protein quality. Protein-dense foods are especially important for young infants, whose small gastric capacity limits the amount they can eat, and for elderly people with poor appetite. Evaluation of a food's protein concentration must be done for ready-to-eat preparations because food processing and cooking can result in significant changes relative to raw foods. Meats, poultry, and fish usually have a higher concentration of protein after cooking or frying, whereas vegetable food preparations contain more water and less protein than the raw products (**Table 8**).

Protein/Energy Ratio

The percentage of protein energy in the diet (P/E ratio) has been used to describe whether a diet provides adequate

amounts of protein. The reasoning is that energy requirements are the main driving force for food intake. Therefore, a diet is adequate if it satisfies the requirements for all nutrients when it is eaten in amounts that will satisfy energy needs.

P/E ratio is calculated by dividing the amount of metabolizable energy derived from dietary protein (grams of protein 16.7 kJ or 4 kcal) by the total amount of metabolizable energy in the diet, multiplied by 100 to avoid using fractional values. However, the use of P/E ratio as an index of food's protein adequacy may be misleading because it only gives information about protein concentration and does not indicate the biological value or quality of the proteins. Its usefulness improves when amino acid score is taken into account to calculate what can be defined as a desirable P/E ratio, as in the examples discussed later.

The P/E ratio indicates the amount of protein that the diet provides relative to energy and does not imply a constant relationship between protein and energy requirements. For example, the lower limit of the desirable P/E ratio of a diet with an amino acid score of 85% is 6.2 for a young child whose daily requirements are 16 g protein and 5.1 MJ energy $((16 \text{ g} \times 16.7 \text{ kJ}/0.85)/5100 \text{ kJ} \times 100)$. For an adult male with daily requirements of 55 g protein and 12.8 MJ, the desirable P/E ratio is 8.4 $((55 \times 16.7/0.85)/12800 \times 100)$. Obviously, the critical modifiable factor in these calculations is the energy requirement, which is linked to the habitual physical activity level. Therefore, in adults with a lower energy requirement, such as the elderly, the P/E requirement ratio will be higher.

Diets, especially those eaten by adults, often provide protein in amounts that surpass requirements, which elevates the P/E ratio. For example, almost all adult populations eat diets with P/E ratios between 10% and 15%. This is related to culture and food availability and does not reflect a biologically optimal ratio. Consistent with the calculations in the preceding paragraph, P/E ratios of 10 and 15 are adequate and it cannot be argued that one is nutritionally better than the other.

Improvement of Protein Quality

Amino Acid Profile

The amino acid profile of a food or diet can be improved by increasing the amount of constituent amino acids in its proteins, adding specific amino acids, or combining foods in proportions that result in a better amino acid pattern.

- Genetic handling. This has resulted in cereals with higher contents of the amino acids that limit their protein quality.

Table 7 Calculation of protein quality of a mixed diet based on whole wheat, polished rice, and chicken breast

Raw ingredients	Data from analysis of literature						Quantities calculated for the mixed diet						
	Weight (g)	Protein (g per 100 g)	Lys	SAA	Th	Trp	True digestibility (%)	Total protein (g) $H = A \times B/100$	Lys (mg) $I = H \times C$	SAA (mg) $J = H \times D$	Thr (mg) $K = H \times E$	Trp (mg) $L = H \times F$	
A	B	C	D	E	F	G							
Whole wheat	300	11	28	37	29	11	86	33	924	1221	957	363	
Polished rice	200	7	36	38	33	13	88	14	504	532	462	182	
Chicken breast	150	19	83	38	40	12	95	28.5	2366	1083	1140	342	
Total								75.5	3794	2836	2559	887	
M Weighted mean digestibility of the mixed diet (sum of $(G \times H)$ for each food component divided by total protein, H)													0.90
N mg amino acid per g protein (total for I, J, K , or L divided by total H)													
P Amino acid scoring pattern, mg amino acid per g protein													
Q Score for each amino acid in the mixed diet (N/P)													
R Amino acid score adjusted for digestibility (Q of the limiting amino acid multiplied by M)													
													$0.86 \times 0.90 = 0.77$ (or 77%)
													0.86
													58
													50
													38
													34
													25
													1.52
													1.00
													11
													12
													1.09

Lys, lysine; SAA, sulfur-containing amino acids; Thr, threonine; Trp, tryptophan.

For example, varieties of Opaque-2 corn have approximately 50% more lysine and 35% more tryptophan than native corn, both of which are limiting amino acids in this cereal.

- **Fortification and enrichment.** The addition of synthetic amino acids eliminates or reduces the magnitude of limiting amino acids, for example, in lysine-enriched wheat flour.
- **Complementation.** The combination of a food that has one or more limiting EAAs with another food(s) that has a surplus of these amino acids results in an improved combined amino acid profile. A double complementation effect has been achieved in the formulation of vegetable mixtures based on protein sources in which one has a surplus of the EAA that is limiting in the other and vice versa (Figure 2).

Table 8 Protein concentration in selected raw and ready-to-eat foods (g protein per 100 g food)

Food	Ready-to-eat	Raw
Beef, lean	36.8 (cooked)	21.4
Fish	31.8 (fried)	20.0
Wheat flour	12.0 (white bread)	11.0
Egg, hen	11.3 (hard-boiled)	11.3
Lentils	7.1 (cooked)	23.7
Common beans	6.2 (cooked)	22.0
Maize	4.2 (tortilla)	9.4
Milk powder, cow	3.2 (12% in water)	26.1
Rice	2.5 (boiled)	7.2
Potato, no skin	1.1 (cooked)	1.8

Digestibility and Bioavailability

Various food processing procedures can improve protein digestibility by removing food constituents that reduce digestibility (such as dietary fiber), breaking down poorly digestible vegetable cell membranes, destroying or neutralizing antiphenological factors, and increasing the food surface area that can come into contact with gastrointestinal enzymes. For example, soya protein isolate, polished rice, and refined wheat flour have higher protein digestibilities than soya flour, whole rice, and whole wheat, respectively (Table 4).

Food storage and processing in adverse circumstances can reduce protein quality by making some EAAs unavailable for use in the human body. These conditions should be avoided to preserve protein quality. Some examples are the storage of dried milk under mild to moderate heat and humidity, which renders lysine side chains unavailable after reacting with the reducing sugar, lactose (Maillard or 'browning' reaction); the severe treatment of protein with alkali, which causes lysine and cysteine residues to react and form lysinoalanine; and the treatment of proteins with oxidizing agents, which can result in a loss of methionine. Severe heating conditions in the presence of reducing sugars or oxidized lipids can make some food proteins resistant to digestion, thereby reducing the availability of all their amino acids.

Protein Concentration

Protein concentration can increase by genetic selection of protein sources, as in improved varieties of rice that have approximately 30% more protein than native rice, by the use of

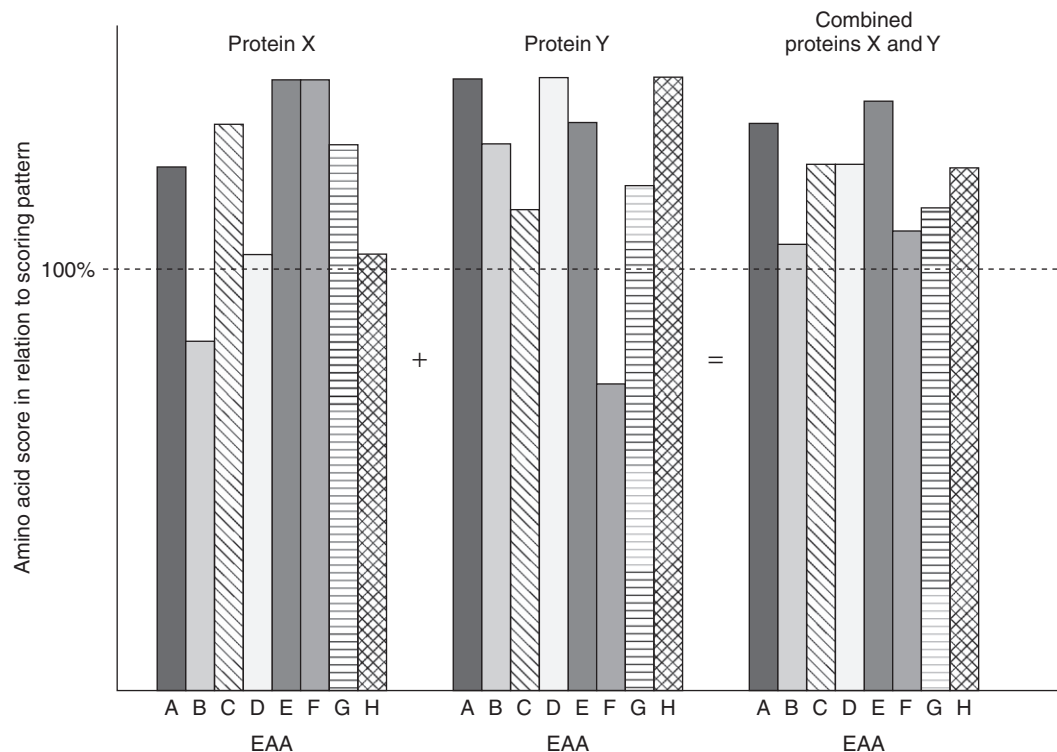


Figure 2 The effect of combining proteins on the amino acid score of the dietary protein intake.

nitrogen-concentrating fertilizers that can raise the protein contents of several cereals or by industrial and home processing that reduce the water content of food preparations. Addition of concentrated protein sources, such as casein, soya protein isolates, soya flour, milk powder, or dehydrated egg, will also increase the protein concentration of foods and diets, as well as their amino acid score in some instances.

Protein Quality and Dietary Sources

Foods of animal origin, such as milk and milk products, eggs, meats, poultry, and fish, have excellent amino acid composition with a score of 100% and true protein digestibility of 95–98%. In addition, their protein concentrations often increase after cooking. Consequently, they are used as the reference for comparison of protein quality, provided that they are processed in ways that will not decrease amino acid bioavailability.

Almost all vegetable foods have one or more limiting amino acids. Soya beans and soya products are notable exceptions. In general, proteins in natural vegetable foods have digestibilities of 70–85%. Vegetable protein isolates, flours, and extruded products have higher digestibilities.

Among vegetables, pulses have the highest protein concentrations, ranging from 20% to 25% in most raw beans and peas to approximately 36% in soya beans. Pulses usually have limiting sulfur-containing amino acids.

Cereals and cereal products are the largest sources of protein in most areas of the world. Cereal grains and flours contain approximately 7–12% protein with a quality that is limited by their lysine content and, in many instances, also by threonine or tryptophan. Although deficient in lysine and threonine, rice has one of the best amino acid compositions among cereals, whereas sorghum and native maize (i.e., not genetically improved) are among the lowest. Most nuts and edible seeds contain 8–18% protein. Many oil seeds have 12–20% protein, and the cake that remains after oil extrusion can have as much as 30–40% protein.

See also: Amino Acids: Chemistry and Classification; Metabolism. Cereal Grains. Energy: Balance. Nuts and Seeds. Protein:

Requirements and Role in Diet; Synthesis and Turnover. Protein Deficiency. Protein Digestion and Bioavailability

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Requirements and Role in Diet

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Defining minimum amino acid and protein requirements is inherently difficult. Humans are exposed to a wide range of protein intakes, which enable full expression of their genotypical lean body mass throughout the range, and identifying the lower limits of this range has proved intractable. Without unequivocal symptoms of deficiency, the adequacy of an intake can only be assessed in terms of nitrogen or amino acid balance, which is unsatisfactory for several reasons. In particular, adaptation causes major difficulties in designing balance studies and interpreting results. Furthermore, nitrogen balance methods are inherently imprecise and logistically extremely difficult, especially to ensure exact energy balance. It is therefore not surprising that there is much debate about both the nature and the extent of protein requirements.

Terminology

Protein requirements are best discussed in terms of metabolic demand, dietary requirement, and dietary allowances. Metabolic demand concerns amino acids and the magnitude and amino acid pattern of this demand is determined by the nature and extent of those metabolic pathways (e.g., net protein synthesis) that consume amino acids and that vary with the phenotype and the developmental and physiological state of the individual. The dietary requirement is the amount of protein or its constituent amino acids that must be consumed in order to satisfy the metabolic demand. The requirement will usually be greater than the metabolic demand. Thus, factors associated with digestion and absorption may limit digestibility (i.e., dietary nitrogen lost in the feces) and biological value (i.e., the availability of the absorbed amino acid pattern in relation to cellular needs), which influences urinary nitrogen excretion. Dietary allowances are a range of intakes derived from estimates of individual requirements taking into account variability between individuals. They are designed to meet the dietary requirements of almost all individuals or the majority of the population. The most recent values of safe intakes for individuals and populations are those published by World Health Organization (WHO)/Food and Agricultural Organization (FAO)/United Nations University (UNU).

Metabolic Demands for Amino Acids

Current evidence supports the representation of the metabolic demands as in **Figure 1**. The metabolic demand for amino acids is to maintain tissue protein at appropriate levels and to provide for all amino acid-derived metabolites and any additional needs during growth, rehabilitation, pregnancy, and lactation. Tissue proteins are diverse, including structural or fibrous insoluble types and soluble globular species, with characteristic properties and functions that are determined by

their amino acid sequence. All proteins are in a dynamic state of constant turnover (i.e., breakdown to constituent amino acids and resynthesis), although for the structural proteins this is slow or minimal. Nonprotein products include nucleic acids and a diverse range of smaller molecules, such as creatine, taurine, glutathione, hormones (e.g., catecholamines and thyroxine), neurotransmitters (serotonin and dopamine), and nitric oxide, a key regulator of blood flow and other physiological processes.

The metabolic demand is supplied from the free amino acid pool, the size of which, for most amino acids, is regulated within narrow limits. This regulation involves supply from three sources: dietary proteins after digestion and absorption from the upper gastrointestinal tract (GIT), tissue protein after proteolysis during protein turnover, and urea salvage in the large bowel. Within the free amino acid pool there are also interconversions of several amino acids, especially during transfer between organs and in the course of amino acid absorption from the GIT. Removal of free amino acids occurs by reactions in which they act as substrates, and these reactions are shown as three pathways, one of which is the metabolic demand. This pathway involves a number of irreversible pathways, including net protein synthesis, and other irreversible metabolic transformations of individual amino acids. The second and quantitatively largest pathway is the removal for protein synthesis during protein turnover. At nitrogen equilibrium, because turnover involves the reversible removal of amino acids, with replacement through proteolysis, it does not exert a net metabolic demand (other than for those amino acids irreversibly modified during or subsequent to protein synthesis). The third pathway is the irreversible removal of amino acids by oxidation and nitrogen excretion provoked, for example, by the transient increases in some or all free amino acids after a protein meal. This would represent an inefficient utilization. Although amino acid oxidation and urea synthesis is assumed to be irreversible, this is not entirely true because of urea salvage in the lower GIT. Thus, the rate of urea synthesis is usually in excess of the rate of urea excretion because some urea enters the large bowel and is hydrolyzed by bacteria. Most of this nitrogen is utilized by bacteria, and since little is lost as fecal nitrogen, it is eventually returned to the systemic pool as ammonia and amino acids after bacterial death and proteolysis, including indispensable amino acids. Although the extent and nature of this salvaged urea nitrogen are poorly understood, it does include ammonia which can contribute to the *de novo* synthesis of dispensable amino acids and may provide nutritionally important amounts of indispensable amino acids.

The metabolic demand for amino acids appears to involve obligatory and adaptive components. The obligatory component for subjects at equilibrium (i.e., maintenance) comprises conversion of some individual amino acids into important metabolites that are further transformed into

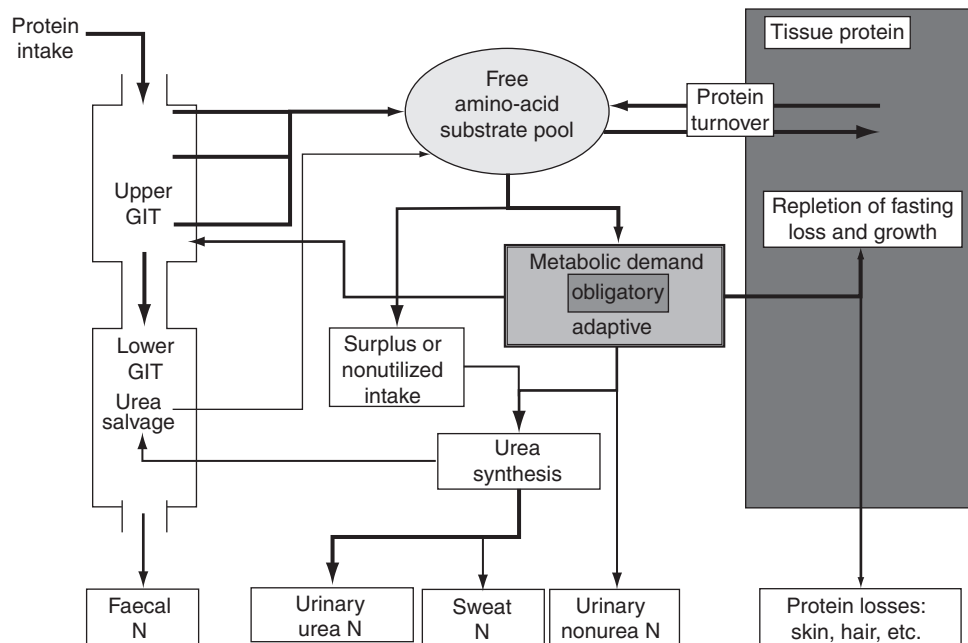


Figure 1 Schematic representation of the metabolic demands for amino acids.

nitrogenous end products, mainly urea and other compounds in urine, feces, or sweat, as well as net synthesis of proteins lost from the body as skin, hair, and any other secretions including that within the upper GIT involving mucin proteins lost into the large bowel. These diverse biological demands for amino acids for maintenance represent an essential but probably quite small intrinsic metabolic demand for an equivalent amount of protein. The magnitude of this maintenance component is assumed empirically to be equal to the obligatory nitrogen loss (ONL) – that is, the sum of all nitrogen losses from the body observed in subjects fed a protein-free but otherwise nutritionally adequate diet after 7–14 days, by which time nitrogen losses have declined to a stable and reproducible low level with the subjects losing body protein at a constant daily rate. In normal adults, the daily obligatory urinary, fecal, and subcutaneous and other losses are approximately 32, 10.5, and 4.8 mgN kg⁻¹, respectively (i.e., 47.1 mg kg⁻¹ d⁻¹), in total equivalent to about 0.3 g protein per kg per day tissue protein mobilized to meet such demands. The ONL is a function of body weight and, as far as is known, varies little with age. After adaptation to a protein-free diet, net tissue proteolysis is assumed to provide for the nonprotein components of the obligatory demand at a rate determined by the metabolic consumption of the rate-limiting amino acid (the amino acid with the highest ratio of molar proportion in the metabolic demand to molar proportion in protein). Because the obligatory metabolic demand is for a mixture of amino acids with a profile that is unlikely to match that of tissue protein, the actual nitrogen content of the metabolic demand is likely to be less than that indicated by the ONL (i.e., less than an equivalent of 0.325 g protein per kg per day). This is because all amino acids mobilized to provide for the metabolic demand must be oxidized and will contribute to the nitrogen excretion, whereas only some of them will serve useful functions. The evidence for this is the lowering of the

ONL in response to feeding selective amino acids, such as threonine, tryptophan, and methionine. In addition to these metabolic demands for maintenance, any net protein synthesis associated with growth, pregnancy, and lactation also constitutes an obligatory metabolic demand.

The adaptive component of the metabolic demand represents amino acid oxidation at a rate varying with the habitual protein intake that occurs as a result of the increasing activities of the pathways of oxidation of amino acids that regulate free amino acid pool sizes. Although this aspect of amino acid metabolism is least understood, it is likely to occur as a consequence of the fact that humans grow slowly or maintain constant weight on diets that contain protein considerably in excess of minimum needs. Thus, to be able to rapidly dispose of excess protein and maintain the very low tissue concentrations of those amino acids, such as the branched chain, aromatic, and sulfur amino acids, that may be toxic at higher concentrations, pathways of oxidative amino acid catabolism adapt (increase their V_{max}), enabling them to operate at the appropriate rate set by habitual protein intakes. Importantly, the adapted rate of amino acid oxidation, characteristic of habitual intake, changes only slowly in response to either a change in dietary protein intake level or feeding and fasting. This has two main consequences. First, when intake falls below habitual intake mobilization of tissue protein occurs with a negative nitrogen balance for as long as it takes to adapt to the lower level of intake. This was previously identified as the labile protein reserves. It can be assumed that for intakes greater than the minimum protein requirement (MPR), full adaptation to the new level will include not only a change in the adaptive metabolic demand to match intake but also repletion of most tissue nitrogen lost during the adaptive transition, i.e., a period of positive nitrogen balance. Second, because the adaptive rate of amino acid oxidation continues to some extent into the postabsorptive state, there are varying

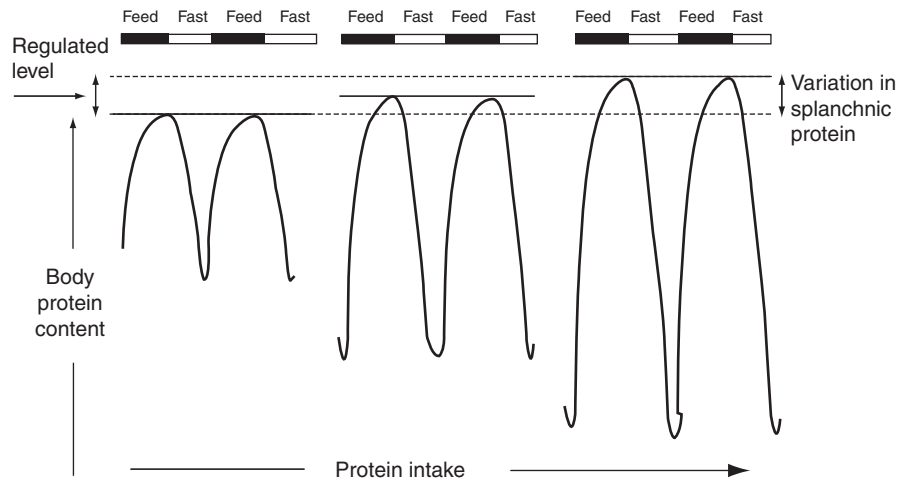


Figure 2 Balance regulation throughout the diurnal cycle with increasing habitual protein intakes.

postabsorptive losses of tissue protein and nitrogen excretion with varying habitual intake – that is, a diurnal cycle of postabsorptive losses and postprandial gains with an amplitude that increases with the increasing habitual level of protein intake as shown in **Figure 2**. As such, the adaptive metabolic demand includes a component of net protein synthesis that repletes postabsorptive losses. The magnitude of this varies in a complex way with eating pattern and with the amount and quality (amino acid score) of the habitual protein intake.

The main practical implication of this adaptive metabolic demands model is that the true MPR will be that associated with the lowest possible adaptive metabolic demand, and it is not known with any certainty how long such adaptation would take. However, studies that have examined balance responses to changes in protein intakes suggest it is likely to be longer than the periods employed in short-term balance studies. This implies that short-term balances from which our estimates of the MPR derive may overestimate the value and also that some of the variability in protein requirements between studies may reflect variable completeness of adaptation to the test diets. Another consequence of this metabolic model is that intakes and requirements are correlated, which has implications for the definition of risk of deficiency and safe intakes.

Qualitative Aspects of the Metabolic Demand: Plant versus Animal Sources

The nutritional requirement for protein will be the minimum intake that satisfies metabolic demands and that maintains appropriate body composition and growth rates, after taking into account any inefficiency of digestibility and metabolic consumption. With continuous and extensive amino acid interconversion, the pattern of dietary amino acids need not match that of the composition of tissue proteins or the maintenance metabolic demand exactly because some amino acids (aspartic acid, asparagine, glutamic acid, alanine, and serine) are dispensable and can be replaced by sufficient total

amino acid nitrogen supplied from other amino acids or sources of nonessential nitrogen. However, there will be a minimum dietary requirement for those amino acid that are not interconverted, classified as indispensable amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine), and for those that are formed only slowly from other amino acids and become indispensable under specific physiological or pathological conditions (conditionally indispensable; e.g., cysteine, tyrosine, taurine, glycine, arginine, glutamine, and proline). Traditionally dietary proteins have been classified by their nutritional value (quality) measured in terms of their ability to provide for tissue growth in rapidly growing rats. In this case, marked differences are observed between most animal and individual plant protein sources when tested as the sole source of protein, with relative nutritional value reflecting mainly relative amounts of specific indispensable amino acids. The similarity between overall tissue protein amino acid composition and that of most animal dietary protein sources and the contrast with plant protein sources resulted, in clear distinctions between their quality in these animal growth assays. However when different plant protein sources are combined in these assays, such as cereals and legumes, near maximal growth can be observed and this gave rise to the concept of complementation, in which the appropriate balance of essential amino acids is provided from combinations of plant proteins.

However, in human nutrition with growth occurring very slowly after the first few months of life, the nutritional demand for indispensable amino acids for tissue growth is much less and may be minimal. Little of the metabolic demand for amino acids is generated by protein turnover because of amino acid recycling. Some net protein synthesis is associated with skin and hair growth and with gastric secretions (e.g., threonine-rich mucus glycoproteins) that pass into the colon to be utilized for bacterial metabolism. The metabolic demand for maintenance of normal function and composition is a poorly understood pattern of amino acids utilized in the various metabolic pathways other than protein synthesis, but this pattern is almost certainly different from that required for growth (i.e., mainly the amino acid pattern of tissue protein) and may contain a much lower overall

amount of indispensable amino acids. As such, a distinction between animal and plant dietary protein sources is much more difficult to demonstrate at least for mixtures of plant proteins as consumed in vegetarian diets, so that protein quality in terms of the amino acid profile may be less relevant in human nutrition. However there has been considerable controversy regarding the magnitude of the requirements for indispensable amino acids in the human diet with different views about the relative importance of dietary protein quality which has not been entirely resolved by the most recent report from WHO/FAO/UNU. This reassessment of the reported values for each indispensable amino acid clearly shows quite significant differences in the requirement values determined by different methodologies. In the absence of agreement on the most appropriate methodology, for each amino acid, the requirement value selected is the average value. Since for some amino acids the overall range of reported values is quite large, (e.g., more than two-fold for lysine), this means that considerable uncertainty remains about the amino acid requirement pattern which is used to judge the quality of dietary protein sources. This is clearly unsatisfactory.

Nitrogen Balance

Nitrogen balance studies were initiated in the mid-nineteenth century by Carl Voit, and such studies have been central to the definition of protein requirements within a model of nutrient balance: i.e., an intake which balances all losses observed in a healthy weight-stable subject. Nitrogen is the proxy measure of protein intake and losses, and health is identified only in terms of an appropriate lean body mass at a healthy body weight. Thus the MPR, is determined as the intake for nitrogen equilibrium, (zero balance), as indicated in nitrogen balance studies. There are no other measureable physiological or biochemical indicators of health which are exclusively and unequivocally indicators of protein intake and no disease states which have been identified as having a quantifiable relationship with protein intakes.

The aim of nitrogen balance studies is simple – to define the relationship between intake and all losses (urinary, fecal, and surface – mainly sweat, skin, hair, breath ammonia, nail clippings, etc.) so that the intake that allows equilibrium and provides for all losses can be identified. Thus, when the intake equals the requirement,

$$\text{balance} = \text{intake} - \text{losses} = 0.$$

As indicated previously, the lowest level of losses observed, the ONL, is approximately equivalent to a daily loss of 0.325 g protein per kg per day. When such subjects are re-fed with protein, losses of body protein decrease as the dietary protein provides for some of the metabolic demand. However, nitrogen losses increase with intake so that the required intake for balance is more than the ONL. The main objective of nitrogen balance studies has been to define how much extra protein above the ONL must be fed to achieve equilibrium. The literature on human nitrogen balance studies has been assessed in a meta-analysis, with all the reported individual balance points and the analytical principle shown in **Figure 3**. A linear regression of balance against intake will allow prediction of the ONL as the zero intake intercept. The slope of the balance curve ($a/b=e$) will indicate the efficiency of

utilization, and the maintenance requirement (i.e., the amount that must be fed to balance all losses and produce equilibrium) is ONL/e . Thus, the currently accepted maintenance requirement for adults ($0.66 \text{ g kg}^{-1} \text{ d}^{-1}$) derives from an analysis of the data shown in **Figure 3** (the median requirement calculated from linear regression for each individual studied on at least three levels of protein intake). Although the majority of the studies were young men studied with animal proteins, some studies with mixed plant proteins, with women and with older adults were included, but no significant influences of gender, age, or dietary protein source were observed. Subsequent nitrogen balance studies examining age and gender specifically have confirmed that these factors do not influence the MPR.

Inherent Difficulties with Nitrogen Balance Studies

This apparently simple but laborious approach, which is currently the main method for investigating protein and amino acid requirements, is in fact beset with a large number of quite serious problems, as listed in **Table 1**.

The lack of precision results in balance being a small value compared with the much larger values of nitrogen intake and nitrogen excretion, resulting in considerable error. The various systematic errors mean that balance is usually overestimated, often with unrealistic positive balances (protein gains) at high intakes. The nonlinearity of the balance curve as losses increase to match intakes when body protein reaches the maximum level means that there is no simple term to define the overall shape of the balance curve allowing prediction of the requirement (as the zero balance intake). In practice, prediction of a zero balance–intake intercept is made from a few balance points by linear regression, and this will result in requirement values that will vary according to where the intake values lie on the balance curve; that is, studies conducted using low intakes will underestimate requirements, whereas studies conducted with supra-maintenance intakes will overestimate requirements. The logic of this is that (i) reliable balance studies are those that are conducted with intakes very close to the actual requirement, and (ii) studies with intakes based on preconceived requirement values will tend to confirm such preconceptions.

Protein–Energy Interactions

Body protein equilibrium can be influenced by intakes of energy, and ensuring that energy intakes are sufficient is difficult. Excess energy intake leads to weight and some lean tissue gain, whereas with too little energy intake, weight loss occurs and dietary or body protein is oxidized as an energy source. This means that the protein requirement is a function of the state of energy balance and the actual influence is quite marked. According to one analysis of nitrogen balance (NB) on intakes of energy (EI; kcal kg^{-1}) and N (NI; mg N kg^{-1}), $\text{NB} = 0.171\text{NI} + 1.006\text{EI} - 69.13$, implying that the intake for N equilibrium (the requirement) will vary from 1.4 to $0.32 \text{ g kg}^{-1} \text{ d}^{-1}$ according to whether energy intakes are equal to the resting metabolic rate (RMR) or equal to twice the RMR. In fact, most, (85%), of the estimated between individual variability used to calculate the safe protein intake shown in **Figure 4** could be accounted for by an error of only $\pm 10\%$ of

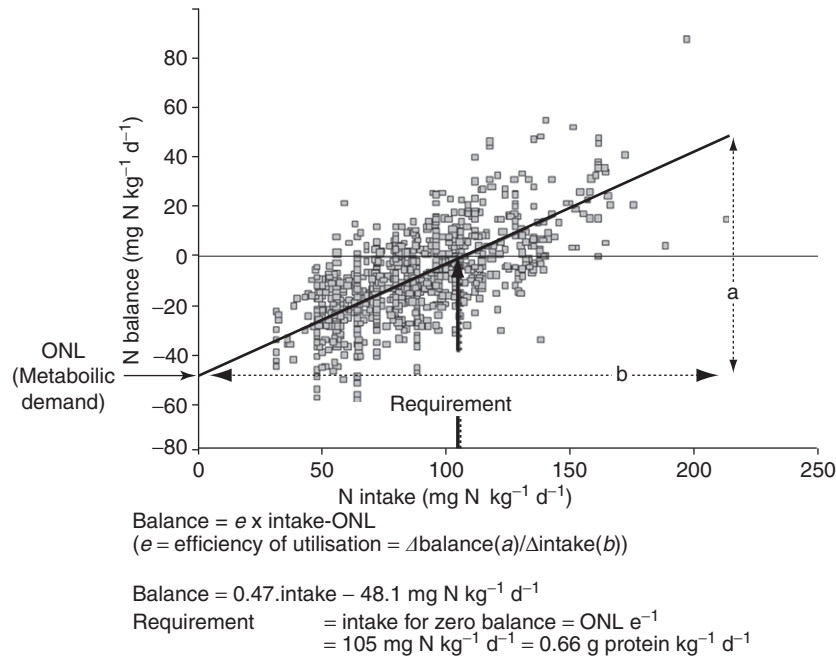


Figure 3 Meta analysis of N balance studies.

Table 1 Potential problems relating to nitrogen balance methodology

Imprecise
Systematic errors: intake overestimated, loss underestimated owing to problem of accounting for all losses, for example,
Skin surface and secretions
Loss of N_2 gas
Expired ammonia
Endogenous NO production gives urinary nitrate, fecal ammonia, and nitrite
Changing size of the body urea pool
Nonlinearity of the balance curve
Design
Dietary energy intake and physical activity influences balance
Accounting for adaptation

basal metabolic rate (BMR) in estimating the true energy needs of a subject. Such errors might not be observed in terms of weight change during the few days in which nitrogen balance is measured.

Since nitrogen balance varies as a function of energy intake, it may be argued that protein requirements can only be defined in terms of a specified energy intake level, but what is the appropriate energy intake? Should populations with low protein staples consume more energy to achieve body protein equilibrium? Will this predispose to obesity? To what extent does variation in energy intakes at energy balance (i.e., with increasing levels of physical activity) influence nitrogen balance? These are difficult and currently unanswered questions.

Adaptation

With the metabolic demand for amino acids including both fixed and variable demands, the relationship between intakes and balance will be a function of time and the rate of

adaptation. This is undoubtedly why the determination of human protein requirement by nitrogen balance has proved to be so difficult. Thus, when protein intake changes, the metabolic adjustments involved with matching amino acid oxidation and urea excretion rates to the new intakes take considerable time to adapt to the new level of intake. The actual time taken for complete adaptation is poorly understood and controversial.

In practice, most balance studies are short term, with dietary periods of 2 weeks at each intake studied and with diet periods randomized to minimize metabolic carryover of prior diets. Two weeks is comparable to the time taken to stabilize excretion in subjects fed a protein-free diet while establishing the magnitude of the obligatory nitrogen losses. It may be that adjustment to a protein-free diet, an extreme metabolic change, occurs more rapidly than the adjustment from one intake to another, with evidence of changes over several months to an intake similar to the ONL, and more than 1 month is required to adjust to a lower but adequate intake ($1 \text{ g kg}^{-1} \text{ d}^{-1}$) after 2 months of a high-protein diet of $3 \text{ g kg}^{-1} \text{ d}^{-1}$. As discussed below incomplete adaptation in multi-level nitrogen balance studies will increase the apparent intake for equilibrium and decrease the slope: i.e., underestimate the true efficiency of utilization. It is the case that an efficiency of utilization of 0.47 as shown in Figure 3 for diets which were mostly based on high quality protein is quite inconsistent with standard concepts of protein digestion and amino acid utilization. Furthermore when measured by stable isotope studies, protein utilization of high quality protein is very efficient.

Dietary Protein Allowances for Individuals and Populations and Implications of Adaptation

Nitrogen balance studies aim to determine an individual MPR, and allowances for protein are calculated from the distribution

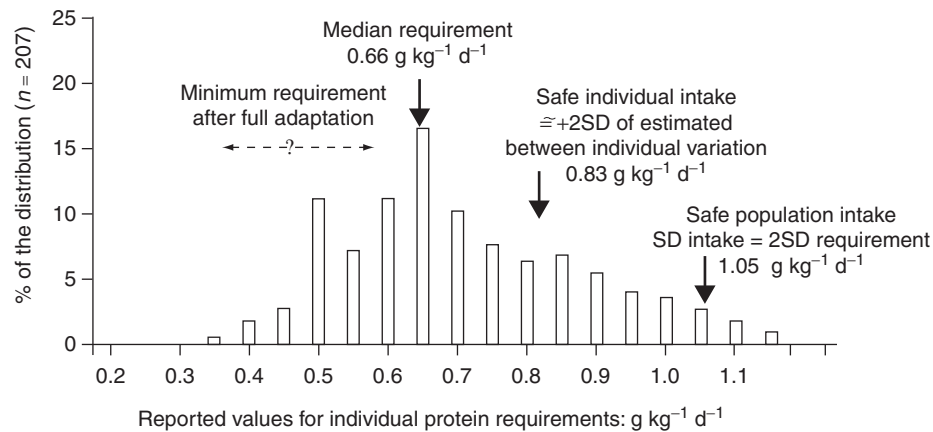


Figure 4 Distribution of reported values for the protein requirements showing values for safe individual and population intakes. The bars are the distribution of individual values of intakes for nitrogen equilibrium expressed as protein equivalents ($n = 224$ individual subjects from $n = 32$ studies, after a 5% trim of outliers) from a meta analysis of N balance data reported by Rand *et al.* (2003). The minimum requirement after full adaptation is not known but likely to be less than the median value within the range shown.

and interindividual variability of these values as shown in **Figure 4**. The average or median requirement is the mid point of the range of individual requirements. The safe individual intake (Recommended Nutrient Intake in the UK) is defined as the 97.5th percentile of the distribution, nominally the average + 2SD. Thus any individual receiving such an intake will have a very low (<2.5%) risk of deficiency (intake < requirement). However it is not generally appreciated that the safe intake for a population will differ from the safe individual intake. This is because calculation of a safe population intake, needs to take into account the distribution of individual intakes as well as requirements. Because the variability of intake is usually greater than that of the requirement, the safe population intake is greater than the safe individual intake, usually approximating to the average requirement + 2SD of intakes or plus 3–5SD of requirements. In fact the average requirement can be used to judge the adequacy of intakes of populations because, on the assumption that intakes and requirements are not correlated, the prevalence of deficiency approximates to the proportion of the population with intakes below the average requirement, which, for a safe population intake, would be about 2.5%, (this is called the cut point method of calculating the approximate deficiency prevalence). A tolerable upper limit (TUL) has not been identified for protein but the evidence suggests that intakes of 3–4 times the safe individual intake are consumed without obvious harm. This would suggest that the TUL may be quite high and certainly higher than the value of $2 \times$ safe intake often assumed in the past.

The serious implication of lack of complete adaptation in short term multi-level balance studies is that because of the very wide range of protein intakes in the human diet, mainly through variable meat intake, the apparent requirement indicated in a study may still reflect the prior habitual diet: i.e., the actual metabolic demands are higher than minimum levels because of the adaptive component of amino acid oxidation set to balance previous intakes. This may explain the very wide range of reported apparent requirements shown in **Figure 4** from below 0.4 to greater than 1.1g protein per kg per day. If adaptation does account for the variability rather than variation in actual protein requirements than a quite different

analytical model would be implied in which the MPR and safe intake are much lower. In this case risk of deficiency for fully adapted individuals would remain low until intakes fell to quite low levels. Such adaptive models pose difficult questions for public health nutrition.

Protein Requirements for Growth and Special Needs

For infants, children, and pregnant and lactating women, protein requirements are derived by a semifactorial analysis of the components of the metabolic demands shown in **Figure 1**, with an assumed efficiency of utilization, all adjusted for individual variation to give the safe intake. The main components of the protein requirements for infants children and adults as reported in the recent WHO/FAO/UNU report are shown in **Figure 5**. The metabolic demands are for maintenance, assumed to be the ONL as derived for adults (as shown in **Figure 3**), and assumed to be the same for all age groups, and the demand for growth derived from measured rates of protein accretion in infants and children. The additional amounts needed to account for the low dietary efficiency of utilization to meet these metabolic demands come from balance studies in adults and children which indicate efficiencies of 47% and 58%. To account for interindividual variability, the safe intakes includes the addition of 2 SDs for maintenance and dietary growth needs, calculated from a coefficient of variation (CV) that is the weighted mean of the CVs for maintenance and growth. Overall the safe intake falls from $1.31 \text{ g kg}^{-1} \text{ d}^{-1}$ at 6 months to $0.83 \text{ g kg}^{-1} \text{ d}^{-1}$ in adults.

Pregnancy Requirements

The values reported by WHO/FAO/UNU allow for protein retention in the products of conception and in the maternal tissues associated with an average gestational weight gain in a healthy women of 13.8 kg estimated from body composition analyses, and for the increased maintenance costs of the increased body weight. The efficiency of dietary protein

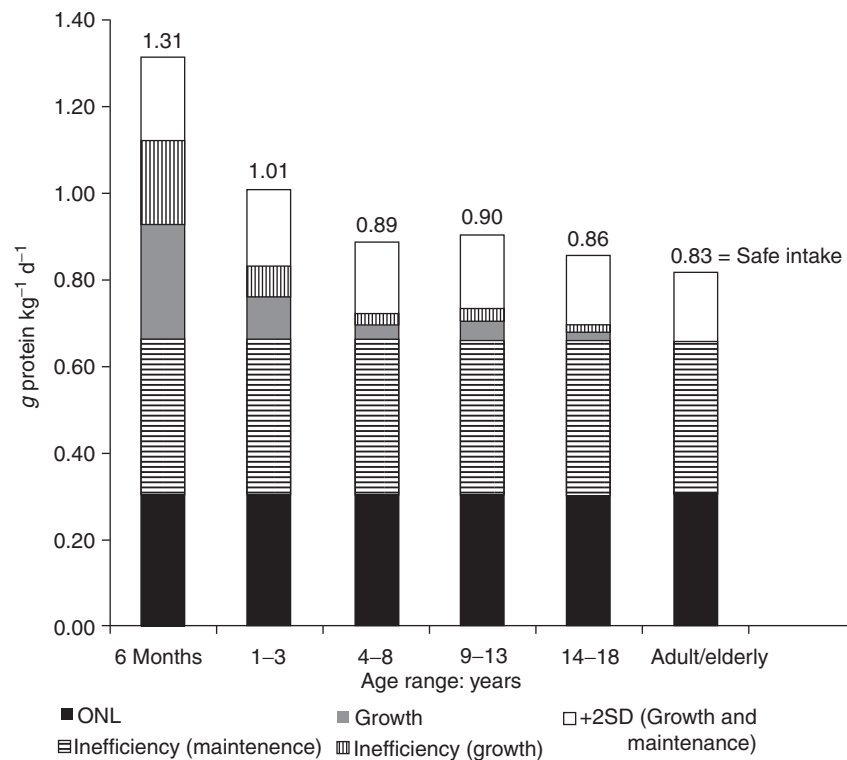


Figure 5 Factorial estimates of protein requirements throughout the lifecycle. Overall values are averages of the separate values for boys and girls.

utilization to meet these growth costs is assumed to be 42% a value observed in nitrogen balance studies in primiparous teenagers, and the safe intake is calculated assuming a CV of 12%. This equates to additions of 0.7, 9.6, and 31.2 g protein per day. Lactation requirements of 19 g d⁻¹ and 12.5 g d⁻¹ derived from the demand observed as average milk protein output of well-nourished women exclusively breastfeeding for the first 6 months and partially breastfeeding after this time adjusted for an efficiency of utilization of 47% as observed in N balance studies in adults shown in Figure 3.

Areas of Uncertainty

Requirements for Infants and Children, Pregnancy and Lactation

Definition of protein requirements has historically been problematic and successive reports although dealing with some areas of criticisms have never been without new areas of controversy. In the most recent report a major consequence of the difficulty associated with interpreting nitrogen balance studies is not only uncertainty about the adult protein requirement as discussed above but also the possibility of overestimation of requirements for the other population groups for whom factorial models have been used to calculate the requirements. As is apparent in Figure 5 for children and adolescents the metabolic demand for maintenance and growth accounts for only half of the average requirement because the assumed efficiency of dietary utilization is only about 50%, a low efficiency for which there is no biological

explanation. A similar low efficiency is assumed in calculating the requirements for pregnancy. If the actual efficiency is higher, as assumed in previous reports, then the true requirement values would be lower. Although overestimation of requirements for infants and children is unlikely to result in harm, this may not be the case for pregnancy. In fact there is evidence that excessive protein intakes in pregnancy can have adverse effects on pregnancy outcomes so that if any additional dietary intakes are recommended for pregnant women these should comprise of a normal healthy diet. In the WHO/FAO/UNU report advice is given that the additional protein during pregnancy should consist of additional normal food, rather than high protein supplements. During the third trimester an extra 31 g d⁻¹ of protein would represent an extra 3.6 MJ of a mixed diet assuming it contains 15% protein energy. This is considerably more additional energy than is recommended at this stage of pregnancy by any agency. Indeed given the concern for adverse influences of overweight and obesity on pregnancy outcomes and given that pregnant women often have successful pregnancy outcomes without any increase in food intake, it is likely that this amount of additional food would result in excessive weight gain.

Optimal Protein Intakes and Implications of Adaptation for Nutrition Policy

In general, protein requirements serve two purposes. One is as a basis for prescription (i.e., advice on safe diets through recommending appropriate dietary intakes). Adaptation implies a low but difficult to define MPR. Indeed, since natural diets, providing sufficient energy and other nutrients, usually

provide considerably more than the minimal amount of protein, the magnitude of the MPR becomes to some extent an issue of scientific curiosity only. Formulation of policy in relation to prescriptive matters will inevitably and correctly be most concerned with satisfying the upper range of demands for protein and, where there is uncertainty, include positive margins of error. In this case, it is arguably unwise to adopt an adaptive model and reduce the MPR, even if agreement could be reached on the likely lower limit of adaptation. Indeed, an adaptive model does not imply that protein is an unimportant nutrient for the maintenance of human health and well-being but that indicators other than balance (nitrogen, protein, or amino acid) need to be identified. Thus, the most relevant measure is an optimal requirement allowing balance and supporting both optimal body function and minimum risk of chronic disease. There is increasing experimental evidence for the potential benefit of protein intakes considerably higher than the current MPR for bone health in the elderly and epidemiological evidence for benefit with respect to hypertension and ischemic heart disease. However, such influences are unproven, with no plausible mechanism identified in the latter cases. In any case, there are no quantifiable indicators. This results in a dilemma for those attempting to frame prescriptive dietary guidelines. From this perspective, it is probably wise to retain current values as an operational expedient until it becomes possible to quantify the benefits (and any risks) of protein intakes within the adaptive range.

The other purpose of requirement recommendations is as a diagnostic indicator of deficit risk, often within an epidemiological context in which population groups rather than individuals are considered. In this case, indicators used to estimate prevalence of disease states or deficit risk are carefully chosen so as to strike an acceptable balance between false positives and false negatives. The main implication of adaptation for estimating risk of deficiency as intakes become less than requirements is a dramatic reduction in the prevalence of risk for most populations compared with that assessed according to the traditional model, which does not account for adaptation. This occurs because the requirement and intake can be assumed to be correlated and because the actual MPR and safe intake calculated from it will be lower. As in the prescriptive context, this low risk of deficiency applies only to that of being unable to maintain nitrogen balance after full adaptation with otherwise nutritionally adequate diets satisfying the energy demands. Whether such populations enjoy optimal protein-related health in terms of immune function, bone health, or any other function is a separate issue and needs to be addressed as such. From this perspective, it follows that maintenance of nitrogen balance can no longer be used as

a surrogate of adequate protein-related health, and that current lack of quantifiable alternative indicators is no excuse for ignoring the issue of adaptation.

See also: Amino Acids: Metabolism; Specific Functions. Children: Nutritional Requirements. Nutritional requirements of Infants. Older People: Nutritional Requirements. Protein: Quality and Sources; Synthesis and Turnover. Pregnancy: Safe Diets. Protein Deficiency. Protein Digestion and Bioavailability

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Synthesis and Turnover

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Whole-Body Protein Homeostasis

The regulation of the protein mass of the body requires mechanisms that control the protein content within cells, organs, and tissues and that coordinate this control during growth and body weight maintenance. Because all intracellular proteins exhibit turnover, regulation of the cellular protein content involves control of both protein synthesis and proteolysis. For some proteins, such control is understood in considerable detail. Less is known about the coordinated control of intracellular protein turnover to maintain an appropriate cellular composition, and even less is known about whole body coordination. There are two aspects of control: acute control during feeding and fasting and chronic control during growth and long-term maintenance.

The diurnal cycle of feeding and fasting characteristic of human nutrition results in gains and losses of body protein in overall nitrogen balance. Furthermore, such diurnal cycling occurs with an amplitude that increases with increasing dietary protein intakes. These oscillations in body protein content involve changes in whole body and tissue protein synthesis, proteolysis, and amino acid oxidation. Much effort has been invested in identifying the control mechanisms, particularly those that mediate the anabolic drive of dietary protein, i.e., essential amino acids and the anabolic hormones stimulated by dietary protein, and how these influences control the efficiency of postprandial protein utilization.

Long-term homeostasis is a less well understood phenomenon. For the slow-growing long-lived human, most of the life span involves a constant body weight and the

remarkable phenomenon of this long-term constancy of body protein at a characteristic mass is a particularly challenging problem. Regulatory mechanisms exist that both allow restoration of body weight and especially protein content to its target size after an insult that induces wasting, (i.e., catch-up growth), and that prevent the continuation of growth after that target size has been reached. However such mechanisms are poorly understood.

One approach to the problem has involved the concept of a protein-stat mechanism, the central feature of which is an interaction between linear growth of bone, protein deposition in skeletal muscle, and dietary protein intake, with the growth of most other organs secondary to this interaction (**Figure 1**). Within this context whole body protein content is controlled through an amino-static appetite mechanism, acting primarily to maintain skeletal muscle mass at a level set by the linear dimensions of the organism. Bone lengthening occurs at rates determined by genetic programming and an appropriate hormonal anabolic drive, exerted by dietary protein. Bone lengthening controls, by passive stretching, net protein deposition in skeletal muscle mainly through the regulation of new connective tissue synthesis that controls muscle volume. Some level of muscle activity is also required for maximal muscle size. Provision of amino acids to allow muscle to accumulate myofibrillar protein and increase to its phenotypic size is regulated through appetite stimulation that in some way monitors net amino acid flow into muscle. This is most obvious in catch-up growth. After muscle wasting with loss of myofibre protein there is potential for expansion within the pre-existing connective tissue framework. Muscle growth

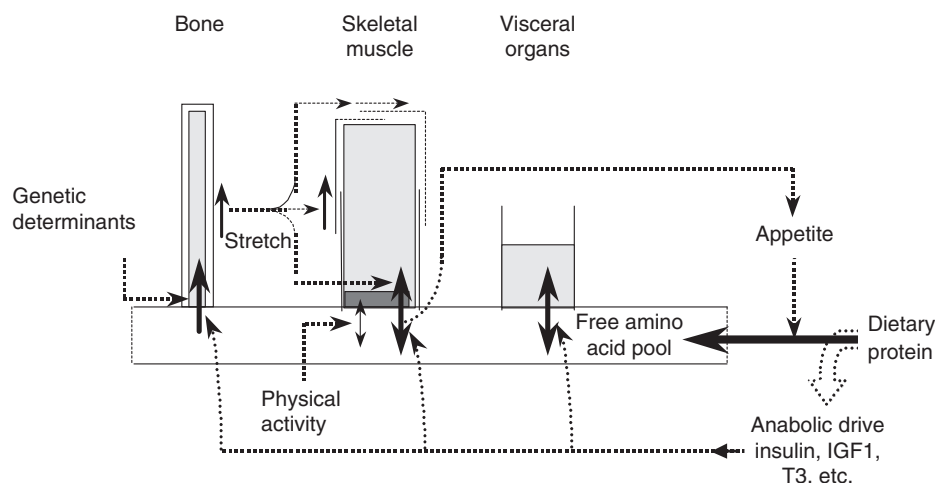


Figure 1 Protein-stat mechanism for coordinated control of body protein growth and maintenance. The protein content of skeletal muscle is controlled by long bone growth mediated through a passive stretch mechanism and anabolic signals in response to dietary protein intake, with the growth of most other organs secondary to this interaction, i.e., growth rate for these organs is a function of metabolic and functional demand in response to food intake. For details of regulatory mechanisms see Millward DJ (1995) A protein-stat mechanism for regulation of growth and maintenance of the lean-body mass. *Nutrition Research Reviews* 8: 93–120.

ceases in the absence of passive stretch when bone length growth ceases. The growth of most other organs is secondary to this main interaction, determined primarily by the level of protein intake and the consequent metabolic work and functional demand for the organ and is not specifically limited in size. Such a mechanism would mean that any reduction in bone length will result in loss of muscle mass and this may be one explanation of sarcopenia, the loss of appendicular muscle in old age. Although long-bone shortening in old age has not been described in detail, some shortening may accompany loss of mineral mass with osteoporosis and given the sensitivity of muscle mass to changes in bone length, (muscle mass \approx length⁴), it may be that only very small reductions in length are sufficient to mediate measureable sarcopenia.

Protein Turnover

Protein turnover occurs because of the presence within cells of proteolytic systems that degrade proteins for a variety of reasons ranging from the removal of proteins with an incorrect primary amino acid sequence (error proteins) to the provision of free amino acids during nutrient deprivation. However the half-lives of individual proteins vary over at least three orders of magnitude within the same cells, identifying the process as specific. The nature and control of proteolysis is poorly understood. The physico-chemical structure, especially hydrophobicity and ionic charge influences susceptibility to proteolysis. Also an amino acid sequence (PEST; proline, glutamate, serine and threonine) influences susceptibility to proteolysis and occurs in several rapidly degraded proteins. The molecular basis for this remains largely unknown. With heterogeneous turnover of proteins within structures such as mitochondria, myofilaments, and multienzyme complexes although maintaining functional integrity, complex co-regulation is required with evidence for at least three different systems involved in the case of skeletal muscle.

The lysosomal-autophagic system is present in all cells and involves acid proteinases (cathepsins), within a distinct vacuolar structure capable of engulfing and degrading complete organelles, ribosomes as well as individual intracellular proteins and proteins entering cells *via* endocytosis. Lysosomal proteolysis is complete and most is known about hepatic macroautophagy where hepatic protein mass appears to be regulated by a receptor-mediated amino-acid dependent inhibitory process.

The ubiquitin-proteasome system is widely distributed among tissues, with a relatively broad protein specificity, catalyzing the hydrolysis of protein to peptides averaging about eight amino acids long, and exhibiting an adenosine triphosphate (ATP)-dependency. It involves two components. One is a recognition system involving an ATP-dependent formation of a covalent link between the protein and a short polymer of ubiquitin, which is responsible for targeting the protein substrates toward proteolysis. This phase involves three separate reactions: ATP-dependent activation of ubiquitin; conjugation of ubiquitin to cellular proteins, and proof-reading of the conjugates to either regenerate the target protein by removal of ubiquitin or commit the target protein to proteolysis by further ubiquitinylation. Proteolysis is

mediated by the giant multifunctional protease, the proteasome. This comprises a Core Particle made of duplicate sets of at least 14 different proteins, assembled in groups to form rings that are in turn stacked to form a donut like structure within which the ubiquitin conjugated proteins are unfolded by another ATP-dependent process and proteolytically cleaved to form peptides. A regulatory particle both delivers the ubiquitin conjugated proteins to the Core Particle and also removes ubiquitin from the peptides released after proteolysis. Proteolysis of the peptides is achieved by other systems, including the lysosomal system, since degradation of ubiquitinated proteins can also be achieved by the lysosome, or other poorly described proteinases and peptidases including the giant protease tripeptidyl peptidase II (TPP II) and various aminopeptidases. The relative importance of the two main systems capable of complete proteolysis, the lysosome and proteasome, in various tissues remains uncertain. Most work on the regulation and activation of the ubiquitin-proteasome system has involved the accelerated proteolysis in skeletal muscle atrophy, antigen processing, and removal of aberrant proteins, rather than basal and nutritionally sensitive proteolysis.

The third proteolytic system involves calpain and calpastatin, a calcium-activated proteolytic pathway that can initiate but not complete proteolysis. This comprises a highly conserved family of nonlysosomal calcium dependent cysteine proteases comprising two ubiquitous isoforms (calpain I and II), several tissue specific isoforms, and a 28 kDa regulatory subunit (calpain 4). *In vivo* calpain activity is tightly regulated by its endogenous and highly specific inhibitor, calpastatin. There is little evidence to suggest a role in nutritionally sensitive basal proteolysis.

Models and Tracer Methods for the Study of Protein Turnover

Study of protein turnover has utilized isotope tracer techniques, radioactive tracers (³H, ¹⁴C, and ³⁵S) in animals and stable isotopes (¹³C, ¹⁵N, and ²H) in human studies. Most studies utilize simplified models; the simplest and most widely used (Figure 2) based on the measurement of the amino acid flux through the plasma amino acid pool. An example is the primed continuous intravenous infusion of ¹³C-1 labeled leucine. During the infusion, the tracer isotopic enrichment is diluted by unlabeled amino acid from proteolysis (D) and the diet (I). At isotopic equilibrium, constant labeling of the tracee is achieved, the magnitude of which, (tracer/tracee ratio), in relation to the infusion rate, (i), indicates the flux, (Q), that is the total entry or exit rate of leucine through the pool. With the free leucine pool relatively small and turning over rapidly, isotopic equilibrium can be reached in 2–4 h if a priming dose is given as a bolus injection at the start of the infusion.

At isotopic and metabolic equilibrium, rates of entry and exit from the free leucine pool are equal for both labeled and unlabeled leucine so that Q is the rate of appearance or irreversible loss. Appearance is partitioned into dietary intake that is known, (I), and entry from proteolysis of body protein, (D), (i.e., no *de novo* synthesis of leucine occurs). Loss is

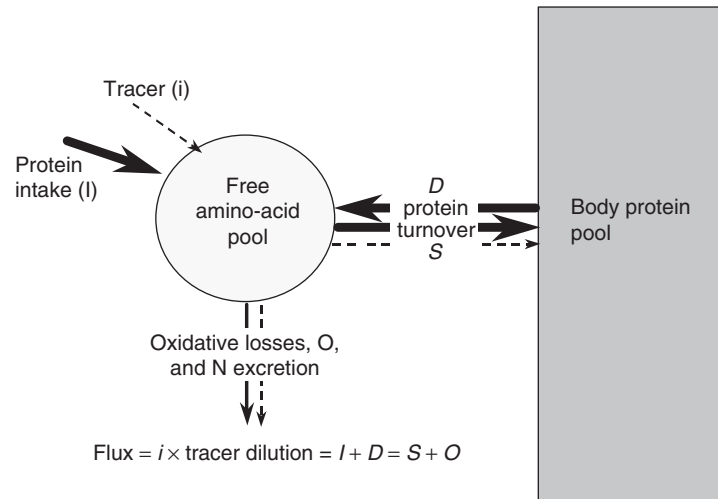


Figure 2 Single pool model for study of amino acid flux and protein turnover by tracer labeled amino acid infusion. Movement of tracer is shown as dotted lines.

partitioned into protein synthesis (S) and oxidative catabolism (O) to CO_2 and urinary N . The rate of leucine oxidation (O) is calculated from measurement of the production of labeled $^{13}\text{CO}_2$ in the breath and the labeling of the leucine or its keto acid in the plasma. This allows the components of protein turnover (D and S) to be calculated. Using the leucine content of tissue proteins, rates of leucine appearance and loss can be converted into rates of whole-body protein synthesis and proteolysis.

^{13}C -1 leucine is especially useful since its essentiality enables D to be calculated; it has a small pool enabling equilibrium to be achieved in a short period; decarboxylation is the first irreversible step in its catabolism releasing $^{13}\text{CO}_2$ quantitatively; and its transamination product α -ketoisocaproate appears in the plasma and can serve as a measure of the labeling of the intracellular pool.

This latter advantage of leucine is especially important since the problem of definition of isotopic enrichment of the precursor amino acid pool for protein synthesis is the most serious problem in these studies. Thus, amino acid pools are compartmentalized in the body, and the isotopic enrichment of transfer RNA (tRNA)-bound amino acids is lower than the measurable extracellular amino acid pool. Because it is difficult to measure labeling of amino acyl tRNA, a variety of indirect approaches have been used in an attempt to circumvent the problem. Equilibrium labeling of apolipoprotein B-100 has also been used to measure isotopic enrichment of hepatic amino acids. Thus, because apo B-100 turns over with a half-life of less than 1 h, during an infusion of several hours the protein labeling will reach a plateau level representative of the hepatic precursor pool, and this has indicated a complex relationship between plasma and precursor enrichments for phenylalanine and leucine.

Flux rate measurements define whole body rates of protein synthesis and proteolysis and each measurement is subject to error associated with the precursor assumption. Alternative methods have attempted to measure protein synthesis and proteolysis separately. In animal studies, a flooding large dose measurement of protein synthesis has been developed that

enables all free pools to become equally labeled. Protein synthesis rates are calculated from measurement of isotope uptake into protein and free amino acid labeling during short periods after the dose. The method has been adapted for human use with a stable isotope but there is evidence that the large quantities of the single amino acid stimulates protein synthesis.

Quantification of rates of proteolysis is especially problematical. In animal studies proteolysis can be estimated from rates of synthesis and growth of tissue protein and with careful design proteolysis rates can be measured over relatively short periods (e.g., at 6-hourly following the administration of an endotoxin). Urinary excretion rates of 3-methyl histidine, a post-translationally modified amino acid not metabolized in the organism and excreted quantitatively in the urine, were proposed as a measure of myofibrillar protein degradation. Although the substantial contribution of small rapidly turning over pools in microfilaments invalidates this approach as far as whole body studies are concerned, its release from incubated or perfused muscle can be used to determine myofibrillar protein degradation.

Simultaneous determination of protein synthesis and degradation can be made, in principle at least, from organ tracer balance studies, (i.e., measurements of concentrations and isotopic enrichments of tracer amino acids across tissues like leg or forearm that have been combined with measurements of 3-methyl-histidine release). Such studies have identified a selective inhibitory effect of insulin on nonmyofibrillar protein degradation and stimulatory influences of amino acids on muscle protein synthesis.

All of these methods allow study of turnover of individual amino acids in protein and measurement of their nonprotein metabolic fate (e.g., oxidation). A different approach is to use ^{15}N glycine to study overall amino nitrogen turnover. Because of nitrogen exchange between amino acids by transamination, this label acts as a tracer for total free amino nitrogen, rather than for any individual amino acid. The whole body nitrogen flux is estimated from the relative proportion of administered tracer excreted in the end product.

This is then resolved into protein synthesis and proteolysis from measurements of N intake and excretion. The application of the method can be made simple by giving the ^{15}N label orally as a single dose. Although simple in concept this approach is metabolically complicated with two urinary end products of nitrogen metabolism, urea and ammonia, each deriving from different pathways and each giving different flux values.

The choice of method must depend on the questions asked and circumstances of the subjects under study. ^{13}C carbon labeling is more suited to short-term (e.g., 3 or 4 h to 24 h) clinical measurements for which frequent blood and breath sampling is possible. Thus, the efficiency and mechanisms of postprandial protein utilization during meal feeding can be measured by means of ^{13}C leucine balance and turnover measurements. ^{15}N methods are more suitable for free living subjects and patients, when urine sampling is possible but regular blood and breath sampling are inconvenient. The most famous example is use of this method in an unassisted Antarctic crossing. Both methods involve many assumptions but in practice the two approaches have been shown to give similar results.

Applications

Extent and Physiological Implications of Protein Turnover

In the human adult about 300 g of protein turnover occurs each day, ($4 \text{ g kg}^{-1} \text{ day}^{-1}$) that is, three or four times the daily dietary intake. Rates vary between tissues with rapid turnover in visceral tissues and slow turnover in muscle. Liver and intestine account for about 8% of the lean body mass (LBM) and up to 50% of whole body protein turnover with skeletal muscle, at 55% of the LBM, accounting for only about 25% of total protein turnover. Thus, whole body protein turnover varies with body composition and this largely explains developmental changes. In the infant, turnover rates are much higher, ranging between 10 and 20 g protein turnover per kilogram per day, consistent with the higher proportion of metabolically active tissue and lower muscle mass. However animal studies indicate a developmental fall in protein turnover in specific skeletal muscle types that may be an additional component of the marked fall in protein turnover with age. It is not clear whether such changes occur in adult skeletal muscle with evidence suggesting only small changes in protein turnover, if any, in the elderly other than that associated with the fall in lean body mass.

Protein turnover constitutes an appreciable fraction of the maintenance energy expenditure. On the basis of 5 moles ATP/guanosine triphosphate (GTP) per mole of protein turnover, (4 moles per peptide bond with an extra mole for amino acid transport, RNA turnover and proteolysis), an energy cost of 22 kcal mol^{-1} ATP, and a molecular weight of 110 per mole of peptide bond, this is equivalent to about 1 kcal g^{-1} protein turnover. Thus, in the normal adult, protein turnover at 300 g d^{-1} accounts for about 20% of the basal metabolic rate. Therefore, changes in the protein turnover and metabolic rate would be expected to occur in parallel to some extent, and this is observed. Thus the fall with age in both protein turnover

and metabolic rate from birth to adulthood involve a factor of 3–4 in each case.

Regarding protein turnover and protein requirements there is no *a priori* reason for any interrelationship and little evidence of any. Thus turnover does not consume amino acids and amino acid catabolism and oxidation is not linked to turnover. Maintenance protein requirements decrease relatively little with age, ($<20\%$) compared with the three- to four-fold fall in turnover.

Regulatory Mechanisms of Protein Turnover Control

The physiological importance of protein turnover is undoubtedly the regulatory flexibility it allows. With opportunities for control of both synthesis and proteolysis the number of potential control sites is increased. In addition because of the continuing turnover in the steady state, changes in amounts of protein can be achieved with low energy costs through inhibition of proteolysis to allow growth or inhibition of synthesis to allow mobilization.

At the molecular level, regulation of protein synthesis is necessarily complex at both transcriptional and translational levels. Advances in molecular biology have revealed many examples of transcriptional control to the extent that changes in specific messenger RNA (mRNA) concentrations have become a surrogate measure of changes in rates of synthesis for specific proteins. Notable nutritional examples include control of hepatic export protein synthesis. Thus the down regulation of albumin synthesis in response to either protein deficiency or the proinflammatory cytokine-mediated acute-phase response is mediated largely at the level of transcriptional control of mRNA levels, with reductions in mRNA for albumin and other hepatic export proteins and increases in mRNA for acute phase proteins.

The concentration of ribosomes in tissues determines the capacity for protein synthesis and in this way controls overall tissue protein turnover rate and the changes associated with postnatal development. Cellular ribosome concentrations can change both acutely, e.g., during the diurnal cycle of feeding and fasting, and chronically in response to protein and energy intakes, increased functional demand, and hormones such as insulin, thyroid, growth hormone, and the glucocorticoids. Furthermore these influences are tissue specific with glucocorticoids, for example, increasing hepatic ribosome concentrations (as part of the hepatic acute phase response) and decreasing ribosome concentrations in muscle. In contrast thyroid hormones increase ribosomes (and proteolytic enzymes) in both muscle and liver in association with a generalized increase in protein turnover.

Acute regulation of translation is exerted mainly through initiation, with reversible phosphorylations known to regulate at least four separate steps of the initiation cycle enabling very rapid changes in protein synthesis. Peptide hormones, (insulin and insulin like growth factor (IGF)-1), glucocorticoids, and amino acids have all been implicated in such regulation although the specific targets of such control remains uncertain. Furthermore major differences exist between the mechanisms observed in the young rapidly-growing compared with the adult animal. Thus in skeletal muscle in the young

rat, an insulin-mediated stimulation occurs. In adult human muscle insulin is relatively ineffective with amino acid levels the main stimulatory influence. Indeed with insulin inhibiting proteolysis and lowering amino acid levels, insulin alone appears to inhibit protein synthesis in human muscle.

Regarding the nutritional regulation of proteolysis, most is known about lysosomal proteolysis especially hepatic autophagy, with both amino acids and insulin having inhibitory roles. Leucine, alanine, and insulin interact in regulating this pathway, with a leucine sensitive receptor-mediated inhibitory pathway identified in liver. In the case of the ubiquitin-proteasome system its activation in skeletal muscle during fasting and following glucocorticoid treatment does support a role on the physiological regulation of protein turnover. On the other hand both lysosomal and calcium-activated proteolysis are activated under the same conditions. Similarly in response to protein deficiency when protein turnover rates generally fall in tissues, in part through the fall in thyroid-hormone levels, the activities of all three systems fall. One control mechanism involves changes in cell volume. Thus, swelling acts like a proliferative anabolic signal, inhibiting proteolysis whereas cell shrinkage is catabolic, stimulating proteolysis. These effects have been shown in liver and there is some evidence for such a mechanism in skeletal muscle.

Postprandial Protein Utilization

Overall nitrogen homeostasis within the lean body mass is maintained within a diurnal cycle of postprandial protein gain and postabsorptive loss. The amplitude of these diurnal changes increases as habitual protein intakes increase with implications for the qualitative nature of the metabolic demands for amino acids and hence dietary protein. The key questions are how both acute and chronic protein intakes mediate such responses and, most importantly, what influences the efficiency of postprandial protein utilization and consequent protein requirements. Nutritional regulation of dietary protein utilization, protein turnover, and amino acid oxidation involve both hormonal responses to food intakes and direct substrate influences. Whereas interactions between insulin, thyroid hormones, and IGF-1 mediate the anabolic drive of dietary protein on muscle and bone growth in the growing animal, the control mechanisms involved in the transient gains and losses of protein during diurnal cycling differ since neither thyroid hormones nor IGF-1 levels vary from meal to meal or in relation to habitual protein intakes. On the basis of several studies with either insulin or amino acids alone or variation in meal protein levels it would appear that insulin and amino acids act as main acute regulators.

The mechanisms involved are best understood in the context of the interrelationships between the free and protein bound amino acid pools. Many indispensable amino acids are potentially toxic and are maintained at very low concentrations in tissues so rapid and regulated postprandial disposal is important. After a protein meal two pathways for amino acid disposal exist. The first comprises the various high-capacity, finely regulated oxidative pathways activated by a protein meal. In most cases rates of amino acid oxidation are influenced by their tissue concentrations (generally similar to the

K_m of the rate limiting enzymes), together with substrate activation and covalent enzyme modification. Examples are phenylalanine hydroxylase, and branched-chain α -keto dehydrogenase, which are both regulated by substrate binding and reversible phosphorylation and dephosphorylation. The second pathway is net protein deposition. This can be achieved by stimulation of protein synthesis or inhibition of proteolysis so that a regulatory link between postprandial hyper-amino acidemia and protein synthesis and proteolysis might be expected and does indeed exist.

For protein synthesis, amino acids cannot exert simple kinetic concentration-related influences, since the low K_m of amino acyl tRNAs synthesis means they are usually fully charged. Nevertheless there is ample evidence for a regulatory stimulation by amino acids. One key protein in the amino acid mediated signal transduction pathway is the mammalian target of rapamycin (mTOR), part of the mTOR complex 1 (mTORC1) which regulates muscle protein synthesis *via* phosphorylation of 4E-BP1 and p70s6k consequently activating initiation activators eEF2K and eIF4G. However stimulation of protein synthesis through increased intracellular amino acid levels may also stimulate amino acid oxidation pathways as discussed above and although this allows effective removal of amino acids, in the context of an efficient protein utilization this would not be a preferred mechanism unless signal transduction could commence in the extracellular amino acid pool, i.e., increases in plasma amino acid concentrations can signal an increase in protein synthesis whereas intracellular amino acids need not increase. There is increasing evidence that this is the case.

For proteolysis, amino acids exert an inhibitory influence as described above, and this inhibition will reduce endogenous amino acid supply. This will prevent undue increases in amino acid levels and will therefore minimize amino acid oxidation and maximize dietary protein utilization. Furthermore since inhibition of proteolysis and lowering of intracellular amino acid levels can be achieved by receptor mediated mechanisms involving insulin as well as specific amino acids (e.g., leucine) this allows the postprandial increases in plasma amino acids to mediate substantial amino acid transport into cells, allowing protein deposition without any increase in intracellular amino acid levels and with minimal increases in amino acid oxidation. Thus as a strategy for mediating postprandial protein utilization, inhibition of proteolysis would be predicted to be more efficient.

^{13}C leucine studies have provided clear experimental support for such a mechanism. The meal protein-dependent responses of protein synthesis, proteolysis, and leucine oxidation are shown in [Figure 3](#) based on measurements in adult subjects fed isoenergetic meals of increasing protein intake from 0.36 to 2.07 g protein per kg per day. An inhibition of proteolysis occurs at all levels of protein intake, but to an increasing extent with intake. However, the direction and magnitude of the response of protein synthesis reflects the level of dietary protein intake, with slight inhibition or no change at low intakes and stimulation at high intakes. Such studies clearly establish the importance of proteolysis as a regulator of tissue protein balance in the postabsorptive and postprandial state. These and other ^{13}C leucine studies of postprandial protein utilization have allowed the separate

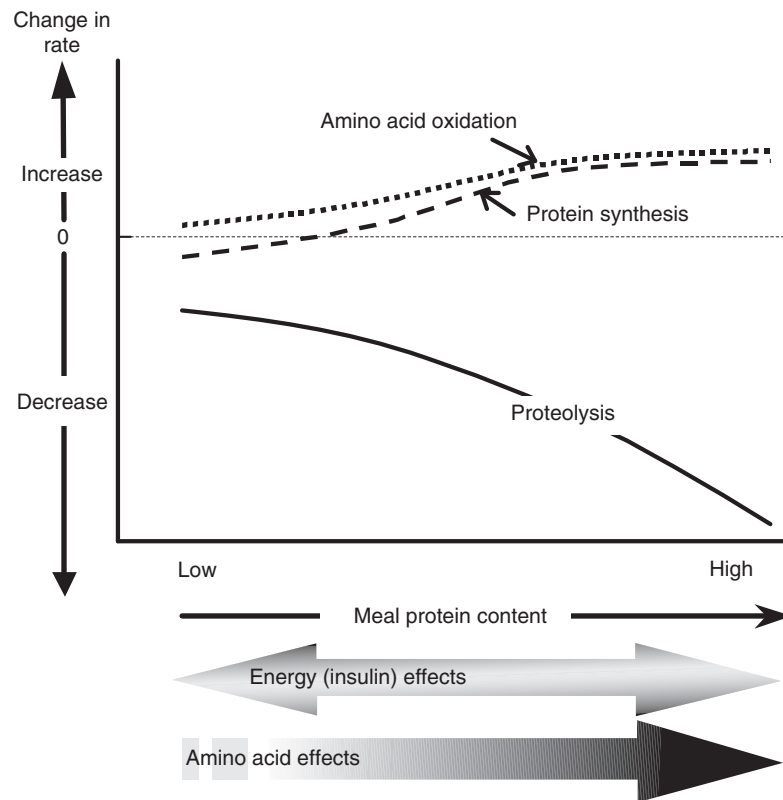


Figure 3 Feeding induced responses of leucine kinetics shown as protein synthesis, proteolysis, and leucine oxidation. Patterns of responses reflect actual changes observed in ^{13}C -1 leucine infusion studies of feeding responses to frequent small meals of increasing protein intake, equivalent to daily intakes between 0.3 and $2.0 \text{ g kg}^{-1} \text{ d}^{-1}$. Adapted from Pacy PJ, Price GM, Halliday D, Quevedo MR, and Millward DJ (1994) Nitrogen homeostasis in man: 2. The diurnal responses of protein synthesis, degradation and amino acid oxidation to diets with increasing protein intakes. *Clinical Science* 86: 103–118, and Gibson NR, Fereday A, Cox M, Halliday D, Pacy PJ, and Millward DJ (1996) Influences of dietary energy and protein on leucine kinetics during feeding in healthy adults. *American Journal of Physiology* 33: 282–291.

influences of dietary energy and amino acids protein to be identified as shown in **Figure 3**. The response to energy alone involves insulin-mediated changes allowing leucine balance to become less negative through an inhibition of proteolysis with minimal changes in protein synthesis or in amino acid oxidation. In fact because this tends to lower amino acid levels, there is a fall in protein synthesis. With increasing amino acid supply as the dietary protein intake increases there is a further inhibition of proteolysis by amino acids with increases in protein synthesis and to some extent amino acid oxidation, allowing net protein deposition as tissue protein.

The increase in amino acid oxidation with protein feeding is an unwanted response that reduces the efficiency of protein utilization. Although utilization of proteins such as milk is higher than that of wheat, as would be expected because of the lysine limitation of wheat gluten utilization, there is variability between individuals in the efficiency of postprandial protein utilization, ranging from 50% to 100% with milk protein. **Figure 4** shows the results of studies designed to examine this variation. Efficient utilization involves maximal inhibition of proteolysis by protein feeding with minimal increases in free intracellular amino acid concentrations and consequent amino acid oxidation and with stimulation of protein synthesis mediated by an extracellular amino acid sensing mechanism, indicating that the efficiency of protein

utilization in individuals is determined mainly by the sensitivity of the insulin-mediated inhibition of proteolysis to amino acid supply.

Current understanding suggests a mechanism indicated in **Figure 5** in which the major target of insulin is inhibition of proteolysis with extracellular amino acids acting to both enhance the inhibition of proteolysis and stimulate protein synthesis and with intracellular amino acids stimulating their own oxidation. With extracellular amino acid levels controlled by diet, and tissue levels controlled by endogenous supply from proteolysis, inhibition of proteolysis will minimize any increase in amino acid levels, minimize oxidation, and maximize protein utilization. Since protein synthesis and amino acid oxidation appear to be stimulated in parallel, the optimum strategy for maximum efficiency of postprandial protein utilization would appear to involve maximal inhibition of proteolysis ensuring minimal postprandial increases in tissue amino acid levels with a stimulation of protein synthesis mediated from extracellular amino acid supply.

In summary, postprandial food protein utilization appears to be mediated by both insulin and amino acid supply, the insulin effect mediated mainly by the energy (especially carbohydrate) component of the food and exerting a protein-conserving influence that inhibits proteolysis, lowers amino acid levels, and reduces oxidation, with dietary amino acids

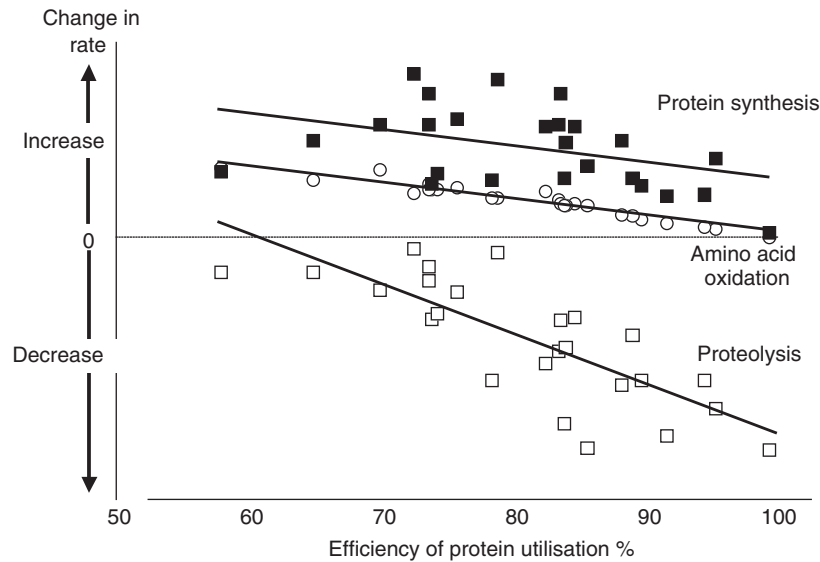


Figure 4 Relationships between the efficiency of protein utilization ($\Delta\text{balance}/\Delta\text{intake}$) and responses of leucine turnover and oxidation to protein feeding observed in 24 normal adults fed frequent small meals containing protein intakes similar to the habitual intakes of the subjects. Adapted from Fereday A, Gibson NR, Cox M, Pacy PJ, and Millward DJ (1998) Variation in amino acid mediated, insulin activated inhibition of proteolysis determines the efficiency of protein utilization. *Clinical Science* 95: 725–733, with permission from Portland Press.

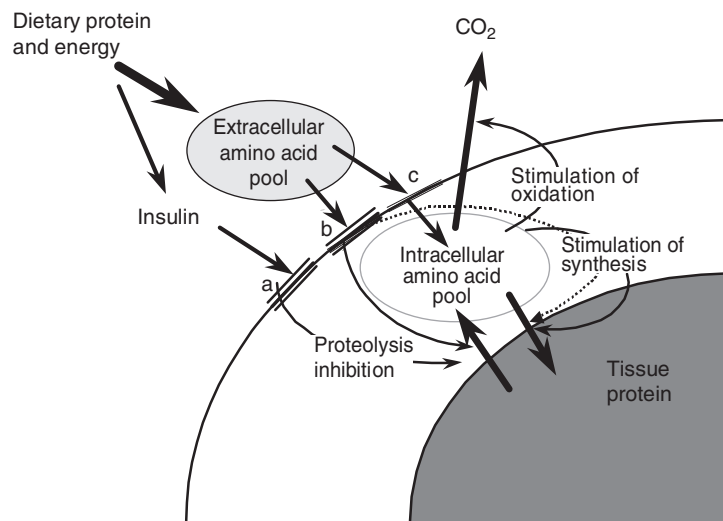


Figure 5 Scheme for the action of insulin and amino acid supply on postprandial protein utilization. Insulin and extracellular amino acids exert inhibitory influences on proteolysis and protein synthesis through receptor mediated mechanisms (a and b) whereas amino acid uptake (c) and proteolysis regulate intracellular amino acid levels, amino acid oxidation, and protein synthesis in parallel. Maximal inhibition of proteolysis and maintenance of low intracellular amino acid levels is the optimal response. Reproduced from Millward DJ, Fereday A, Gibson NR, and Pacy PJ (1996) Postprandial protein metabolism. *Baillier's Clinical Endocrinology and Metabolism* 10: 533–549.

augmenting the inhibition of proteolysis and increasing protein synthesis. To maximize protein synthesis without stimulating amino acid oxidation intracellular amino acid levels need to be maintained at a sufficient level to enable availability for net protein synthesis with a falling endogenous supply following insulin mediated inhibition of proteolysis but not an excessive level which would stimulate their oxidation. This is ensured by extracellular signaling by the postprandial increases in plasma amino acid levels. When amino acid dietary supply exceeds the capacity for its net deposition

intracellular amino acids will rise stimulating their disposal via oxidation.

See also: Amino Acids: Metabolism; Specific Functions. Children: Nutritional Requirements. Nutritional Requirements of Infants. Older People: Nutritional Requirements. Pregnancy: Safe Diets. Protein Deficiency. Protein Digestion and Bioavailability. Protein: Quality and Sources; Requirements and Role in Diet

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R

REFUGEES

Nutritional Implications

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Glossary

Community therapeutic care program (CTC) This program is a new approach to managing malnutrition at the community level. A CTC program has the same initial metabolic stabilization phase as a traditional feeding program, and life-threatening infections are identified and treated in the same way. However, once the patient is stabilized they move directly to an outpatient therapeutic program that operates through existing health structures and initiates nutritional rehabilitation with ready to use therapeutic foods (RUTFs). When there is no longer a risk of severe malnutrition, they are referred to supplementary feeding programs for recuperation.

Internationally displaced persons (IDPs) These are persons fleeing from war, civil disturbance, and violence of any kind but who do not cross international boundaries.

Ready to use therapeutic foods (RUTFs) These foods are ready to eat, and high in energy and protein. They also contain micronutrients and electrolytes. Their main use is for treatment of malnutrition. Their use to prevent stunting and wasting is also being evaluated.

Special feeding program This program deals with provision of high-quality foods to be consumed in addition to the usual diet, with either targeted (to prevent persons with moderate acute malnutrition from becoming severely malnourished) or blanket (to prevent nutritional deterioration of a larger population) distribution.

Introduction

Since World War II, more than 100 million people have been forced to flee persecution or the violence of war to seek refuge in neighboring countries or in different areas of their own countries. The optimism following the end of the Cold War was short-lived because an epidemic of civil conflicts erupted in several areas of the world. In 1993, 47 conflicts were active, of which 43 were internal. Armed conflicts have increasingly affected civilian populations, resulting in high mortality, widespread human rights abuses, forced migration, famine, and total collapse of governance in some countries.

The 1951 United Nations Convention defines a refugee as

any person who owing to a well-founded fear of being persecuted for reasons of race, religion, nationality, membership of a particular social group, or political opinion is outside the country of his nationality and is unable, or owing to fear is unwilling to avail himself of the protection of the country.

In 1969, the Organization of African Unity expanded this definition to include persons fleeing from war, civil disturbance, and violence of any kind.

'Refugees' cross international borders, but 'internally displaced persons' (IDPs) do not. However, both groups have been forced to leave their homes and undergo physical and mental trauma as they settle in harsh and unhealthy environments, where they are often unable to take responsibility for their own welfare. The terms 'refugee' and 'internally displaced person' have major implications for the people concerned, particularly regarding their rights to protection and assistance, which are embedded in international law. The United Nations High Commissioner for Refugees (UNHCR) is mandated by the international community to protect and provide assistance to refugees. Owing to state sovereignty, the internally displaced are not included within UNHCR's mandate. Only on an *ad hoc* basis, at the request of the secretary general of the United Nations and the nation concerned, does UNHCR provide assistance to IDPs.

Trends

The escalation in crises and numbers affected since the early 1990s has had a dramatic impact on the nutrition and health of refugees. The number of people affected by natural disasters increased from 50 million in 1980 to 250 million in 2000. Similarly, approximately 30 million people were affected by conflict each year during the 1990s in more than 60 countries. The number of refugees has steadily increased from approximately 5 million in 1980 to a peak of more than 20 million in 1994, with a slow decline by 2003 to approximately 10.4 million. This is primarily due to the fact that more refugees are repatriating than are being forced to leave their countries, and new refugee flows have declined. The large numbers of repatriated refugees from Afghanistan, Angola, and Sierra Leone have contributed to the reduction in the number of refugees. In addition to the large numbers of refugees, in 2003 UNHCR assisted approximately 5.8 million of the estimated 20–25 million IDPs worldwide.

The largest numbers of refugees have been in Asia, which is also the region from which more than half the world's refugees originated. Included in this region is Afghanistan, which in 2001 accounted for an estimated 3.8 million refugees, or one-third of the global refugee population. Africa is the second largest refugee region. Approximately 48% of the UNHCR persons of concern are female, 12% are children younger than the age of 5 years, and half of the population is between 18 and 59 years of age (Figure 1).

Some 43 million people were forcibly displaced worldwide at the end of 2009, the highest number uprooted by conflict and persecution since the mid-1990s, according to UNHCR's annual 2009 Global Trends report. At the same time, the number of refugees voluntarily returning to their home countries has fallen to its lowest in 20 years.

The report indicates that overall refugee numbers remained relatively stable at 15.2 million, two-thirds of whom come under UNHCR's mandate although the other third fall under

UN Relief and Works Agency for Palestine Refugees. The report also notes that more and more refugees are living in cities, primarily in the developing world, and this remains a big challenge. The number of people known to be stateless at the end of 2009 was some 6.6 million.

Nutrition Implications of Displacement

The public health and nutrition consequences of war and population displacement have been well documented during the past 25 years. The major determinants of high mortality among affected populations and the major priorities intervention have also been identified. Access to safe water, sanitation, shelter, and immunizations (measles) are essential, but an adequate and diverse diet remains central to management of refugee operations. Today, it is acknowledged that acute malnutrition is a strong predictor of excess mortality among young children, and micronutrient deficiencies contribute significantly to diseases in emergencies.

Undernutrition results from a lack of food and/or prolonged inadequacies of food consumption, infection, or both. Undernutrition comprises a broad range of clinical conditions in children and adults that result from deficiencies in one or a number of nutrients. It has been defined as a state in which the physical function of an individual is impaired to the point at which he or she can no longer maintain adequate bodily performance processes, such as growth, pregnancy, lactation, physical work, and resisting and recovering from disease. The link between acute malnutrition and excess mortality has been documented for decades. The close correlation between these two factors was demonstrated during a Somali refugee operation in Ethiopia in 1988–89 and later in other emergencies. During the period of peak incidence of mortality and high prevalence of acute undernutrition, access to adequate food was less than 1400 kcal person⁻¹ day⁻¹ instead of the recommended 1900 kcal person⁻¹ day⁻¹ at the time and

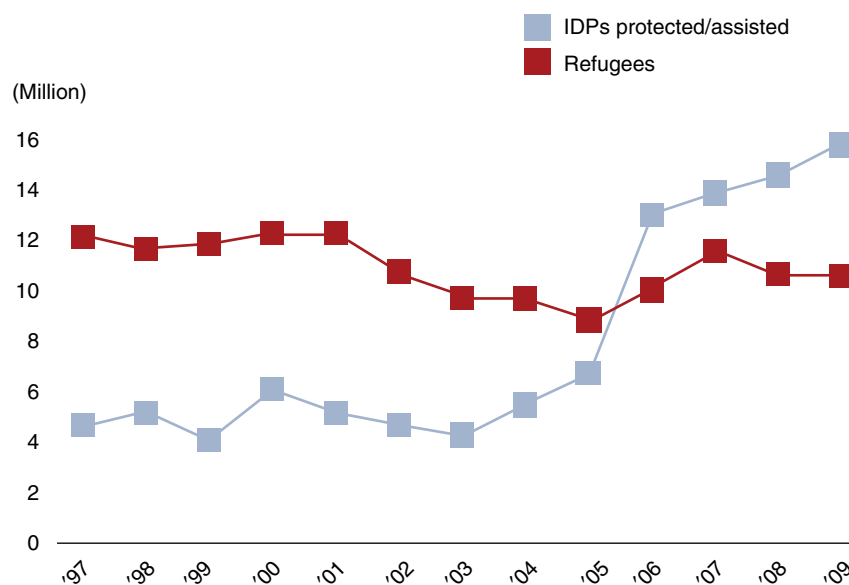


Figure 1 Refugees and IDPs protected/assisted by UNHCR 1997–2009 (end-year).

limited access to safe water, sanitation, shelter and densely crowded conditions. (Figure 2).

Although the immediate aim of most food assistance programs in refugee emergencies is to prevent excess mortality, there is also increasing evidence that undernutrition during critical periods of life has long-lasting effects. Undernutrition, or the risk of being undernourished, may be carried from one generation to another in an intergenerational cycle. Undernourished women give birth to undernourished infants who, in turn, are more likely to become undernourished adolescents and adults. Therefore, the nutritional status of refugees can have long-lasting effects on future health for individuals and generations (Figure 3).

Macronutrients

An inadequate supply of macronutrients and micronutrients (protein, fat, carbohydrates, and vitamins and minerals) results in protein-energy malnutrition (PEM), the most com-

mon form of malnutrition, especially among infants and young children. There are two types of growth failure associated with PEM: wasting (acute undernutrition) and stunting (chronic undernutrition). Wasted individuals (children, adolescents, or adults) are extremely thin, whereas stunted individuals are short for their age as a result of impaired growth during childhood. Severe PEM has a high case fatality rate and is often classified into two forms: Marasmus and kwashiorkor. Both are identifiable by severe weight loss; however, the oedema associated with kwashiorkor can mask the otherwise dramatic skeletal appearance of marasmic individuals. Nutritional issues among refugees vary greatly from one region of the world to another. Prevalence of wasting, defined as weight for height less than -2 standard deviations of the reference population, have been as high as 50% in the Horn of Africa and as low as 5% in Southeast Asia, Malawi, and the Persian Gulf. Mortality rates in some of these populations during the acute phase of displacement have been extremely high – up to 60 times the expected rates.

Micronutrients (Vitamins and Mineral Deficiencies)

Micronutrients, although needed in small amounts, are as essential as macronutrients in addressing nutrition requirement of the populations through food assistance programs. Micronutrient deficiency diseases are key in nutrition-related morbidity and mortality. There is a misconception that people do not die of micronutrient deficiencies because one does not often see the signs and symptoms as visibly as those for PEM. Nonetheless, these deficiencies can be fatal. In addition to deficiency diseases of vitamin A, iron, and iodine, conditions widely described as diseases of public health importance, epidemics of scurvy, beriberi, and pellagra, have been frequently reported among refugee populations, primarily because of limited access to a diverse diet and overreliance on one or two commodities (i.e., maize or polished rice) (Table 1). The importance of micronutrient deficiencies among the refugee population has only been documented

*Excludes unknown locations

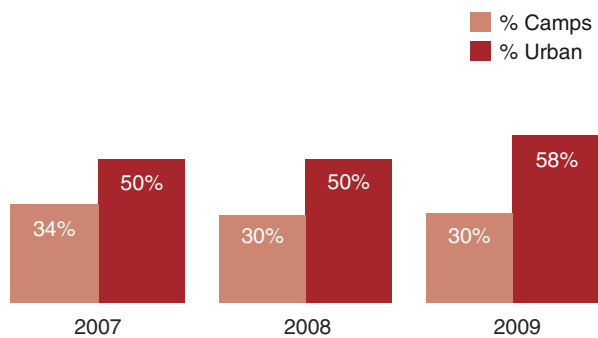


Figure 2 Relationship between malnutrition and mortality as seen in Ethiopia 1987–90. Reproduced from Figure 8 in Centers for Disease Control (1992) *Morbidity and Mortality Weekly Report*, vol. 41, 10 pp. Atlanta, Georgia.

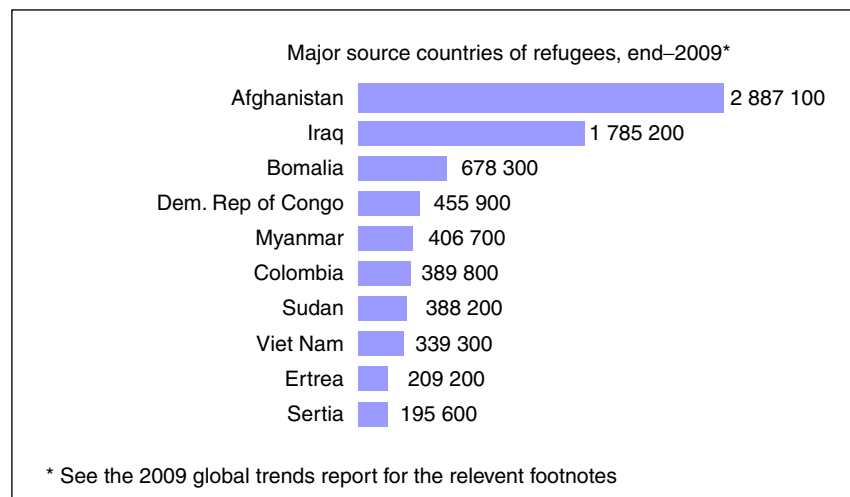


Figure 3 The life cycle and intergenerational transmission of malnutrition. Adapted with permission from James, et al. (2000) *The 4th Report on the World Nutrition Situation: Nutrition Throughout the Lifecycle*. Geneva, Switzerland: ACC/SCN.

Table 1 Micronutrient deficiencies in refugees

<i>Micronutrient</i>	<i>Deficiency disease</i>	<i>Symptoms</i>
Iron	Anemia	Pallor, tiredness, headaches, breathlessness
Iodine	Goitre	Swelling of the thyroid gland in the neck
	Cretinism	Severe mental and physical disability that occurs in the offspring of women with severe iodine deficiency
Vitamin A	Night blindness	Inability to see well in the dark – an early sign of vitamin A deficiency
	Xerophthalmia	Including Bitot spots and corneal ulceration and night blindness
Niacin	Pellagra	Affects the skin, gastrointestinal tract, and nervous system and is sometimes called the 3Ds: dermatitis, diarrhoea, and dementia
Thiamin	Beriberi	Loss of tendon reflexes; drooping of arms and feet; wet or cardiac beriberi resulting in heart failure
Vitamin C	Scurvy	Painful joints, swollen and bleeding gums, and slow healing or reopening of old wounds

since the late 1980s, and more attention is being paid to the usefulness of inclusion of micronutrient-rich foods and/or supplements in the management of refugee nutrition. There are additional innovations for delivering micronutrients for home fortification. This represents one of the best potential opportunities to increase impact on child development and saving lives. Micronutrient powders, for example, known under the trade names MixMe and Sprinkles, are provided to fortify foods after preparation, just before consumption, to ensure an adequate intake of micronutrients essential for body functions. These come in small individual sachets containing one full recommended nutrient intake of 15 vitamins and minerals in one gram of powder for one person per day. These are currently used in many refugee and emergency operations.

Addressing Nutrition in Refugees

Owing to the nature of displacement and the loss of livelihoods, refugee populations are extremely vulnerable. Often, refugees settle in camps with support from the international community and host government. In some cases, refugees may live in open situations in which they integrate into the local community. In almost all cases, refugees are dependent on outside assistance, although the level of need depends on the level of self-reliance the refugees are able to achieve. In some instances, refugees are able to bring some assets with them when they flee and/or have some sort of income-generating activity, such as access to land and labor and employment. However, this very much depends on the policies of host governments. In these cases, refugees are not totally dependent on external assistance, and nutrition management response takes these factors into account by adjusting humanitarian assistance and the food assistance in particular to meet the assessed needs. There has been an evolution in the standards of food energy required for refugee populations. These were based on estimates of energy requirements from parameters such as body weight, demographic composition, environmental temperature, and activity levels. In the 1980s, the standard was approximately 1500 kcal person⁻¹ day⁻¹, the minimum deemed adequate for survival. In the late 1980s, this was recalculated to 1900 kcal person⁻¹ day⁻¹ as a preliminary standard in order to include expenditure of energy for light activity as opposed to merely the basal metabolic rate. In the 1990s, the benchmark value was modified to a more

realistic 2100 kcal person⁻¹ day⁻¹. This was based on an increase in energy required for physical activity, some adjustments to the demographic composition, and an increase in the proportion of pregnant and lactating women in the population. It should be recognized that this recommended value is the average of the individual requirements based on developing nation population demographics and is not a specific provision for individual needs. In addition to the recommended kilocalorie content of the daily food ration, nutritional science has determined that the ration should have an optimal balance of fat and of protein (17 and 12%, respectively).

Food Baskets for Populations (General Food Distribution)

The sudden and massive reduction in food availability associated with displacement immediately affects the nutritional status of refugees. The first response is intervention through the implementation of adequate food distribution to all to ensure all refugees have access to the required food ration. A general food distribution is the first line of intervention and the highest priority when a refugee population does not have access to sufficient food to meet its nutritional requirements. If the recommended adequate ration of 2100 kcal person⁻¹ day⁻¹ and the quality of food basket is not available, malnutrition levels may escalate.

Even if the overall food needs of refugees are adequately met, inequities in the distribution system, disease, and various social factors may contribute to a high level of malnutrition among certain groups. Children younger than 5 years of age, pregnant and lactating women, the chronically ill (e.g., tuberculosis and HIV/AIDS patients), and the elderly are considered vulnerable groups since they have specific nutritional requirements. A special nutrition intervention program targets these nutritionally vulnerable groups through supplementary feeding programs and those in need of nutritional rehabilitation through therapeutic feeding programs. Malnutrition prevalence, as well as an assessment of aggravating factors in the environment, are used as guidelines to determine if a nutrition intervention program needs to be initiated. Aggravating factors that influence the nutritional situation include an elevated crude mortality rate; epidemics of communicable diseases such as measles, diarrhoeal

diseases, and respiratory infections; and an unstable social, political, or environmental situation.

Nutrition programs are primarily managed by non-governmental organizations (NGOs) that have specialization in the management of refugee nutrition. Other humanitarian partners have roles to play in response to refugee situations, including host country authorities, United Nations agencies such as the World Food Programme, UNHCR, and UNICEF; and other multisectoral NGOs. There exist memorandums of understanding and partnership agreements among agencies to provide assistance to the affected populations. The need for partnership is essential for management of refugee nutrition and health programs. The Sphere Project was launched in 1997 to develop a set of universal minimum standards in vital sectors of humanitarian assistance. The aim of the project is to improve the quality of assistance provided to affected populations and to enhance the accountability of the humanitarian system in emergency response.

Addressing Acute Malnutrition among young children

Management of Moderate Acute Malnutrition

The most common nutrition intervention is supplementary feeding programs (SFPs) to address moderate acute malnutrition in emergency situations. SFPs provide a high-quality food as a nutritional supplementation to the daily diet of malnourished populations. There are two main types of SFP – targeted and blanket. The goal of targeted supplementary feeding is to prevent people who are moderately malnourished from becoming severely malnourished. Blanket supplementary feeding, which provides all members of a vulnerable group with a food supplement, is intended to prevent the deterioration of nutritional status among a larger population group rather than narrowly defined individuals at specific nutritional risk. Implementation of SFPs can take two forms: Prepared meals consumed on site (wet rations) or food rations issued weekly or monthly to take home for preparation (dry rations). Food supplements usually consist of a fortified blended food (FBF) mixed with oil, and sometimes sugar is included. Wet rations should provide 500–700 kcal, whereas the recommended dry ration is doubled to 1000–1200 kcal in order to account for sharing at home. There have been many advances in improving the food commodities to address nutritional needs for SFPs. The composition of fortified blended food has been enhanced by including animal proteins (dried milk) and additional vitamins and minerals. In addition ready to use lipid nutrient products – such as plumpy doz, supplementary plumpy, nutri butter, and other ready to use supplementary foods for children are proven to be more effective for improving nutritional status and speed of recovery.

Addressing Severe Acute Malnutrition

Therapeutic feeding programs (TFPs) provide the severely malnourished with their full nutritional requirements in addition to medical care. They are initiated to reduce excess mortality among individuals facing severe malnutrition and

have played an important role in reducing malnutrition-related mortality in emergencies. The first phase of a TFP focuses on treatment of infections, management of other medical complications, and metabolic stabilization. This phase has the highest mortality rate of all nutrition interventions due to the poor state of the patients and the intensive treatment required. The second phase of a TFP is a rapid weight gain period designed to rehabilitate the patient's nutritional status.

Recognition of severe acute malnutrition as a complex nutritional condition during the 1990s led to the development of certain foods defined explicitly for therapeutic treatment of malnutrition with the appropriate balance of energy, protein, and micronutrients in order to avoid overloading the body's metabolism, which potentially may lead to cardiac shock. These products include F-75, F-100, and BP-100 biscuits and other ready-to-use therapeutic foods (RUTFs) such as 'plumpy nut'.

Community-based care is a recently developed public health approach to deal with severe malnutrition and aims to treat the majority of people suffering from severe acute malnutrition in their homes. A community therapeutic care (CTC) program initially is set up complementary to traditional TFP components and represents a new approach to managing malnutrition at the community level. A CTC program has the same initial metabolic stabilization phase, and life-threatening infections are identified and treated just as in a TFP. However, once the patient is stabilized, he or she moves directly to an outpatient therapeutic program that operates through existing health structures and, with the use of RUTFs, nutritional rehabilitation is initiated. When patients are no longer at risk of severe malnutrition, they are referred to SFPs for recuperation. This phase is followed by greater emphasis on community mobilization to increase the population's involvement and training of mothers. CTC is an innovative approach and is proven to be successful. Proposed benefits of this method are the improved coverage to increase the number of people treated and reduce overall mortality rates. In addition, local production of RUTFs has been initiated in few countries to reduce the cost of treatment, and shorten the length of stay in centres away from the family. Finally, the decentralized nature of CTC can enable earlier detection of malnutrition, thereby reducing the incidence of severe malnutrition (Table 2).

Challenges

Nutrition interventions alone are not adequate to address the multiple causes of undernutrition in refugees. The access to public health inputs is essential in preventing and reducing excess mortality and malnutrition and in ensuring that nutrition interventions have the desired effects.

Although the quality of nutrition assistance and interventions has improved considerably since the 1970s, the international community is still searching and moving forward to improve the quality of food products provided to address undernutrition. This is an exciting time in the field of nutrition to develop appropriate strategies, interventions, and products to address undernutrition. 1,000 days is a global effort for addressing undernutrition during pregnancy and early childhood. This refers to a special window of

Table 2 Milestones in addressing nutrition in refugees

1960s:	Food response based on commodities available (donated) Limited recognition of relevance of nutritional content of rations Food provided based on resources rather than nutritional needs
1970s:	Focus on protein deficiency (in protein-energy malnutrition) Food ration comprised mainly cereal, pulses/beans, and oil Fortified blended foods (FBFs) used only in supplementary feeding
1980s:	Major relief agencies raise planning figure from 1500 to 1900 kcal person ⁻¹ day ⁻¹
1990s:	Relief agencies raise planning figure for fully food aid-dependent populations from 1900 to 2100 kcal person ⁻¹ day ⁻¹ FBF included in most rations for completely dependent populations Basic six-commodity food basket becoming common: cereal, pulses, oil, salt, sugar, FBF UNHCR/WFP Memorandum of Understanding signed with clear roles and responsibilities Development of multi-UN agency and NGO policies and guidelines on common approaches to addressing malnutrition in emergencies Fortification of oil, salt, and flours, on international market Development of therapeutic foods for treatment of malnutrition (F100-F75) Local production of fortified blended foods
2000s:	Development of community management of acute malnutrition (CMAM) Creation of inter-agency clusters (IASC) – Nutrition, food security, health, wash, education, protection, logistics, and telecommunication Development of capacity in nutrition in humanitarian staff Pilot testing of on-site milling and fortification in a refugee camp Development of ready to use foods Provision of multimicronutrient powders Provision of cash and or cash vouchers to address malnutrition

opportunity to take action to combat undernutrition. 1,000 days refers to the time from start of a mother's pregnancy until the child turns 2 years old. Research shows that children who are undernourished during this period are far more likely to suffer from long-term health problems, poorer education performance, and lower economic prosperity.

Because so many factors – food, health, care, and environment – interact to determine nutritional well being, a partnership among agencies with different mandates is essential to effectively address and correct nutritional issues. Refugee nutrition must be addressed in tandem with other services in order to ensure that underlying factors of malnutrition are being met and that nutrition interventions are effective.

See also: Malnutrition: Secondary, Diagnosis and Management.
Supplementation: Developed Countries; Developing Countries

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RELIGIOUS CUSTOMS, INFLUENCE ON DIET

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Glossary

Ahimsa The practice of nonviolence in all aspects of life, including taking the life of living creatures for food.

Brahmin The highest caste among Hindus, with the greatest number of food restrictions.

Eucharist The celebration of the Mass in Roman Catholicism, in which wine is said to miraculously

transform into the Blood of Jesus and bread, normally a consecrated wafer, changes into His flesh.

Halal The food laws of Islam, which forbid pork and alcohol. It's opposite is haram.

Kosher The complex food laws of Judaism, which originate in the book of Leviticus, which are still practiced by devout Jews today, with some modifications.

Introduction

Religion is among the most pervasive forces influencing the human diet, even among those who do not actively practice a particular faith. Religion shapes individual and group preferences in the form of taboos, celebratory foods, and ritual offerings, it prescribes the modes of commensality and attitudes toward indulgence and abstinence, and throughout history religious leaders have carefully defined the meaning of alimentary sustenance in ways that continue to resonate into the present, having a direct impact on people's dietary choices. This article will recount attitudes toward food in the major world religions, and their origin and historical development as necessary for a full appreciation of the complexity of how religion shapes food cultures today.

The origin of the deep connections between food and faith lie without doubt in the earliest polytheistic religions. Since pre-historic times, the Gods were invoked to ensure good harvests and thanked for their bounty; they also had to be appeased with sacrifice. Ancestral spirits were provided with offerings to maintain their good will and a cycle of festivals marked the agricultural and religious calendar. Many religious festivals retain features of these original agricultural rites. With the discovery of alcohol and other intoxicants, communities could enter into an ecstatic union with the Godhead, a numinous state of oneness, bridging the earthly and otherworldly planes. To this day all these primal elements of the relationship between food and religiosity survive as rudiments, altered in form, but still discernible among modern practices.

Judaism

Perhaps more than any other civilization, the ancient Hebrews defined their relationship to God in terms of what they ate, what was considered clean and unclean and what was sacrificed to God. A succession of dietary codes in various historical epochs also explains how that relationship changed. In the biblical narrative, following creation, in the first epoch humans were intended to be not only vegetarian in the state of

innocence, but fruitarian, meaning that Adam and Eve's Edenic diet was obtained without killing any living thing. They ate only fruits and seeds and leaves, which could be taken without killing the plant. Plants also produced fruit spontaneously without human effort. This initial ban on murder will help explain the internal logic of the complex rules of kashrut and sacrifice, which were anything but arbitrary or capricious. Following the fall, as punishment for their disobedience in eating from the Tree of Knowledge the couple were forced to subsist through labor. In this second epoch, Adam had to earn his bread by the sweat of his brow, i.e., by planting crops, and for Eve it meant pain in childbirth as well as subjection to man.

Although it is not clear exactly what was eaten in this second epoch, the professions of Cain and Abel, a shepherd and farmer, closely match the early economy of the Fertile Crescent after the Neolithic Revolution. It is not until after the flood (the third epoch) that Noah is given explicit permission to kill for food. "Every creature that lives and moves shall be food for you; I give you them all, as once I gave you all green plants." It is an admission on God's part that humans are faulty. Only the blood, which contains the life, must be poured on the ground, as it belongs to God. This blood prohibition remains in effect as central to Judaism, and kosher meat must be salted to drain it of all traces of blood. It was also at this time that the first sacrifice was made. It is clear that the burning fat and entrails provide a soothing odor to the Lord, that He is in a sense sustained by the smoke. More importantly, the sacrifice, explained only in later books of the bible, reinstates justice in the universe. To compensate for the act of killing, some creature must be punished, normally an unblemished "scapegoat."

It is only with the giving of the law under Moses, the fourth and final historical epoch, that the Hebrews were given a complex dietary code, which is still for the most part in effect among observant Jews. At its root is still the prohibition against murder. Unclean animals are those, which kill in order to eat. Rather than catalog all clean animals, the Levitical

Priests devised a shorthand way to recognize the innocent herbivores: those which chew their cud and have a cloven hoof. Thus the omnivorous pig was banned along with other creatures, which did not seem to fit into this scheme, such as camels and hares. It has been suggested that the real origin of this ban was an informal knowledge of trichinosis, or perhaps the fact that pigs are inefficient in the desert, being unable to sweat and thus requiring watering holes, and more importantly competing for resources with humans because pigs are unable to convert grass (indigestible to humans) into food, the way ruminants can. The Hebrews may have banned pigs in order to maintain distinction from their pig-eating neighbors and keep their identity intact, for to eat with someone is one step away from intermarriage. Yet none of these explanations is as convincing as the original ban on murder, which translated into considering all predatory animals unclean.

More difficult is explaining the ban on shellfish and other creatures, which seems to defy the categorical schema of the priests. According to their logic, a fish must have scales and swim, birds must have feathers and fly, animals must have legs and walk. Scaleless fish, flightless birds, etc., defy the rational categorization and are thus also unclean. Another stricture demands that milk and meat must never be mixed, which by tradition stems from not seething a calf in its own mother's milk. This means that a cheeseburger is not kosher. Even among nonobservant Jews, through cultural conditioning foods categorized as *trayf* may be unappetizing or even repugnant.

In addition there are numerous holidays, which revolve around food, or lack thereof. Most important is the fast, which takes place from sundown to sundown on Yom Kippur, a time to reflect on and atone for one's sins in the preceding year. Passover is also essentially a food holiday, though no leavened bread (*chometz*) may be eaten in commemoration of the Jews' escaping Egypt who had no time to let their bread rise. The seder plate also recalls other aspects of the captivity in Egypt: salt water for tears and bitter herbs for suffering, *charoseth* – a thick fruit and nut paste that reminds one of mortar. The passover seder ritual revolves around food stories drawn from Exodus. The dining practices of the meal itself however date from the period when the Holy Land was ruled by Seleucid Greeks, and is essentially a form of symposium, particularly in the reclining while eating, drinking four glasses of wine, and hiding the *afikomen* (i.e., *epicomium*, outside the meal) – a *matzoh* that the children search for at the end of the ritual. Today, sticking to the exact letter of the law, *matzoh* meal is used to make a kind of passover bread, cakes, and other products, which because not technically leavened, are still allowed.

In general, modern Judaism retains the kosher rules, though sacrifice ended with the destruction of the second temple in AD 70. However, a good proportion of practicing Jews, particularly in the Reformed tradition no longer adhere to any dietary restrictions, and there is also a broad range of levels of compliance, from those who keep kosher only in the home, to those who merely avoid pork, but follow no other restrictions. Nonetheless, food is so intimately bound to Jews' cultural identity, that active or not, certain foods are absolutely requisite at family gatherings and holidays, which at least for Ashkenazi Jews include familiar items such as bagels and lox, corned beef and pastrami, knishes, gefilte fish, and *matzoh* ball soup.

Christianity

Christianity grew directly from Judaism and in an effort to distinguish itself therefrom, the early church made an explicit point of abandoning the kosher rules. It is not what goes into the mouth but what comes out that defiles a man, as Jesus himself explained, meaning food cannot pollute a person, only cruel words. There is also a scene recounted by Matthew in which Jesus is asked why he doesn't fast and he responds that "when the Bridegroom will be taken from them, then shall you fast" meaning that once he is gone there will be occasion to fast, but fasts should not be regularly scheduled and habitual as among standard Jewish practice. Regarding clean and unclean food, there is also a vivid dream told by Peter in which a huge net teeming with creatures lowers from heaven and he is ordered by God to kill and eat. Not that the early church was entirely bereft of attitudes toward food, though.

The letter to the Corinthians resolves the question of whether it is alright to consume meat that had been sacrificed to pagan gods. Although technically permissible, Paul counsels to avoid it lest one lead fellow Christians astray. In this time the central sacrament of the church also developed, based on the Last Supper when Jesus was sitting at a Passover seder with his disciples and asked them to remember him when they ate bread and drank wine, alluding to the fact that he would be gone the next day, and reminding them, "this is my body; this is my blood." The sacrament of the holy communion is based on these words, and in 1215 the Lateran Council decreed that the bread and wine literally transform into the flesh and blood of Christ, which is consumed by communicants. It is in this way that the faithful obtain grace, forgiveness of sins, through the act of eating.

Early Christians also fasted, not only in miraculous ways as had Moses and Jesus who ate nothing at all for 40 days, but as a whole community during impending disaster to ask for God's mercy, or as individuals as an act of penitence. The early church also saw the development of monastic orders, which followed ascetic regimens limiting the amount of food eaten and frequently abstaining from meat, which was believed by medical theorists to stimulate the production of blood and sperm and ultimately incite the libido (in both men and women). Celibate orders naturally restricted meat consumption, though the *Rule of St Benedict*, which generally set the pattern for orders in the West is not entirely abstemious, allowing for example, the equivalent of a few glasses of wine, though ideally it would be best if monks could abstain. Certainly an antipathy toward the sin of gluttony pervades Christian thought, for it was not only the first sin in Eden, and leads to other sins like sloth and lust, but prevents one from exercising virtues such as charity.

The general ascetic attitude toward meat did eventually, in the course of the institutionalization of the church, lead to a number of official fast days. These were every Saturday (starting on Friday night, hence fish on Friday), the vigils of saints days and the entire 40-day period of Lent stretching from Ash Wednesday to Easter, minus Sundays. The fast was defined as abstention from meat and all meat products such as butter, eggs, and milk, though typically a dispensation could be obtained for children, pregnant women, the infirm, and for special cases. Fasts alternated with feast days,

the best known being Mardi Gras, the celebration preceding Lent when all remaining meat has to be consumed, as well as eggs and butter – in pancakes and other confections. In general this was a time for ritual subversion, serving as a safety valve for society, which would naturally return to the status quo once the celebrations were over. Most places abolished these celebrations in the course of the sixteenth century though, the rare exceptions being Venice and New Orleans where Carnival is still celebrated.

These fasting regulations remained uniform in the Roman Catholic Church until the 1960s and the Vatican II Council, when adherents were asked to give up something important as a sacrifice, but not necessarily meat. However by custom certain days do remain fish days, including Christmas Eve among Italians. Fasting regulations also remain in place in the Eastern Orthodox Church and are even more extensive, and for certain fasts a broader range of foods is prohibited, such as olive oil.

The Protestant denominations returning to scriptural authority over tradition took a number of different positions on official fasts. The Church of England kept a “Political Lent” requiring fish consumption as a way to support the fishing industry and the navy. Eventually the practice fell into abeyance, likewise in Lutheran Churches. In the Reformed tradition (i.e., The Swiss, Dutch, Scots, English Puritans, and French Huguenots) fasting once again took its biblical form, as a complete abstention from food as an act of penitence or a communal fast to avert God’s wrath. In general, however the practice ceased, though many evangelical Christians still fast for religious purposes. Nonetheless, the Reformed churches did adopt a new attitude toward food stressing frugality, simplicity, and at times abstinence from alcohol. The Prohibition of alcohol in the US in the early twentieth century sprung directly from the work of the Women’s Christian Temperance movement, which may seem ironic given that Jesus drank wine and even miraculously provided guests with a bounteous supply on one occasion.

A number of unique attitudes toward food developed among more recent Christian sects, for example, the modern vegetarian movement sprang from Bible Christian Societies on both sides of the Atlantic, and the 7th Day Adventists, following the visions of Ellen White are the only sect that demands total abstention from meat, alcohol, and tobacco, though Mormons (Church of Jesus Christ of Latter Day Saints) do abstain from the latter two as well. Historically Christian sects have expressed their ethical positions in ways that have a direct bearing on food practices. For example, Quakers who opposed slavery in the nineteenth century abstained from products made by the plantation economy, including sugar, molasses, and rum. More recently there have been explicitly Christian weight loss diets, Christian groups advocating fair trade or ethical treatment of animals. Suffice to say that although all Christian sects promote charity, any number of ethical positions and dietary regimes may fit under its umbrella today.

Islam

The food tenets of Islam also bear a relation to the previous two faiths. The most obvious similarity with Judaism is the ban on pork. In general there is not the great number or complexity of food rules, though birds of prey and similar animals are not considered halal (legal to eat). Animals to be

consumed must however be ritually slaughtered without pain, thanking the animal and giving praise to Allah. The most important food custom is the month-long fast of Ramadan, when the faithful must eat and drink nothing between sunrise and sundown. The meal in the evening, to break the fast, traditionally eaten on the floor and with fingers of the right hand only, may be quite elaborate and sumptuous; some even report gaining weight during the holy month. The fast is also broken with Eid al-Fitr, a resplendent feast with special foods for the occasion, such as dates, which are a traditional food of the Arabian Peninsula.

A unique prohibition in Islam is the ban on alcohol. This, as much of the religion, stems from the personal experience of the prophet Mohammed, who after having witnessed a scene of drunken violence, understood that it would be best if alcohol is never consumed. Some Muslim countries forbid the sale of alcohol entirely, whereas others are more lax and even produce excellent spirits such as Raki in Turkey or Arak elsewhere. It has been suggested that the importance of coffee and coffee houses in the Muslim world is a result of the ban on alcohol, and caffeine is also purported to keep holy men awake for long hours of prayer.

Perhaps the most pervasive custom in the Muslim world is the charge to perform acts of charity and show effusive hospitality to guests. Originating in the very practical need to feed strangers who would otherwise starve in the desert, generosity with food has become an essential part of the practicing Muslim daily life. Devout Muslims will say a prayer before eating and thank Allah when finished. There are also a number of religious festivals celebrated with food. Eid-al-Adha commemorates the willingness of Abraham to sacrifice his son Ishmael (not Isaac as in the biblical tradition). On this day a ram is sacrificed and a third is kept for the family, a third given to friends and neighbors, and the last third given to the needy.

A unique phenomenon among African-Americans is the movement founded by Elijah Mohammed known as the Nation of Islam. Its tenets include rejection of what its founder considered slave food; pork and offal meats, black eyed-peas and greens stewed with fatty meat. Mohammed was not only interested in dietary reform but wrote an entire book on the topic, which also counseled abstinence from debilitating intoxicants which cause dependency and subjection. Though many members have gravitated toward traditional Sunni Islam, the group still survives.

Hinduism

The origins of Hinduism can be traced to the Indo-European invaders (Aryans) who arrived in the Indian subcontinent after approximately 1500 BC. They were originally sacrificers and consumers of cattle and how exactly they came to ban cows for food is among the more hotly debated topics among scholars. First it is important to understand that the ancient Vedic texts regard all living creatures as manifestations of the first primordial principle, known as Atman, which translates as “self.” Atman, having divided and subdivided gave rise, therefore, to all living beings, which are manifestations of the original Atman. In daily life people often have difficulty recognizing the unity of all creation, which is how abstinence and yogic

practices, including meditation, help us to recognize the self – which is every creature. Moreover, on death all creatures are reincarnated in a different form in accordance with their conduct in the previous life. These ideas, at least to start with were not implemented in a way that determined diet.

Second and equally important was the division of society into separate castes, seemingly at odds with the idea of the unity of creation, but separating people by various professions with no possibility of social mobility. Unless you were demoted by marrying below your caste, you and your descendants were always and forever in the same caste. This social structure had a profound impact on food customs, because one could not eat with or accept food from those of a lower caste, which would become polluted. The highest of castes, the priestly Brahmins had, as we shall see, the most restricted diet, with the greatest possible sources of pollution.

At first the Brahmins were eaters of meat, but apparently a period of widespread famine challenged their position at the top of society as the lower castes threatened them with violence. The Brahmins maintained their status by implementing the full ramifications of their sacred texts into practice. That is, they asserted that because the cow is the highest order of reincarnation, it should be sacred, revered, and never consumed. Hindus went from cow consumers to ardent reverers of cows. Because the priests had the most abstemious diet, they could no longer be the object of envy or violence from the lower castes, as they ate less and their diet was much more restricted. In fact the lower the caste, the more foods were allowed. The idea that cattle are more efficient as providers of traction for plows, dairy products, and manure for fuel than they would be if used for meat, may be true but it does not explain the origin of cow reverence.

Although the caste system has for the most part broken down in the modern era, beef is still prohibited for Hindus, and certain cow products like ghee (clarified butter) are considered the most sacred. A countless number of holy festivals also revolve around food. It is important to remember that Hinduism is a polytheistic faith, and thus festivals worship any number of different gods, the three main gods Brahma, Vishnu and Shiva, but also their many avatars and family members. Offerings to the gods, *prasad*, often consist of foodstuffs: fruits, sweets, or milk products. The offerings are consumed after the ritual. There are an extraordinary number of festivals throughout the year as well, seasonal, honoring particular deities, all of which include eating particular festival foods. For example, Mahashivarati celebrates the marriage of Shiva to Parvati when the temples flow with offerings of milk, yoghurt, honey, and ghee, but devotees fast through the day and the fast is broken by a special vegetarian meal made with no grains. Janmashtami celebrates the birth of Lord Krishna, Ganesh Chaturthi the birthday of the elephant-headed Ganesh. Diwali, the Festival of Lights features sweets, which are given to children and even fed to cattle.

Buddhism

Buddhism developed directly from Hinduism when Siddhartha Gautama in the c. 6th BC was searching for the meaning of life and was suddenly enlightened with the

realization that suffering in the world comes from never being satisfied with what one has, from being far too attached to one's self. Neither extreme asceticism nor indulgence, both inherently selfish, lead to happiness, but rather the middle way, eating enough to survive. The key to ending suffering is simply to recognize the self as an illusion and to break the chain of causation, stop the endless cycle of reincarnation and achieve the state of nirvana, or nothingness.

Before this, however, were very practical Buddhist directions for living life in this world. Most important is the principle of nonviolence or *ahimsa*, not to cause suffering to any other creature. By logical extension, this meant also not killing them for food, and vegetarianism is thus at the core of Buddhist belief. There are varying degrees of practice among the many forms of Buddhism though. Normally monks are the only strict vegetarians and they created an elaborate non-meat cuisine featuring items like high protein tofu. Though many modern Buddhists around the world are vegetarian, among most varieties fish is eaten regularly, and even meat in some.

Interestingly, when Buddhism became the state religion in India under the ruler Ashoka in the third century BC, meat eating became illegal as well. Although the religion did not survive in India it spread northward through Asia influencing places as far away as Japan, where Zen Buddhists traditionally abstained from meat. Ironically the largely vegetarian population of southern India, although mostly Hindu, were most likely influenced by Buddhist practices. In East Asia, Buddhism is not considered an exclusive faith, meaning that in China, for example, it may be mixed with elements of Taoism and Confucianism, or in Japan with traditional Shinto.

Although Confucianism does not have any explicit food-related rules, the practice of filial piety does require that people act with deference to superiors and care for inferiors. This often translates into a practical requirement to feed elders first, without neglecting to provide for those below one in status.

Another religion arising in India, the Jains, adopted an extreme vegetarian position, such that to harm any living creature, even accidentally is forbidden. They are naturally pacifists as well. In terms of diet, this also means avoiding root vegetables, the harvesting of which kills the entire plant and they may harbor many microorganisms. Jains often filter water to prevent destruction of the same. Monks and nuns in this tradition may also practice forms of asceticism, and among all Jains there are set times for fasting, though one may also elect to fast individually for a variety of reasons. There are also varying degrees of fasting, some lasting many days.

Conclusion

Religion continues to shape foodways around the globe, not only in traditional forms of fasting and celebrations or food taboos, but in particular in the ethical attitude toward our fellow creatures as well as our responsibility for stewardship of the environment, and most importantly for the responsibility we owe to perform works of charity to help sustain our fellow humans.

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RIBOFLAVIN

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Glossary

Coenzyme A low molecular-weight substance that acts at the active site (i.e., catalytic centre) of an enzyme, thereby participating in a key (essential) role in the chemical reaction that is catalysed by the enzyme. B-vitamins either constitute, or become converted to, coenzymes in the body.

Deficiency (clinical or biochemical) Arises when the amount of an essential nutrient in (tissues of) the body declines below a critical minimum level. Biochemical (tissue) depletion then occurs, and may be followed by clinical (i.e., pathological) signs and symptoms of deficiency. Deficiency of an essential nutrient in the diet may lead to tissue- and clinical-deficiency, but impaired absorption, increased losses, increased tissue demands, etc., may also lead to a functional (tissue) deficiency, even when the diet content is adequate.

Estimated average requirement (EAR) Similar to RDA and RNI, except that this is the mean (i.e., average) nutrient requirement of the individuals in a defined population group (USA and UK).

Nutrient (e.g., vitamin) status Is commonly assessed by measuring the concentration of the nutrient or a derivative in an accessible body fluid such as serum or urine or else the functionality of an enzyme or a biochemical pathway (functional status). Published normal ranges enable the result to be classified as (e.g.,) deficient, low, normal, or high.

Recommended dietary allowance (RDA) The amount of a nutrient (per day) that covers the needs of the majority (usually *ca.* 97.5%) of the individuals in a defined population group (e.g., adult males), in the USA. The term: 'Reference Nutrient Intake (RNI)' is used for a similar concept in the UK.

Absorption, Transport, and Storage

Riboflavin (vitamin B₂) is not synthesized by higher animals, in which it is an absolute dietary requirement for the synthesis of certain essential coenzymes needed for intermediary metabolism. It is transported from food sources in the gastrointestinal tract, across the gut wall into the circulatory system, and thence via the blood to the tissues. This occurs against a concentration gradient, thus retrieving even small amounts from food and from the low concentrations in plasma to the higher concentrations inside cells.

Gut riboflavin transport systems have been studied, for example, using partly isolated segments of the small intestine within an anesthetized animal, by isolated everted gut segments, or by vesicles prepared from the intestinal brush border.

These model systems have shown that the transport of riboflavin at low (e.g., micromolar) concentrations is temperature and energy-dependent (it is inhibited by inhibitors of adenosine tri phosphates (ATP) production from energy substrates), it becomes saturated as the concentration of riboflavin increases, and it is sodium ion dependent. These characteristics are shared with many other types of small molecules that are actively transported across the gut wall. More specifically for riboflavin, the active transport mechanism involves phosphorylation (to riboflavin phosphate, also known as flavin mononucleotide (FMN)), followed by dephosphorylation back to riboflavin, both steps occurring

within the intestinal cells (**Figure 1**). This is one of a number of strategies that the gut uses to entrap essential nutrients and then transfer them in a controlled manner. A similar strategy is used at other sites in the body, to ensure entrapment of circulating riboflavin by cells whose nascent flavin-dependent enzymes need a regulated supply of the vitamin.

Although the active transport of riboflavin across the gut wall and across other cell membrane barriers within the animal is a saturable process, if pharmacological amounts are present, then the slower and less efficient, but nonsaturable, process of passive absorption predominates. The active transport process is increased in riboflavin deficiency and is decreased if the riboflavin content of the tissues is high, and it involves calcium and calmodulin, but not sodium. Specific riboflavin receptors and a role for the microtubules have been identified.

Although some of the riboflavin in foods is present as the free vitamin, a larger fraction is in the phosphorylated coenzymes FMN and flavin adenine dinucleotide (FAD), and there may also be small amounts of a glucoside. These forms are all efficiently converted into free vitamin by enzymes secreted into the gut lumen and thus become available for absorption. There are also small amounts of covalently bound and thus unavailable forms of riboflavin, present in enzymes such as succinate dehydrogenase (succinate: ubiquinone oxidoreductase EC 1.3.5.1), monoamine oxidase, and gulonolactone oxidase, which cannot be released by the hydrolytic enzymes in the gut. Also unavailable (or very poorly available)

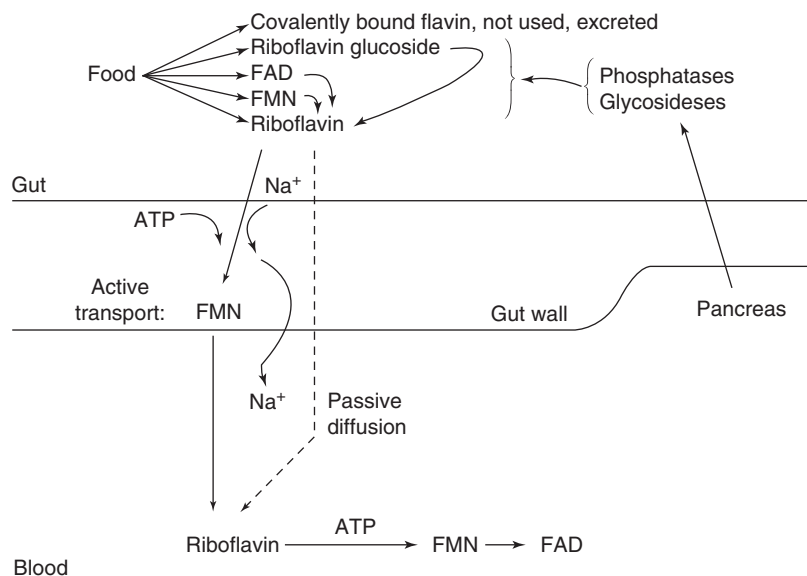


Figure 1 Characteristics of the absorption process for riboflavin and its coenzymes.

in humans is the riboflavin synthesized by the gut flora of the large bowel. Certain animal species such as rodents can utilize this riboflavin source by coprophagy.

Analogues of riboflavin have been prepared to explore riboflavin economy. Some of these have riboflavin-like activity; others are inactive, whereas some are antagonists and can cause functional deficiency. These structural variations can affect absorption and the conversion of riboflavin into its coenzyme forms. Certain drugs such as phenothiazines (antipsychotics) have sufficient structural similarity to riboflavin to act as antagonists.

Absorption by Human Subjects

Studies of riboflavin absorption by human subjects require a test dose taken by mouth, and a sampling procedure to estimate the amount absorbed and its subsequent fate. The sampling compartment is generally urine, because plasma is unsatisfactory (see the last paragraph of this section and the section on Assessment of Riboflavin Status below), and fecal sampling is useless because of the synthesis of riboflavin by bacteria in the large bowel. Riboflavin labeled with radioactive or stable isotopes has not yet been widely used in human studies. Instead, most studies have relied on relatively large bolus oral doses of several milligrams of riboflavin, with urinary monitoring over a few hours. Riboflavin can be quantified in urine by its characteristic fluorescence, by a microbiological assay, or high-performance liquid chromatography (HPLC). The duration of exposure in the upper ileum is critical, because this is the region of greatest absorptive efficiency. Slow-release forms of the vitamin do not enhance its absorption, but there does appear to be some absorptive advantage for synthetic lipophilic esters, such as the tetrabutrylate ester, which becomes hydrolyzed to free vitamin during or after absorption. These esters possess beneficial (e.g., antioxidant) properties in some model systems. Food can enhance absorption, possibly by increasing bulk transit time.

The efficiency of absorption does not vary markedly with age or sex in humans.

The large intestine is now known to possess efficient and specialized carrier-mediated systems that are capable of mediating the absorption of bacterially synthesized B-vitamins, including riboflavin, and this is likely to be especially important for local colonocyte nutrition.

Plasma is of little use as a sampling fluid because redistribution to other tissue sites and urine occurs too rapidly. Although the urinary response to a test dose is preferred, it has the disadvantage that physiological intakes, and especially intakes from poor food sources, cannot be measured accurately. A more sensitive biochemical marker of riboflavin status at low intakes is the index known as the 'erythrocyte glutathione reductase activation coefficient' (EGRAC).

Riboflavin Transport at other Sites and Storage

Free riboflavin is trapped as one of its phosphorylated coenzyme forms, which are then associated (and in a few cases covalently linked) to the protein chains of catalytic flavoenzymes. If not covalently linked, the flavin coenzyme can often be released, for example, by extremes of pH. At some locations, such as mature red cells, flavoenzymes such as glutathione reductase (NADPH: oxidized-glutathione oxidoreductase EC 1.6.4.2) may exist partly in their apoenzyme form, i.e., without the flavin coenzyme and therefore without enzyme activity. An increased supply of riboflavin will allow the depleted coenzyme (in this case FAD) to be synthesized so that holoenzyme and activity can be restored.

Different enzymes and different tissue sites differ in the tenacity with which they retain flavin coenzymes during riboflavin deficiency; thus, there is a characteristic pecking order for flavoenzyme protection, reflecting the metabolic importance of the pathways affected. However, there is no repository of unused or nonfunctional riboflavin that can act as a store in times of dietary deficiency. Although some organs

(such as the liver) have relatively high concentrations of flavin enzymes, all of the flavin is present as coenzyme in flavin holoenzymes. Each tissue has a characteristic ceiling level of riboflavin at saturation and a floor level characteristic of severe depletion, and these are determined, respectively, by the total amount of apoflavoprotein and the irreducible amounts of the holoenzymes, which cannot be depleted of their cofactor complement.

Riboflavin is secreted into milk, whose concentration is species-specific and dependent on maternal status and intake. There is active transport from the maternal to the fetal circulation in pregnancy, the flavin concentration being greater on the fetal side. Studies from India have identified a riboflavin carrier protein (RCP) present in bird (e.g., chicken) eggs, which is specific for riboflavin, and essential for normal embryological development. If it is rendered ineffective (e.g., by immunoneutralization), embryonic development ceases and the embryo dies. A genetic mutant lacking RCP was infertile. A homologous protein, which was rendered ineffective by the antibody to pure chicken riboflavin carrier protein, was found to occur in several mammalian species, including two species of monkeys, and in humans. Circulating RCP levels and the immunohistochemical staining of RCP in biopsy specimens may provide new markers for breast cancer diagnosis and prognosis. Termination of pregnancy occurred after immunoneutralization of RCP in monkeys. The role(s) of RCP in humans, however, remain controversial, and other, less specific riboflavin binders in blood, including gamma-globulins, also seem to play an important role. Further evidence of the flavin needs of developing embryos has been provided by the demonstration that riboflavin analogs can cause teratogenic changes in the absence of any detectable damage to maternal tissues.

Metabolism and Excretion

The riboflavin coenzymes are depicted in **Figure 2**. ATP is a cosubstrate and driving force (in energy terms) for both stages of the conversion of riboflavin into FAD. Some flavoenzymes specifically require FAD, whereas others specifically require FMN. **Table 1** lists the broad categories (by two alternative classification options) of flavoenzymes found in living tissues: all revolve around redox processes. The central biochemical reaction of the flavin coenzymes involves the interconversion of the reduced, dihydro form of the flavin ring and the oxidized form. One of the most important sites of action of flavoenzymes in higher animals is the electron transport chain in the mitochondria. Flavins in succinic dehydrogenase and NADH dehydrogenase form essential redox links between the oxidizable energy-rich substrates of aerobic metabolism and the cytochrome chain, leading to molecular oxygen, which can generate approximately 38 mol of ATP per mole of glucose oxidized.

Hormone status can affect riboflavin economy and riboflavin status can affect hormone production. One important control point for riboflavin economy is thyroid hormone status: hypothyroidism leads to lower tissue levels of flavin coenzymes, and hence to inactivation of certain flavoenzymes, thus resembling the effects of dietary riboflavin deficiency.

Both flavokinase (ATP: riboflavin 5'-phosphotransferase EC 2.7.1.26) and FAD pyrophosphorylase (ATP: FMN adenyltransferase EC 2.7.7.2) are regulated by thyroid hormone status. In the kidney, the synthesis of flavokinase and hence of flavin coenzymes is controlled by aldosterone.

The amount of absorbed riboflavin that remains in the circulation (in blood plasma) is regulated by glomerular and tubular filtration, and tubular reabsorption, in the kidneys. This is an active, saturable, transport process, with characteristics similar to those of active transport in the gastrointestinal tract. It is mainly responsible for the very sharp and characteristic transition between minimal urinary excretion of riboflavin at low intakes, and a much higher level of excretion, proportional to intake, at higher intake levels. This transition point has been used to define and to measure riboflavin status and requirements and to allow studies of intestinal absorption *in vivo*. Excretion of riboflavin is affected by some chemicals (such as boric acid, which forms a complex with it) and by certain diseases and hormone imbalances.

In addition to the excretion of unchanged riboflavin, there are small urinary amounts of hydroxylated breakdown products of the vitamin, which arise through normal turnover, either within the tissues of the body or in the gastrointestinal tract from bacterial action, before absorption. The rate of destruction of riboflavin by this turnover pathway is low in all

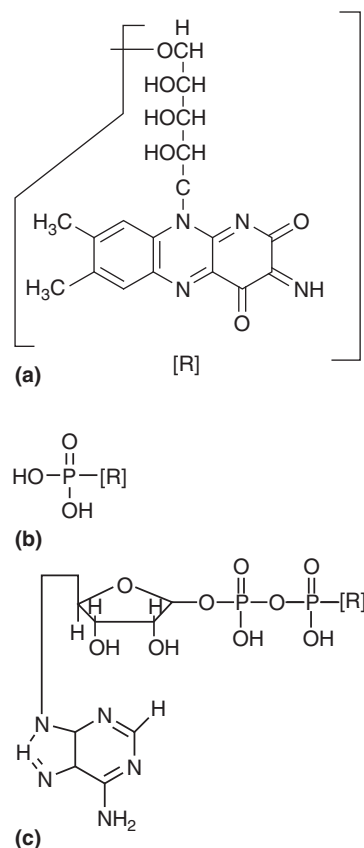


Figure 2 Structure of riboflavin and its coenzyme derivatives. (a) Riboflavin; (b) riboflavin phosphate (flavin mononucleotide, FMN); and (c) flavin adenine dinucleotide (FAD).

Table 1 Two classification options for flavoenzyme categories

Category	Example
<i>Classification by reaction-type</i>	
1-Electron transferases	Mitochondrial electron-transfer flavoprotein
Dehydrogenases	Mitochondrial NADH dehydrogenase and succinate dehydrogenase; (cytosolic) glutathione reductase
Dehydrogenase-oxygen reductases (with O ₂ reduction to H ₂ O ₂)	Monoamine oxidase
Flavoprotein monooxygenases	Bacterial lactate monooxygenase, microsomal FAD-containing monooxygenase
<i>Classification by flavin-type</i>	
Enzymes with FMN	NADH-FMN oxidoreductase
Enzymes with FAD	Glutathione reductase, methylene tetrahydrofolate reductase
Enzymes with both FMN and FAD	NADPH-cytochrome P-450 reductase
Enzymes with covalently bound flavin (formed by posttranslational flavinylation)	Succinate dehydrogenase

species examined and riboflavin within the mammalian body is efficiently conserved.

Metabolic Function and Essentiality

The following section addresses the biochemical and physiological actions of flavins that are responsible for the characteristic functional effects of riboflavin deficiency.

Fatty Acid Oxidation

An early effect of serious metabolic disturbance seen in moderate riboflavin deficiency is a disturbance of fatty acid oxidation. The normal first stage in the spiral process of beta-oxidation of fatty acids within the mitochondria is the removal of two hydrogen atoms from the two carbons located alpha and beta to the activated carboxyl end of the fatty acid chain. The fatty acyl coenzyme A substrate is acted on by one of several fatty acyl CoA dehydrogenase flavoprotein enzymes (e.g., long-chain acyl-CoA:(acceptor) 2,3-oxidoreductase EC 1.3.99.13), each of which is specific for a narrow range of acyl chains. The second stage involves transfer of the electrons via another flavoenzyme, known as electron-transferring flavoprotein dehydrogenase (electron-transferring flavoprotein: ubiquinone oxidoreductase EC 1.5.5.1) and thence to the mitochondrial cytochrome chain and to oxygen. These flavoenzymes, unlike the flavoenzymes that are linked to carbohydrate oxidation, are highly sensitive to dietary riboflavin depletion. Characteristic disturbances of lipid

metabolism therefore arise in riboflavin-deficient tissues and organisms.

Disturbances in fatty acid oxidation by isolated mitochondria, for example, from the livers of deficient animals, have been demonstrated, and one of the most characteristic metabolic changes, observed even in a mild deficiency state in experimental animals, is the appearance of abnormal dicarboxylic acids and their derivatives in the urine. These products may arise because fatty acyl intermediates become diverted away from the usual pathway of mitochondrial beta-oxidation, toward abnormal partial oxidation in the peroxisomes.

Humans normally do not accumulate these urinary products but individuals with an abnormal gene resulting in dicarboxylic-aciduria (as in multiple acyl CoA dehydrogenase deficiency or glutaric aciduria type II) do respond to riboflavin supplements quite frequently, showing a reduction in their excretion and clinical improvement. High-dose riboflavin can thus overcome the genetic abnormality, by providing more coenzyme, thereby ensuring that the residual fatty acid oxidation pathway works at optimum capacity. The accumulation of dicarboxylic acids in urine is characteristic of riboflavin-deficient mammals but not of birds; thus, chick embryos deprived of riboflavin via a genetic lesion affecting riboflavin carrier protein seem to die of hypoglycemic shock, but do not exhibit dicarboxylic-aciduria.

Iron Economy

An important interaction of riboflavin with iron economy is indicated by the fact that iron-deficient animals fail to respond to iron supplements if they are also riboflavin deficient and that the redox system involving riboflavin and its coenzymes interacts readily with the redox system between ferric and ferrous iron.

Studies in experimental animals indicate impairment of iron absorption in riboflavin-deficient animals, changes in its distribution between body compartments; and an increase in rate of iron loss from the intestinal mucosa, resulting in impaired retention of the body iron stores. This enhanced rate of iron loss is accompanied by hyperproliferation of crypt cells, and increased cellular transit along the villi, leading to an excessive proportion of immature villi and a reduction in absorptive area. These studies help to explain how a combination of iron deficiency and riboflavin deficiency, which is frequently encountered in human populations in developing countries, may lead to a gradual deterioration of iron status, which is often accompanied by other intestinal lesions and impaired gut function.

Riboflavin enhances the hematological response to iron, and deficiency may account for at least some of the anemia seen in human populations. Unlike iron-deficiency anemia, the anemia of riboflavin deficiency is usually normocytic and normochromic.

Malaria

Low-dietary riboflavin intakes are frequently encountered in malarious areas of the world, and in some studies, biochemical riboflavin deficiency is associated with a reduced

level of blood cell parasitemia. Although neither animal nor human studies have indicated that riboflavin deficiency protects from the life-threatening sequelae of malaria, there may be an interaction between the parasite and flavins within cells. Some prophylactic drugs used to prevent malaria infection have riboflavin-like structures.

Cataracts and Photoreceptors

Several micronutrients, especially those with antioxidant-type functions in living tissues, might provide some protection against degenerative eye diseases, such as cataract. Animal models, epidemiological studies, and an intervention study in China support the suggestion that good riboflavin status, or riboflavin supplements, may be protective and this possibility deserves further study.

Another intriguing role of flavoproteins in the eye involves a photoreceptor function that synchronizes circadian rhythms with the solar light–dark cycle, acting via cryptochromes 1 and 2, which contain FAD and function as blue light-sensitive photoreceptors.

Interaction with Vitamin B₆

Riboflavin and vitamin B₆ are metabolically interrelated. The conversion of pyridoxine or pyridoxamine phosphates into pyridoxal phosphate is catalyzed by a flavoenzyme (pyridoxaminephosphate oxidase EC 1.4.3.5) and a deficiency of riboflavin can, at certain sites, result in a secondary deficiency in vitamin B₆-dependent pathways.

Effect on Folate Metabolism

FAD is an essential coenzyme for 5,10-methylene tetrahydrofolate reductase, a key enzyme of the folate activation pathway, catalyzing the interconversion of 5,10-methylene tetrahydrofolate and 5-methyltetrahydrofolate. Of several single nucleotide polymorphisms affecting this enzyme, the best known are the C699T and A1298C variants. The former confers thermolability and lowered reductase activity in the TT homozygote, apparently explained by enhanced loss of the FAD cofactor. Marginal riboflavin status is associated with increased plasma homocysteine levels (possibly predictive of increased vascular disease risk), arising from the reduced activity of this key enzyme in TT subjects. The same polymorphism appears to modulate the risk of some cancers, notably colorectal cancer. Gene–nutrient interactions, in which synergism arises between a common genetic subtype and a marginal nutrient deficiency or imbalance, can thus modulate functional risks.

Assessment of Riboflavin Status

Assessment of riboflavin status is closely linked to the estimation of its dietary requirement and the monitoring of human populations for intake adequacy. It is often cheaper, easier, and more accurate to collect a sample of blood or urine from an individual and then perform biochemical analyses

that determine status, than attempting to measure food intakes, because the latter requires more cooperation from the subject and has the limitations of imprecision and poor applicability of food table riboflavin values.

Riboflavin status estimates are generally based on urinary excretion or measurements of erythrocyte glutathione reductase (NADPH: oxidized-glutathione oxidoreductase EC 1.6.4.2) and its reactivation with FAD in red cell lysates. Other biochemical indices, such as plasma or red cell flavin concentrations, have been less widely used, but their potential may increase with new assay techniques such as capillary electrophoresis with laser-induced fluorescence detection. Functional indices linked to flavin-requiring pathways *in vivo* are rarely used, except for the investigation of errors of metabolism or of rare diseases. The two principal status tests are as follows:

Urinary Excretion

The amount of riboflavin excreted in the urine is negligible at low intakes of the vitamin. As the dietary level increases, there is slow increase to a transition point, above which the slope of the excretion rate increases very sharply and then remains proportional to intake until absorption is saturated. For population studies, it has been found convenient to use the creatinine excretion rate as the denominator, and the suggested interpretation of urinary riboflavin excretion rates is <27 µg riboflavin per gram creatinine as deficient; 27–79 µg g^{−1} as low; and >80 µg g^{−1} as acceptable. This index is sufficiently sensitive to distinguish riboflavin requirements between individuals on low-fat, high-carbohydrate diets and the slightly higher requirement associated with high-fat, low-carbohydrate diets. However, one serious drawback of the urinary excretion index is that it is relatively insensitive to intake variations at low to moderate riboflavin intakes. Another is that 24-h urine samples are not easy to collect and excretion rates may fluctuate over short time periods. In addition, metabolic states associated with tissue catabolism may release riboflavin during cell turnover, resulting in increased urinary excretion even when the dietary intake is low.

The Glutathione Reductase Test

A more reliable status index is the degree of unsaturation of the red blood cell enzyme, glutathione reductase (NADPH: oxidized-glutathione oxidoreductase EC 1.6.4.2), with respect to its flavin cofactor, FAD (Figure 3).

Inadequate dietary riboflavin results in low circulating levels and hence a gradual progressive loss of cofactor from this red cell flavoenzyme over a period of several weeks. Because the enzyme protein (apoenzyme) remains intact and is re-activatable by FAD, it is possible to assess riboflavin status by measuring glutathione reductase activity with, and without, its FAD cofactor, in washed red cells. If riboflavin replete, FAD has little effect and the activation coefficient or the ratio of FAD stimulated to unstimulated activity (EGRAC) is between 1.0 and 1.3–1.4. If deficient, FAD produces a larger stimulation and the activation coefficient is much higher. For individuals living in communities with very low intakes of

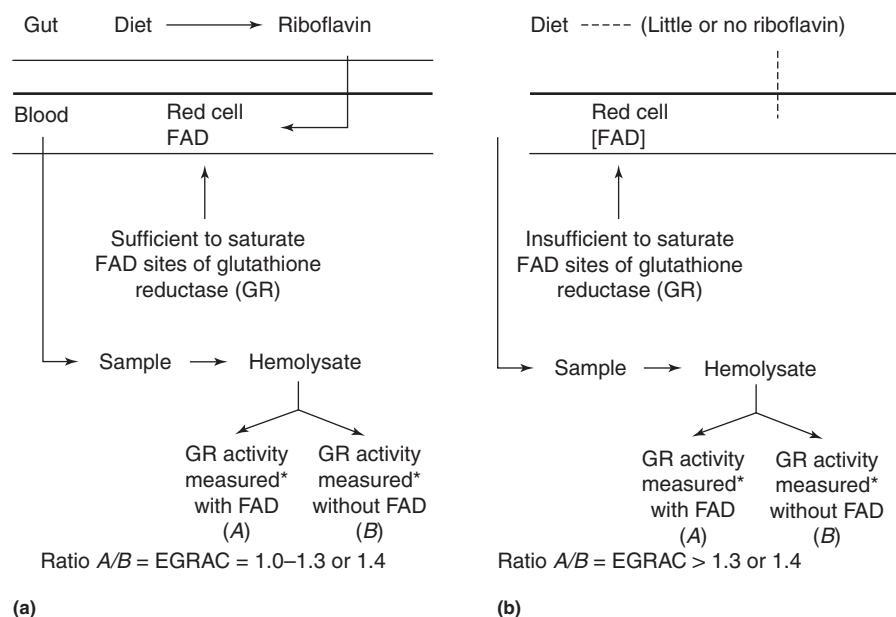


Figure 3 Basis of the glutathione reductase for riboflavin status: (a) riboflavin sufficient; (b) riboflavin deficient. *Reaction of oxidized glutathione with reduced nicotinamide adenine dinucleotide phosphate.

riboflavin and a significant prevalence of clinically recognizable deficiency, activation coefficients as high as 2.0–3.0 are common. In Western countries, few values as high as 2.0 are encountered. However, recent population surveys in the UK have indicated that the proportion of values between 1.3 and 1.8 is considerable across all age ranges. Whether this reflects suboptimal intakes of riboflavin-rich foods, such as cow's milk, remains uncertain.

This blood test is highly sensitive to, and predictive of, the extent of tissue depletion in the range of severe to moderate deficiency. It is robust and requires only a small sample of blood and can be automated by commercial enzyme rate reaction analyzers. When deficient subjects are provided with riboflavin supplements, there is rapid restoration of saturation of the enzyme, and graded supplements can be used to estimate human requirements.

There are minor operational differences among different published versions of the analytical procedure for EGRAC, which result in small between-laboratory differences in the definition of the normal range, and there are external factors that may cause ambiguity of interpretation. One of these is the genetic variant, glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP+ 1-oxidoreductase EC 1.1.1.49) deficiency. Both homo- and hetero-zygotes are affected, and their erythrocyte glutathione reductase is almost saturated with FAD, even when their tissues are riboflavin deficient. Alternative tests of status, such as HPLC measurement of riboflavin in blood fractions, are then required.

Some groups of individuals have increased requirements for riboflavin. There is, for instance, a progressive increase in requirement during pregnancy, followed by a gradual decrease during lactation. Babies exposed to phototherapy for neonatal jaundice have increased requirements. Oral contraceptives may increase requirements, but the evidence is conflicting. Individuals with inborn metabolic errors leading to

dicarboxylic-aciduria and associated clinical abnormalities may have a functional deficiency. Some drugs affect riboflavin status indices, but their need for riboflavin supplements is uncertain.

Requirements

As for all micronutrients, the evidence on which requirement estimates are based can be subdivided into the following broad classes of criteria:

1. prevention of clinical (pathological) deficiency;
2. attainment of specified blood levels or tissue stores of riboflavin;
3. titration to the urinary excretion threshold;
4. tests based on cofactor saturation of one or more accessible, diet-sensitive, flavin-dependent enzymes, such as erythrocyte glutathione reductase; and
5. optimization of riboflavin-dependent physiological functions.

Of these five classes of criteria, the first has been useful in defining 'minimum' requirements, but as a practical test of status, it has several drawbacks. Clinical signs of deficiency in human communities tend to be nonspecific and multifactorial and signs such as angular stomatitis and cheilosis do not always correlate closely with, or respond rapidly to, changes in dietary riboflavin supply or biochemical evidence of deficiency. Factors such as local infection are also likely to be critical.

The use of physiological functional indices in relation to riboflavin deficiency (analogous to dark adaptation for vitamin A; clotting factors for vitamin K, etc.) has not proved possible, because the analogous riboflavin-sensitive physiological processes are insufficiently specific for use in

population studies. Of the biochemical indices, urinary excretion and reactivation of erythrocyte glutathione reductase are the most favored for human studies.

For avoidance of clinical deficiency signs in normal healthy adults, the basic requirement for riboflavin is 0.55–0.8 mg day⁻¹. The UK reference nutrient intake (RNI) for riboflavin is 1.3 mg day⁻¹ for men and 1.1 mg day⁻¹ for women, increasing to 1.6 mg day⁻¹ during pregnancy and lactation. For formula-fed infants, the reference intake is 0.4 mg day⁻¹. The requirements may increase to some extent as a result of heavy exercise or dieting, and abnormal status has been observed in anorexia nervosa.

In the US, the current recommended dietary allowances (RDAs) are 1.3 mg day⁻¹ for men and 1.1 mg day⁻¹ for women, increasing to 1.4 mg day⁻¹ in pregnancy and 1.6 mg day⁻¹ in

lactation, with proportional amounts, based on metabolic body weights and growth requirements, for children and adolescents. RDAs are set 20% higher than the estimated average requirement (EAR) for each group.

Dietary Sources and High Intakes

Table 2 lists the riboflavin contents of some commonly consumed foods in Western countries. As is the case with most other B vitamins, the richest food sources comprise items such as offal and yeast extract, with meat and dairy products such as milk also providing generous amounts. (In the UK, milk intake by children and young adults has tended to decline in recent decades, being affected by changes in government policy on provision of free milk to school children, and by the increasing popularity of manufactured soft drinks.) Fruit and vegetables provide modest amounts of riboflavin, and ungerminated grains and seeds, such as nuts, are relatively poor sources. There is an enormous difference in intakes and in status observed between most Western countries, on the one hand, where the dietary intake tends to be relatively generous, and many developing countries, on the other, where the common staples tend to be riboflavin-poor foods such as polished rice. Although riboflavin deficiency is not as life threatening as some other types of malnutrition that are commonly encountered in the Third World, it can nevertheless cause debility, through skin lesions and metabolic dysfunctions. Therefore, optimization of riboflavin nutrition deserves a place in public health improvement programs.

As with most other B vitamins, riboflavin and its cofactors are remarkably nontoxic even at high intakes. The reasons for this are probably the upper limit on absorption and very rapid urinary excretion of any absorbed vitamin that exceeds cellular requirements. Some recent studies have suggested that high-dose riboflavin may benefit certain medical conditions, such as migraines, lactic acidoses, myopathies, and Leigh disease.

See also: Amino Acids: Metabolism. Antioxidants. Iron: Physiology, Dietary Sources, and Requirements

Table 2 Riboflavin content of selected foods

Food	mg per 100 g fresh wt	mg per MJ
<i>Meat, offal, and fish</i>		
Stewed minced beef	0.19	0.22
Grilled pork chop	0.16	0.21
Calf liver, fried	2.89	3.94
Lamb's kidney, fried	3.10	3.95
Cod, grilled	0.06	0.15
<i>Dairy products</i>		
Cows' milk, full cream	0.23	0.84
Cheese, cheddar	0.39	0.23
Yogurt (whole milk, plain)	0.27	0.81
Boiled chicken's egg	0.35	0.57
Human milk	0.03	0.10
<i>Fruits</i>		
Apples, eating flesh and skin	0.02	0.10
Oranges, flesh	0.04	0.25
Pears, flesh and skin	0.03	0.18
Strawberries, raw	0.03	0.27
Dried mixed fruit	0.05	0.04
<i>Vegetables</i>		
Potatoes, boiled, new	0.06	0.19
Carrots, boiled, young	0.01	0.11
Brussels sprouts, boiled	0.09	0.59
Cauliflower, boiled	0.04	0.34
Onions, fried	0.01	0.01
<i>Grains, grain products, nuts</i>		
White bread	0.06	0.07
Wholemeal bread	0.05	0.05
Rice, boiled, white	Trace	Trace
Cornflakes (Kellogg)	1.3	0.81
Baked beans in tomato sauce	0.06	0.35
Peanuts, plain	0.10	0.04
<i>Other</i>		
Marmite (yeast hydrolysate)	11.9	15.6
Bovril (beef hydrolysate)	8.5	11.2

Note: Compiled and calculated from data in the Food Standard Agency's Integrated Data set (CoF IDS): <http://tna.europarchive.org/20110116113217/http://www.food.gov.uk/science/dietarysurveys/dietsurveys/> (accessed May 2011), based on McCance and Widdows's *The Composition of Foods*, 6th Summary edn. (2002) Cambridge: Royal Society of Chemistry. © Crown copyright material is reproduced with the permission from the Controller of HMSO and Queen's Printer for Scotland.

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SALT

Epidemiology

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Glossary

DASH Refers either to a major trial involving highly controlled single or multiple changes in diet to assess the effects on blood pressure or a DASH diet which is taken to mean diets complying with the greatest induced changes in salt, fruit and vegetable, and fat intakes used in the original trials.

Discretionary salt The salt used in the home where the person/family determines how much salt is used rather than inadvertently taking it in purchased food products.

Hypertension An increase in blood pressure usually considered to be present in adults when either of the two

measures of arterial blood pressure attain or exceed consistently either 140 mmHg pressure for the upper reading (systolic pressure) or 90 mmHg for the lower reading (diastolic pressure).

Intersalt The name given to an international population study relating urinary electrolyte excretion and blood pressure measurements made in the same individuals in 52 communities around the world.

Migration This simply means a substantial change of residence, e.g., from country to a city and does not necessarily mean moving to a different country.

Introduction

This article describes the historical importance of salt use; its production and trade throughout the centuries; and its significance in food preservation, flavor enhancement, and food processing. Man developed complex mining and salt drying systems, which are still in use as the demand for salt grows. Humans and other animals, exposed throughout evolution to very limited salt sources, have developed an intrinsic biological drive for salt with salt-specific taste receptors and highly effective hormonal and cellular transport systems for minimizing any salt loss from the intestine, kidney, and skin. The use of highly salted food unfortunately then induces a series of physiopathological responses including changes in blood volume, hormonal, and cellular changes that lead, in conjunction with other dietary and environmental factors, to a range of disorders including high blood pressure with its increased risks of stroke, coronary heart disease, and heart failure. Excess salt intakes also seem to promote the development of osteoporosis, gastric cancer, and bronchial reactivity. The relationship of salt intakes to these conditions will be described and the options for limiting intakes will be outlined.

Occurrence in Nature

The terms salt, sea salt, or table salt relate primarily to the compound sodium chloride. Sodium is the sixth most abundant element in the Earth's crust, of which it constitutes 2.8%. Sodium is a reactive element and is always found in compound form. There are a huge variety of salts containing sodium and many of these are found in food, but in most societies the dominant form of sodium is as sodium chloride. Sodium chloride is very soluble in water and in seawater comprises approximately 80% of the dissolved matter.

A History of Salt Intake

The fundamental drive to obtain salt has been recognized from the earliest times when humans evolved in a hot African environment with scarce sources of salt. Evidence has been found of salt use during the Neolithic Age and the Egyptian, Babylonian, and Chinese civilizations, all had special culinary uses for salt that are well documented. In China for centuries the production of salt was a major industry. Salt sources were highly valued and were often protected. A tax on salt in the

form of a head tax provided the Chinese government with a reliable resource of revenue from approximately 2200 BC. For centuries the only method of extraction practiced by coastal salt-workers was to boil sea water and this technique was employed in every maritime province of China as late as 1830. Solar evaporation was also used or alternatively shallow salt fields were filled with sea water, which was shifted from field to field daily until salt crystallization began. A third method used in areas either far from the sea or on higher ground involved digging wells to tap sea water or salt-enriched aquifers.

Evidence for the exploitation of saline slicks in the Austrian Tyrol dates from the Bronze Age and to this day the salt mines of Salzburg in Austria and Krakow, Poland, are still in use. For the Indians in Central America salt was so precious that to please their gods they abstained from eating salt and Mexican civilizations offered sacrifices to the goddess of salt, Vixtocioatl. Arab cultures still offer salt to visitors as a sign that their guest is safe; even a Bedouin robber will not violate the laws of hospitality once he has tasted his host's salt.

In pre-Roman times, the principal Italian road started at the saltworks near the mouth of the Tiber River and cut through the Italian peninsula toward the Adriatic. In North Africa the caravan route linked the salt oases, whereas salt roads were a feature of several South American countries. From remote parts of South America, such as the Amazon and Argentina, trails of more than 1500 km were linked to form the famous 'Cerro de Sal'. In the sixteenth century, sea salt crystals were traded from the sea through the Andes, gradually becoming more expensive further from the sea so that at distances of more than 300 km it was used only by tribal chiefs. The common people made do with salt processed from palms and human urine. Salt from springs near Bogota was traded over a distance of 200 km to the north and south. Columbus' voyages were financed by the wealthy proprietors of the Mata Salt region of Spain and when the first Spaniards arrived in South America in 1537, they found Indians exploiting local salt reserves on a large scale.

The financial structure of Venice was also substantially affected by the salt trade, which contributed to the emergence of Venetian capitalism and the vast fortunes of some Venetian merchants. In France, salt became a political issue in the fourteenth century; the tax on salt was the most hated of all taxes and a major issue before the French Revolution. At that time England, Germany, and Italy also taxed salt and in Britain the control of the world salt markets was a substantial contributor to wealth in the seventeenth and eighteenth centuries. Liverpool, a minor tobacco port in the early eighteenth century also became a major trading city in part because of its role in the salt trade.

During the earliest period of British rule in India the supply of salt was often tightly controlled and then taxed. Gandhi emphasized the essential nature of common salt for human and animal well-being, especially in a tropical country like India. Gandhi's 'salt march' to the sea broke the monopoly on salt use and led to his arrest and jailing. The following revolt, with 100 000 arrests, brought a change in the law to allow people to produce salt for their own use.

The production of salt currently depends on the same range of methods as have been used for centuries with substantial amounts being obtained by dry mining. Solution mining still involves water being pumped into rock salt

deposits and the resulting brine being pumped back up to the surface for purification and evaporation. Solar evaporations, the oldest of the methods, is still used in hotter climates with the salt pools then allowing the evaporation of the salt or sea water in the sun. Currently world salt production is more than 210 million tons a year with 60% of the production being used to manufacture chlorine, caustic soda, and synthetic soda ash. Approximately 20% of the world's production is for food use.

Salt in Food Technology

Salt enhances and modifies flavor, controls microbial growth, and alters nutrient availability and the texture/consistency of food. It also aids extraction methods, food formulation, and helps in the malting and the fermenting of foods. In the production of some foods, for example, pickles, cheese, and fermented sausages, salt induces the withdrawal of water and various nutrients from the pickled tissue and provides an appropriate environment for growing the specific salt-resistant bacteria required for the fermentation or pickling process.

Sodium is also important in forming the texture of cheese, limiting bacterial growth, and dehydrating cheese, thereby helping to form the rind. Most processed meats, for example, ham and bacon, have added salt to season and cure the meat. Salt also inhibits bacterial growth and helps to emulsify the fat in sausages.

Sodium nitrate is used as a curing agent to prevent botulism as well as to provide the cured taste and red color of such meat products. Sodium polyphosphate is often added to poultry and fish fingers to increase their water holding capacity and to bind the product. Salt is also effective in binding meat together by altering protein structures and dissolving some proteins. Salting fish has both flavoring and a preservative role and fish may be treated in brine before being smoked.

In baking, salt enhances other flavors in the product; it also controls the rate of fermentation of yeast-leavened products and prevents the development of undesirable 'wild' types of yeast, which would lead to uncontrolled fermentation rates and variable products. Salt also strengthens the gluten in bread doughs, thus helping to ensure good dough-handling and reducing the rate of water absorption. Sodium acid pyrophosphates are used in many industrial baking powders for speciality products. Salting of canned vegetables is primarily for flavor, but it can be used to separate mature, starchy green beans or peas, which will sink, from the younger, fresher beans, which float.

Processed 'snacks' are often heavily salted as a marketing feature, as are processed cereals and sodium-containing ingredients are added to many processed foods (Table 1), with sodium chloride accounting for approximately 90% of the sodium used by the food industry.

Other Uses of Salt

The universal use of common salt has allowed it to be used as a vehicle for combating widespread iodine deficiency by fortifying the salt with iodine, and fluoride has also been added as a preventive measure against dental caries. Chloroquine or

Table 1 Sodium-containing additives used in food processing

Additive	Use
Sodium citrate	Flavoring, preservative
Sodium chloride	Flavoring, texture preservative
Sodium nitrate	Preservative, color fixative
Sodium nitrite	Preservative, color fixative
Sodium tripoliphosphate	Binder
Sodium benzoate	Preservative
Sodium erythroate	Antioxidant
Sodium propionate	Preservative
Monosodiumglutamate	Flavor enhancer
Sodium aluminosilicate	Anticaking agent
Sodium aluminium phosphate acidic	Acidity regulatory emulsifier
Sodium cyclamate	Artificial sweetener
Sodium alginate	Thickener and vegetable gum
Sodium caseinate	Emulsifier
Sodium bicarbonate	Yeast substitute

pyrimethamine salt mixtures have been used to suppress the sporozoites responsible for vivax malaria.

The Impact of Refrigeration on Salt Intakes

Salt intake varies widely across the world. Some agricultural communities, for example, the Yanomano Indians from Brazil and the Chimbus of New Guinea, do not consume salt other than that found in natural food sources. The Kamtschadales and the Tungouses nomadic tribes from the north of Russia and Siberia are also averse to added salt, whereas the Japanese have traditionally consumed large quantities of salt in pickled salted fish and vegetables.

Without some form of food preservation it would be impossible to supply urban populations with food in any systematic way. Refrigerators were introduced on a mass scale from the 1960s onward and this was accompanied by a fall in salt consumption in most countries (Table 2) and refrigeration has taken over from salting as a method of preserving food. In Roman times salt use was estimated to amount to 25 g day⁻¹ on average but more recently in Japan, intakes as extreme as the 60-g intake of a farmer were observed in 1955 with an average consumption of 27–30 g per person per day. However, this had fallen dramatically to 8–15 g per person per day by 1988. However, in the USA salt intake probably started to decline in the 1920s as ice-boxes and refrigerators became widely available.

There are also analyses of salt intake in children, which show that by the age of 4 years intakes of salt are already, for example, in the UK 5 g day⁻¹ on average with boys taking in approximately 10% more salt than girls. As the children grow and eat more their salt intakes rise so that in UK teenage boys aged 15–18 years were consuming 8.3 g day⁻¹ and girls 5.8 g day⁻¹ on average in 1997.

Changes in Mineral Composition of Food Induced by Industrialization and Migration to Urban or Affluent Communities

The process of industrialization and urbanization has affected the nutritional value of many of the more traditional foods as

illustrated for Mexico in Table 3. Although corn and maize tortillas, together with beans, formed the staple traditional diet, tortillas are now being produced differently, both industrially and by individuals at small market stalls in the cities.

The concentrations of the major nutrients sodium, potassium, calcium, magnesium, and phosphorus in unprocessed foods vary within narrow limits, but in processed or cooked foods, where salt (NaCl) or additions of other sodium-containing ingredients are common, the concentration range of sodium is higher. A large proportion of processed food has salt added; as more processed foods are eaten, the saltier the diet becomes. Table 3 shows that the maize in its original form contains a very small concentration of sodium but is rich in potassium. Once the grain is milled, fractionated, and processed to produce tortillas, then the nutrient composition alters. Potassium is also lost during the initial washing procedure. Limestone is added to release the niacin from its bound form; this also induces a threefold increase in calcium content. Salt is not commonly added during tortilla preparation in the country, but a remarkable 70- to 200-fold increase is found in breakfast cereals and processed maize snacks as well as substantial potassium losses. Almost no calcium is found in modern breakfast cereals whereas traditionally prepared tortillas have almost 60-times more calcium.

Migration studies have also revealed the marked effects of transferring into cities from traditional rural areas or migrating to modern affluent environments.

Rural–Urban Differences in Salt Intake and Blood Pressure (BP)

Migrant studies are useful in assessing the impact of environmental changes on BP in different ethnic groups. Shaper's original study on Samburu men recruited from Kenyan villages to military camps was associated with a 12 mm Hg increase in systolic BP (SBP) within weeks and similar findings were obtained in Ugandan villagers who had migrated to an urban environment. Table 4 shows some of the differences between individuals living in their original rural African environment and those who had migrated to a more complex urban environment. In both countries the urban dwellers' BPs were higher and the Ugandan analyses evaluated the rate of rise in BP with age in the two communities and showed marked differences.

Beaglehole also found that the BP of Polynesian children migrating to New Zealand rose simultaneously with dietary changes and this increase was not explained simply by an increase in body weight. More recent studies, for example, in Mexico (Table 5), show the effect of migration on both sexes with in this case a rise in potassium as well as in sodium intakes in both sexes.

Conversely the Japanese migrating to the US showed marked reductions in the prevalence of hypertension and stroke mortality consistent with the known markedly lower salt intake in association with other environmental changes in the US and Tunisians migrating to France and slowly adapting

Table 2 Salt intake as NaCl (g day⁻¹)

<i>Before 1982^a</i>	<i>Year</i>	<i>Intake</i>	<i>From 1988^b</i>	<i>Year</i>	<i>Intake</i>
<i>Communities not using added salt</i>					
Brazil (Yanomano Indian)	1975	0.06			
New Guinea (Chimbus)	1967	0.04			
Solomon Island (Kwaio)		1.20			
Botswana (Kung Bushmen)		1.80			
Polynesia (Pukapuka)		3.60			
Alaska (Eskimos)	1961	<4.00			
Marshall Islands in Pacific		7.00			
<i>Salt-using communities</i>					
Kenya (Samburu nomads)		5–8	Mexico (Tarahumara Indians)		3–10
Mexico (Tarahumara Indians)	1978	5–8	Mexico Rural, men ^d	1992	6.0
			Mexico Rural, women ^d	1992	5.4
			Mexico Urban, men ^d	1991	7.7
			Mexico Urban, women ^d	1991	6.7
Denmark		9.8	Denmark	1988	8.0
Canada (New Foundland)		9.9	Canada		8–10
New Zealand		10.1			
Sweden (Göteborg)		10.2			
USA (Evans County, Georgia)		10.6	USA (Chicago)		7.7
Iran		10.9			
Belgium	1966	11.4	Belgium ^k	2010	10.3 ^l
UK (Scotland)		11.5			
UK ^c				1990	9
Australia		12.0			
India (North)		12–15	India		9–11.4
Federal Republic of Germany		13.1			
Finland (East)		14.3	Finland ^j	2010	9.3 g (men) 6.8 g (women)
Bahamas		15–30			
Kenya (Samburus, Army)	1969	18.6			
Korea		19.9			
Japan					
Japan (farmer)	1955	60.3	Japan	1988	8.15
Japan (Akita)		27–30			
Japan	1964	20.9			
			Portugal ^e	2006	12.3
			Denmark ⁱ	2009	10.6 (Men) 7.1 (Women)
			Turkey ^f	2010	18.01
			Slovenia ^g	2010	13.0 (Men) 9.9 (Women)
			Spain ^h	2011	9.8

^aSource: Intersalt cooperative research group (1988) *British Medical Journal*: 297: 319–328.

^bSource: Pietinen PJ (1981) *Journal of the Agricultural Science Society of Finland* 53: 275–284.

^cThe 1987 Dietary and Nutritional Survey of British Adults.

^dSánchez-Castillo, *et al.* (1996) *Archives of Medical Research* 27:559–566.

^ePolónia J, *et al.* (2006) *Revista Portuguesa de Cardiologia* 25:801–817.

^fErdem Y, Arici M, Altun B, *et al.* (2010) The relationship between hypertension and salt intake in Turkish population: SALTURK study. *Blood Pressure* 19: 313–318.

^gRibič CH, Zakotnik JM, Vertnik L, Vegnuti M, and Cappucco FP (2010) Salt intake of the Slovene population assessed by 24 h urinary sodium excretion. *Public Health Nutrition* 13: 1803–1809.

^hOrtega RM, *et al.* (2011) *British Journal of Nutrition* 105: 787–794.

ⁱAndersen, *et al.* (2009) *European Journal of Clinical Nutrition* 63: 598–604.

^jPietinen, *et al.* (2010) *Public Health Nutrition* 13: 920–924.

^kVandevijvere, *et al.* (2010) *European Journal of Clinical Nutrition* 64(11): 1260–1265. Epub (2010) Aug 18.

^lThe average is from two different regions.

Table 3 Effects of industrialization on the composition of Mexican foods

Food	Salt content (mmol per 100 g fresh weight)		
	Na	K	Ca
Corn	4	284	55
Tortilla (traditional)	11	192	177
Processed wheat tortilla	620	73	11
Breakfast cereals	866	101	3
Processed snacks	838	197	102
<i>Beans</i>			
Beans, home cooked	14	470	67
Beans, processed	354	371	26

Source: Adapted from Sanchez-Castillo, *et al.* (1997) *The Journal of Food Composition and Analysis* 10: 312–333.

Table 4 Migration studies: Rural–urban differences in Africa

	Uganda ^a		Ghana ^b	
	Village	Urban	Village	Semiurban
SBP/age slope	0.15	0.64	–	–
SBP (mmHg)	–	–	121.5	129.2
Urinary Na (mmol l ⁻¹)	82.4	108.6	99.0	103.1
Urinary K (mmol l ⁻¹)	67.4	38.4	58.1	39.7
Na/K	–	–	1.91	2.83

^aPoulter, *et al.* (1984) *Journal of Cardiovascular Pharmacology* 6(supplement 1): S197–S203.

^bKerry, *et al.* (2005) *Ethnicity and Disease* 15: 33–39.

Table 5 The urinary 24-hour output of electrolytes and the associated BP differences in rural and urban Mexico

	Men		Women	
	Rural n = 24	Urban n = 19	Rural n = 54	Urban n = 58
Na (mmol day ⁻¹)	103.3	133.1	93.3	114.7
K (mmol day ⁻¹)	41.6	56.7	36.9	50.4
Na/K	2.64	2.51	2.67	2.44
NaCl (g day ⁻¹)	5.99	7.72	5.41	6.65
SBP (mm Hg)	110.4	114.3	104.4	113.8
DBP (mm Hg)	73.3	75.6	67.0	72.8
Body mass index	25.5	25.1	24.1	26.6

Source: Adapted from Sánchez-Castillo CP, Solano ML, Flores J, *et al.* (1996) Salt intake and blood pressure in rural and metropolitan Mexico. *Archives of Medical Research* 27: 559–566.

to the French more Mediterranean cuisine dropped their salt intakes by a quarter.

Salt and Disease

The Roman word from which the name ‘salt’ was derived is Salus, goddess of health. Gandhi also argued that salt was

“essential for human wellbeing, especially in a poor country like India where its inhabitants eat vegetables and rice which contain low salt”. However, as time passed, although its name evoked health, a long-term excess intake of salt came to be recognized as a major cause of hypertension and thus a risk for stroke and coronary heart disease. An excess of dietary salt may also affect three other conditions/diseases: gastric cancer, osteoporosis, and bronchial hyper-reactivity. Evidence also suggests that high-salt intake causes left ventricular hypertrophy independently of BP effects.

Salt Intake and BP

When salt is ingested it is readily absorbed in the small intestine in association with other molecules such as glucose. The intestinal secretions also contain sodium at concentrations similar to those found in the plasma but the colon has a highly effective active transport system for absorbing practically all the sodium in the colonic contents; only approximately 1 mmol of sodium is normally excreted in the feces unless a child or adult has severe diarrhea. Once the sodium is absorbed the body ensures that the tonicity of the body fluids is finely maintained; so water is retained by the kidney and the blood volume tends to expand until the hormonal responses, for example, from the atrial natriuretic hormone (released in response to changes in atrial pressure) and in the renin-angiotensin system lead to a fall in the kidney and sweat glands’ reabsorption of sodium and therefore a greater sodium urinary excretion and loss in sweat. Salt is also taken up in the interstitial tissues of the skin without water and this skin sodium seems to precipitate an increased polymerization and sulfation of glycosaminoglycans. Subcutaneous tissue macrophages then react and express the transcription factor tonicity enhancer binding protein and secrete vascular endothelial growth factor C, which stimulates lymphatic formation and endothelial nitric oxide synthase expression. Nitric oxide is a well-recognized endothelial dilator so the balance of secreted molecules, which includes transforming growth factor β production seems to determine to some extent the individual’s BP response to higher salt intakes. These new insights into the role of extracellular sodium also suggest that the immune system has a role in the regulation of water and tissue volume and BP homeostasis. The adjustments in vasomotor tone and the exchange of sodium and potassium across cellular membranes interact with the neuronal responses and the kidney may then reflexly demand a higher BP in order to limit the body’s extracellular volume expansion.

The degree to which the BP rises in response to dietary salt also depends on a range of interacting genetic factors and other environmental influences including the intake of potassium, magnesium, and calcium. The suppressive effects of these minerals partly explain the BP-lowering effects of a diet rich in fruit and vegetables but recently nitrate associated with vegetable intake has also been shown to generate nitrite by oral bacterial action with this nitrite serving as a substrate to produce the endothelial nitric oxide and a fall in BP. Higher fat intakes have been shown to amplify resting BPs whereas moderately intense exercise is followed by a lower BP. As body

fat increases as a consequence of excess weight gain, the greater storage of fat leads to changes in a range of hormonal secretions from the fat cells including angiotensinogen, a precursor of the renin-angiotensin axis affecting the kidney's excretion of sodium. Adiponectin secretion from expanding adipocytes falls thereby making the blood vessels much more sensitive to plaque formation, medial hypertrophy, and fibrosis. Salt-induced increases in BP also involve an array of other hormonal responses including the potent vasoconstrictor endothelin-1 and the vasodilator bradykinin, these being potentially involved in the BP-independent effects of higher salt intakes on arterial thickening, cardiac ventricular hypertrophy and the synthesis of elastin and collagen in the artery. This makes them progressively thicker and less pliable. There is increasing evidence that the salt-induced rise in BP is markedly increased if the subjects are obese and that the pliability of vessels can adjust quite rapidly to changes in salt intake. Obese individuals also tend to have a higher salt intake because of their relatively high-food intake for their physical activity level.

Given this complex of interacting factors it is not surprising that the selective effect of salt intakes on BP has been hard to define. The role of salt in inducing high BP is based on extensive animal experiments at the cellular and physiological levels, on clinical studies and dietary intervention trials as well as on major population analyses of BP in relation to salt intake. Meta-analyses of longer-term intervention trials to investigate the effect of salt reduction on hypertension also demonstrate that a modest reduction in salt intake has a significant effect on BP in normotensive individuals and an even greater effect in those with preexisting hypertension.

The response of neurohumoral mechanisms to salt loading varies in different individuals and for many years investigators sought to define what they termed 'salt-sensitive' individuals. There are rare genetic mutations associated with extreme salt-sensitivity but within the general population there appears to be a more or less continuous variation in responsiveness consistent with multiple gene-environmental interactions. So perhaps it is not surprising that the choice of cutoff points for defining 'salt-sensitivity' is rather arbitrary. Patients with advanced renal failure do have an increased response of their BP to salt loading but this is due to a loss of functioning nephrons.

In all the analyses of population migration, several dietary changes as well as altered salt intakes have occurred, for example, in potassium and calcium intakes together with weight gain, altered intensities of physical activity and doubtless psychosocial stress from entering an unfamiliar environment. Experimental, epidemiological, and clinical evidence suggests that dietary deficiencies of potassium or calcium potentiate the sodium induction of high BP. Potassium loading prevents or ameliorates the development of sodium chloride-induced hypertension in several animal models and epidemiologically the ratio of urinary sodium to potassium (Na:K) is a stronger correlate of BP than either sodium or potassium alone. Results of clinical trials also suggest that an increased potassium intake decreases BP in patients with hypertension and the anti-hypertensive effect of potassium is more pronounced in persons consuming a high sodium chloride intake. With

acculturation, primitive societies tend to both increase the sodium intake and reduce the potassium content of their diet so the combination of a high-potassium with a high-salt diet is, therefore, somewhat unusual. However, high-potassium intakes were found in the Aomori prefecture of Japan where there was a lower BP and a reduced mortality from strokes despite high salt intakes.

There is also an inverse association within and among populations between dietary calcium and BP. A low-calcium intake may amplify the effect of a high sodium chloride intake on BP, and calcium supplementation blunts the effect of a high sodium chloride intake on BP. High dietary calcium also preferentially lowers BP or attenuates the development of hypertension in sodium chloride-sensitive experimental models.

Given all these dietary effects discerning the impact of salt intake changes as such is not easy. The migrant studies are crude compared with the analyses of controlled dietary changes in the sodium intakes of volunteers. More robust analyses can also be obtained from the relationship between sodium intakes and BP across a whole spectrum of different societies where account is taken of the possible effects of sodium intakes at different ages, of other dietary and environmental effects as well as of differences in body size. The ability to reduce BP by selectively limiting dietary sodium intake has also been assessed in a series of meticulous meta-analyses.

Genetic Influences

Primary hypertension has a well-known familial aggregation and has been calculated to be approximately 40% genetically determined. Children with a family history of hypertension are 30% more likely to remain in the upper quartile of SBP than their peers. Young adults from families with hypertension have a greater rate of sodium excretion after a salt load than adults from normotensive families. Twin studies also provide convincing evidence for a hereditary component to salt responsiveness. However, the effect of family history decreases with age as other environmental factors, for example, weight gain modify the risk. Studies have suggested that polymorphisms in certain genes, such as the angiotensinogen gene, might be implicated in the BP response to a high salt intake and genes whose products function prominently in the renin-angiotensin-aldosterone system, are potential candidate genes contributing to essential hypertension. However, two meta-analyses assessed the relation of both insertion/deletion (I/D) polymorphisms of the angiotensin-converting enzyme (ACE) gene and in the M235T angiotensinogen gene with primary hypertension and cardiovascular diseases and found no association with hypertension in ACE I/D gene polymorphism. Individuals homozygous for the deletion allele seem to have a higher risk of macrovascular and microvascular complications and the T allele encoding angiotensinogen may be a marker for hypertension, at least in white subjects but great caution is needed before inferring that a single set of genes has a substantial impact on the development of higher BPs in response to increases in salt intakes as so many neurohormonal mechanisms are involved. New analyses suggest that particular

genes may be important in Asian and in African population's sensitivity to salt-induced hypertension.

Age-Related Changes in BP

In most populations BP increases with age but there are a few small groups who have not been exposed to modern environmental conditions and they do not show a rise in BP with age. The Kuna indigenous populations who live on islands in the Panamanian Caribbean were among the first communities described showing almost no age-related rise in BP or hypertension. Other populations in Africa, the Americas, Asia, and the Pacific Region have the same characteristics. In many of these communities, the primary evidence that the protective factor is environmental rather than genetic was the BP rise following migration to an urban environment. Among the many lines of evidence suggesting a role for salt intake in the pathogenesis of hypertension, particularly compelling has been the identification of these isolated communities where salt intake is low, hypertension is rare, and BP does not rise with age. Salt intake in such communities generally provided less than 40 mmol of sodium per day, and typically much less. The age-related rise is rare at mean sodium excretion rates of $< 100 \text{ mmol day}^{-1}$ but clearly there are many other dietary and environmental differences.

Intersalt Studies

A major transnational study of more than 10 000 men and women described the association between urinary excretion of sodium chloride (as a measure of salt intake) and BP. After

adjustments for body weight, alcohol intake, sex, and age, a higher sodium intake of $100 \text{ mmol day}^{-1}$ was linked with a SBP rise of 3–6 mm Hg in adults aged 40 years but one of 10 mm Hg when aged 70. Updated results suggest that the association between sodium excretion and BP is stronger when not adjusted for body weight, but the relationship is present whether or not the adjustment is made.

Figure 1 summarizes the relationships between sodium intake and BP in the intersalt study. Different populations may show different responses depending on the host of other environmental factors that may be involved. The figure also illustrates the fact that individuals within any population may show very different effects and that appreciable changes in salt intake may be needed before a clear change in BP is evident. Part of the problem in displaying the relationship arises for the difficulty in establishing what the prevailing BP of individuals is given the remarkable variation in BP during the day and night; difficulty also arises because it takes many complete 24-h urinary collections to obtain a reasonable estimate of the customary sodium intakes. The age-related incline also implies longer-term amplification of the pathophysiological changes in hormonal controls and in blood vessel reactivity and plasticity - thus as the BP increases the tendency to further increases is enhanced in an accelerating process. This emphasizes the potential importance of early interventions when the BP is tending to rise. It also implies that interventions to alter the diet of the young may be particularly valuable. This is borne out by the observation in the Netherlands that new-born babies fed a reduced salt content in their formula milk for the first 6 months of life had very much lower BPs when reassessed at the age of 15 years. Early influences are also suggested by the observation that small babies are more likely to have hypertension than adults.

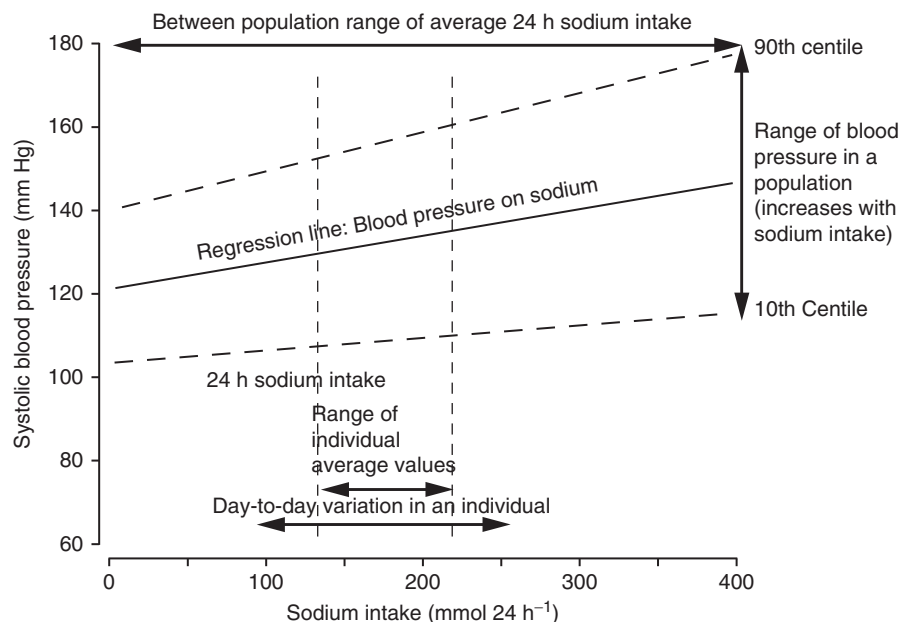


Figure 1 The relationships between sodium intake and BP in the intersalt study. Reproduced with permission from Frost CD, Law MR, and Wald NJ (1991) By how much does dietary salt reduction lower blood pressure? II. Analysis of observational data within populations. *British Medical Journal* 302: 815–818.

The range in BP responses to a 100-mmol higher sodium intake can, as shown in the figure, be wide and becomes greater with age. Thus, it has been calculated that the fifth centile response in SBP in a child aged 15–19 years exposed to 100 mmol more sodium per day will be 3 mm Hg whereas a similar increase when aged 60–69 years will amount to 6 mm Hg at the same fifth percentile range. The corresponding 95th centile in the teenagers for the same 100 mmol sodium increase amounted to 7 and 15 mm Hg in the 60–69 year olds. There is an increasing consensus among epidemiologists that salt is an important causal factor determining the steady increase in average BP with age and the increasing prevalence of hypertension.

Adults with episodic high BP, for example, as a response to mental stress, have a greater tendency to develop persisting hypertension. The higher the BP level becomes, the greater the further increase in BP. Thus, the age-dependent increase in BP may be a particularly important factor to measure in both individuals and the community.

On a population basis it has been estimated that in affluent societies, where average population BPs are high, a reduction of 2 mm Hg in diastolic blood pressure (DBP) would result in a 15% reduction in the risks of stroke and transient ischemic attacks and a 6% reduction in risk of coronary heart disease. There may also be a reduction independent of the effects on BP on other conditions such as left ventricular hypertrophy.

A higher frequency of salt responsiveness has been observed in adults with hypertension. Estimates of the prevalences of this sensitivity have ranged from 29 to 60% in hypertensive populations to 15–46% in normotensive populations, although the larger studies have indicated that more than 50% of a hypertensive population and approximately 25% of a normotensive population are clearly salt responsive. Longer-term, for example, 27-year long studies have shown that those with initially normal BP but a marked responsiveness to salt had an increased risk of cardiological events and death as had those with preexisting hypertension. In the absence of a consensus on defining either the genetic polymorphisms relating to hypertension or robust parameters of salt sensitivity the greatest benefits are likely to be achieved by taking the current national approaches to reducing the whole population's salt intake.

Intervention Trials

Two controlled intervention trials: the dietary approaches to stop hypertension (DASH) and the follow-up DASH sodium-trial compared three precisely controlled different types of eating patterns: (1) the 'control diet,' (2) extra fruit and vegetables; and (iii) the 'DASH or combination diet,' which was lower in saturated fat, total fat, and cholesterol as well as having higher intakes of fruits, vegetables, and low-fat dairy products. All three eating plans used 3 g sodium per day. Great care was taken to ensure that the subjects remained weight stable. The combination diet or 'DASH diet' decreased SBP by 11.4 mm Hg below the control diet and decreased DBP by 5.5 mm Hg in adults with hypertension. In adults without

hypertension the decreases were 3.5 mm Hg (SBP) and 2.1 mm Hg (DBP).

When the selective effects of salt were examined without weight changes then reducing the salt intake from 9 to 3 g significantly reduced BP by 6.7/3.5 mm Hg on the controlled diet and on the higher potassium DASH diet by 3.0/1.6 mm Hg.

Thus, the combined effects on BP of the DASH diet and low-salt intake were greater than either of the interventions alone. The effects were observed in both sexes, across racial groups and were more marked in those more than 40 years of age.

One objection to these studies is that, although meticulously controlled, they were of very short duration so perhaps the effects might wear off. However, to keep children or adults on precisely controlled diets for months or years is clearly impossible. However, there are meticulous chimpanzee studies with groups of animals fed small, moderate, or large amounts of salt for several years. A clear dose-related increase in BP was observed on increasing salt intakes and then when salt was again restricted the BP fell. These studies therefore prove that in primates high-salt intakes do induce high BP.

The impact of salt reduction in populations with either hypertension or with acceptable or 'normal' levels of BP has also been tested. A meta-analysis, which related studies of modest salt reductions for at least 4 weeks showed that there were 17 trials in hypertensive subjects and 11 trials in normotensive groups for analysis. The combined and pooled estimates found significant reductions in BP of 4.96/2.73 mm Hg in those with hypertension and 2.03/0.97 mm Hg in normotensive individuals. A further analysis of 57 trials with hypertensive subjects and 58 trials with normotensive subjects confirmed the greater fall in SBP (4.18 mm Hg) in those with hypertension placed on a low-salt diet compared with those with normotension (1.27 mm Hg) and both these effects were highly significant ($P < 0.0001$). A meta-analysis of 10 trials of children and three trials of infants also showed that salt reduction invariably reduced BP. Although these BP changes seem small on a population-wide basis they could have significant effects.

Whether these salt-related falls in BP can actually reduce cardiovascular disease is the next step to be considered although it has long been accepted that very small reductions in BP will normally lower the risk of strokes, heart attacks, and cardiovascular deaths. The trials of hypertension prevention (TOHP) study prospectively followed adults with prehypertension in a randomized trial lasting 1–3 years while testing the effects of various lifestyle interventions including reductions in salt intake. Data collected 10–15 years after enrolment showed a 25% reduction among those who had reduced their salt intake between 33 and 44 mmol per 24 h (equivalent to a 2.5–3 g reduction in salt intake) compared to the controls. A higher potassium intake was also associated with a reduction in the frequency of cardiovascular disease.

The medical response to these trials has changed with the recognition of the importance of avoiding high BP. New diagnostic thresholds to define hypertension were set out in the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High BP. A new category designated 'prehypertension' is defined as SBP

values between 120 and 139 mm Hg and DBP values of 80–89 mm Hg. Individuals within this group require health-promoting lifestyle modifications to prevent cardiovascular disease because they are at increased risk for progression to hypertension. The thresholds for Stage 1 hypertension are BP values of 140–159 mm Hg (systolic) and 90–99 mm Hg (diastolic) with Stage 2 hypertension being defined when BP values are ≥ 160 mm Hg (systolic) and ≥ 100 mm Hg (diastolic) values, respectively. Both categories require lifestyle modifications as well as drug therapy. Individuals with diabetes, who are recognized as at greater cardiovascular risk, should keep their BP below 130/80 mm Hg.

Salt Reduction in Preexisting Hypertension

Salt deprivation became the major means of treating hypertension in the early part of the twentieth century. The low-salt diets were notoriously unpalatable, so patients reduced their intake and the consequent weight loss helped to reduce further the BP. A large number of trials of salt restriction have been conducted since then on both hypertensive and normal subjects and the overall analyses show that the greater the BP, the more marked the fall in BP, particularly if the sodium intake reduction persists. These data have been interpreted to suggest that the effect of a universal moderate reduction in dietary salt would substantially reduce a population's mortality from stroke and ischemic heart disease with an impact far greater than that achieved by drug treatment of those with high BP. Thus, the World Health Organization (WHO) and most national dietary guidelines now call for a lowering of salt intake to 5–6 g day⁻¹ on average or less and the UK National Institute of Health and Clinical Excellence guidelines for cardiovascular prevention propose a longer term target of an average of 3 g salt per day.

Gastric Cancer and Stroke

There is a strong geographical correlation between stomach cancer and stroke mortality, both of which correlate with salt intake. There are four recognized major etiological factors for gastric adenocarcinoma: infection with *Helicobacter pylori*, excessive salt consumption, and low intakes of ascorbic acid, carotenoids or more generically of vegetables and fruits. Sodium chloride induces atrophic gastritis and enhances the mutagenic effect of foods preserved with nitrite. Salt may also play a role in the later steps involving the transformation of mucosal dysplasia to carcinoma. The salted pickles and salted fish of Japanese cultures appear to be strongly linked to the development of stomach cancers and the second World Cancer Research Fund report concluded that there was convincing evidence that fermented salty fish, as consumed in Japan and parts of China was a cause of the higher prevalence of gastric cancer in Asia.

Osteoporosis

It has been known for many years that sodium intake is one of the major determinants of urinary calcium excretion. It has

been estimated that urinary calcium losses increase by approximately 1 mmol per 100 mmol sodium intake. Experimentally sodium intake increases calcium excretion but also induces markers of bone resorption. Trabecular demineralization may occur, leading to postmenopausal changes and an increased risk of vertebral fractures and cortical erosions. An increasing number of studies show an inverse relationship between urinary sodium excretion as an index of sodium intake and a reduced bone density with a greater propensity to osteoporosis and bone fracture. Salt has also been shown to have a significant negative impact on isotopically assessed bone calcium balance in postmenopausal women on a high calcium diet.

Bronchial Hyperreactivity

There have been no large-scale epidemiological studies, but a positive relationship between asthma mortality and regional purchases of table salt per person have been shown. In a randomized double-blind crossover trial in subjects with moderately severe asthma, the airway response to histamine was related to urinary excretion of sodium in a dose-response way, but only in men. The data so far available suggest that dietary salt restriction reduces airway hyperresponsiveness in asthmatics but the data are not clinically convincing. There are no longer term analyses as yet of a low-sodium diet on the prevalence or severity of asthma or on exercise-induced breathlessness.

Sources of Salt Intake

Various approaches to measuring the daily salt intake in individuals have been tried. Salt comes from: (1) salt in natural products; (2) salt added during industrial processing; (3) salt from catering; (4) other sodium-containing sources; (5) discretionary salt cooking and table salt; and (6) sodium in drinking water. Traditional methods of estimating salt intake, for example, with economic data, lead to marked errors and usually substantial overestimates. These are now set aside in favor of more modern methods.

Estimating Salt Intakes and Their Sources

The principal and most accurate method for estimating sodium intake is to measure sodium excretion rates in individuals who are asked to collect one or more complete 24-h urinary outputs. To measure absolute amounts a marker for completeness of collections is required. Measurement of intake from dietary assessment methods alone is considered unreliable. Assessing how much of the 24-h sodium intake is derived from different sources requires the use of the lithium marker technique whereby lithium carbonate (Li₂CO₃) as a tracer is fused with the salt. One preliminary 24-h collection and three full 24-h urinary collections are required. The lithium technique provides new opportunities for studying the sources of salt intake and shows that reliance on weighing techniques markedly originally overestimated discretionary salt intakes.

Gains and Losses of Salt During Cooking

Only a small proportion of the salt, for example, 24% added to water for cooking foods is, in practice, eaten in the cooked food.

The Assessment of Total Discretionary Salt Use

Figure 2 compares the traditional and lithium marker techniques for assessing both total salt intake and the distribution of its sources. When table and cooking salt are combined to form a single value, then the percentage contribution of these discretionary sources to the total intake measured by the lithium marker technique is significantly lower (15%) in the UK compared with the 39% as assessed by traditional methods, which do not consider salt losses during cooking and at the table. This proportion of discretionary salt intake in the UK seemed unusually low but has now been confirmed in Denmark where even lower percentages of discretionary salt (table and cooking salt), for example, 10.2% in men and 8.7% in women are observed. However, when discretionary sources were assessed in various regions of Italy using the lithium marker technique, discretionary salt intake varied between 31% and 41% of total intake because these households used traditional cooked meals rather than relying on purchased processed foods. In rural Benin the use of discretionary sources in women was higher, 52%, in Indonesian women 51% and in rural Guatemala was as much as 77%. Thus, the more industrialized the food system the greater is the proportion of nondiscretionary salt intake, which then makes it more difficult for individuals to reduce their salt intake. In Japan, salt is ingested in large amounts as pickled and salted fish and

vegetables but these distinctive items may be considered discretionary sources of salt. Similarly, there are specific discretionary salted meat and vegetable extracts that are used for flavoring in Western societies.

Implications of the Salt–Disease Relationships in Relation to Population and Individual Strategies for Improving Health

A population-based approach to reducing disease by reducing salt intakes is a public health strategy directed at the whole population rather than those individuals considered to be at high risk. Such a strategy is based on the observation that a small reduction in risk of a large number of people with average risk may result in a large reduction in risk for the entire population. However, this does not mean that individualized strategies cannot be used to help individuals considered at high risk because of preexisting hypertension. Indeed the greater reductions in BP in those with hypertension on reducing salt intakes imply that there should be a special focus on this vulnerable group. So ideally both strategies are needed: the whole-population strategy because the risk from cardiovascular disease associated with higher BPs is not confined to those who are considered clinically hypertensive, but includes large numbers of people in the upper ‘normal’ BP range. Furthermore, many surveys in different countries show that a large proportion of the hypertensive people are not in receipt of any treatment. This emphasizes the value of dealing with a population, which overall may be at a relatively high risk of premature mortality. Such measures should cause a downward shift in the

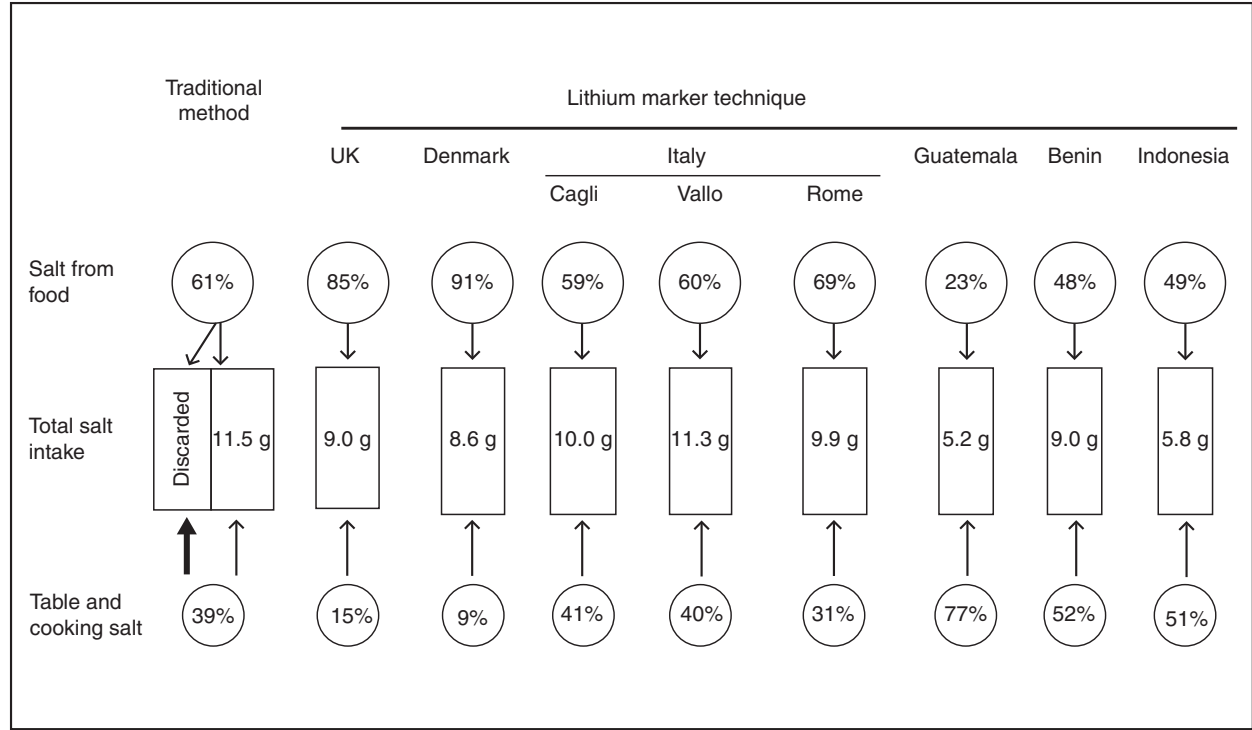


Figure 2 The assessment of total discretionary salt use.

population distribution of BP, which would also benefit high-risk groups.

Individualized Approaches

Individuals with hypertension can take steps to reduce their salt intakes by modifying their diets. If these individuals come from societies where a substantial amount is eaten as discretionary salt then those responsible for adding salt to the cooking either in the home or in catering establishments need to be persuaded to take progressive measures to limit salt use and substitute herbs and other flavors. Individuals can also be asked to eliminate the addition of salt at the table but this can only make a minor contribution in most cases to reducing their salt intake. In theory it is possible for patients to select foods low in salt but this usually means selecting relatively unprocessed foods. Multimineral mixes may also be found to be more acceptable for use in households as these are a mix of different salts with, for example, the addition of potassium and calcium salts to the sodium chloride thereby both diluting the amount of sodium used and adding elements, which counter sodium's effects. Theoretically, food labels can be used to choose lower salted foods but this needs far too much sophisticated understanding for most consumers. The simplest test of somebody's ability to alter their diet and reduce salt intake by avoiding salted and pickled foods, heavily salted breads, prepared meats, and snacks is to check their urinary excretion of sodium. The great difficulty in permanently changing diets is shown by longer-term analyses of intervention studies, which reveal very modest long-term reductions in urinary sodium.

This emphasizes the need for population approaches such as that developed in Finland where children were taught at school how to select less salted foods and to alter the use of salt in cooking within the home. There was also a multi-pronged drive to persuade catering organizations and restaurants to limit the salt in cooking and the food manufacturing sector was persuaded to alter their product composition and limit salt addition as well as altering their fat and fatty acid content. As a result of these measures the average SBP of the adults of North Karelia in Finland fell by 10 mm Hg in more than a 15-year period and this has been accompanied by a dramatic fall in stroke and coronary artery disease deaths of more than 85%, helped substantially by the simultaneous falls in the average total blood cholesterol levels of the population.

The importance of altering the salt content of foods – especially bread, which is often a major source of salt – was shown in Portugal where a village baker was persuaded to reduce the salt content of his bread. Two years later the average BP of the villagers was significantly lower than that of a neighboring village where no changes had been made.

Thus, governments have a major role in persuading their health services to take a systematic approach to reducing salt in hospital foods and to engage in systematic patient and public health educational initiatives. The problem is that the salt industry and other components of the food industry often do their utmost to contest the evidence and find reasons why they should not progressively reduce the salt content of their

products. We need to see major improvements in food labels and when a traffic light type warning system is used high-salt products can readily be identified. Several countries have undertaken steps to reduce the salt content of processed foods. It is also important to recognize that in the past some soft drink companies with snack food products oversalted them so that this would stimulate thirst and therefore the demand for their drinks! Only when countries follow the Finnish lead can we expect to see an appreciable fall in salt intakes and a concomitant reduction in hypertension rates and cardiovascular disease.

Conclusions

The evidence suggesting that sodium intake is an important determinant of BP in the population as a whole, and influencing the rise in BP with age is increasingly robust.

The predominant source of salt varies from country to country. In Western societies, the greatest potential benefit involves reducing the salt content of manufactured food. A different public health approach will apply where processed foods are not the dominant dietary feature and this may require a variety of measures including teaching caterers and others how to use alternative flavorings for their food. The benefit from salt reduction measures, however, is a highly cost-effective measure for governments throughout the world.

See also: Cancer: Epidemiology and Associations Between Diet and Cancer. Nutritional Considerations for the Management of Hypertension. Potassium. Sodium: Physiology

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National Institutes of Health; National Heart, Lung, and Blood Institute.

SEASONALITY

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Introduction

The agroclimatic characteristics of some areas of the planet with lower technological development lead to seasonal fluctuations in food production and food availability, labor demand, and incidence of disease that affect the nutritional status of rural populations. Such cyclical stress has a negative impact on the well being, productivity, and potential for development in individuals with a preexisting marginal nutrition. Approximately 400 million adults and 500 million children in the world may be affected.

Definition and Measures of Seasonality

Seasonality is defined as the cyclical change of food availability and agricultural labor induced by climatic changes in rural areas of the least developed countries (LDC). In seasonal climates a bad, lean, or slack season, also known as the *saudure* (French, meaning junction between two agricultural cycles), is present for 2–3 months a year, often coinciding with rains, leading to cyclical stress on the health and nutrition of rural populations. Agroclimatic seasonality is relevant in populations practising subsistence agriculture and among hunter-gatherers, or in other agricultural systems in which the background food security is poor; such as in areas where cash crops are mainly planted. Human interventions can alter this pattern, by changing the water and sun exposure conditions, with irrigation and greenhouses, but these techniques are not accessible to the majority of peasants in LDC.

Changes in rainfall, temperature, exposure to winds, and relative humidity, with respect to the water retention capacity of the soil, are responsible for a cyclical change of water balance that may restrict the period for plant growth to some parts of the year. The proportion of dry months in a year, named absolute seasonality, can vary between 0 (sufficient rains all year long) to 1 (lack of a period suitable for plant growth). If the vegetative cycle of corn (maize) is considered (120 days), then the areas of the world can be classified as follows: low seasonality when there are more than 200-day vegetative season per year and two harvests are possible; moderate seasonality with 120–200-day vegetative season and one to two corn harvests possible; and severe seasonality with less than 120-day vegetative season and barely one corn harvest possible. With even shorter vegetative periods, agricultural production is impossible in the absence of irrigation. The

different areas of agroclimatic seasonality in the world are shown in **Figure 1**.

Factors aggravating the climatic seasonal effects may be the occurrence of pests, for example, the arrival of locusts along with the rainy season in Sahel. Furthermore, seasonal patterns of food production are often superimposed on longer term cycles, which leads to the periodic appearance of drought and famines in sub-Saharan Africa and in Central Asia.

Effects of Agroclimatic Seasonality on Food Availability and Dietary Intakes

Subsistence farmers store their harvest and use it progressively until the next season, so that a fluctuation of food stocks can be observed during the year. Stores decrease as a result of human and animal consumption, losses due to pests, rodents and microbiological contamination, and sales, barter or donations. The market price of food staples is also subject to great seasonal fluctuations, which are usually inversely related to the size of domestic stocks.

As a result, both energy supply and dietary quality may be affected. The dietary changes observed in slack seasons may involve eating foods that are less preferred, but are more affordable, an option that is not biologically dramatic, but perceived by people as stressful. Households that are close to exhaustion of staple foods stocks may use alternative food sources (root crops, gathered leaves and fruits, hunted small animals), may consume immature grains, or may reduce food intake by limiting portion size, reducing the number of meals, or skipping meals for an entire day. Dietary changes may also be due to reduced time available for food collection and preparation.

In areas of high climatic seasonality the magnitude of the reduction of energy intake can be in the order of 400–500 kcal and is associated with a reduction in protein and micro-nutrient intake. In Cameroon, the rainy season is associated with a 20% reduction of energy intake, a 50% reduction of protein intake, and a 25% reduction of fat intake. However, the reduction in food intake is not a universal characteristic of areas prone to agroclimatic seasonality. In Mali energy intake in adults had minimal fluctuations between 11.7 MJ in the harvest season and 11.4 MJ in the rainy season.

Changes in dietary intake may affect household members unequally. For economic or social reasons some household members may be protected. In Northeastern Thailand energy intake in the rainy season is reduced in women but is constant in men. Children may be protected by maternal buffering, but

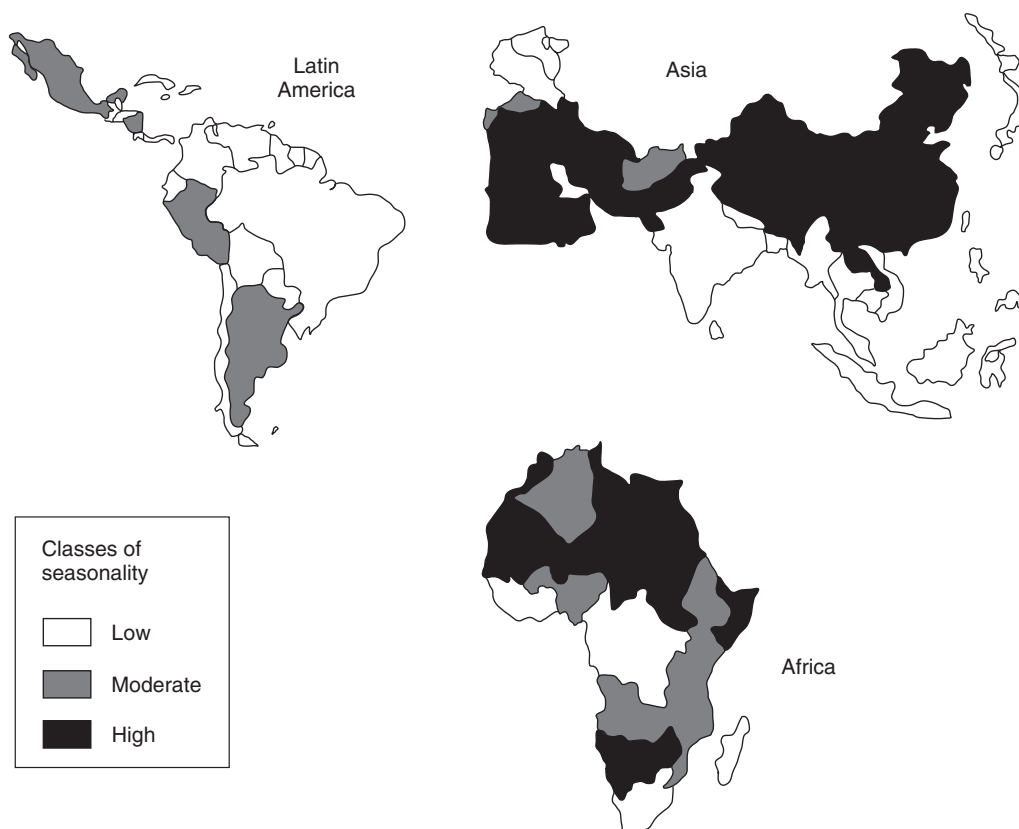


Figure 1 Agroclimatic seasonality in different world regions. Seasonality is calculated taking into account soil characteristics and water balance and is an expression of the vegetative period of a food crop allowed by such conditions. Reproduced from Ferro-Luzzi A, Branca F, and Pastore G (1994) Body mass index defines the risk of seasonal energy stress in the Third World. *European Journal of Clinical Nutrition* 48(supplement 3): S165–S178.

may also be discriminated against in favor of male workers, who have to ensure the family's food supply. A study in Bangladesh showed that energy and protein intakes were significantly different in male and female adults and in 1–4-year-old children, but were unchanged in older children. In another Bangladesh study (Figure 2) dietary energy intake was reduced in all age groups, including pregnant and lactating women, but the reduction in women (–28%) was far greater than that in men (–18%).

It seems that women consistently get the least food during seasonal shortages, regardless of their physiological status. In The Gambia a 12% reduction of intake has been observed in pregnant women and 29% in lactating women during the rainy season.

Seasonality does not spare weanlings, who in Bangladesh have a 33% difference in energy intake between the highest and lowest intake seasons, or breast-fed babies, in whom a decrease in breast-milk intake has been observed. A decrease in breast-milk output in the early postpartum period (2–6 months) has also been observed in The Gambia during lean seasons: the daily output was 850 g day^{–1} in a cohort of women who gave birth during the dry season and 540 g day^{–1} in a second cohort who delivered during the wet season. Furthermore, breast-milk fat concentration was 3.95 g 100 ml^{–1} in the first cohort and 3.52 g 100 ml^{–1} in the second cohort, with a resulting decrease in energy content. The decrease in breast-milk output is not

necessarily related to the deteriorating nutritional status of the lactating mothers, but rather to the limited time available for childcare, with a resulting reduction in suckling time. This latter circumstance has been specifically documented in Bangladesh.

The reduction in diet diversity may affect micronutrient intake, although not always in the same direction. In The Gambia fruit and vegetable intake was found to be seasonal and vitamin C intake varied from nil in the rainy season to approximately 100 mg day^{–1} in the dry season, affecting both plasma ascorbate and breast-milk ascorbate. In Cameroon, during the wet season calcium intake was reduced by 30%, iron intake by 20%, and thiamin by 15%. However, the consumption of leafy vegetables and fruit increased during the wet season, as did carotene (+100%) and ascorbic acid (+50%).

Effects of Agroclimatic Seasonality on Time Allocation and Energy Expenditure

The second important consequence of agroclimatic seasonality is the concentration of agricultural practices at certain times of the year. More time has to be devoted to often intense efforts, sometimes regardless of the actual physical capacity to perform them. Thus, although adult males are primarily involved, women and even pregnant women also have to spend more time in the field.

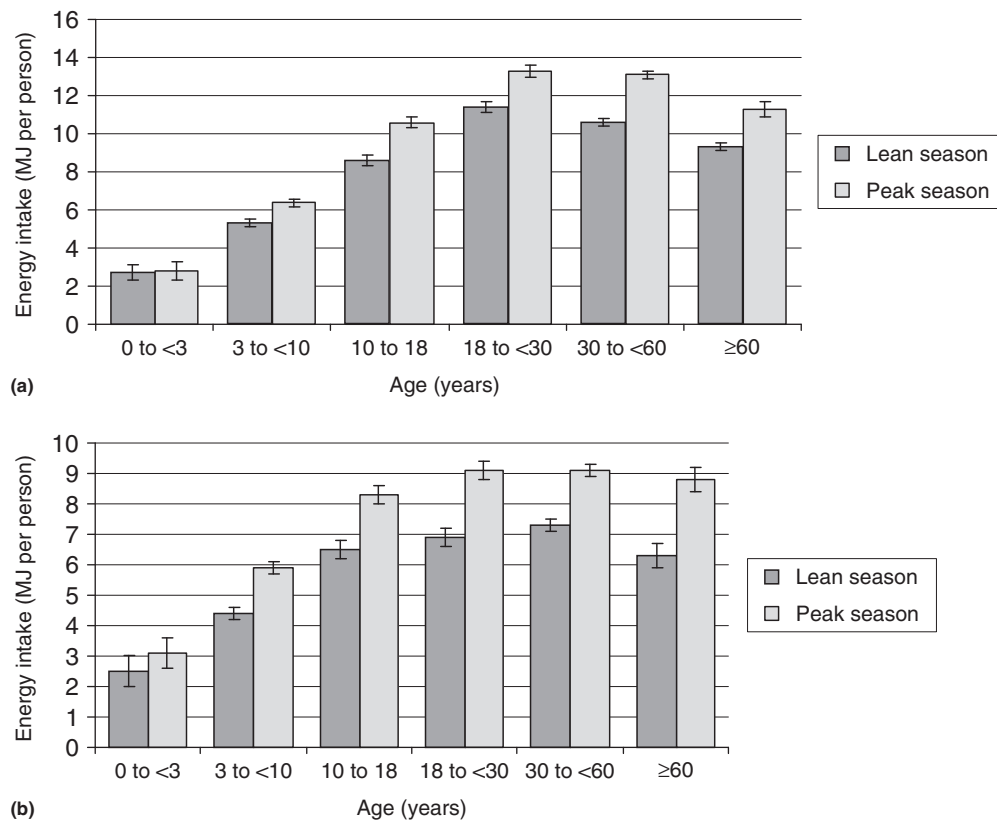


Figure 2 Seasonal changes in dietary energy intake in different age groups in Bangladesh: (a) males and (b) females. Data from Tetens I, Hels O, Khan NI, Thilsted SH, and Hassan N (2003) Rice-based diets in rural Bangladesh: How do different age and sex groups adapt to seasonal changes in energy intake? *American Journal of Clinical Nutrition* 78: 406–413.

The increase in energy expenditure reported by different studies during the peak agricultural season amounts to 320–1050 kcal day⁻¹. In Mali, during the rainy season the energy expenditure from agricultural work is double that during harvest and triple that in the dry season, although the difference is smaller for women.

As a result of increased demands on time for agricultural labor, time spent on activities is reallocated. Northeastern Thai men spend 2 h less resting and 1.5 h less on domestic work than in other seasons. Women not only spend less time sleeping and in leisure, but also spend less time cooking, carrying out household tasks such as cleaning, collecting food and water, caring for the children, or carrying out income-generating activities such as handicrafts. The reallocation of women's time may have important consequences for their own health and well being and for the health of all the household members needing care. (Figure 3). Where possible, such household tasks may be reallocated to older children. In adolescent girls in Senegal the total energy expenditure measured by accelerometer was 5% higher in the rainy than in the dry season (100 kcal difference). In the rainy season the girls spent 1.5 h more on vigorous activities and more time in domestic activities.

Seasonal Patterns of Disease

A third factor subject to seasonal changes is morbidity. In most cases the slack season is also a wet season and the

environmental changes may lead to seasonal outbreaks of diseases, such as acute respiratory infections, gastrointestinal tract infections, but also other infectious diseases such as measles, malaria, and guinea worm. Overall mortality also increases. A study in Mali showed that the duration of disease episodes in the rainy season was more than double that in other seasons and that the morbidity episodes in the rainy season accounted for more than half the yearly episodes, particularly fever, diarrhea, and respiratory illness.

Increased morbidity is a consequence of epidemic cycles and environmental factors, but is also related to increased susceptibility to infections, as a result of decreased food intake and increased stress. Impairment of immune function has been documented in a study of undernourished Gambian children aged 6½–9½ years, in whom seasonality influenced antibody responses to different vaccines. Some behaviors that are typical for the season also determine increased risk of disease. For example, food may be prepared only once a day and left over for the second meal, thus increasing the risk of contamination; there is less time for personal and household hygiene and this leads to easier spread of gastrointestinal and skin disorders.

Morbidity seriously affects the nutritional status of children and sometimes endangers their lives, but it also impairs labor capacity and imposes further time and financial burden on the households, who have to care for the sick and pay for their treatment.

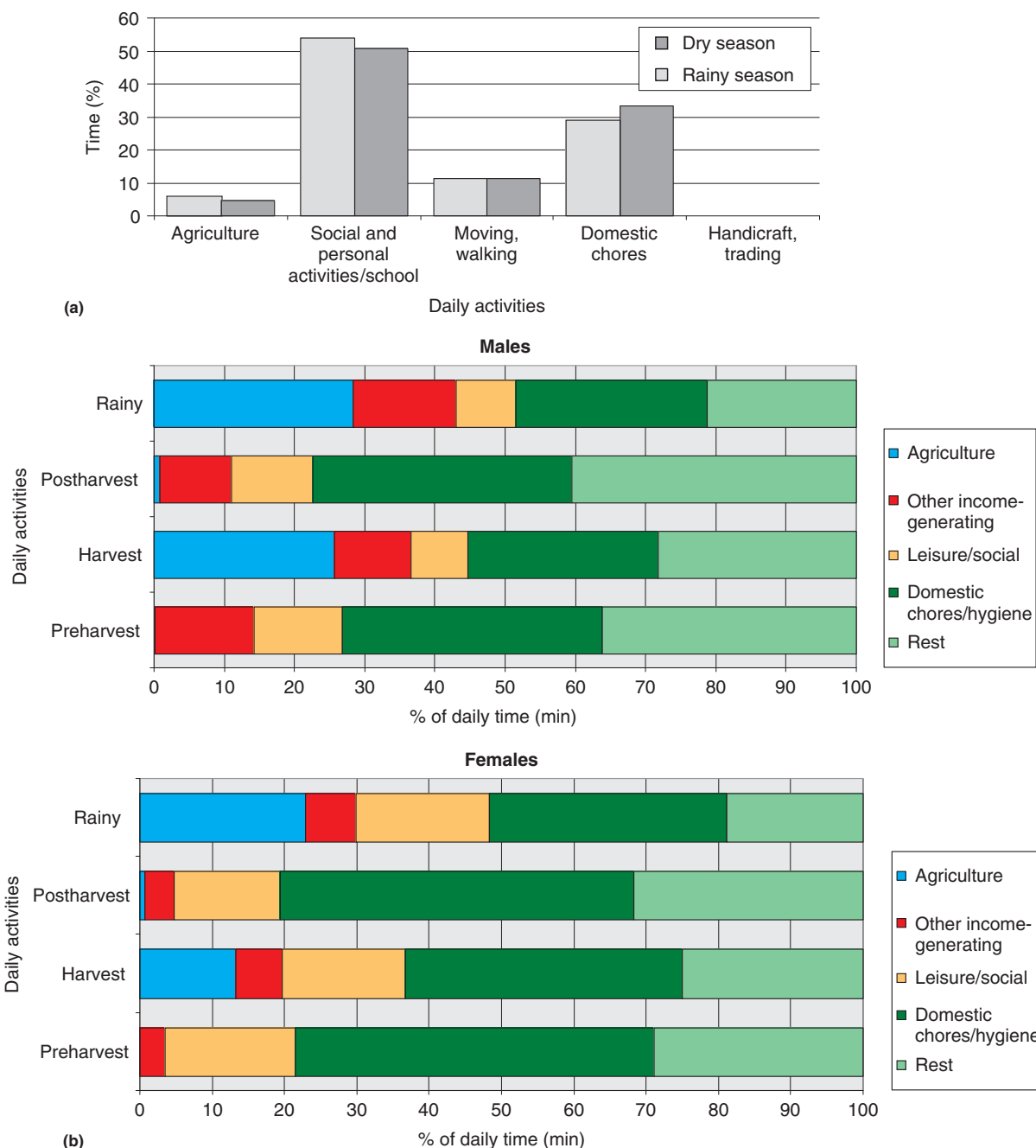


Figure 3 (a) Time allocation of Senegal rural adolescents in different seasons. Data from Benefice E, Garnier D, and Ndiaye G (2004) *European Journal of Clinical Nutrition* 58(2): 292–301. (b) Time allocation of a Thai rural population in different seasons. Duration of daytime activities (night sleeping excluded) in four seasons. Data from Murayama N and Ohtsuka R (1999) *European Journal of Clinical Nutrition* 53(1): 39–49.

Coping Strategies

People living in areas with agroclimatic seasonality have developed different strategies to cope with the environmental challenge. The earliest and most successful are aimed at maintaining an adequate level of food stocks, but when these fail, other more costly strategies are put in place and their presence indicates that a crisis is occurring.

The negative effects of seasonality can be prevented to a certain extent by selecting the appropriate crops, namely by

using varieties with a shorter vegetative period and lower demand for water and by using reserve crops such as root crops, leguminous plants, and groundnuts. Manioc (*Manihot esculenta*) is used as a reserve crop in the Guinean zone, enset (*E. ventricosum*, false banana) is used in southern Ethiopia, and groundnuts (*Arachis hypogaea*) in Sahel. Reliance on a single crop is highly risky, as failure of the crop due to a pest, natural disaster, or war may lead to permanent indebtedness and poverty. In areas where cash crops are common, the fluctuation of international market prices may also disappoint the

expectations of farmers. The coffee crisis is a typical example. Furthermore, diversification allows people to shift some of the labor burden to different times of the year and to obtain a second harvest in the year.

To secure a regular income, landless people who live on seasonal labor, will have to seek off-farm labor opportunities. At times other than the peak agricultural season labor demand and wages fall and migration of the labor force to different climatic zones may take place. Migration may involve both adults and children, who in some cultures may be sent out to work as servants. At times of food shortage, children may also be sent out to stay with relatives with better resources.

Mutual support groups are created and donations in cash or food may be received by families who may be affected by crop failure or by the occurrence of an unexpected event, such as an illness or death of a family member. Food or money to buy food may be borrowed, although this strategy leads to permanent indebtedness. The final resort is the sale of household assets, particularly animals. This is a high-cost strategy, as the inability to maintain a permanent asset base traps people in poverty and permanent food insecurity.

Nutritional Impact of Seasonality

Body Weight, Body Composition, and Growth

People living in areas of high climatic seasonality are well aware of the nutritional impact of seasonality, as indicated by the language they use to define such seasonal stress periods. The Massa of Cameroon call the month of July in the middle of the wet season the month of 'Did you call me for food?' and they have a word to define 'hunger with threat,' when food shortage has been too long and life is in danger. However, it was not until the 1950s that the scientific community started to appreciate the presence of a nutritional impact of seasonality, and its functional significance is still a matter of discussion.

As described earlier, seasonal climates may affect nutritional status via a combination of reduced dietary intake, increased physical activity, and increased disease incidence, and may occur to a variable extent in different populations and socioeconomic groups. As a result, both energy balance and micronutrient status may be affected, and this is confirmed by the observation of changes in body weight and body composition in adults, growth performance in children, pregnancy weight gain, and birth weight as well as by changes in micronutrient status.

Body weight changes between 1 and 4 kg, corresponding to 2–5% of body weight have been observed in adults in areas of medium and high seasonality. Larger body weight changes occur in drought years or among pastoralists. Remarkable interindividual differences also feature in parallel with such mean values. Smaller changes are usually suffered by women and by people with a lower body mass index (BMI). Socioeconomic differences may go in both directions: wealthier people may either lose more weight, because they own land and have the opportunity and the need to perform more intense agricultural work, or lose less, because they rely on hired labor.

The composition of the tissue lost varies according to the size of the loss and to the initial energy stores. In a rural population in Mali the mean BMI was low (19.8 kg m^{-2} in men and 19.3 kg m^{-2} in women), the weight loss in young active men (16–35 years) was 2.6 kg, corresponding to a 3.8% change in body weight, and changes in body fat were in the order of 1.5%. The arm muscle area was also reduced. In Northeast Thailand, where BMI in men was similar (19.8 kg m^{-2}) but the weight change smaller (1 kg), a 1.5% reduction of the fat mass was also observed, but no reduction in the fat-free mass could be detected by anthropometry.

The vulnerable groups of the population are not spared by seasonal stress. During the wet season, energy imbalance leads to the utilization of fat reserves in pregnant women and the women's own energy requirements compete with that of the fetus, leading to increased reproductive risk. In Sierra Leone, at the time of planting and harvesting, pregnant women are expected to continue working and are also more affected by malaria, anemia, and pregnancy-induced hypertension; as a result, in this season birth weights are the lowest in the year. In Taiwan predelivery skin-fold thickness of a cohort of women measured in the cold season was greater than that of a comparable cohort measured in the warm/wet season and a 150-g difference in mean birth weight was observed. In The Gambia a $0.4 \text{ kg month}^{-1}$ weight gain was observed among pregnant women during the rainy season, as opposed to $1.4 \text{ kg month}^{-1}$ during the dry season. Dry season mean birth weight was 160 g higher than in the wet season, and the prevalence of low birth weight was 13% in the dry season and 35% in the wet season. Perinatal and infant mortality were also higher in wet season cohorts.

Seasonal stress continues after birth for both mothers and children. In The Gambia lactating women lost on average $0.74 \text{ kg month}^{-1}$, at the same rate as nonlactating women. As shown earlier, during the wet season very young children get less attention and less breast milk from their mothers and their growth is affected. In Taiwan, children born in the hot, wet, summer season were smaller, but could catch-up in the following 3 months, whereas those born in the dry season had a larger birth weight but had a slower postnatal growth.

Seasonal impact is more evident at critical times when a more intense growth effort is required, in order to catch-up from previous delays or at the mid-infancy growth spurt. In a rural area of The Gambia the lower weight and height gains observed in the wet season were not followed by corresponding increases at other times of the year. In Malawi the weight-for-age Z-scores and height-for-age Z-scores declined more rapidly during the rainy season among 1–6-month-old babies and among 13–36-month-old children, but not among the 7–12-month-old babies.

Weight and height increments are more sensitive indicators than achieved weight and height. In Bangladesh monthly height gain in children under 5 years ranged from a minimum of 12–20% of the reference value to 200–240% (respectively, in boys and girls), while height gain fluctuated between 52–60% and 165–180%. In Ethiopian children, height growth velocity showed a marked seasonal pattern, with values close to normal (-0.2 SD units) in July to December, a period characterized by better food availability, and lower values (-3.0 SD units) in January to June, a period characterized by

intensive farm labor and heavy rains. Therefore, there was never an opportunity to recover from growth faltering and stunting was a continuous process in the first 5 years of life.

Unlike younger children, seasonal variables did not have a permanent effect in older children and adolescents. In the Ethiopian study, girls above the age of 10 years showed accelerated growth in the first semester of the year and delayed growth in the second semester, characterized by the wet season, so that the mean yearly growth rates were normal overall. This was also observed in Senegalese adolescents, in whom arm circumference and triceps skin fold were significantly lower during the rainy season, followed by a recovery in the postharvest season, but no change in the growth rate was observed.

In young children, labor burden is not a critical variable, but the reduction of food availability, combined with greater incidence of infectious diseases, especially diarrhea, leads to impaired growth. This is supported by the observation that seasonality may also affect urban children. In an urban area of The Gambia height-for-age showed little seasonal variation, but weight gain was poor during the rains and was not compensated by catch-up growth during the dry season. In older children and adolescents, the seasonal effects may instead be related to increased physical activity.

Figure 4 illustrates the magnitude of seasonal effects on mean weight changes observed in adult men and women in different regions of the world. In both sexes the observed values range between 1 and 5 kg, although the values greater than 4 kg have been documented during extreme environmental stress, as in drought.

Changes in Micronutrient Status

Few studies document the changes in micronutrient status that can be expected from seasonal changes in dietary quality. In The Gambia both plasma ascorbate and breast-milk ascorbate had seasonal fluctuations connected with fruit and vegetable intake. Carotenoids also showed a three-fold fluctuation, while retinol was unchanged.

In another study in undernourished children in The Gambia significant seasonal changes were observed for hemoglobin levels and plasma concentrations of vitamin C and α - and β -carotene. Hemoglobin levels decreased with the wet season, whereas vitamin C and α - and β -carotene plasma levels were highest during the mango season (April–May) and during the rainy season (September–October) when green leafy vegetables are abundant. Zinc and retinol plasma levels were not significantly affected by seasonality.

Metabolic Adaptation

Adaptation to reduced energy intake is a possible biological mechanism to cope with seasonal energy stress. A small reduction in basal metabolic rate (BMR) has been observed. In a multicenter study carried out in India, Benin, and Ethiopia the energy debt generated by seasonal changes in food intake and labor pattern was accounted for by the mobilization of fat stores in Benin, abundant in this population, and by a combination of a modest reduction of body weight in India and

Ethiopia (0.3 and 1.6 kg, respectively), together with a reduction in BMR that allowed a 30–50% saving of total energy expenditure. The adaptive response occurred at a relatively low level of energy deficit, i.e., 70 kcal day⁻¹ in India and 90 kcal day⁻¹ in Ethiopia.

Functional Consequences

The size of the nutritional impact is dependent on the magnitude of seasonal stress and baseline nutritional status, as this sets the limit of tolerable stress. Lean people will lose more fat-free mass than fatter people. At a BMI of 21, 50% of the weight lost is lean tissue. Therefore, in populations with a lower mean BMI there is a greater impact on their fat-free mass, with greater consequences for their productivity and fitness. A meta-analysis of the body weight change/body weight relationship indicates that farmers tend to maintain the loss at below 2% of their fat-free mass; people with lower BMI will lose less weight, but they will also have to limit their physical activity, which can have socioeconomic consequences. In some populations, body weight lost can be as high as 4% of the fat-free mass, but this is probably the maximum stress cyclically tolerable.

Some authors maintain that the observed fluctuations may be regarded as an acceptable physiological response to energy imbalance or even a successful adaptive response. Indeed, having a maximum body weight at the beginning of the heavy work season may be more advantageous than keeping a constant body weight, because this minimizes the farm storage losses.

However, in some subgroups of the population living in seasonally prone areas such compensatory mechanisms may irreversibly affect other physiological functions such as reproductive performance and growth. While men have time to recover after the seasonal imbalance of energy, women are permanently undergoing stress and a greater impact on health, well being, and function should be expected. Furthermore, women's seasonal stress also has an impact on the early growth and development of young children, such that seasonal cycling may be considered as one of the factors responsible for the intergenerational cycle of malnutrition (Figure 5).

Young children suffer a double seasonal burden: one imposed on themselves by disease and the other imposed on their mothers. Seasonal changes in children's weight and height velocity have also been observed in more developed societies. However, in high seasonal areas of LDC periods of retarded growth in the youngest age groups are not followed by periods of adequate catch-up growth, such that height-for-age decreases progressively. Therefore, it is suggested that stunting is listed among the functional consequences of seasonality, along with its correlates of impaired cognitive and metabolic function, and decreased productivity. The impact is going to be highest in younger children, and the age of 5 years may be arbitrarily taken as a limit for increased seasonal vulnerability.

Extension of the Problem

Approximately one billion people live in areas of moderate and severe seasonality. Taking into consideration the BMI

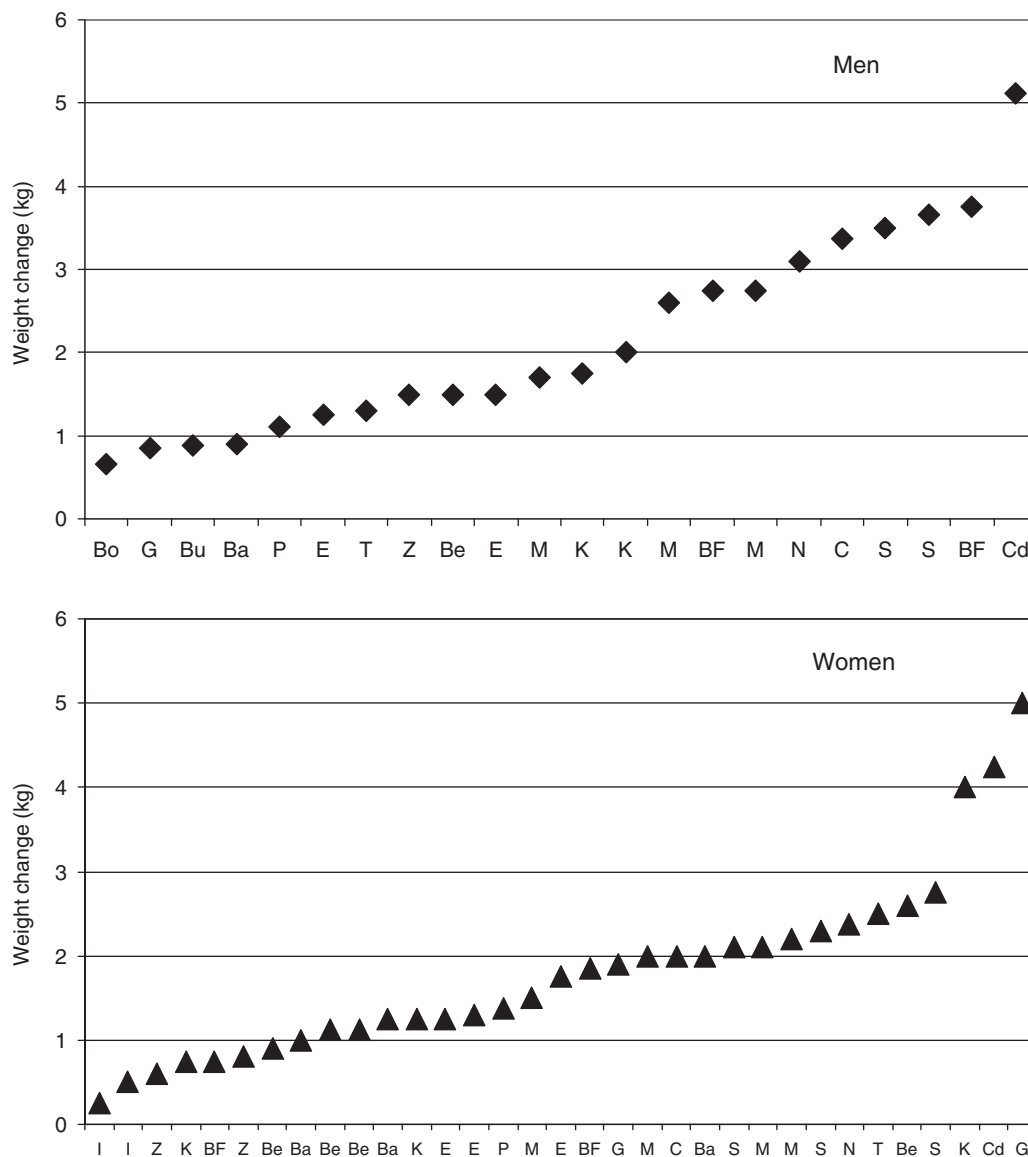


Figure 4 Range of body weight changes between lean and good seasons documented among adult men and women in different countries. Legend of countries: Ba, Bangladesh; Be, Benin; BF, Burkina Faso; Bo, Botswana; Bu, Burma; C, Cameroon; Cd, Cameroon during drought; E, Ethiopia; G, Gambia; K, Kenya; I, India; M, Mali; N, Niger; P, Papua; S, Senegal; T, Thailand; Z, Zaire. Reproduced from Ferro-Luzzi A, Branca F, and Pastore G (1994) Body mass index defines the risk of seasonal energy stress in the Third World. *European Journal of Clinical Nutrition* 48(supplement 3): S165–S178; Adams AM (1995) Seasonal variations in energy balance among agriculturalists in central Mali: Compromise or adaptation? *European Journal of Clinical Nutrition* 49: 809–823; Ategbo E-AD, van Raaij JMA, de Koning FLHA, and Hautvast JGAJ (1995) Resting metabolic rate and work efficiency of rural Beninese women: A 2-y longitudinal study. *American Journal of Clinical Nutrition* 61: 466–472, and Murayama N and Ohtsuka R (1999) Seasonal fluctuation in energy balance among farmers in Northeast Thailand: The lack of response of energy intake to the change of energy expenditure. *European Journal of Clinical Nutrition* 53: 39–49.

distribution of those populations, it is possible to calculate that 65% of the adults living in rural areas, i.e., 408 million people, are at risk of severe stress, most of whom are in Asia (90%) and the remaining 10% in sub-Saharan Africa.

Pregnant women and young children should, however, be added to this count. A rough calculation indicates that in those areas, the number of children under 5 years is approximately 500 million and the number of pregnant women

approximately 20 million. They should also be included among the victims of seasonal climatic changes in LDC, thus bringing the estimate to about a billion people.

Nutrition interventions aimed at accelerating growth rates should then preferably be carried out at the time of the highest seasonal stress in these vulnerable population groups, particularly in younger children, in order to achieve the maximum long-term benefit.

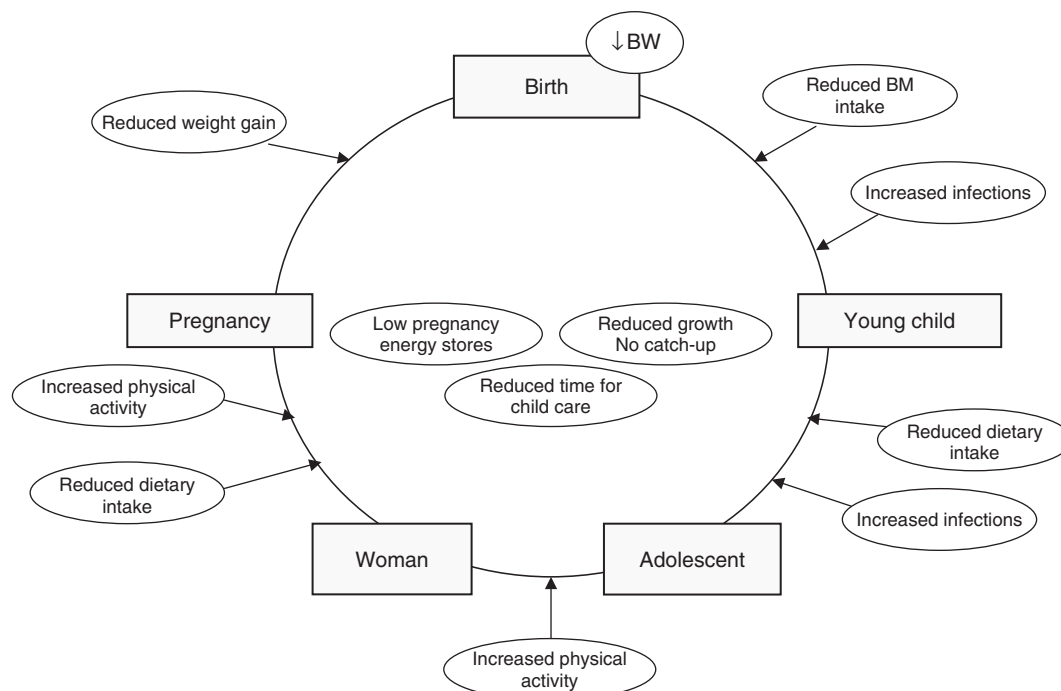


Figure 5 Model of the effects of seasonal stress on the intergenerational cycle of malnutrition. Seasonal factors are drawn outside the circle; biological and behavioral effects are drawn inside the circle. BW, body weight, BM, breast milk.

See also: Bioavailability. Breast Feeding. Energy: Adaptation. Energy Metabolism. Energy Requirements. Lactation: Dietary Requirements. Supplementation: Developing Countries

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SELENIUM

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Glossary

Antioxidants Antioxidants are molecules which can safely interact with free radicals and terminate the chain reaction, thereby inhibiting the oxidation and damage to cells that may play a role in heart disease, cancer and other diseases.

Kaschin–Beck disease A selenium-responsive endemic osteoarthropathy with necrosis of joints and epiphyseal plate cartilage.

Keshan disease An endemic cardiomyopathy responsive to selenium with clinical features of cardiac insufficiency and enlargement, electrocardiographic changes, and fibrosis.

Selenium Selenium is a trace element with an atomic mass of 78.96, which is an essential nutrient for good

health but required only in very small amounts. Selenium is incorporated into selenoproteins, which have a variety of functions including antioxidant, redox and thyroid function regulation.

Selenoproteins Functional proteins requiring selenium for functionality and containing the selenoamino acid, selenocysteine, at the active site.

Single nucleotide polymorphisms A single-nucleotide polymorphism (SNP) is a DNA sequence variation occurring when a single nucleotide – A, T, C, or G – in the genome (or other shared sequence) differs between members of a biological species or paired chromosomes in an individual.

Introduction

Selenium was first considered to be a toxic trace element for animals that consumed plants grown on high selenium soils, causing loss of hair and blind staggers. The realization that selenium may be an essential micronutrient arose in 1957 when it was shown to be essential for mammalian life by preventing liver necrosis in rats, and later white muscle disease in cattle and sheep, hepatosis dietetica in swine, and exudative diathesis in poultry. In 1973 selenium was identified as an integral component of the selenoenzyme glutathione peroxidase (GPx), and this was followed by characterization of many other selenoproteins, now known to number 25 in humans. The identification of Keshan and Kashin–Beck diseases as endemic selenium-responsive conditions, occurring in a central belt of China and areas of Russia, demonstrated conclusively that not only is selenium an essential element for man but also that deficiencies occur naturally. On the other hand, high soil selenium concentrations result in the opposite condition of selenosis, or selenium overload. A large body of research over the past decades has provided information on the metabolism of selenium and its importance to human health, and has led to the establishment of recommended dietary intakes based on amounts required for maximal plasma selenoproteins. The focus of research has now turned to possible benefits of selenium in preventing certain types of cancer and cardiovascular disease (CVD), and in maintenance of an optimal immune system. In this area of research, genomics is rapidly advancing our knowledge of functions and requirements for selenium.

Metabolism of Selenium

The main forms of selenium in foods are the selenoamino acids, selenomethionine, and selenocysteine. Selenium appears not to be essential for plants, but is normally taken up readily into their tissues and substituted in place of sulfur. Selenomethionine is present in general proteins in plant and animal foods and selenocysteine in selenoenzymes in animal foods. Selenite and selenate, inorganic forms of selenium, are used in experimental animal diets and as supplements. Selenium metabolism is dependent on the chemical form ingested and on other interacting dietary factors, and varies with species.

Absorption and Bioavailability

Selenium is readily absorbed, especially in the duodenum but also in the cecum and colon. Selenoamino acids are almost completely absorbed (>90%): selenomethionine *via* the gut methionine transporter and selenocysteine probably *via* the cysteine transporter. Inorganic forms are absorbed with approximately 50–90% efficiency, selenite more readily than selenate, and there is competition with sulfate transport. Absorption does not depend on selenium status, indicating that there is no homeostatic regulation of absorption.

Bioavailability of a nutrient also includes retention in the body and transformation to functional forms, which for selenium is assessed by monitoring changes in tissue

selenium concentration is responsive to changes in maternal selenium intake.

Excretion

Urine is the main route of selenium excretion, and the urinary excretion pathway is very important for selenium homeostasis of tissues. Urinary selenium tends to reflect recent intake rather than tissue status, and accounts for approximately 50–60% of the total amount excreted. Fecal selenium is mainly unabsorbed selenium.

Excess selenium is methylated to methylated selenium metabolites from the intermediate selenide. The major urinary metabolite is 1 β -methyl seleno-*N*-acetyl-D-galactosamine (selenosugar). Another urinary metabolite, trimethylselenium ion appears to be excreted in response to very high intakes of selenium and may be used as a biological marker for excessive doses. Excess selenium is also excreted in expired air as volatile dimethylselenide.

Functions of Selenium

Selenium functions as a constituent of over 30 selenoproteins, 25 in humans. These selenoproteins have a number of functions, including antioxidant defense and redox metabolism (GPxs, thioredoxin reductases), thyroid metabolism (iodothyronine deiodinases), immune function, reproductive function, and storage and transport activities (SEPP). Many selenoprotein functions are incompletely understood, whereas others have clinically important implications in diseases such as cancer and autoimmune thyroid disease. **Table 1** describes the selenoproteins that have been identified in mammals.

Selenium is in the active site of all selenoproteins as selenocysteine, which is inserted into proteins cotranslationally through the process described in the Section on Metabolism and distribution.

GPx was the first selenoprotein to be characterized, and is now known to exist in at least five different forms, all using glutathione to catalyze the reduction of hydrogen peroxide or phospholipid hydroperoxides. Three of these (GPx1, GPx2, GPx3) are tetramers, consisting of four identical subunits, whereas GPx4 is a monomer. These enzymes may function *in vivo* to remove hydrogen peroxide in cells (GPx1), gastrointestinal tract (GPx2), and plasma (GPx3), thereby preventing the initiation of peroxidation of membranes and oxidative damage. GPx1 is one of the more highly responsive selenoproteins to changes in selenium status and deficiency. Phospholipid hydroperoxide GPx (GPx4) differs from other GPxs in that it can metabolize phospholipid hydroperoxides in cell membranes, and thus may protect biomembranes against oxidation. GPx4 is also involved in redox signaling and regulatory processes such as inhibition of lipoxygenases and apoptosis. GPx4 also acts as a structural protein required for sperm maturation.

There are three distinct thioredoxin reductases in humans (TR1, TR2, TR3). These are NADPH-dependent flavoprotein oxidoreductases that reduce the disulfide of thioredoxin. They support antioxidant defense, redox-regulated signaling cascades and cell proliferation and may be involved in spermatogenesis, embryonic development, and other redox-related aspects of health and disease.

The three iodothyronine 5'-deiodinases (DI1, DI2, DI3) are involved in synthesis and metabolism of thyroid hormones, which regulate most metabolic functions and are essential for growth and development. The deiodinases catalyze the conversion of thyroxine (T_4) to its active metabolite triiodothyronine (T_3) and severe selenium deficiency results in an increase in plasma T_4 and a decrease in T_3 . If selenium and iodine are deficient in a human population, the thyroid deficiency is more severe (and goiters are larger) than if only iodine is lacking, a situation which occurs in some areas of Central Africa.

SEPP is the major selenoprotein in plasma, providing 40–50% of total plasma selenium. In selenium deficiency the concentration in rat plasma falls to 10% and SEPP is therefore a useful biomarker of selenium status. SEPP is a glycoprotein that contains multiple selenocysteine residues. The functions of SEPP are still unclear, but there is evidence for an antioxidant role and a transport role in facilitating uptake of selenium into testis and retention of selenium in the brain.

Further functions for selenium are suggested by the identification of several other selenoproteins in microorganisms and in animal tissues.

Selenoprotein W (SEPW), found in muscle and other tissues, derived its name because it is missing from heart and muscle of lambs suffering from white muscle disease. SEPW concentration in muscle decreases during selenium deficiency, but it is retained in the brain. Its functions are unclear, but it may have antioxidant/redox function involved in cell immunity.

Selenoprotein N (SEPN) is another selenoprotein that may be involved in muscle function and disease. Mutations of the SEPN1 gene cause a group of neuromuscular disorders referred to as SEPN-related myopathy.

Selenoprotein R (SEPR), also known as methionine-R-sulfoxide reductase, is involved in antioxidant function, regulation of enzyme activity and cell signaling, and may protect the brain from oxidative damage.

Other selenoproteins include a 15-kDa selenoprotein and selenoproteins H, I, K, M, O, S, T, and V, the functions of which are unclear or unknown. Another selenoenzyme is selenophosphate synthetase 2 required for formation of tRNA-bound selenocysteine during synthesis of selenoproteins.

A growing body of recent research indicates that mutations or single nucleotide polymorphisms (SNPs) in selenoproteins most likely affect the efficiency of incorporation of selenocysteine, which in turn may contribute to the etiology of diseases such as cancer, CVD, and autoimmune diseases.

Deficiency and Excess

Selenium Deficiency Diseases

Keshan disease, an endemic cardiomyopathy occurring in low-selenium areas of China and Russia, is associated with low selenium intake and low blood and hair levels, and affects mainly children and women of childbearing age. The main clinical features of Keshan disease are cardiac insufficiency and enlargement, electrocardiographic changes, and fibrosis. In 1979 Keshan disease was reported to be responsive to supplementation with sodium selenite and was initially thought to be a simple selenium deficiency. However, some features of Keshan disease (e.g., seasonal variation) cannot be explained

Table 1 Selenoprotein description and functions

<i>Selenoprotein</i>	<i>Location/expression</i>	<i>Function</i>
<i>Glutathione peroxidases (GPx)</i>		
GPx1	Location: cytosol. Expression: ubiquitous in cells.	Antioxidant. Catalyzes reduction of H ₂ O ₂ and soluble organic peroxides. Recovery of cells after oxidative stress.
GPx2	Location: cytosol. Expression: gastrointestinal tract.	Antioxidant. Catalyzes reduction of various peroxides. Protects intestinal epithelium from oxidative stress.
GPx3	Location: plasma and extracellular. Expression: kidney, plasma, other tissues.	Antioxidant. Catalyzes reduction of H ₂ O ₂ and soluble organic peroxides. Regulates bioavailability of nitric oxide.
GPx4, phospholipid hydroperoxide	Location: cytosol and membrane. Expression: various tissues including brain, testis.	Antioxidant. Reduces phospholipid hydroperoxides. Structural protein involved in sperm maturation. Redox signaling, apoptosis.
GPx6	Location and expression: Embryonic and olfactory epithelium.	Antioxidant.
<i>Thioredoxin reductases</i>		
TR1	Location: cytosol. Expression: ubiquitous.	NADPH-dependent flavoprotein oxidoreductases.
TR2	Location: mitochondria. Expression: liver, kidney, heart.	Reduce oxidized thioredoxin. Regulate intracellular redox state, gene expression, cell growth, cell survival and apoptosis.
TR3	Location: cytosol of testes. Expression: testes.	
<i>Iodothyronine deiodinases</i>		
DI1	Location: plasma membrane. Expression: liver, thyroid, kidney, pituitary.	Synthesis and metabolic regulation of thyroid sulfated hormones (T ₃ , T ₄ , T ₂). Converts thyroxine (T ₄) to bio-active triiodothyronine (T ₃). Converts T ₄ to bio-inactive 3',3',3' reverse T ₃ .
DI2	Location: endoplasmic reticulum. Expression: thyroid, brain, heart, spinal cord, intestine, skeletal muscle.	Converts thyroxine (T ₄) to bio-active triiodothyronine (T ₃).
DI3	Location: plasma membrane. Expression: brain, placenta, skeletal muscle.	Converts T ₄ to bio-inactive 3',3',3' reverse T ₃ .
Selenophosphate synthetase (SPS2)	Location: cytosol. Expression: ubiquitous.	Catalyzes conversion of selenide to selenophosphate in the biosynthesis of selenocysteine and selenoproteins.
15-kDa selenoprotein (SEP15)	Location: endoplasmic reticulum. Expression: prostate, thyroid, parathyroid.	Function unclear. Thioredoxin-like.
Selenoprotein P (SEPP)	Location: extracellular, plasma. Expression: liver, other tissues.	Selenium-transport protein. Antioxidant in endothelium.
Selenoprotein W (SEPW)	Location: cytosol. Expression: Skeletal and heart muscle.	Function unclear. Antioxidant. Involved skeletal and cardiac muscle metabolism.
Selenoprotein R or X (SEPR or SelX)	Location: cytosol. Expression: pancreas, liver, kidney, leukocytes.	Also known as methionine-R-sulphoxide reductase (MsrB1). Antioxidant, methionine metabolism, protein repair.
Selenoprotein N (SEPN)	Location: endoplasmic reticulum membrane. Expression: ubiquitous.	Function unknown. Important in muscle development.
Selenoprotein S (SEPS)	Location: endoplasmic reticulum. Expression: ubiquitous.	Involved in inflammatory response
Selenoproteins H, I, K, M, O, T, V.		Functions unclear or unknown.

solely by very low selenium status. Involvement of a viral factor is likely, as a strain of a Cocksackie B virus has been isolated from infected individuals. This hypothesis is strengthened by the demonstration that Cocksackie B3 virus, in the presence of selenium deficiency in mice, mutates to a more virulent form that compounds the heart condition.

Another condition that has been associated with severe selenium deficiency is Kaschin-Beck disease, with clinical features of osteoarthropathy and necrosis of joints and epiphyseal plate cartilage. Kaschin-Beck disease occurs during preadolescent or adolescent years in rural areas of China,

Tibet, and Siberia. However, other factors such as iodine deficiency or presence of mycotoxins may be more important than selenium deficiency.

Severe selenium deficiency in combination with inadequate iodine status contributes to the pathogenesis of myxedematous cretinism. Even mild to moderate selenium deficiency appears to be responsible for initiation and progression of autoimmune thyroid disorders.

Selenium deficiency has also been reported in patients on long-term intravenous nutrition, because of previously negligible amounts of selenium in the fluids. Cardiomyopathy,

muscle pain, and weakness in these patients are responsive to selenium supplementation, but are not seen in all patients with low selenium status, such as children on low selenium synthetic diets for inborn errors of metabolism, indicating that there may be other interacting factors.

Selenium Toxicity

Toxicity of selenium or selenosis may occur from consuming high-selenium foods grown in seleniferous areas in Venezuela, Colombia, northern USA and Enshi county in China. Loss of hair and nails is the most common sign of poisoning, and changes in hair and nails are currently the only diagnostic technique for selenium toxicity. Other overt signs of selenosis include skin damage, mottling of teeth, nerve lesions, nausea, weakness, and diarrhea. Garlic odor on the breath from breathing out dimethylselenide also indicates excessive selenium exposure. Effects of selenium toxicity are seen at daily dietary intakes of above 900 µg.

Selenium and Human Health

Immune Function

Selenium is important for optimal function of both innate and acquired immune systems, and is involved in defense of animals against bacterial and viral infections. The mechanisms for this role of selenium are likely to be related to its antioxidant function through the selenoproteins GPx, thioredoxin reductases or SEPP. Selenium supplements can improve several indices of immune function, even in individuals whose selenium status is not severely deficient.

Studies in mice of strains of Coxsackievirus B3 showed that selenium deficiency and vitamin E deficiency increased the cardiotoxicity of myocarditic strains. In addition non-myocarditic strains of the virus caused heart lesions as a result of changes in the viral genome in selenium-deficient mice but not in selenium adequate mice. This is relevant to the etiology of Keshan disease, which has been attributed in part to a viral factor. Selenium deficiency also causes mutational changes in another RNA virus, influenza A, and in the protozoan parasite *Trypanosoma cruzi* and *Heligmosomoides polyus*, enhancing the intensity of infection. In HIV-infected individuals, progression to AIDS and decline in T helper (CD4) cell counts are accompanied by a parallel decrease in blood selenium levels. Selenium deficiency appears to increase the probability of mortality in HIV-infected subjects.

Cancer

A considerable body of evidence suggests a possible link between increased selenium intakes or status and protection against certain cancers. *In vitro* and animal studies provide evidence for a role of selenium as an anticarcinogenic agent at high levels of intake. Evidence from prospective studies investigating the link between low selenium status and increased incidence of cancer at various sites is conflicting, but there is reasonably strong evidence for prostate and breast cancer. Several intervention trials in humans of the effect of selenium,

alone or with other nutrients, on cancer incidence or biomarker concentration have been carried out, but again results of these trials are conflicting. The Nutritional Prevention of Cancer Trial that investigated the efficacy of high selenium yeast in preventing skin cancer provides the strongest evidence. Selenium supplementation had no effect on skin cancer, but there was a significant reduction in total cancer (50%), and cancer of the prostate, lung, and colorectum. The effect was strongest in subjects with the lowest selenium status. In contrast, the Selenium and Vitamin E Cancer Prevention Trial (SELECT) was discontinued early because of lack of beneficial effect on prostate cancer incidence and an increase in the incidence of diabetes in the selenium group and prostate cancer in the Vitamin E group. Observations from all these studies suggest that a U-shaped curve in the association between selenium status and risk of some cancers, and that the protective level of plasma selenium is approximately 120 to 160 µg l⁻¹, which may be achieved by an intake of 100–150 µg day⁻¹.

If there is indeed a protective effect of selenium, the intake required appears to be higher than that required to maximize selenoproteins, suggesting other processes are involved. A role for anticarcinogenic methylated selenium metabolites such as Se-methyl selenocysteine is possible. On the other hand, selenium's role in reducing DNA damage and oxidative stress, and the increasing volume of research indicating effects of genetic variations in selenoprotein genes on cancer risk, suggest that selenoproteins are also involved.

Cardiovascular Disease

Attempts to demonstrate a reduction of CVD by selenium intervention have proven disappointing. A moderate inverse relationship between plasma selenium and coronary heart disease was demonstrated in a meta-analysis of 25 observational studies. However, clinical trials show no evidence of an effect of selenium supplementation on cardiovascular protection in populations with adequate selenium status. Confirmation of this came from the SELECT trial in which selenium had no beneficial effects on atherosclerosis progression. Of concern are observations from several studies that higher selenium status is associated with increased total and LDL cholesterol, higher fasting plasma glucose and glycosylated hemoglobin levels and higher prevalence of diabetes and hypertension. On the other hand, low activity of GPx1 in erythrocytes was shown to be a predictor of cardiovascular events in patients with coronary artery disease. GPxs protect against processes relevant to atherosclerosis such as inhibition of LDL oxidation, inhibition of proatherogenic 15-lipoxygenase by GPx4, and alteration of expression of adhesion molecules induced by cytokines. Further controlled intervention trials are needed to clarify this situation.

It appears from these conflicting observations on associations between selenium and chronic disease in general that a U-curve represents the effects of selenium status as outlined above for cancer risk. In light of recently identified adverse effects of intakes higher than those recommended – diabetes, hypertension, skin cancer, and hypercholesterolemia – we should be cautious about recommending selenium supplementation for chronic disease prevention.

Assessment of Selenium Status

Selenium status can be measured by concentrations in plasma or serum, whole blood or erythrocytes, or in platelets, hair, or nails. Plasma or serum selenium reflects short-term status, erythrocyte concentration is a medium-term index, whereas hair and nail concentrations reflect longer-term status. Urinary excretion of selenium can be used to assess daily dietary intake, estimated as twice the daily excretion.

GPx enzymatic assay in plasma or red cells is another frequently used approach to measurement of status because of the close relationship between plasma GPx3 or erythrocyte GPx1 activity and selenium concentrations. In situations of severe to marginal deficiency, this is a sensitive and responsive index, however, once an adequate supply is achieved, at plasma/serum selenium concentrations above $80\text{--}90\ \mu\text{g l}^{-1}$, a plateau of activity is reached that does not respond to further increases in selenium intake. Therefore, if a population exhibits a strong correlation between plasma (or erythrocyte) selenium concentrations and GPx activity, or there is a significant increase in GPx activity after supplementation, this can be taken as evidence of suboptimum selenium status. If there is little evidence of a correlation or a response to supplementation, the population is likely to have adequate selenium intake. Absolute values of GPx activity are more difficult to interpret because many different versions of the assay are in use, and interlaboratory quality control harmonization has rarely been undertaken.

SEPP accounts for more than 50% of selenium in blood and has been shown to be a reliable marker of selenium status in populations with low-to-moderate selenium status, but like GPx, not with higher selenium status. Other selenoenzymes may also be measured as functional markers, but their use is limited because of a lack of simple assay techniques. Because of different responses of tissues and selenoproteins to deficient, adequate, or high levels of selenium, conclusions

drawn from measurement of one selenoprotein may not apply to all biological functions of selenium. It may be necessary to measure several markers of functional selenium status, in particular those that apply to specific problems associated with suboptimal selenium status.

Dietary Intake of Selenium

Dietary intake of selenium varies greatly with geographic source of foods as a result of large differences in soil selenium concentrations. In particular, plant foods reflect selenium content of soils and availability for uptake from the soils, as plants generally do not require selenium for growth; the selenium content of cereals and grains grown in soils poor or rich in selenium may vary 100-fold. Cereals provide a significant proportion of daily selenium intake in most countries. Some plants, such as garlic, mushrooms, and broccoli, can accumulate selenium from soil and therefore may contain high levels of selenium. Brazil nuts are also exceptionally good sources of selenium, but again the content varies greatly depending on where they are grown. The selenium content of animal foods varies less as animals have an absolute requirement for selenium, which they must get from food or supplements. Rich animal sources of selenium include organ meats, shellfish, and some other types of fish.

Average daily intakes of selenium range from $10\ \mu\text{g}$ in low-selenium areas of China, to medium intakes in New Zealand of $40\text{--}50\ \mu\text{g}$ to over $200\ \mu\text{g}$ or more in seleniferous areas in Venezuela and parts of USA. In the UK, selenium intakes have declined considerably during the past 25 years because of substitution of North American wheat imports by European wheat with lower selenium content. In contrast, intakes in New Zealand have increased as a result of greater consumption of imported foods and increases in selenium

Table 2 Reference values for intakes of selenium for selected countries ($\mu\text{g day}^{-1}$)

	EAR/AR/ANR		RDA/RDI/RNI		UL
	F	M	F	M	
USA/Canada, 2000 (EAR, RDA)	45	45	55	55	400
UK, 1991 (RNI)			75	75	450
Australia/New Zealand, 2005 (EAR, RDI)	50	60	60	70	400
Germany/Austria/Switzerland, 2000 (RI)			30–70	30–70	400
Nordic, 2004 (AR, RI)	30	35	40	50	
Japan, 2005 (EAR, RDA)	20	25–30	25	30–35	350, 450
FAO/WHO, 2004 (ANR, RNI)	20	27	26	34	400

EAR, estimated average requirement; AR, average requirement; ANR, average normative requirement; RDA, recommended dietary allowance; RDI, recommended dietary intake; RNI, reference nutrient intake (UK), recommended nutrient intake (FAO/WHO); RI, reference intake, recommended intake (Nordic); UL, tolerable upper intake level (USA/Canada, Germany/Austria/Switzerland) or upper level (Australia/New Zealand).

Reproduced from Department of Health (1991) *Dietary Reference Values for Food Energy and Nutrients for the United Kingdom, Report on Health and Social Subjects No. 41*. London: HMSO; Food and Nutrition Board, Institute of Medicine (2000) *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids*. Washington, DC: National Academy Press; German Nutrition Society, Austrian Nutrition Society, Swiss Nutrition Society & Swiss Society for Nutrition Research (2000) *Reference Values for Nutrient Intakes*. Frankfurt am Main: Umschau/Braus; Ministry of Health Labour and Welfare Japan (2005) *Dietary reference intakes for Japanese. The Report from the Scientific Committee of Dietary Reference Intakes for Japanese- Recommended Dietary Allowance*; NHMRC (2006) *Nutrient Reference Values for Australia and New Zealand Including Recommended Dietary Intakes*. Canberra/Wellington: NHMRC/Ministry of Health; Nordic Council of Ministers (2004) *Nordic Nutrition Recommendations: Integrating Nutrition and Physical Activity*. Copenhagen: Nordic Council of Ministers; World Health Organization (2004) *Vitamin and mineral requirements in human nutrition, second edition. Report of a Joint FAO/WHO Expert Consultation*. Bangkok, Thailand. Switzerland: World Health Organization.

concentrations in animal foods due to supplementation of commercial fertilizers and animal feeds.

Requirements and Recommended Dietary Intakes

The requirement to prevent selenium deficiency is based on comparison of intakes in endemic and nonendemic Keshan disease areas of China. However, dietary reference intakes in most countries are based on an estimate of the intake at which saturation of plasma GPx activity occurs, obtained from studies in China and New Zealand. This estimate indicated a physiological requirement of approximately $45 \mu\text{g day}^{-1}$ (Estimated Average Requirement, EAR for USA/Canada), which translated into a recommended dietary allowance (RDA) of $55 \mu\text{g day}^{-1}$. An increment of $5 \mu\text{g day}^{-1}$ was added for pregnancy and $15 \mu\text{g day}^{-1}$ for lactation. Committees of other countries have considered additional factors, which have resulted in a range of recommended intakes (Table 2). For example, recent data obtained from studies of SEPP were considered as well as GPx in revising the Australian/New Zealand Nutrient Reference Values (NRV) resulting in recommended daily intake (RDI) of 70 and $60 \mu\text{g day}^{-1}$ for males and females, respectively. On the other hand, the World Health Organization (WHO) recommendations are lower ($40 \mu\text{g day}^{-1}$) based on the premise that full saturation of GPx is unnecessary and two-thirds saturation is probably adequate. Intakes of selenium that may reduce risk of cancer and CVD have also been considered, which would probably result in higher recommendations. However, specific values have not been set as evidence is considered to be limited and confusing and very recent research has not clarified this situation.

The upper safe limit of dietary intake (UL) was set at $400 \mu\text{g day}^{-1}$ by the US/Canadian, Australian/New Zealand and FAO/WHO committees, based on a no-adverse-effect-level of $800 \mu\text{g day}^{-1}$ divided by an uncertainty (i.e., safety) factor of 2.

Conclusion

The essential role of selenium in human nutrition and its discrete biochemical functions are rapidly being characterized. Although the number of selenoproteins in the human body is now finite, the extent and diversity of their functions are yet to be discovered. As these roles are identified, their relationship to known consequences of selenium deficiency or to chronic disease is being clarified. This is being accelerated by the vast amount of recent and current research in genomics, which

indicates that SNPs in selenoproteins may influence susceptibility to the etiology of chronic diseases. Optimum human intakes of selenium are still a matter of debate because some studies have reported benefits (e.g., anticancer and immunological effects) when supplements are given, even to populations that appear to be generously supplied with the nutrient, whereas others have identified adverse effects. The distinction between nutritional and pharmacological benefits is unclear, and further trials to determine risk–benefit balance at different intake levels are needed in a range of populations and age and gender groups.

See also: Antioxidants. Cancer: Epidemiology and Associations Between Diet and Cancer. Iodine: Physiology, Dietary Sources, and Requirements. Nutrient-Gene Interactions: Health Implications; Molecular Aspects

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SKELETAL MUSCLE

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Glossary

Adenosine triphosphate (ATP) An ester of adenosine and triphosphoric acid, $C_{10}H_{12}N_5O_4P_3O_9$, formed especially aerobically by the reaction of adenosine diphosphate (ADP) and an orthophosphate during oxidation, or by the interaction of ADP and phosphocreatine or certain other substrates, and serving as a source of energy for physiological reactions, especially muscle contraction.

Allosteric Pertaining to regulation of the rate of an enzymatic process.

Anabolism The synthesis in living organisms of more complex substances from simpler ones.

Glycolysis The catabolism of carbohydrates, as glucose and glycogen, by enzymes, with the release of energy and the production of lactic or pyruvic acid.

Heparin A polysaccharide, occurring in various tissues, that when injected into the blood prevents coagulation.

Lipolysis The hydrolysis of fats into fatty acids and glycerol, as by lipase.

Oxidation To convert (an element) into an oxide; to combine with oxygen.

Proteolysis The breaking down of proteins into simpler compounds, as in digestion.

Sarcopenia The age-associated loss of skeletal muscle mass and function.

Stem cell A cell that on division replaces its own numbers and also gives rise to cells that differentiate further into one or more specialized types, as various satellite cells.

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Introduction

There are approximately 650–850 muscles in the human body. These include skeletal (striated), smooth, and cardiac muscles. The approximation is based on what some anatomists consider separate muscle or muscle systems. Muscles are classified based on their anatomy (striated vs. smooth) and whether they are voluntarily or involuntarily controlled. The simple distinction is between muscle cells attached to the skeleton and those in the walls of hollow organs. The functions of muscle include movement, stability and posture, heat production, circulation, and digestion. As a result of the diverse and abundant research that has been or is currently being conducted on muscle tissue, not all important aspects of the different types of muscle are discussed here. This article will primarily focus on recent research pertaining to skeletal muscle adaptation to nutrition, exercise, aging, and chronic disease. This will be accomplished by (1) introducing skeletal muscle's structure and function, (2) summarizing its adaptive responses to nutrition and contractile activity, and (3) reviewing changes with aging and chronic disease.

Skeletal muscle is a highly malleable tissue that is a central factor in whole-body health and is essential for maintaining energy homeostasis. Skeletal muscle accounts for approximately 45–50% of body mass in non-obese individuals and plays a fundamental role in locomotion, O_2 consumption, whole-body energy metabolism, and substrate turnover and storage. In addition to its role in locomotion and metabolism, skeletal muscle is also the largest store for glucose, lipid, and protein. Skeletal muscle is responsible for approximately 20–30% of resting oxygen consumption. Unlike the metabolic rate in tissues such as the brain and kidney, which are constantly sustained and fluctuate little throughout the course of one day, skeletal muscle metabolism changes considerably from resting to maximal physical activity, during which muscle O_2 consumption can account for up to 90% of the whole-body oxygen uptake. Furthermore, in healthy individuals skeletal muscle accounts for approximately 80% of whole-body insulin-stimulated glucose uptake but in normal-weight subjects with insulin resistance, total body glucose metabolism is reduced by up to 40%. These data highlight the key role of skeletal muscle in overall energy storage and metabolism.

Structure and Function

Overview

Purposeful movement is a major characteristic of higher animals and is a result of the activity of voluntary skeletal muscle. The understanding that muscle is the central organ of voluntary movement and force production dates back as far as

Galen of Pergamon (AD 129–201), whose detailed dissections of muscles helped to establish the science of muscle (myology). Over the following 2000 years, research encompassing skeletal muscle has enabled us to identify the ultrastructure of skeletal muscle and the processes involved in the activation and contraction of muscle fibers. Because this information has previously been well described in most physiology texts, in this article only a brief overview is provided.

Structure

Skeletal muscle is often considered as being organized in a hierarchy. It is composed of bundles of muscle fibers called fascicles, which in turn are composed of muscle fibers or cells. The term cell and fiber can be used interchangeably because a fiber is a single multinucleated cell. The cell membrane surrounding the muscle cell is the sarcolemma, and immediately below the sarcolemma is the sarcoplasm, which contains the cellular proteins, organelles, and myofibrils. The myofibrils contain the contractile apparatus, which is composed of a thin filament and thick filaments. The filaments are organized into repeating contractile units called sarcomeres, whose lengths span from one Z disk to the next (**Figure 1**). The arrangement of these two protein filaments gives skeletal muscle its striated appearance.

Actin and Myosin

The thin filament is made principally of actin and the regulatory proteins tropomyosin and troponin. Actin is a globular protein (G-actin) with a molecular weight of 42 kilodaltons (kDa), that polymerizes into a double helix (F-actin). The polymerization from G-actin to F-actin involves the hydrolysis of adenosine triphosphate (ATP) and binding of adenosine diphosphate (ADP) to actin. Of interest, 90% of the total ADP in the body is bound to actin. Actin has a myosin-binding site that, when exposed, attaches to the myosin crossbridge. The cycling of the crossbridges causes the development of muscle force.

The major component of the thick filament is myosin, a highly conserved protein found in both the animal and the plant kingdoms. Myosin is a hexameric molecule consisting of two identical heavy chain subunits (MHC), each with a molecular weight of approximately 200 kDa and four regulatory light chain subunits (MLC) with a molecular weight of

approximately 16–28 kDa. Importantly, both MHC and MLC are found in various isoforms influencing contractile function. In mammalian muscle four MHC isoforms have been identified and they are designated as slow type I, fast type IIa, fast type IIb, and fast type IIx. Each of these isoforms differs in the amount of energy transduction kinetics, ATPase activity, and crossbridge activity. The myosin molecule can be split into two major fragments. The S1 fragment, called the globular head, contains ATPase activity and binds to actin. The S2 fragment includes the tail and the flexible region of the molecule that binds the myosin tails together to make the thick filaments. Thick myosin filaments in muscle are arranged so that the thin actin filaments can slide between them.

Classification

Skeletal muscle is made up of functional units called motor units, defined as a group of muscle fibers and the motor neuron that innervates them. Within a motor unit the muscle fibers have similar contractile and biochemical properties that are determined primarily by the motor neuron. Based on work using biochemical methods and histochemical staining, muscle fibers are commonly classified as red, type I, slow-twitch (ST) oxidative (stain dark), and white, type II, fast-twitch (FT) glycolytic (stain light) (**Table 1**). In humans, a further subdivision of the type II fibers is made whereby the more oxidative type II fiber is designated type IIa (FTa), and the more glycolytic fiber is termed type IIb (FTb). In rodents there is a large degree of homogeneity within individual skeletal muscles. However, this is not the case for humans where the heterogeneity of fiber type composition between individuals might explain the significant variation in metabolic potential and exercise capacity observed in athletes.

Energetics

Under normal physiological conditions, skeletal muscle relies on both carbohydrate and lipid-based fuels for oxidative metabolism. The degree to which skeletal muscle utilizes one fuel or another is dependent on several factors (e.g., nutrient availability, nutrient-induced hormone secretion, and contractile status). Skeletal muscle fuel metabolism is a highly regulated process that ensures that ATP supply is always closely matched to ATP demand. There are two physiological components of skeletal muscle metabolism that play underlying roles in the timing of substrate use. The first is fuel selection during the postabsorptive (before a meal) and the postprandial (after a meal) condition. The second is the oxidation of fuels that are either obtained from a meal or mobilized from energy stores (glycogen or triglycerides). Randle and colleagues hypothesized that fuel selection depends primarily on the availability of lipids, and this is the key factor determining the fuel mix to be oxidized during resting conditions (glucose–fatty acid cycle). More recently, it has been observed that hyperglycemia can inhibit fatty acid oxidation, a concept named the ‘reverse Randle cycle’. Without doubt, carbohydrate- and lipid-based fuels are the most important energy sources for matching ATP supply to ATP demand in skeletal muscle.

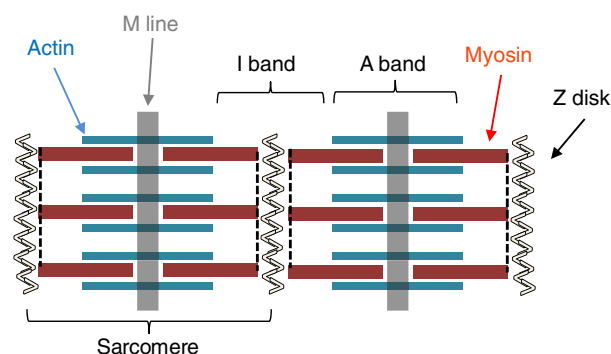


Figure 1 Representation of a skeletal muscle sarcomere. Anisotropic (A)-band, Isotropic (I)-band, Zwischenscheibe (Z)-disk, Mittelscheibe (M)-line.

Table 1 Characteristics of skeletal muscle fiber types

Characteristics	Fiber type		
	Phasic high frequency		Tonic low frequency
Morphology	FTb	FTa	ST
Color	White	White/red	Red
Fiber diameter	Large	Intermediate	Small
Capillaries mm ⁻²	Low	Intermediate	High
Mitochondrial volume	Low	Intermediate	High
Major Fuel Storage	PCr, Glycogen	PCr, Glycogen	Triglycerides
Histochemistry and biochemistry	IIB	IIA	I
	FG	FOG	SO
Myosin ATPase	High	High	Low
Calcium-handling capacity	High	Medium/high	Low
Glycolytic capacity	High	High	Low
Oxidative capacity	Low	Medium/high	High
Function and contractility	FF	FR	S
	FT	FT	ST
Speed of contraction	Fast	Fast	Slow
Speed of relaxation	Fast	Fast	Slow
Fatigability	High	Moderate/high	Low
Contraction strength	High	Intermediate	Low
Myosin heavy chain, genes	MYH4	MYH2	MYH7

FT, fast-twitch; ST, slow-twitch; FG, fast, glycolytic; FOG, fast, oxidative, glycolytic; SO, slow, oxidative; FF, fast-contracting, fast-fatigue; FR, fast-contracting, fatigue-resistant; S, slowly contracting.

Adaptations to Nutrients and Exercise

Substrate Utilization in Skeletal Muscle

Lipids, carbohydrates, and amino acids (AAS) are all important fuels for aerobic metabolism. However, in well-fed individuals, the contribution of AAS to resting energy metabolism is minimal (1–2%). Therefore, this section will focus on the transport and utilization of lipids and glucose in skeletal muscle, including a discussion of the molecular pathways involved in the regulation of lipid and glucose metabolism in skeletal muscle.

The transport of glucose into the cell is the rate-limiting step in carbohydrate metabolism (its subsequent uptake and oxidation). This is achieved by the facilitated diffusion of glucose through glucose transporters (Glut1–12) during postabsorptive and postprandial conditions. In skeletal muscle this process is mediated by the translocation of the insulin-sensitive glucose transporter 4 (Glut4). This is accomplished by two distinct signaling pathways: one pathway is mediated by insulin (i.e., the insulin-dependent pathway) and the other is activated independently by muscle contraction.

Insulin-Dependent Glucose Uptake

The postprandial uptake of glucose into muscle cells is initiated by the binding of insulin to the extramyo cellular α -subunit of the insulin receptor (IR), which results in IR β -subunit autophosphorylation and the subsequent tyrosine

phosphorylation of the insulin receptor substrate (IRS). Phosphorylated IRS then binds and activates phosphoinositide 3-kinase (PI3K), leading to the activation and phosphorylation of Akt and protein kinase C (PKC). The activated Akt subsequently phosphorylates and inhibits its downstream substrate, the Akt substrate of 160 kDa (AS160). The phosphorylation of AS160 allows its binding to the 14-3-3 protein and the untethering from the Glut4 vesicle. These initial signaling events converge on several downstream effectors that promote the metabolic actions of insulin, including the translocation of Glut4 to the myocellular membrane, allowing for the disposal of plasma glucose.

Insulin-Independent Glucose Uptake

Investigations using aerobic exercise or electrically stimulated muscle contraction have revealed that glucose disposal is activated to a similar extent as with insulin. Winder and Hardie first observed that exercise/contraction increased the activation of the 5' AMP-activated protein kinase (AMPK). Subsequently, when using the AMP mimetic 5'-aminoimidazole-4-carboxamide ribonucleoside (AICAR), these researchers proposed a role for AMPK activation in increased glucose uptake into skeletal muscle. Since these studies, the role of AMPK in contraction-stimulated glucose metabolism has been well researched. AMPK is a cellular energy sensor that restrains energy-consuming processes and concurrently increases energy-producing processes, like β -oxidation. AMPK is highly sensitive to and allosterically activated by 5' AMP. This activation is

inhibited by ATP, which makes the AMP/ATP ratio a good indicator of cellular energy status and AMPK activation. However, AMPK is not the only contributor to glucose metabolism and adaptation in this tissue. Indeed, muscle contraction induces the activation of several signaling mechanisms that have been associated with increased Glut4 translocation including Ca^{2+} /calmodulin, nitric oxide, PKC, and interleukin-6 (IL-6) (**Figure 2**).

In humans, during times of low-energy status (i.e., fasting, strenuous muscle contraction), insulin levels decrease considerably and the concentration of free-fatty acids (FAs) increases 5–10-fold above resting levels. Most of the resting energy requirements are obtained from lipolysis in adipose tissue. At rest, the amount of FA released from adipose exceeds the amount oxidized and a large portion is reesterified back into triacylglycerol by the liver. During aerobic exercise, the amount of triacylglycerol reesterified decreases by approximately 50%. In a low-energy state (i.e., fasting, exercise), circulating FAs are the predominant fuel and are readily taken up by skeletal muscle.

The transport of FAs from the blood circulation to the cytosol of skeletal muscle cells is likely the combined result of passive diffusion across the myocellular membrane and protein-mediated membrane transport. FAs are translocated into the cytosol triggering the formation of fatty acyl-CoA (FA-CoA). FA-CoA is targeted for FA oxidation and must first cross the mitochondrial membrane in a rate-controlling step that is regulated by carnitine palmitoyl transferase I (CPTI). During times of low energy (e.g., fasting and prolonged exercise),

malonyl-CoA levels decline and the reliance on FA oxidation increases. When FAs enter the mitochondria, they proceed through a repetitive biochemical process called β -oxidation. During this process the FA is broken down into acetyl-CoA. Acetyl-CoA is then disposed of by way of the tricarboxylic acid (TCA) cycle. β -Oxidation and the TCA cycle produce energy-rich donors (NADH, succinate) for the synthesis of ATP in the electron transport chain.

Changes to Fiber Type Composition

Contractile activity induces considerable increases in the size, number, and oxidative capacity of mitochondria (**Figure 2**). This mitochondrial biogenesis is a well-established muscle adaptation that can be easily observed using electron microscopy. A single bout of exercise is a sufficient stimulus to induce mitochondrial biogenesis, and this mitochondrial proliferation can be maintained by repeated bouts of exercise. Endurance exercise training results in an increase in the oxidative capacity of skeletal muscle by increasing the expression of proteins involved in mitochondrial biogenesis such as PGC1, PPAR α , nuclear factor of activated T cells (NFAT), and nuclear respiratory factor 1 (NRF1) (**Figure 2**). In addition, exercise induces the conversion of muscle fibers from the more glycolytic type IIx (humans/rodents) and IIb (rodents) (white, glycolytic, fast-twitch) to the more oxidative type IIa (white, oxidative, medium twitch), which has a more type I phenotype (red, oxidative, slow-twitch) (**Table 1**). Type I fibers are

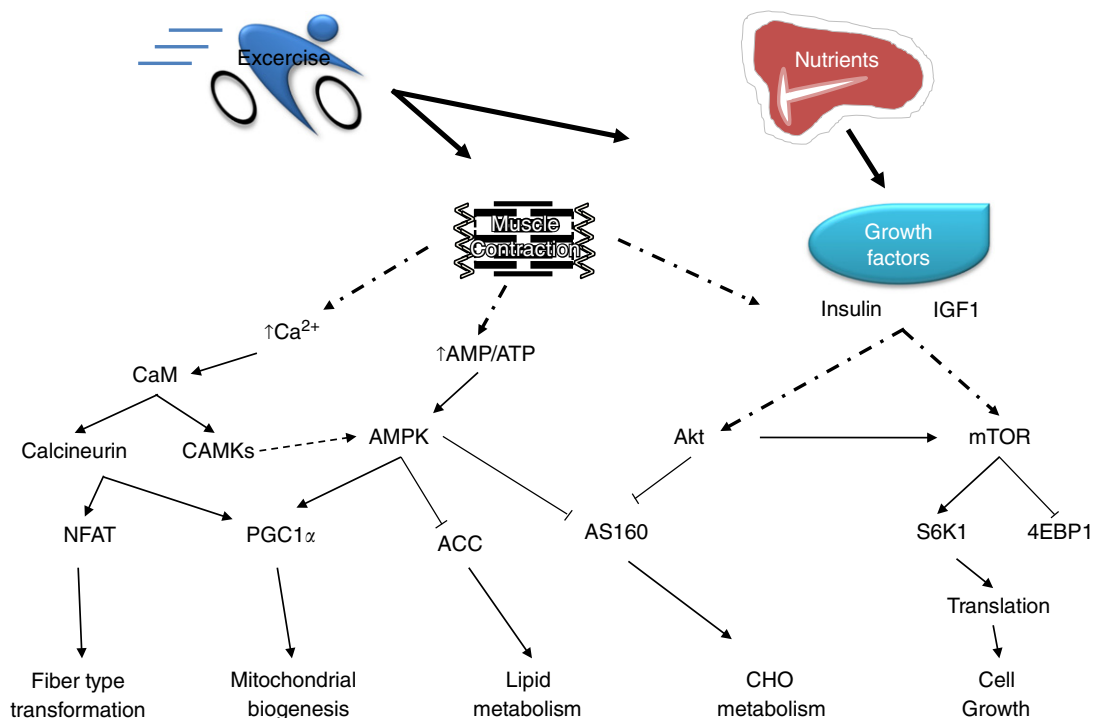


Figure 2 Representation of signalling pathways associated with skeletal muscle adaptations to nutrients and contractile activity. Ca, Calcium; CaM, calmodulin; CaMK, calmodulin kinase; NFAT, nuclear factor of activated T cells; PGC1; AMPK, AMP-activated protein kinase; ACC, acetyl-CoA carboxylase; Akt substrate of 160 kDa (AS160); CHO, carbohydrate; mTOR, mechanistic target of rapamycin; ribosome protein S6K1, S6 kinase; eukaryotic translation initiation factor 4EBP1, 4E-binding protein 1.

characterized by an increased mitochondrial number and oxidative state as well as a specific set of contractile proteins such as troponin I slow and myoglobin (Table 1). The ability to shift skeletal muscle fibers from a more glycolytic fiber to a more oxidative fiber could have a profound effect on metabolic health.

Muscle Growth

The maintenance of muscle mass is regulated by a balance between protein synthesis and protein degradation and is associated with rates of anabolic and catabolic processes, respectively. Under conditions of atrophy, there is evidence for a shift toward myofibrillar and nonmyofibrillar protein degradation and a corresponding reduction in protein synthesis. When protein synthesis exceeds protein degradation, there is increased muscle mass (hypertrophy). In contrast, if protein degradation exceeds protein synthesis, there is muscle loss (atrophy).

Anabolic stimulators, such as insulin, insulin-like growth factors (IGF1), AA, and muscle contraction, rapidly and significantly increase skeletal muscle protein synthesis in young healthy tissue. Increased rates of protein synthesis are a key feature of hypertrophy driving muscle growth. The effect of essential AA on the dose-dependent stimulation of muscle protein synthesis is even observed when circulating insulin concentrations are clamped ($10 \mu\text{IU ml}^{-1}$) or when somatostatin is used to inhibit insulin and insulin-like growth factors in human subjects. The mammalian target of rapamycin (mTOR) signaling kinase, which can be activated by Akt, has emerged as a necessary effector of skeletal muscle growth in response to contraction and anabolic stimuli. Insulin, AAS, and acute contractile activity have all been observed to increase the phosphorylation of mTOR and its downstream targets, p70 ribosomal protein S6 kinase 1 (S6K1) and 4E-binding protein 1 (4EBP1), leading to the initiation of protein synthesis through the activation of S6 ribosomal (rpS6) protein and other components of the translational machinery.

Endurance- and resistance-type exercises have been shown to enhance insulin sensitivity and training-specific adaptation in skeletal muscle. Similarly, acute increases in mTOR phosphorylation are observed in the hours following bouts of diverse contractile activity associated with both aerobic endurance and heavy resistance exercise. Endurance exercise is based on movements performed with a high number of repetitions and low resistance. In contrast, resistance exercise is based on movements performed with high resistance and a small number of repetitions over a short period of time. However, endurance and resistance exercises appear equally capable of enhancing mTOR activity, and the diverse contractile stimuli might initiate both reciprocal and independent pathways to promote specific adaptation in skeletal muscle.

Muscle Regeneration

The regenerative capacity of muscle fibers depends on a pool of myogenically specified undifferentiated mononuclear precursor stem cells called 'satellite cells (SCs)' that appear to function as 'reserve' myoblasts. SCs are the primary stem cells

in adult skeletal muscle and are responsible for postnatal muscle growth, hypertrophy, regeneration, and repair. SCs were identified ultrastructurally and were named for their peripheral location beneath the basal lamina of the myofiber. SCs are primarily in a quiescent, nondifferentiating state, dividing infrequently under normal conditions in the adult but activated (reenter the cell cycle) by regenerative cues such as injury or exercise. Once activated, the cells will proliferate and increase in number, and the daughter cells (myoblasts) will repair damaged skeletal muscle by fusing to existing myofibers or generating new myofibers by fusing together.

It is believed that muscle hypertrophy requires the addition of nuclei to existing myofibers. This follows the premise that increases in fiber size must be associated with a proportional increase in myonuclei for the control of mRNA and protein production per volume of cytoplasm. Growth factors, such as IL-6, testosterone, IGF1, and the IGF isoform, mechanogrowth factor, have been identified as playing a role in postexercise hypertrophy.

Adaptation with Age and Chronic Disease

Changes in Muscle Composition

It is well understood that with advancing age, a sedentary lifestyle, and obesity, there is a change in the composition of skeletal muscle. Skeletal muscle mass normally contributes up to 50% of the total body weight in young adults but declines with age to 25% at 75–80 years. The loss in lean muscle mass is usually offset by gains in fat mass. Longitudinal studies have shown that fat mass increases with age, peaking at approximately 60–75 years. Aging and obesity are associated with the increased accumulation of intramuscular fat as well as with an increase in the incidence of metabolic disorders such as insulin resistance and impaired lipid metabolism. Researchers have observed the defects in lipid metabolism, such as increased intramuscular and circulating lipids, even in lean and otherwise healthy elderly persons. Furthermore, studies have found significant differences in protein metabolism between obese and non-obese humans. The concomitant age-related changes in body composition, obesity, impaired metabolism, and low muscle mass have led to the hypothesis that there may be a causal link between obesity and low strength.

Insulin resistance is also highly coupled with obesity and aging and results in decreased insulin-stimulated glucose uptake, protein synthesis, and the inability to inhibit lipid uptake. It was recently observed that obese humans have a decreased fractional synthetic rate during an AA infusion and insulin clamp in the basal and insulin-stimulated state compared with their age-matched controls. In addition to the evidence showing that high-fat feeding and obesity inhibit protein synthesis in response to an anabolic stimulus, there is also evidence of altered mTOR signaling in the basal and insulin-stimulated state. However, studies report that there is no relationship between acutely increased circulating free-fatty acids (artificially induced with heparin treatment) and decreased protein synthesis or impaired mTOR signaling in skeletal muscle. Although there is some contention regarding the role of increased circulating free-fatty acids and reduced protein synthesis, the increased storage of fat in muscle during

aging has been clearly demonstrated to play a role in reduced muscle mass and functional impairment.

Muscle Loss

Protein Synthesis

Aging and obesity are associated with an inability of insulin to stimulate muscle protein synthesis and AA uptake in otherwise healthy, glucose-tolerant persons. The decline in muscle protein anabolic response to insulin is likely to be responsible for the observed reduction in postprandial muscle protein anabolism in obese and older individuals. It has been observed that protein synthesis does not increase in response to hyperinsulinemia in older adults, in contrast to young subjects. Insulin resistance of muscle protein metabolism with aging may induce a slow but progressive decline in muscle protein content, thereby contributing to the loss of muscle mass in older adults.

It is well established that within a few hours of muscle contraction, there is an increase in protein synthesis even in the fasted state. The contraction-induced effects on muscle protein synthesis have been previously shown to be decreased in older than in young humans. This effect has even been observed after a 3-week strength exercise program in male and female human subjects. This same effect has been further observed in obese and old rodents: there is inhibition of an anabolic signaling in response to muscle contraction and overload in aging skeletal muscle. However, the anabolic resistance attributed to aging muscle has not been observed in all studies. Therefore, more studies are needed to elucidate the significance of anabolic resistance to sarcopenia.

Protein Degradation

There are three known major proteolytic pathways that play a role in skeletal muscle: the lysosomal pathway, the Ca^{2+} -dependent pathway comprising the μ - and m-calpains, and the ubiquitin/proteasome-dependent proteolytic pathway. Of these, the pathway that has recently received the most interest is the ubiquitin–proteasome pathway. In skeletal muscle, this pathway is involved in the breakdown of long-lived myofibrillar proteins. In a variety of conditions such as cancer, diabetes, denervation, disuse, and fasting, skeletal muscles atrophy through degradation of myofibrillar proteins via the ubiquitin–proteasome pathway. The induction of the muscle-specific ubiquitin E3-ligases (atrophy gene-1/muscle atrophy F-box (Atrogin-1/MAFbx) and muscle ring-finger protein 1 (MuRF-1)) is thought to be the common mechanism associated with these diseases. The roles of Atrogin-1 and MuRF-1 in age- and chronic-disease-related muscle loss are not as clear cut. For example, some studies reported a small increase, no change, or even a down-regulation of Atrogin-1 and MuRF-1 mRNA in muscle.

Diet-Induced Metabolic Dysfunctions

Obesity and elevated levels of circulating nutrients (i.e., glucose, AA, FFA) are strongly associated with the development of insulin resistance and type 2 diabetes. These conditions are exacerbated by excess nutrient intake and a sedentary lifestyle. Increased dietary fat consumption has long been associated

with the metabolic abnormalities that are associated with overnutrition. However, increases in the intake of other dietary nutrients, such as protein and carbohydrates, can also have negative effects on whole-body insulin action. The so-called ‘western diet’ is characterized by the consumption of large quantities of processed meats, refined sugars, and fats and is closely linked with obesity and insulin resistance. In addition to nutrient-induced impairments as a consequence of increased lipid availability, hyperglycemia is also known to impair whole-body glucose disposal and glycogen synthesis. Exposure to chronically high glucose levels (glucose toxicity) also plays a role in the insulin resistance of skeletal muscle in part by the inhibition of glucose transport/phosphorylation. However, the treatment of hyperglycemia in patients with type 2 diabetes only partially improves insulin sensitivity, strongly suggesting that insulin resistance might be a cause rather than a consequence of hyperglycemia. Because skeletal muscle is responsible for the majority of insulin-mediated glucose disposal and the muscle’s sensitivity to insulin is highly responsive to changes in circulating nutrients, significant scientific effort has been devoted to elucidating the mechanisms by which overnutrition leads to impaired insulin signal transduction in skeletal muscle.

Summary and Conclusions

In summary, purposeful movement is a major characteristic of higher animals and is a result of the activity of voluntary skeletal muscle. Skeletal muscle is a highly malleable tissue that is a central factor in whole-body health and is essential for maintaining energy homeostasis. In this article, the authors have introduced the structure and function of skeletal muscle, summarized its molecular responses to nutrients and contractile activity, and discussed changes with aging and chronic disease. Understanding the processes involved in maintaining skeletal muscle mass and energy homeostasis is important in the preservation of whole-body metabolic health.

See also: Adipose Tissue: Structure, Function and Metabolism. Aging. Amino acids: Metabolism; Specific Functions. Carbohydrates: Regulation of Metabolism. Diabetes Mellitus: Etiology and Epidemiology. Energy: Adaptation. Energy Metabolism. Fatty acids: Metabolism. Glucose: Glucose Tolerance; Metabolism and Maintenance of Blood Glucose Level. Physical Activity: Beneficial Effects. Protein: Synthesis and Turnover

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Physiology

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Physiological, Clinical, and Nutritional Importance of Sodium

Although the body contains more calcium and potassium, sodium is arguably the most important cation (positive ion): It dictates the volume of extracellular fluid (ECF) and its concentration affects osmotic concentration of both ECF and intracellular fluid (ICF). Abnormalities of ECF sodium concentration cause movement of water into or out of cells, altering the osmotic concentration of ICF and causing swelling or shrinkage of cells. Osmotic shifts affect all cells and tissues but the brain is susceptible to damage from changes in intracranial pressure as it swells or shrinks in response to these osmotic fluid shifts.

Sodium depletion can result from enteric, renal, or adrenal disease, sodium retention from renal disease; healthy kidneys readily excrete excess sodium but chronic ingestion of excess salt, whether or not it increases ECF volume, predisposes to or exacerbates hypertension. Until the 1980s, knowledge of the regulation of body sodium mainly concerned defences against depletion. The 1990s brought insight into mechanisms that excrete excess sodium. In fact most mammals, especially humans, dogs, and laboratory rats, routinely have dietary sodium intakes well above their nutritional requirement which reflects their obligatory losses (maintenance) and the needs of growth, pregnancy, and lactation. Abnormal losses through disease, or excessive sweating in animals such as humans and horses raise the requirement. The impact of equine sweating is different from that in humans. Human sweat sodium concentration is well below plasma (and when aldosterone secretion is raised, sweat sodium becomes very low); horse sweat is hypertonic but this helps to offset the osmotic effect of increased respiratory water loss during exertion, i.e., it defends against hypernatremia, rather than causing sodium depletion. Aldosterone does not reduce equine sweat sodium. In other species (e.g., sheep, rat) hypernatraemia induces 'dehydration natriuresis' – another appropriate defense.

The physiology of sodium embraces its distribution in the body, regulation of total content and concentration, causes of and responses to depletion or excess, and their nutritional implications.

Distribution

Sodium is a cation; its distribution and physiological effects are fairly independent of the anions that accompany its

ingestion though they may affect its absorption and excretion. Most sodium is kept in ECF (Table 1), by the sodium pump, an enzyme system, Na^+/K^+ -exchanging adenosine triphosphatase, which uses substantial amounts of energy to keep intracellular sodium low and intracellular potassium (K^+) high. Sodium transport is the crux of the physiology of sodium:

1. It helps to maintain the ionic environment of ICF and the volume of ECF.
2. It prevents cell swelling (the Na^+ efflux exceeds the K^+ influx).
3. It establishes gradients which, in various tissues, allow transport of other cations in exchange, other anions in parallel or organic solutes – these are often cotransported with sodium down concentration gradients which are secondary to the low sodium environment created by the pump.
4. It establishes the membrane voltages on which excitability and secretory activities frequently depend.
5. The energy expenditure of the pump is a substantial portion of total metabolic activity and contributes to thermogenesis.
6. Sodium transport underlies the retention and loss of sodium in the kidney, gut, salivary, and sweat glands but also influences the excretion or retention of many other solutes. For example, diuretics intended to promote sodium excretion may also cause unintentional losses of potassium and magnesium. Similarly, when renal sodium excretion increases appropriately following ingestion of excess salt, there may also be unwanted losses of calcium

Table 1 Summary of sodium (Na) distribution and requirements

Typical plasma Na concentration (mmol l^{-1})	145 (130–160)
Typical body Na content (mmol kg^{-1})	50–55
Typical proportion (%) of total Na	
ICF	10
ECF	50
Bone	40
Maintenance requirement in mammals ($\text{mmol kg}^{-1} \text{ day}^{-1}$)	
Sheep	0.1
Cattle and goats	0.3–0.7
Pigs	0.6
Rats	0.6
Dogs	0.2–0.5
Cats	0.4
Humans	? < 0.6

1 mmol = 23 mg Na^+ , 58.5 mg NaCl.

and in postmenopausal women these may reduce bone mineral.

Bone contains large quantities of sodium but, as yet, its significance is unknown because it seems not to respond to sodium depletion. Gut fluids contain substantial sodium, mostly secreted rather than ingested, and mostly reabsorbed in distal regions of the intestine.

Sodium in Extracellular Fluid

ECF is mostly interstitial fluid (ISF) in the tissue spaces, providing the transport medium between capillaries and cells. Plasma sodium concentration is slightly higher than ISF because plasma proteins, notably albumin, do not readily escape into ISF across the capillary membranes and their negative charges hold more positive ions, notably sodium, in circulation (Gibbs–Donnan equilibrium). The main effect of inadequate ECF volume is to reduce plasma volume and thus to compromise cardiovascular function, in extreme cases by causing circulatory shock.

Excess ECF volume, mainly expanded ISF, shows clinically as edema (or ascites, fluid accumulated in the abdomen rather than the tissue spaces). Mild edema is merely cosmetic but pulmonary or cerebral edema, or severe ascites, are potentially serious. Although ingestion which overwhelms excretory capacity will result in sodium accumulation, this does not result necessarily in edema unless retention is massive. This is because accelerated lymphatic clearance of excess ISF provides a 'safety factor'. The shifts of sodium and water from the matrix to give pitting edema can occur simply because of massive overload but more often are the result of other physicochemical forces, eg hydrostatic pressure due to venous incompetence or heart failure. Edema suggests excess retention of sodium (overall expansion of ECF) or 'leakage' from plasma to ISF, with plasma volume continuously replenished by renal sodium retention. Such maldistribution of ECF could reflect very low plasma albumin (renal leakage, hepatic impairment, or severe malnutrition), or excessive capillary blood pressure (venous blockage, inactivity, heart failure, or arteriolar dilation, e.g., from heat or allergy), capillary damage, or lymphatic blockage. The latter prevents the removal of proteins that have leaked into ISF. Accumulation of protein in ISF undermines the osmotic gradient responsible for water uptake at the venous end of the capillary, where the pressure is lower. Because edema involves the expansion of a larger compartment (ISF) from a smaller one (plasma), it is only possible as long as the latter is replenished; hence the kidney, although seldom the primary cause of edema, is always the enabling cause. The use of diuretics is therefore appropriate in the treatment of non-renal as well as renal causes of edema. Although these explanations are clinically helpful, the exact stimuli triggering salt retention in edema remain enigmatic, leading to the pseudoconcept of changes in 'effective arterial blood volume'.

Regulation of ECF Sodium

In a mature, nonpregnant, nonlactating, healthy mammal, sodium excretion matches sodium intake and is often used to

estimate it, although this is not reliable, especially when intake is low. Dietary sodium is readily available, i.e., readily absorbed; thus the traditional view of sodium regulation emphasizes renal regulation of urinary Na^+ loss. This oversimplifies the more subtle interplay seen, for example, in herbivorous animals, where salt appetite may contribute to regulation by intensifying during sodium depletion. Moreover, in many herbivores the feces, rather than urine, may be the major route of sodium excretion and the gut may therefore be an important regulator of sodium balance. In fact sodium transport mechanisms in the small intestine show considerable similarities to those of the proximal renal tubules (e.g., linked transport of Na^+ , glucose, and amino acids) whereas the colon, like the distal nephron, responds to the salt-retaining (and potassium-shedding) hormone of the adrenal cortex, aldosterone. Recent research on guanilins suggests a role for these ancient vertebrate hormones in co-ordinating renal and enteric sodium excretion. Diarrhea is essentially enteric diuresis; a failure of intestinal sodium and water reabsorption, which exceeds the compensatory capacity of the colon.

Provided that adrenal function is normal, urinary and fecal sodium loss can be reduced virtually to zero. Human sweat sodium can also be very low, although with severe exertion in hot climates the sheer volume of sweat may override the ability of aldosterone to reduce its concentration, so causing net loss of sodium. Aldosterone also reduces salivary sodium (and raises $[\text{K}^+]$).

There are two components to the regulation of ECF sodium: The total amount of sodium retained and its concentration. The former is regulated by mechanisms that regulate sodium balance, whereas its concentration is mainly regulated via water balance. Thus, whatever sodium is retained in ECF is 'clothed' with the appropriate amount of water to maintain the normal plasma sodium concentration within narrow limits; deviations of less than 1% (hard to measure in the laboratory) trigger corrective responses. Elevated plasma sodium concentration (e.g., after water loss) stimulates both thirst and renal water conservation; antidiuretic hormone (ADH) from the posterior pituitary reduces urine output by increasing water reabsorption in the renal collecting ducts. Even one of these mechanisms can defend body water; thus diabetes insipidus (inadequate production or effect of ADH) causes compensatory polydipsia (increased fluid intake; 'thirst' is a sensation) rather than severe dehydration.

Excess salt intake does not raise plasma sodium concentration (hypernatremia) if water is available and the patient can drink; the excess sodium is diluted. The resulting increase in ECF volume then stimulates increased sodium excretion. Sodium also enables ECF to hold water against the osmotic 'pull' of intracellular solutes; sodium is the 'osmotic skeleton' of ECF, the main determinant of its volume.

Plasma sodium concentration is only indirectly related to sodium balance. When ECF volume, notably circulating volume, is severely reduced, hypovolaemia, rather than Na^+ concentration, becomes the main drive for thirst and ADH secretion. Until ECF volume is restored, water is retained (to protect ECF volume) even though this undermines the protection of ECF Na^+ concentration and, as a result, plasma sodium falls. During sodium depletion, contraction of ECF

volume precedes significant reductions of plasma Na^+ , which is therefore a poor index of sodium status.

Sodium-Retaining Hormones

Sodium depletion, by reducing plasma volume and renal perfusion, stimulates renal production of renin, which increases plasma angiotensin which is a vasoconstrictor (protecting blood pressure), stimulating thirst (helping to restore ECF volume), and stimulating sodium retention both directly (renally) and indirectly (by stimulating adrenal secretion of aldosterone); it thus reduces the sodium concentration of urine, feces, saliva, and sweat, but not milk.

Indices of aldosterone secretion (reduced sodium or increased potassium concentration in urine, feces, etc.) are often assumed to indicate sodium depletion or inadequate sodium intake, but they are unreliable:

1. Aldosterone secretion is also stimulated directly by hyperkalemia (elevated plasma K^+) and promotes potassium excretion.
2. Such interpretations equate adequate or excessive sodium intake. For physiologists and clinicians, traditionally more concerned with sodium depletion and the defenses against it, elevated aldosterone secretion becomes a warning signal. If sodium intakes associated with increased aldosterone have no other harmful effects, however, and especially if excess sodium intakes cause concern, low plasma aldosterone might equally indicate excessive salt intake.

Although sodium reabsorption in the distal nephron, influenced by aldosterone, is particularly important because it can produce sodium-free urine and promote potassium loss, most renal sodium reabsorption occurs elsewhere; approximately 25% in the loops of Henle, over 60% in the proximal tubules. The loop is also a main site of magnesium reabsorption, hence loop diuretics can cause hypomagnesemia.

Although the factors controlling proximal reabsorption are incompletely understood, their effect is clear: Proximal reabsorption of sodium increases or decreases according to the need to protect plasma volume. Proximal tubule fluid is similar to plasma, being formed from it by glomerular filtration, so its composition is ideal for this purpose.

Natriuretic Hormones

Excretion of excess sodium involves not only suppression of salt-retention mechanisms but also activation of sodium-shedding (natriuretic) mechanisms. Two types of hormones are involved: Atrial natriuretic peptide (ANP), produced by the cardiac atria when they are overstretched (reduction of ECF volume being an appropriate response to cardiac overload), and active sodium transport inhibitors (ASTIs), probably produced within the brain. These were probably the original ligands for the receptors binding cardiac glycoside drugs and are therefore also called 'endogenous digitalis-like inhibitors' (EDLIs); their exact identity remains uncertain. Atrial natriuretic peptide has various effects that essentially

oppose those of the salt retention induced by aldosterone: It increases sodium excretion, lowers arterial pressure, and promotes movement of ECF towards the interstitial compartment.

Other hormones (e.g., sex steroids, parathyroid hormone, calcitonin, thyroid hormone, prolactin) affect renal sodium excretion but are not thought to regulate it.

Adequate, Inadequate, and Excess Sodium

It is unlikely that adult daily maintenance requirement exceeds 0.6 mmol kg^{-1} body weight and could well be below this in many mammals. Newborn, growing, pregnant, or lactating animals have additional requirements. The appropriate sodium intake for humans remains controversial with some cultures managing on less than 1 mmol day^{-1} ($0.01 \text{ mmol kg}^{-1} \text{ day}^{-1}$), although Western intakes may be in the range $100\text{--}200 \text{ mmol day}^{-1}$, more where processed foods are heavily consumed. Physicians and human nutritionists seldom appreciate just how high such intakes are, compared with requirements in other mammals. Although humans, as bipeds with stressful lifestyles do differ, there is no real evidence that human obligatory sodium losses or maintenance requirements are any greater. The problem is an ingrained tradition of regarding salt consumption as a benign pleasure, involving a harmless and healthy dietary constituent. Although low salt cultures may also differ in other factors that affect blood pressure (e.g., adiposity, physical activity) their most consistent and striking characteristic is that they do not even experience the age-related rise in arterial pressure which is generally regarded as normal in industrialized populations; hypertension is almost unknown.

There are now numerous studies that, when rigorously analyzed, relate high salt intake to increased human arterial pressure. Unfortunately, those who wish to reduce their sodium intake are still handicapped by inadequate food labeling and the fact that most dietary sodium is added by manufacturers rather than themselves. In UK some 80% of intake is from salt added during processing: it is 'passive consumption'. National Institute for Clinical Excellence estimates that in the last 5 years a 10% reduction of sodium intake has probably saved £1.5 bn from cardiovascular disease: A reduction to $0.7 \text{ mmol kg}^{-1} \text{ day}^{-1}$ could save £0.7 billion annually. Potassium may ameliorate the hypertensive effects of sodium but humans, other than vegetarians, also have a much lower potassium intake than other mammals.

Because obligatory losses of sodium are so low, dietary sodium depletion is hard to induce and sodium deficiency usually results from losses caused by renal, adrenal, or enteric disease; renal disease may cause either retention or loss of sodium. Globally, both in humans and animals, the most common cause of sodium deficits is acute diarrhea. Fortunately, sufficient gut usually remains unaffected for the uptake of sodium and water to be stimulated by suitably formulated oral rehydration solutions. These essentially restore ECF volume (and acid-base balance), allowing natural defenses to overcome the underlying cause of diarrhea. Despite some species variations, such solutions usually work best if their glucose:sodium ratio (in mmol l^{-1}) is close to unity and they are virtually isotonic (i.e., they have a similar osmotic

concentration to ECF; hypertonic solutions draw water into the gut). The function of glucose in these solutions is to promote sodium uptake; its nutritional contribution is trivial. Anions such as citrate, acetate, propionate, bicarbonate, and amino acids (e.g., glycine and alanine) may further enhance the uptake of sodium and therefore water. Nutritional oral rehydration solutions that provide calories and glutamine (to sustain the form and function of enteric villi) are used in calves, where numerous measurements indicate their superiority to those still used in humans. The extra glucose probably promotes the uptake of sodium sequestered in diarrheic gut.

Sodium is thus central to the management of two of the most widespread human clinical problems; hypertension and diarrhea. The World Health Organization (WHO) regards the discovery of oral rehydration as the main life-saving development in twentieth century medicine.

Unresolved Issues

The control of renal sodium excretion is understood in great detail but the regulation of body sodium is not. Key questions remaining unresolved include how ECF volume is monitored, granted that most is interstitial rather than intravascular, and how the mechanisms regulating ECF volume and arterial pressure are integrated, granted that both use renal sodium excretion as their effector. Currently none of the common forms of general edema is amenable to rigorous explanation, except via abstractions such as 'effective blood volume.'

The key nutritional concern regarding sodium is the human dietary requirement, assuming that excess intake predisposes populations to an age-related rise in arterial pressure. This is regarded as normal but it is not seen in any population whose intake is closer to the likely mammalian requirement. For many individuals, this rise will ultimately destine them to antihypertensive therapy and predispose them to serious secondary hypertensive damage. Although it is encouraging that governments are making serious attempts to reduce salt intake, even the most optimistic targets remain above $0.7 \text{ mmol kg}^{-1} \text{ day}^{-1}$, whereas if humans are like other mammals, the requirement is unlikely to exceed $0.6 \text{ mmol kg}^{-1} \text{ day}^{-1}$ i.e., 40 mmol day^{-1} for a 70-kg human. Those who insist that human requirement is higher must provide evidence that the human renal and colonic sodium conservation are uniquely inefficient or that endocrine responses to lower salt intake, i.e., increased activity of the renin-angiotensin-aldosterone axis, diminished secretion of atrial natriuretic peptide and

endogenous active sodium transport inhibitors, have pathological effects that outweigh moderation of the age-related rise of blood pressure. Indeed because those few human populations on sodium intakes close to or below $0.1 \text{ mmol kg}^{-1} \text{ day}^{-1}$ escape it without exception, the key unknown is arguably the maximum intake which avoids this rise.

See also: Breast Feeding. Electrolytes: Acid-Base Balance. Energy Metabolism. Energy: Balance. Energy Requirements. Energy Expenditure: Indirect Calorimetry. Nutritional Considerations for the Management of Hypertension. Potassium

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SPORT AND EXERCISE NUTRITION

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Glossary

Dietary supplements Dietary supplements may be single elements or nutrients, or may be complex mixtures, including herbals and botanicals of uncertain composition.

Fatigue Exercise, if sufficiently intense or prolonged, will inevitably result in fatigue, which has both subjective and objective components. The nature of fatigue depends on exercise type and on individual training status, but typically includes events localized to the active muscles as well as effects on the central nervous system.

Glycogen Glycogen, a long-chain, highly-branched polymer of glucose, is the storage form of carbohydrate in the human body. It is present primarily in skeletal muscle (up to 300–500 g) and in liver (typically 70–100 g in the fed state).

Hydration Euhydration refers to normal or desirable body water content and electrolyte balance. Positive (hyperhydration) and negative (hypohydration) excursions from euhydration are both associated with impaired performance and with health risks. Dehydration is the process of water loss from the body.

Training Training involves a systematic program of exercise with the aim of achieving an improvement in performance. The key characteristics of a training program are the intensity, duration and frequency of the individual training sessions. Performance outcomes are specific to the type of stimulus applied and proportional to the training load.

Introduction

In 2010, the International Olympic Committee issued a consensus statement on nutrition in sport, which began with the following words: “Diet significantly affects athletic performance”. This is a bold and unambiguous statement, leaving little room for doubt, but there are other, more important factors that will determine the outcome of sporting contests. Talent, motivation, training, tactics, and many other considerations are much more important. It is clear, however, that when all else is equal, nutrition can make the difference between victory and defeat. The role of nutrition, and the eating strategies that should be adopted, will vary greatly between sports and will vary also according to the level of competition, the training load and the competition schedule, so athletes may need the help of qualified professionals to ensure good nutrition strategies. For those who exercise for health and enjoyment rather than in pursuit of fame and wealth, a sound nutrition strategy also has much to offer.

Many different issues arise in considering the interactions between diet and exercise. In considering the role of diet in the athlete's life, two main issues must be considered, each of which gives rise to many subordinate questions. The first question is how the demands of training affect the body's requirement for energy and nutrients: This then has implications for body composition (including the body content of fat, muscle, and bone), for the hormonal environment and the regulation of substrate metabolism, and for various disease states that are affected by body fatness, nutrient intake, and other related factors. The second question is how nutritional preparation can influence performance in competition.

Nutrition for Training

The aim of training is to improve performance, and the effectiveness of a training program will depend on the intensity, duration, and frequency of the training sessions that are completed. Training is designed to induce highly specific adaptations that address the limitations to exercise performance and move those boundaries so that performance improves. It was thought that the primary aim of nutrition support in training was to allow better recovery between training sessions so that the total training load could be increased. It is now recognized, however, that more training is not always better – it brings an increased risk of injury and chronic fatigue. Instead, nutrition strategies are aimed at allowing greater adaptations to the training stimulus, or to allow the same training adaptation with less training.

Sound nutrition strategies can also make exercise feel easier. This is especially important to those who exercise for health: If the exercise feels hard, it is unlikely to be repeated and the duration will be cut short. It is well established that some simple strategies, such as ensuring an adequate carbohydrate (CHO) status and maintaining good hydration status, will reduce the subjective perception of effort.

Protein Requirements

In most cases, training adaptations aim to change the structure and function of the muscles engaged in the exercise task, though all the body's tissues are affected. As the structure and function of muscles are dictated by their protein composition,

the training response involves an increased net breakdown of proteins that are not required and an increased net synthesis of those proteins that contribute to exercise performance. The weightlifter wants bigger muscles with more actin and myosin to increase force-generating capacity. The marathon runner does not want bigger muscles: instead, a greater content of oxidative enzymes and more capillaries to increase oxygen and substrate delivery. Smaller muscle fibers – to reduce the diffusion distance from the capillary to the fiber – and a decrease in total mass are both desirable outcomes.

The idea that protein requirements are increased by physical activity is intuitively attractive, and high-protein diets are a common feature of the diets of many sportsmen and women. The available evidence does show an increased rate of oxidation of the carbon skeletons of amino acids during exercise, especially when CHO availability is low. Protein contributes only approximately 5% of total energy demand in endurance exercise, but the absolute rate of protein breakdown is higher than at rest (where protein contributes about the same fraction as the protein content of the diet, which is typically approximately 12–16% of the total energy intake) because of the higher energy turnover. It is often recommended that athletes engaged in endurance activities on a daily basis should aim to achieve a protein intake of approximately $1.2\text{--}1.4\text{ g kg}^{-1}\text{ day}^{-1}$, whereas athletes engaged in strength and power training may need as much as $1.6\text{--}1.7\text{ g kg}^{-1}\text{ day}^{-1}$. There is no evidence of benefits from a daily intake of more than 2 g kg^{-1} . This compares with an estimated average requirement of approximately $0.6\text{ g kg}^{-1}\text{ day}^{-1}$ in sedentary people and a recommended intake of approximately $0.8\text{--}1.0\text{ g kg}^{-1}\text{ day}^{-1}$ for those who take no exercise.

In strength and power sports such as weightlifting, sprinting, and bodybuilding, the use of high-protein diets and protein supplements is especially prevalent, and daily intakes in excess of $2\text{--}4\text{ g kg}^{-1}$ are not unusual. Scientific support for such high intakes is generally lacking, but those involved in these sports are adamant that such high levels of intake are necessary, not only to increase muscle mass, but also to maintain muscle mass. This apparent inconsistency may be explained by Millward's adaptive metabolic demand model, which proposes that the body adapts to either high or low levels of intake, and that this adjustment to changes in intake occurs only very slowly. This means that individuals, such as strength and power athletes, who consume a high-protein diet over many years, will find that any reduction in protein intake will result in a loss of muscle mass. This is because of an upregulation of the activity of the enzymes involved in protein oxidation to cope with the high intake: activity of these enzymes remains high when there is a sudden decrease in intake, leading to a net catabolic effect.

Protein synthesis and degradation are both enhanced for some hours after exercise, and the net effect on muscle mass will depend on the relative magnitude and duration of these effects. Several recent studies have shown that ingestion of small amounts of protein (typically approximately 20–40 g) or essential amino acids (approximately 10 g) either before or immediately after exercise will result in net protein synthesis in the hours after exercise, whereas net negative protein balance is observed if no source of amino acids is consumed in the immediate postexercise period. These observations have

led to recommendations that approximately 20 g of mixed protein should be consumed immediately after exercise. It is important to recognize, though, that the control condition in most of these studies has involved a relatively prolonged (6–12 h) period of fasting before and after the exercise bout, and this does not reflect the normal behavior of athletes. Individuals who consume foods containing CHO and proteins in the hour or two before exercise may not further increase protein synthesis if additional amino acids or proteins are ingested immediately before, during, or after exercise.

Various high (30%) protein, high (30%) fat, low (40%) CHO diets have been promoted for weight loss, and some diets even suggest almost complete elimination of CHO from the diet. Some of these diets have been specifically targeted at athletes, accompanied by impressive claims and celebrity endorsements. Proposed mechanisms of action of these diets include reduced circulating insulin levels, increased fat catabolism and altered prostaglandin metabolism. The high-protein content of these diets may contribute to increased satiety, but it seems more likely that they may achieve weight loss simply by restricting dietary choice and therefore reducing energy intake. These diets can be effective in promoting short-term weight loss, primarily by restricting energy intake (typically to $1000\text{--}2000\text{ kcal day}^{-1}$). There is not any evidence to support improvements in exercise performance, and what evidence there is does not support the concept.

Fat and CHO

A more recent development has been the suggestion that training on a high-fat diet can enhance endurance performance. The theory is sound, but the experimental evidence does not support the theoretical advantage. CHO is an essential fuel for the brain, red blood cells, and a few other tissues and is also an important fuel for muscle during high-intensity exercise. At rest and during low-intensity exercise, most of the energy demands of skeletal muscle can be met by fat oxidation, but the contribution of CHO, and especially of the muscle glycogen, increases as the rate of energy demand increases. The muscle glycogen stores are small, however, and once the glycogen content of the exercising muscles reaches very low levels, the work rate must be reduced to a level that can be accommodated by fat oxidation. In high-intensity exercise, essentially all of the energy demands are met by CHO metabolism. Repeated short sprints therefore place high demands on the muscle CHO store, most of which can be converted to lactate within a few minutes.

CHO is stored in the body in the form of glycogen, primarily in the liver (approximately 70–100 g in the fed state) and in the skeletal muscles (approximately 300–500 g, depending on muscle mass and preceding diet). These stores are small relative to the body's requirements for CHO. CHO supplies approximately 45% of the energy in the typical Western diet. This amounts to approximately $200\text{--}300\text{ g day}^{-1}$ for the average sedentary individual, and is adequate for normal daily activities. In an hour of hard exercise, however, up to 200 g of CHO can be used, and sufficient CHO must be supplied by the diet to replace the amount used. Replacement of the glycogen stores is an essential part of the recovery

process after exercise: if the muscle glycogen content is not replaced, the quality of training must be reduced, and the risks of illness and injury are increased. Low muscle glycogen levels are associated with an increase secretion of cortisol during exercise, with consequent negative implications for immune function.

Reducing the CHO availability forces the muscle to rely more on fat oxidation for energy supply and restricting the availability of CHO will result in an increased capacity of the muscle to oxidize fat. This can be achieved by feeding a low-CHO diet that meets the total energy demand through an increased fat intake or by feeding an energy-deficient diet. The training studies that have been completed suggest that training on a low-CHO diet impairs some of the adaptations that take place in muscle in response to training, even though the capacity for fat oxidation is increased. Even restoration of the muscle glycogen content by a short period of high-CHO intake does not allow the same performance capacity as when training was performed with adequate CHO availability. The recommendation that athletes consume a high-CHO diet during periods of intensive training therefore remains in place. When rapid recovery is a priority, especially when the interval between successive training sessions is no more than a few hours, replacement of CHO should begin as soon as possible after exercise with CHO foods that are convenient and appealing. Thereafter, the diet should supply sufficient CHO to replace the amount used in training and to meet ongoing demands of other tissues. Some recommendations for CHO intake after training or competition are shown in **Table 1**. It is important to remember that not all athletes need a high-CHO diet at all times: when training consists of mostly technical work, the total energy expenditure and the demand for CHO may be low. For the athlete with very high levels of energy expenditure in training, the exercise intensity will inevitably be reduced to a level where fatty acid oxidation will make a significant contribution to energy supply and fat will provide an important energy source in the diet. Once the requirements for protein and CHO are met, the balance of energy intake can be in the form of fat. Fat also serves other important functions in the diet. As well as providing essential fatty acids, it acts as a vehicle for the transport of fat-soluble nutrients. Some athletes try to minimize their fat intake, but this is not wise.

The high-CHO diet recommended for the physically active individual coincides with the recommendations of various expert committees that a healthy diet is one that is high in CHO (at least 55% of energy) and low in fat (less than 30% of energy). However, where energy intake is either very high or

very low, it may be inappropriate to express the CHO requirement as a fraction of energy intake. With low-total energy intakes, the fraction of CHO in the diet must be high, but the endurance athlete with a very high energy intake may be able to tolerate a higher fat intake. Recommendations, as in **Table 1**, should be framed in absolute amounts relative to body mass, i.e., grams of CHO per kg body mass.

The type of CHO eaten is generally much less important than the amount. It is valuable to choose nutrient-rich CHO foods and to add other foods to recovery meals and snacks to provide a good source of protein and other nutrients. The presence of small amounts of protein in recovery meals may promote additional glycogen recovery when CHO intake is less than optimal or when frequent snacking is not possible. Protein at this time may also stimulate protein synthesis in muscles, as described above. CHO-rich foods with a moderate to high glycemic index (GI) provide a readily available source of CHO for glycogen synthesis, and should be the major fuel choices in recovery meals.

Vitamins and Minerals

Many micronutrients play key roles in energy metabolism, and high rates of energy turnover – up to 20–100 times the resting rate – may be required in the active muscles during hard exercise. Although an adequate vitamin and mineral status is essential for normal health, marginal deficiency states may be apparent only during periods of metabolic stress. Prolonged strenuous exercise performed on a regular basis may also result in increased losses of essential nutrients in sweat or urine, or may increase the rate of breakdown, resulting in the need for an increased dietary intake. An increased food intake to meet energy requirements will generally increase dietary micronutrient in proportion to energy intake, but not all athletes have high-energy intakes. Athletes who restrict food intake to control or reduce body fat levels may have low-energy intakes over prolonged periods. Some athletes may also eat monotonous diets, with a limited range of foods in the diet, thus increasing the risk of an inadequate micronutrient intake. Supplementation with micronutrients may be warranted in some instances, but normally only where specific deficiencies have been demonstrated by biochemical investigations and where dietary modification is not an option.

Individuals who are very active may need to pay particular attention to their intake of iron and calcium. Iron deficiency anemia affects some athletes engaged in intensive training and competition, but it seems that the prevalence is similar in athletic and sedentary populations, suggesting that exercise *per se* does not increase the risk. The implications of even mild anemia for exercise performance are, however, significant. A fall in the circulating hemoglobin concentration is associated with a reduction in oxygen carrying capacity and a decreased exercise performance. Low serum ferritin levels are not associated with impaired performance, however, and iron supplementation in the absence of frank anemia does not influence indices of fitness. Routine iron supplementation is not wise, as too much may be harmful.

Osteoporosis is now widely recognized as a problem for both men and, more especially, women, and an increased

Table 1 Suggested carbohydrate intakes for athletes in training

Immediate post-exercise recovery (0–4 h): 1 g per kg body mass per hour, consisting of several small snacks

Daily recovery (moderate duration/low-intensity training):
5–7 g kg⁻¹ day⁻¹

Daily recovery (moderate–heavy endurance training):
7–12 g kg⁻¹ day⁻¹

Daily recovery (extreme training: 4–6 h or more per day):
Up to 10–12 g kg⁻¹ day⁻¹

bone mineral content is one of the benefits of participation in an exercise program. Regular exercise results in increased mineralization of those bones subjected to stress and an increased peak bone mass may delay the onset of osteoporotic fractures; exercise may also delay the rate of bone loss. Estrogen plays an important role in the maintenance of bone mass in women, and prolonged strenuous activity may result in low estrogen levels, causing bone loss. Many very active women also have a low body fat content and may also have low-energy (and calcium) intakes in spite of their high-activity levels. All of these factors are a threat to bone health. The loss of bone in these women may result in an increased predisposition to stress fractures and other skeletal injury and must also raise concerns about bone health in later life. It should be emphasized, however, that this condition appears to affect only relatively few athletes, and that activity is generally beneficial for the skeleton.

In recent years it has increasingly been recognized that vitamin D may be needed in supplemental form when sun exposure is inadequate, and this may apply especially to those athletes who spend long periods training indoors and to those who live in high latitudes. Intakes of most other nutrients can be met from food sources, but athletes may need advice from qualified professions to identify their nutrition needs and to develop an eating strategy that will meet those needs.

Water and Electrolyte Balance

Prolonged strenuous exercise in a warm environment poses a major challenge to the body's homeostatic mechanisms. Only approximately 20–25% of the energy available from substrate catabolism is used to perform external work, with the remainder appearing as heat. At rest, the metabolic rate is low: Oxygen consumption is approximately 250 ml min^{-1} , corresponding to a rate of heat production of approximately 60 W. Heat production increases in proportion to metabolic demand, and reaches approximately 1 kW in strenuous activities such as marathon running (for a 70 kg runner at a speed that takes approximately $2\frac{1}{2}$ hours to complete the race). To prevent a catastrophic rise in core temperature, heat loss must be increased correspondingly and this is achieved primarily by an increased rate of evaporation of sweat from the skin surface. In hard exercise in hot conditions, sweat rates can reach 3 l h^{-1} , and trained athletes can sustain sweat rates in excess of 2 l h^{-1} for many hours. This represents a much higher fractional turnover rate of water than that of most other body components. In the sedentary individual living in a temperate climate, approximately 5–10% of total body water may be lost and replaced on a daily basis. When prolonged exercise is performed in a hot environment, 20–40% of total body water can be turned over in a single day. In spite of this, the body water content is tightly regulated, and regulation by the kidneys is closely related to osmotic balance.

Along with water, a variety of minerals and organic components are lost in variable amounts in sweat. Sweat is invariably hypotonic relative to plasma and the main electrolytes lost are sodium and chloride, at concentrations of approximately $15\text{--}80 \text{ mmol l}^{-1}$. A range of other minerals, including potassium and magnesium, as well as trace elements, are lost

in small amounts. Sweat rate and sweat composition both vary greatly between individuals. Some athletes may lose up to 10 g of salt (sodium chloride) in a single training session, and may train in these conditions twice per day. Others doing the same training will lose no more than 1 g of salt. High-salt losses may be related to development of muscle cramps in some, but not all, individuals, and additional salt intake may be helpful for susceptible individuals. Salt losses must be replaced from foods and drinks, though the use of salt supplements is seldom necessary.

Failure to maintain hydration status has serious consequences for the active individual. A body water deficit of as little as 1–2% of total body mass can result in a significant reduction in exercise capacity, especially in endurance exercise performed in warm weather. Endurance exercise is affected to a greater extent than high-intensity exercise, and muscle strength is not adversely affected until water losses reach 5% or more of body mass. Hypohydration greatly increases the risk of heat illness, and also abolishes the protection conferred by prior heat acclimation.

Many studies have shown that the ingestion of fluid during exercise can significantly improve performance. Adding an energy source in the form of CHO confers an additional benefit by providing an energy source for the working muscles. Addition of small amounts (perhaps approximately 2–8%) of CHO, in the form of glucose, sucrose, or maltodextrin, will promote water absorption in the small intestine as well as providing exogenous substrate that can spare stored CHO. Recent evidence suggests that addition of fructose in addition to glucose, sucrose, or maltodextrin will increase intestinal absorption of CHO and can enhance performance. The addition of too much CHO will slow gastric emptying and, if the solution is strongly hypertonic, may promote secretion of water into the intestinal lumen, thus delaying fluid availability. Voluntary fluid intake is seldom sufficient to match sweat losses, and palatability of fluids is therefore an important consideration. It is not necessary to consume enough fluid during exercise to match sweat losses, as a body mass deficit of 1–2% is unlikely to have adverse consequences. If exercise is prolonged and sweat losses high, the addition of sodium to drinks may be necessary to prevent the development of hyponatremia. Ingestion of large volumes of plain water is also likely to limit intake because of a fall in plasma osmolality leading to suppression of thirst.

Replacement of water and electrolyte losses incurred during exercise is an important part of the recovery process in the postexercise period. This requires ingestion of fluid in excess of the volume of sweat lost to allow for ongoing water losses from the body. Reestablishment of water balance requires replacement of solute, especially sodium, losses as well as volume replacement. If food containing electrolytes is not consumed at this time, electrolytes, especially sodium, must be added to drinks to prevent diuresis and loss of the ingested fluid.

Dietary Supplements

The use of nutritional supplements in athletes and in the health-conscious recreationally active population is widespread, as it is in the general population. Many different

supplements are used by athletes with the aim of improving or maintaining general health and exercise performance. In particular, supplement use is often aimed at promoting tissue growth and repair, promoting fat loss, enhancing resistance to fatigue, and stimulating immune function. Most of the supplements that are sold to athletes have not been well researched, and both safety and efficacy remain open to question for many of these products. Anyone seeking to improve health or performance would be better advised to ensure that they consume a sound diet that meets energy needs and contains a variety of foods. A recent development of concern to athletes is the finding of various prohibited doping agents in what should be legitimate sports nutrition products. A wider concern are the recent reports of serious health issues related to the use of various supplements.

Supplements for which there is good evidence of beneficial effects on performance in some specific situations include caffeine, creatine, and buffering agents, but the risk of an inadvertent positive doping result must always be considered. Supplement use in young athletes is discouraged, and the focus should be on choosing a varied, nutrient-rich diet to provide all the nutrients essential for growth while maintaining a healthy body composition.

Nutrition for Competition

A detailed consideration of nutrition strategies for competition in all sports is beyond the scope of this brief review. For athletes preparing for competition, a reduction in the training load and the consumption of a high-CHO diet in the past few days are recommended: This will maximize the body's CHO stores, and should ensure optimum performance, not only in endurance activities, but also in events involving short-duration high-intensity exercise and in field games involving multiple sprints. Beginning competition in a well hydrated state is generally beneficial, and is essential in events lasting longer than approximately 30 min in warm environments. Regular intake of fluids should be based on the individual needs identified during training or previous competition.

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STARVATION AND FASTING

Biochemical Aspects

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Glossary

Adapted starvation Relates to the state of steady fat store depletion and minimal proteolysis beyond three weeks of fast during which ketone bodies and fatty acids are the main energy substrates.

AMPK The AMP-activated protein kinase is an important sensor of intracellular energy status. AMP and ATP are bound in a competitive manner. Under conditions of low intracellular energy charge activation of AMPK leads to downregulation of anabolic and upregulation of catabolic enzymatic pathways with the aim to increase intracellular ATP availability.

Cori cycle Describes the metabolic pathway in which lactate – generated through anaerobic glycolysis in the muscle – is used for hepatic glucose synthesis, which then is transported back to the muscle as an energy substrate.

FOXO Forkhead box O is a family of transcription factors. FOXOs are elemental regulators of metabolism, upregulate key enzymes of hepatic gluconeogenesis during early starvation, interfere with adipocyte differentiation and increase protein catabolism. Its activity and cellular localization are controlled by its acetylation (through *SIRT1*) and phosphorylation (through *AMPK*).

Intermediate phase This relates to the necessary transcriptional transition to ketogenesis and ketolysis as well as fatty acid oxidation during two to three weeks after initiation of fasting.

Kwashiorkor Hypoalbuminemic or edematous protein energy malnutrition. Kwashiorkor is one of the major clinical syndromes of severe childhood malnutrition (SCM, see also *Marasmus*). Whilst wasting is characteristic for all syndromes of SCM, additional clinical features such as hypoalbuminemia, generalized edema, skin changes (dermatitis, hypopigmentation) are typical for kwashiorkor. The underlying etiology in comparison to marasmus is still unclear, additional stressors such as infections and environmental toxins might be involved. The overall mortality is high.

Marasmus Uncomplicated or non-edematous protein energy malnutrition. Marasmus is one of the major clinical syndromes of severe childhood malnutrition (SCM, see also

kwashiorkor) characterized by slow weight loss and wasting of muscle protein compartment through months and years. The serum albumin is normal. The associated overall mortality is low following reintroductions of adequate nutrition.

mTOR Mammalian target of rapamycin is a serine/threonine protein kinase with pleiotropic intracellular anabolic effects and functions. mTOR is involved in regulation of protein synthesis, cell proliferation, cell survival and inflammatory reactions. Upstream activators are insulin and insulin-like growth factors (IGF-1), inhibition is mediated through the *AMPK-FOXO* pathway.

PCG1 α PPAR γ coactivator-1 α is a transcriptional coactivator. It is accepted as the central node in starvation-induced transcriptional co-activation and regulates large clusters of genes involved in oxidative phosphorylation and fatty acid metabolism. It is regulated and activated by *AMPK* and *SIRT1* in situations of intracellular energy deficit. It exerts its effect together with transcription factors such as *FOXO* and *PPAR*.

PPAR Peroxisomal proliferator-activated receptor is a family of ligand-activated transcription factors involving three main subclasses, PPAR α , β and γ . PPAR α is one of the master regulators of cellular fatty acid oxidation acting as an intracellular fatty acid sensor.

SIRT1 An evolutionary-conserved NAD⁺-dependent histone deacetylase which has recently been identified as an important element of metabolic homeostasis. SIRT1 activates for example *FOXO* or *PCG1 α* by deacetylation of target proteins. SIRT1 itself is activated by low intracellular NAD⁺-levels that increase with fasting and nutrient restriction: energy-intensive anabolic enzyme pathways are switched off whilst catabolic pathways are induced.

Ubiquitine proteasome system One of the two main pathways to accomplish regulated protein catabolism in prolonged fasting. It acts through a cascade of enzymes involving conjugation of target proteins with ubiquitine chains which are then further degraded in the so-called proteasome. It serves as the primary route for degradation of short-lived proteins and maintains an intracellular pool of glucogenic amino acids during starvation.

The Feeding/Fasting Cycle

Energy Requirements and Metabolism

Energy is essential for many important body functions. These functions include the maintenance of cellular integrity and function, new tissue synthesis and growth, thermoregulation and adaption to physical activity and other metabolic stressors. The energy requirements of an individual vary with age, sex, body composition, physical activity, and stress. In the normal adult at basal state, approximately 75% of energy requirements reflect the energy needs of major organs (brain ~20%, skeletal muscle 18–22%, abdominal muscles ~25%, and heart ~11%). In children, up to 50% of resting metabolic energy is expended by the brain. During normal daily activity, the total energy requirement and the proportion of energy needed by different tissues may vary considerably.

Energy Production

Adequate availability of metabolic fuels for energy production has to be maintained in both the fed and the fasting states. The body derives energy from combustion of carbohydrate, fat, and protein provided exogenously in the fed state with the surplus being stored as glycogen in liver and muscle and as triacylglycerol in adipose tissue. These stores help to endogenously buffer periods of low-energy intake such as the postabsorptive and fasting state. A mixture of metabolic fuels including glucose, triacylglycerols, ketone bodies, non-esterified fatty acids, and amino acids is present in the circulation. The proportion of these energy substrates in the blood at any one time depends on the fed or fasting state of the individual, the extent of fuel stores, and recent or current metabolic demand and stressors. Many of these are interconvertible and thus allow easier adaptation to situations of changing exogenous energy supply. In a normal, nonobese 70-kg adult, there are approximately 500 MJ (120 000 kcal) contained in adipose tissue, 100 MJ (24 000 kcal) stored in

muscle and visceral proteins, and 8 MJ (2000 kcal) stored as liver and muscle glycogen. During a normal day, half of the total energy requirement is met by carbohydrate metabolism. At this rate, glycogen stores would be exhausted after 1–2 days of fasting. However, glycogen stores are maintained for a longer period owing to the production of glucose from gluconeogenesis (**Figure 1**). Gluconeogenic substrates such as lactate, amino acids (alanine, glutamine), glycerol, and β -hydroxybutyrate are shuttled from muscle and adipose tissue toward liver and kidney where they act as core substrates for gluconeogenesis. During prolonged fasting, the liver will account for approximately 80% of total body glucose production, whereas the remaining 20% is made by the kidney.

Carbohydrate Metabolism

Glucose plays a key role in body metabolism. It is the preferred source of energy for the majority of tissues and is particularly important for the retina and the brain where it is the main energy substrate. Owing to a lack of mitochondria, red blood cells and the renal medulla are completely reliant on glucose as the sole metabolic fuel at all times. Therefore, the metabolic changes that occur during fasting and starvation target the necessity to spare blood glucose and liver glycogen for use by brain and other glucose-dependent tissues by providing alternative fuels to other tissues.

Glucose enters the bloodstream through absorption from the intestine, the breakdown of liver glycogen, and hepatic gluconeogenesis from glucogenic precursors (e.g., lactate, alanine). To produce energy from glucose, three metabolic pathways are involved (**Figure 2**). Glucose is first oxidized to form pyruvate via the glycolytic pathway. Pyruvate then enters the Krebs cycle and is completely oxidized to form $\text{NADH} + \text{H}^+$, FADH_2 , and carbon dioxide. The $\text{NADH} + \text{H}^+$ transports hydrogen to the respiratory chain where it is used to reduce oxygen to water through oxidative phosphorylation. The net yield of energy from the metabolism of 1 molecule of glucose is 38 molecules of ATP. As the combustion of glucose within the human body is coupled to the energy-consuming

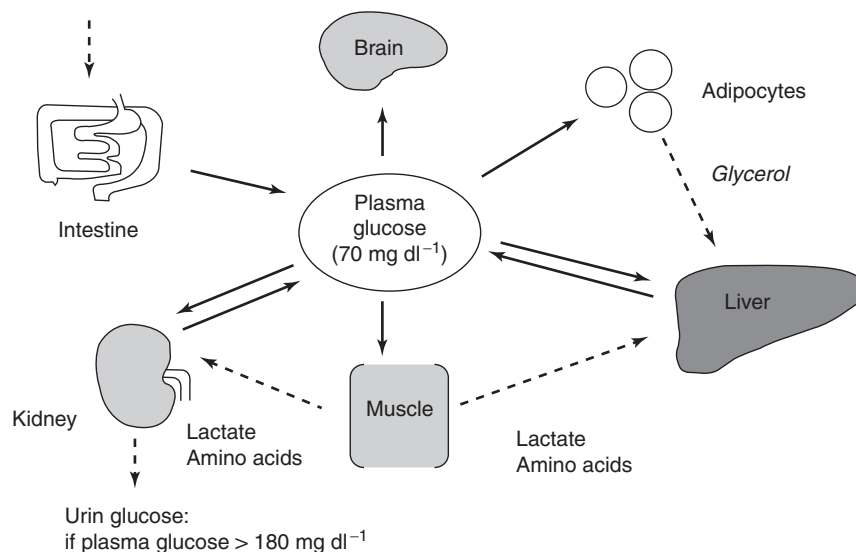


Figure 1 The metabolism of energy substrates to maintain glucose homeostasis.

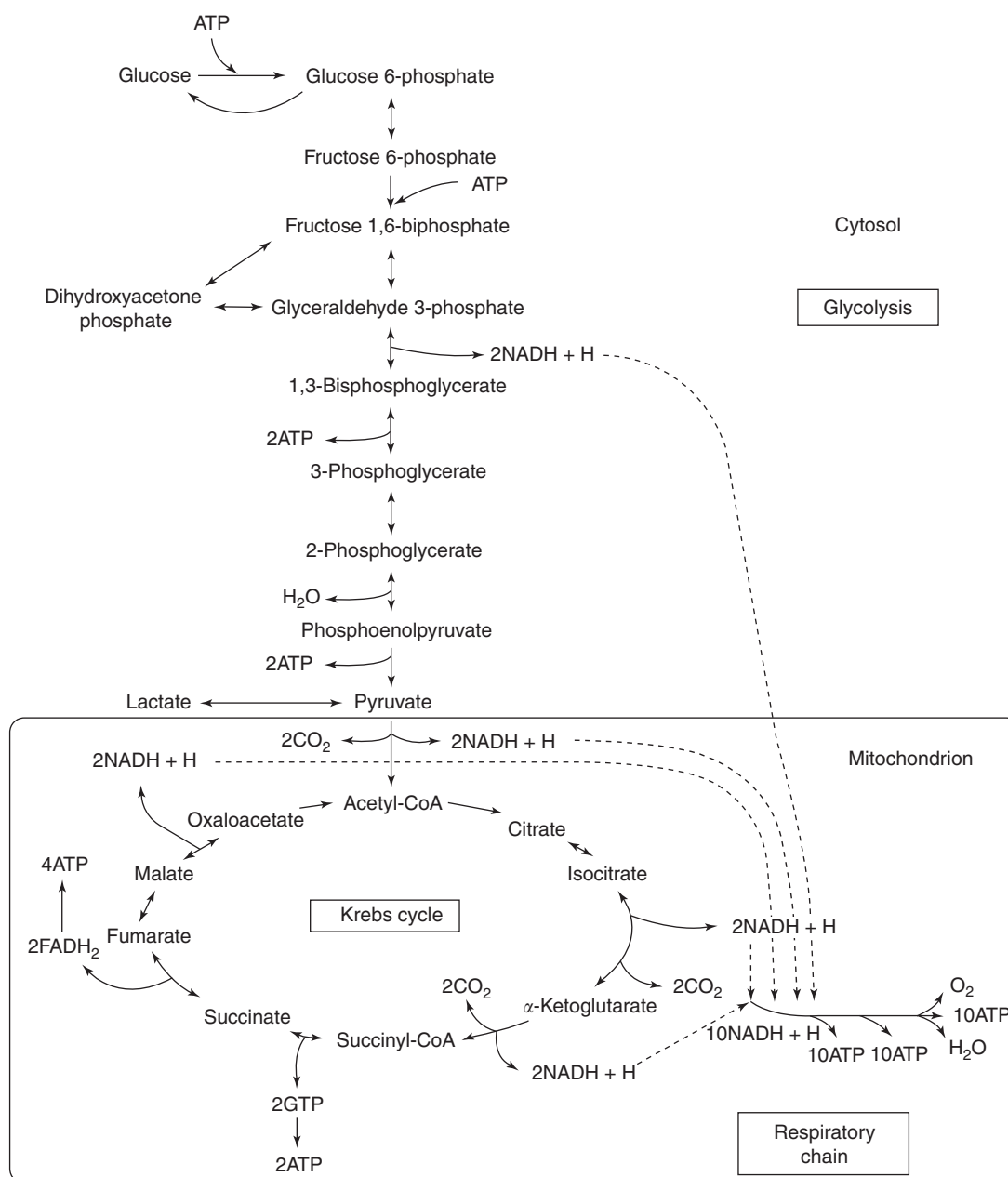


Figure 2 The production of energy from glucose via the glycolytic pathway, the Krebs cycle, and the respiratory chain.

synthesis of ATP, the overall efficiency of energy extraction from glucose within the cell is only approximately 50%.

Of all energy substrates, blood glucose concentration is the most constant. Because of the strict glucose requirements of the brain, the circulating blood glucose pool is tightly controlled at approximately 16 g or 5 mmol l⁻¹. Three important mechanisms are responsible for this regulation.

1. Insulin, as an anabolic hormone, enhances glucose uptake into muscle and fat and stimulates glycogen synthesis. It also inhibits lipolysis, glycogenolysis, and gluconeogenesis. High insulin levels will decrease blood glucose levels.

Conversely, low insulin levels will cause a rise in blood glucose by decreased inhibition of glycogenolysis and reduced peripheral uptake of glucose.

2. Glucagon counteracts the insulin effect and increases liver glycogen breakdown, gluconeogenesis, and ketogenesis from fatty acids. It also stimulates lipolysis from adipocytes in extrahepatic tissue. The net result of glucagon activity is an increase in blood glucose concentration, which helps to maintain blood glucose levels despite the effect of insulin.
3. Neuroendocrine counterregulatory responses to glucose deprivation in the brain with sympathoadrenal upregulation and release of cortisol, catecholamines, and growth

hormone act to rapidly normalize blood glucose by increasing hepatic gluconeogenesis and glycogenolysis and curtailing peripheral tissue glucose uptake and utilization.

The fed state is characterized by increased blood concentrations of glucose, amino acids, and fat. Insulin secretion is stimulated while glucagon levels remain unchanged or are decreased. As a result, there is increased glucose uptake into tissues and enhanced glycogen, protein, and triacylglycerol syntheses. Glucagon balances this effect by stimulating glycogen breakdown to maintain blood glucose levels. By this mechanism, blood glucose levels are controlled during periods of surplus carbohydrate ingestion and excess glucose is stored as glycogen or fat.

Glycogen is a complex, hydrated gel-like polymer of glucose arranged in a highly branched spherical form. It allows glucose to be stored in large amounts and a relatively small volume without causing osmotic shifts and acts as a rapidly available short-term energy buffer in the postabsorptive and fasting state. The terminal glucose molecules within this branching structure are accessible to the enzymes mediating glycogen breakdown to allow the rapid release of glucose in times of stress. The glycogen molecule expands in size after a carbohydrate-rich meal to approximately 40 nm in diameter and shrinks to 10 nm in diameter or less between meals. An adult man receiving a normal carbohydrate-containing diet has approximately 70 g of liver glycogen and 200 g of muscle glycogen. The final enzymatic step required to complete glycogen breakdown to glucose, glucose-6-phosphatase, is present only in the liver. Muscle glycogen is metabolized by anaerobic glycolysis to form pyruvate and lactate. Lactate is then transported to the liver where it acts as a precursor for gluconeogenesis. This is called the 'Cori cycle' (Figure 3). The Cori cycle contributes to approximately 40% of the normal plasma glucose turnover. It has the advantage of providing

energy (net 3 molecules of ATP) without the loss of glucose molecules. The energy required for the resynthesis of glucose in the liver is derived from fatty acid oxidation. The total body glycogen stores can meet the needs of the brain for approximately 3 days. After this period, alternative sources of metabolic fuel must be found.

Protein Metabolism

Body nitrogen resides in two main compartments. Approximately half of the body's nitrogen is contained in extracellular tissues such as collagen. The nitrogen present within these tissues is relatively fixed and does not change significantly with starvation. The nitrogen turnover within this compartment can be assessed by the measurement of hydroxyproline excretion. The remaining nitrogen is present in the lean muscle mass, comprising skeletal and visceral muscles. The proteins within these tissues are constantly being broken down and resynthesized at a rate of between 3 and 3.5 g kg⁻¹ day⁻¹ in a young adult. Measurement of urinary 3-methylhistidine and creatinine excretion can be used to estimate the fractional catabolic rate of skeletal muscle. Similar to fatty acids and glucose, amino acids can be completely oxidized. However, the body's protein compartments – contrary to liver glycogen or adipose tissue – are not primarily energy stores. Although amino acids serve as important energy substrates in gluconeogenesis and Krebs cycle oxidation during times of inadequate energy intake, the loss of functional body protein is the life-limiting factor in prolonged fasting.

In the fed state, amino acids digested and absorbed in excess of the body's immediate requirements for incorporation into proteins or other molecules are either oxidized for energy or metabolized to glycogen or fat. Protein provides approximately 17 kJ g⁻¹ (4 kcal g⁻¹) of energy when metabolized as an energy source.

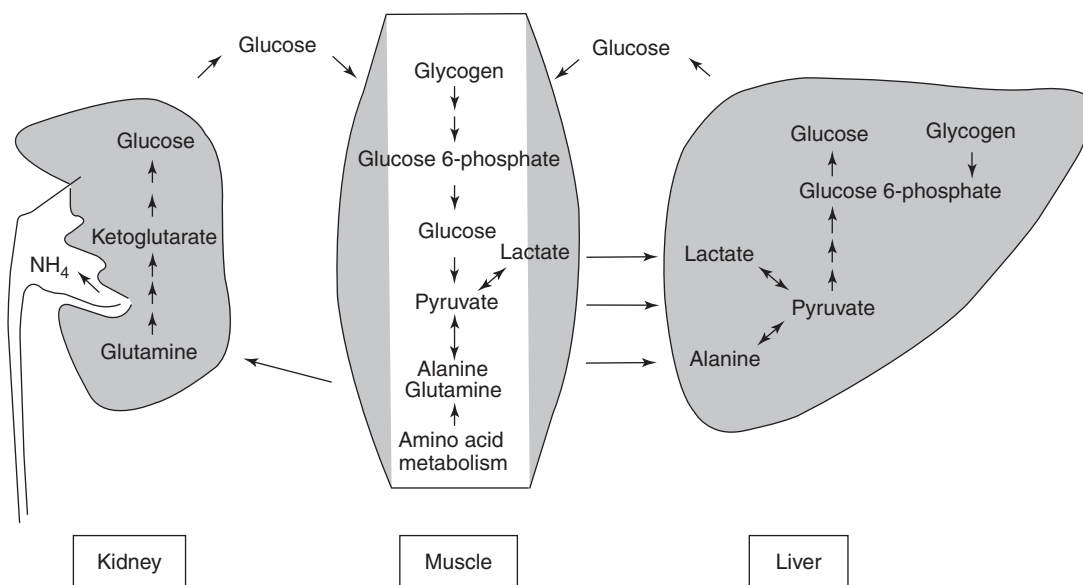


Figure 3 The metabolism of muscle glycogen and protein to form glucose, involving the Cori cycle (lactate to glucose) and the glucose-alanine cycle.

Prolonged fasting results in depletion of liver and muscle glycogen stores. In this clinical setting, the conversion of amino acids to glucose in liver and kidney contributes to the glucose requirements of the brain. The transition to metabolism of amino acids as an energy source is mediated by an alteration in the balance of insulin and glucagon. The breakdown of tissue protein to provide glucose results in a sustained loss of body nitrogen of approximately 12 g day^{-1} . Experimentally, this loss of body nitrogen can be prevented by the administration of glucose. As a result of muscle protein breakdown, amino acids – predominantly alanine and glutamine – are released into the circulation. However, the amount of alanine released exceeds the alanine content of the muscle protein. This is because approximately one-third of the alanine released from muscle originates directly from the muscle protein, whereas the remaining two-thirds is derived from pyruvate. Pyruvate is formed by the metabolism of muscle glycogen or by the transamination of other amino acids contained within the muscle protein. Alanine is then transported to the liver where it is rapidly taken up and converted to glucose: this is known as the glucose–alanine cycle (Figure 3). Despite the increased release of alanine from muscle, plasma alanine levels fall in early fasting. This results from the rapid uptake and conversion of alanine by the liver. Similar to the glucose–alanine cycle between muscle and liver, there is a steady exchange of glutamine and glucose between muscle and kidney: glutamine released from muscle tissue acts as one of the main renal gluconeogenic precursors besides lactate in prolonged fasting.

Two major metabolic pathways accomplish regulated protein catabolism in prolonged fasting: the ubiquitin–proteasome system (UPS) and the autophagy–lysosomal system. The mechanisms and role of the calcium-dependent calpain–calpastatin system and the caspase–proteolytic system in starvation and muscle wasting processes likely also play a role but are less well described. The UPS acts through a cascade of enzymes that conjugate polyubiquitin chains with the target protein that are thereby marked for further degradation by the so-called ‘proteasome.’ The ubiquitinated proteins are bound by two 19S-regulatory proteins, unfolded and transported to the proteolytic centers of the 20S proteasomal core. The UPS serves as the primary route for degradation of short-lived proteins with high selectivity allowing the fine-tuning of the steady state of many regulatory and rate-limiting enzymes and degrading defective proteins. UPS-mediated catabolism is an important adaptation mechanism during acute starvation and serves to maintain intracellular amino acid pools as a substrate source for gluconeogenesis. The enzyme complex is activated by a number of metabolic changes seen in fasting and starvation such as the decrease in insulin and other anabolic hormones, an increased concentration of intracellular reactive oxygen species, a decrease in intracellular amino acid concentration, and a reduction in AMP/ATP ratio (Figure 6).

Autophagy describes a catabolic process in which cell constituents (organelles, proteins) are engulfed by a phagophore and delivered to the lysosomal compartment for further degradation. It is primarily responsible for the degradation of long-lived proteins and proceeds at a basal rate in nearly all eukaryotic cells. As such, it plays a housekeeping role and

contributes to constant cellular renewal and turnover. Autophagy also plays a significant role in response to chronic nutrient starvation conditions, responding to metabolic changes such as increased energetic or oxidative intracellular stress or a decrease in intracytoplasmic amino acids. Traditionally, the UPS and autophagy were considered as two independent proteolytic systems. More recently, it has been recognized that these systems are closely interrelated, share activating trigger factors and intracellular signaling pathways, as well as protein substrates, and can compensate for the compromise of the other system.

Fat Metabolism

Fat is an efficient store of energy providing approximately 38 kJ g^{-1} (9 kcal g^{-1}). Fat is predominantly stored as triacylglycerols within adipocytes. The amount of fat stores may vary substantially between individuals. Contrary to carbohydrate metabolism with blood glucose concentrations being tightly regulated, the concentrations of triacylglycerols and non-esterified fatty acids are much more variable. In the fed state, insulin stimulates triacylglycerol synthesis by allowing acyl-CoA from excess carbohydrates, protein, or fat being stored as adipose tissue. During fasting, triacylglycerol is converted to fatty acids and glycerol (Figure 4). Within days, glycerol and palmitate release increases by two to three times fed levels. This release is regulated by hormone-sensitive lipase. Owing to the absence of glycerol kinase in white adipose tissue, glycerol cannot be completely metabolized within the adipocytes and is transported to the liver where it is converted into glucose by gluconeogenesis. The fatty acids either are released from the adipocytes to be oxidized by the liver or other tissues or may be reesterified with glycerol-3-phosphate and reenter the cycle to form triacylglycerol (Figure 4). The energy cost of reesterification of fatty acids in starvation may account for 2–3% of the resting energy expenditure.

Fatty acids delivered to the liver may be oxidized or reesterified into triacylglycerols. Fatty acid oxidation is stimulated by the activation of carnitine/acyl carnitine translocase acyltransferase, which effects the transport of long-chain fatty acids into the mitochondria. Most of the acetyl-CoA produced from fatty acid oxidation is metabolized to acetoacetate, which, in turn, may be converted to β -hydroxybutyrate and acetone. These products are known as ‘ketone bodies’ and are water-soluble intermediates. In the fed state, ketone bodies are only produced in small quantities, are generally metabolized by the liver, and are not released into the circulation. During fasting, the rate of production of acetoacetate and β -hydroxybutyrate significantly increases. These metabolites are released into the circulation and can be used by the brain and other tissues as an alternative energy source.

Metabolic Consequences of Fasting and Starvation

General Considerations

During periods of prolonged inadequate nutrient intake, the human body needs to mobilize preexisting stores of nutrients for continued functioning. After depletion of liver glycogen during early starvation, energy will mainly be provided by

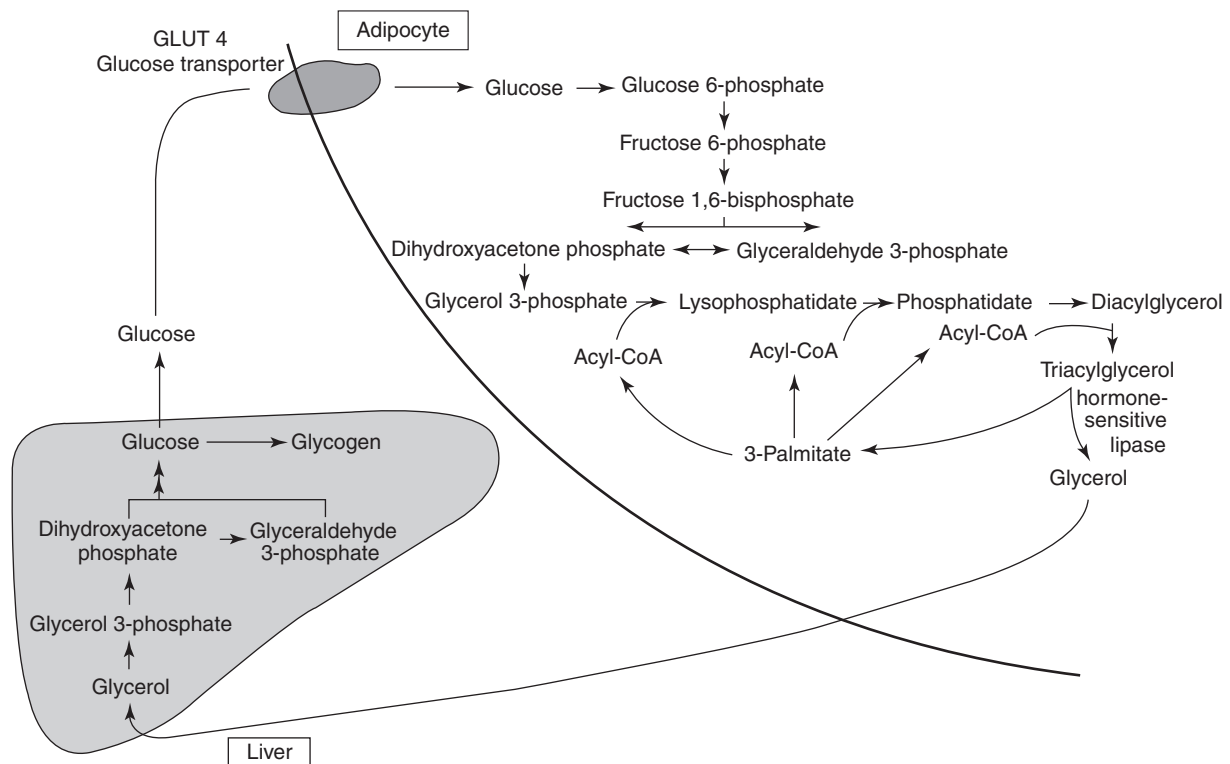


Figure 4 The triacylglycerol/fatty acid cycle.

Table 1 Time course of the adaptive metabolic response to starvation

Metabolic adaptation	Duration	Pathway	Effect
Postabsorptive state	12 h	Glycogenolysis	Carbohydrate mobilization
Early starvation	2–3 days	Glyconeogenesis	Glycogenic AA mobilization
Intermediate phase	2–3 weeks	Ketogenesis/fatty acid oxidation	Transcriptional transition to fatty acid metabolism
Adapted starvation	> 3 weeks	Ketogenesis/fatty acid oxidation	Steady fat store depletion economized proteolysis

breakdown of adipose and muscle tissue (**Table 1**). The physiologic and biochemical changes during starvation are targeted at lowering resting metabolic rate and energy expenditure by a number of glucose-sparing mechanisms in order to economize on the functionally important protein compartment. Low total body energy charge parallels a down-regulation of energy-consuming anabolic processes (fatty acid/triglyceride synthesis, glycogenesis), whereas catabolic reactions are activated (glycolysis, fatty acid oxidation, and proteolysis). The process of metabolic adaptation involves three main steps:

1. Replacement of glucose by fat as the main source of energy.
2. Promotion of ketone bodies as a fuel alternative in glycolytic tissues.
3. Maintenance of minimum of glucose supply by gluconeogenesis from glucogenic amino acids and glycerol, and by the shutdown of irreversible glucose oxidation in the citric acid cycle.

The transition from the postabsorptive state and the early phases of adaptation to the steady state of adapted starvation can take up to 3 weeks. The length of survival (**Figure 5**) will

finally be determined by the total endogenous caloric reserve (calories per kilogram of bodyweight) that is the magnitude of preexisting stores of fat and protein, as well as the rate at which the endogenous substrates are utilized in the fasting state defined by the total energy expenditure (calories per kilogram of bodyweight per day). Owing to a different body composition with a much higher percentage of total body water and smaller subcutaneous fat stores, the neonatal overall caloric reserve is significantly lower compared with that of the adult. The neonate is further disadvantaged in its ability to tolerate starvation by its higher energy expenditure that is negatively related to the gestational age. Thermal stress, as a consequence of a 2.5 to 3 times higher surface area to bodyweight ratio in comparison to an adult, and the limited insulating capacity from subcutaneous fat are important determinants of increased neonatal energy expenditure and thus susceptibility to starvation.

Regulation of Adaptation

Metabolic regulation in starvation is complex and aims at the translation of total body metabolic environment into adaptive

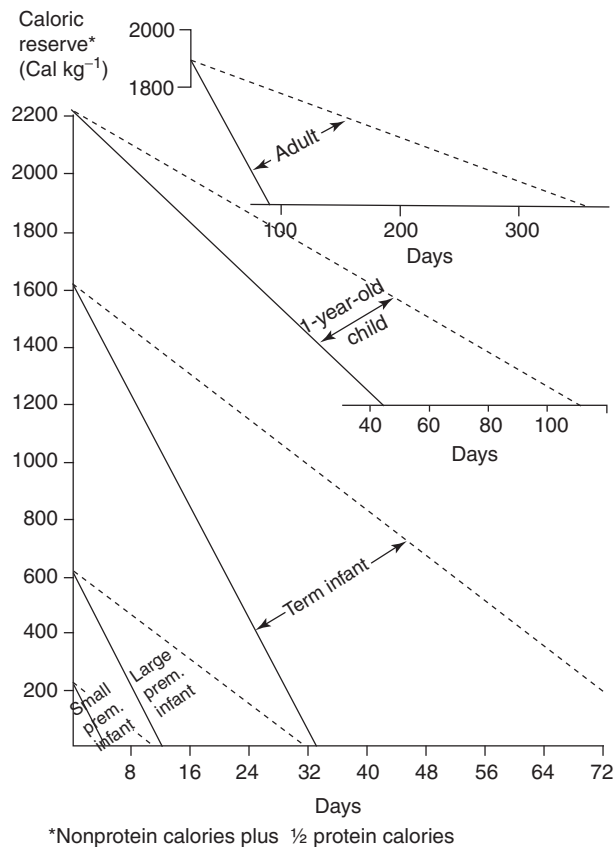


Figure 5 Duration of survival in different age groups expected in starvation (solid line) and semistarvation (dashed line). Reproduced from Heird WC, Driscoll Jr JM, Schullinger JN, Grebin B, and Winters RW (1972) Intravenous alimentation in pediatric patients. *Journal of Pediatrics* 80(3): 353.

responses on a tissue and cellular level, leading to structural, biochemical, and functional modifications. There is an extensive interplay of nutrient sensing mechanisms and neural and endocrine afferent and efferent signaling.

The hypothalamus, in particular the arcuate nucleus (ARC), has been accepted as a major hub for integrating nutritionally relevant information from peripheral organs into an energy code setting the level of whole-body energy expenditure. Reduction in resting energy expenditure during fasting cannot be solely explained by decrease in lean body mass, and coordination of hormonal changes on the hypothalamic level plays an additional important role. Catecholamine secretion and turnover are decreased in uncomplicated starvation. This is clinically recognized as a reduction of core temperature, heart rate, and blood pressure of patients during starvation. Thyroid hormones are known as potent hypothalamic regulators of whole-body energy homeostasis. Fasting results in marked suppression of diencephalic TSH secretion as a consequence of a fall in leptin. Leptin is an adipocyte-derived hormone, which induces biosynthesis and release of hypothalamic TRH under nonfasting conditions through an increased hypothalamic type 2 deiodinase-dependent T_3 production. Decreased activity of 5'-mono-deiodinase in the liver and peripheral tissues resulting in a

reduction in the conversion of thyroxine (T_4) to the metabolically active form, triiodothyronine (T_3), has been observed within hours to days in patients on a starvation diet. However, the mechanism linking low-circulating T_3 levels to decreased resting energy expenditure in starvation is not well understood yet.

Glucagon, a 29-amino acid peptide released from pancreatic α -cells, is an important regulator of glucose homeostasis, counterregulatory to insulin, and is released in situations of low plasma glucose. It stimulates hepatic glucose output by increasing hepatic glycogenolysis and gluconeogenesis through its canonical cAMP/PKA pathway, facilitating phosphorylation and allosteric change of key metabolic enzymes and nuclear factors (e.g., CREB, cAMP response element-binding protein). It thus plays a crucial role in the early phase of adaption to starvation (Table 1).

The coupling of the body's metabolic environment to the energy status of the cell is crucial for its adaptive response to starvation. Cells have energetic sensors detecting limitation of nutrient availability during starvation and initiating homeostatic mechanisms in order to tailor their metabolic needs to nutrient fluctuations. The heterotrimeric AMP-activated protein kinase (AMPK) and the evolutionary-conserved NAD^+ -dependent histone deacetylase SIRT1 have recently been identified as the important signaling backbone in cellular as well as whole-body energy homeostasis, during starvation (Figure 6). AMPK is activated under conditions of low-energy charge sensing intracytoplasmic changes in AMP/ATP ratio in a competitive manner. SIRT1 is induced and activated by low intracellular NAD^+ levels (nicotinamide adenine dinucleotide) that increase similar to AMP with fasting and nutrient restriction. Activation of each enzymatic pathway leads to a similar phenotypic output with restitution of intracellular energy balance by switching off energy-intensive biosynthetic pathways (such as protein synthesis, glycogen synthesis, fatty acid, and sterol synthesis) and favoring ATP production (through, e.g., lipolysis, fatty acid oxidation, and mitochondrial biogenesis). On a structural level, this translates into the observed muscular plasticity during starvation, with a switch from fast-twitching glycolytic type II fibers to slow-twitching oxidative type I fibers.

The AMPK-SIRT1 axis integrates multiple hormonal and nutritional signals (e.g., glucagon, leptin, adiponectin, glycogen, and free fatty acids) and is embedded into a complex self-regulating network aiming to restrict overall energy expenditure in times of starvation (Figure 6). It has pleiotropic intracellular effects resulting in rapid changes through AMP kinase-driven phosphorylation and allosteric changes of principal metabolic enzymes such as acetyl-CoA carboxylase (fatty acid synthesis), hormone-sensitive lipase (triglyceride hydrolysis), and pyruvate dehydrogenase complex (oxidation of pyruvate in the Krebs cycle). Medium- and long-term adaptive mechanisms take effect as a consequence of transcriptional modifications of metabolic enzymes through phosphorylation and deacetylation of downstream nuclear receptors (e.g., FOXO, PPAR) and transcriptional coregulators (PCG-1 α). In addition, deacetylation of lysine residues helps SIRT1 to increase the degree of chromatin compaction, leading to direct repression of transcriptional activity.

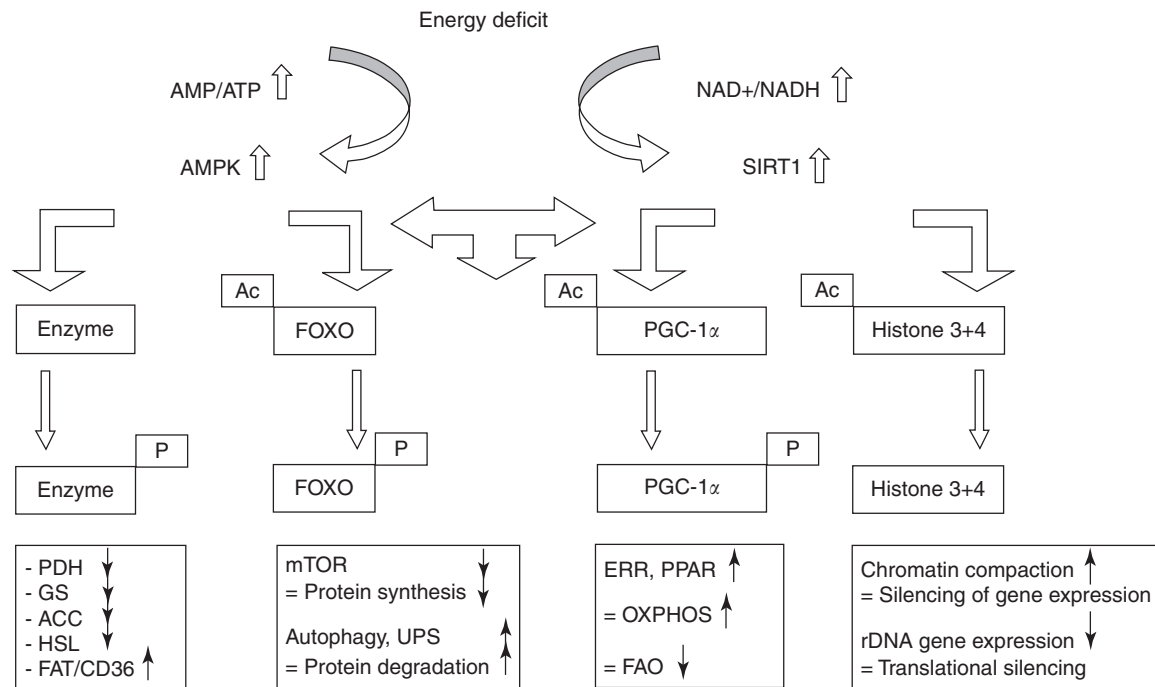


Figure 6 The AMPK-SIRT1 signaling axis. Ac, acetyl group; ACC, acetyl-CoA carboxylase; ERR, estrogen-related receptor; FAO, fatty acid oxidation; FAT/CD36, fatty acid translocase; GS, glycogen synthase; HSL, hormone-sensitive lipase; mTOR, mammalian target of rapamycin; OXPHOS, oxidative phosphorylation; P, phospho group; PDH, pyruvate dehydrogenase; PPAR, peroxisomal proliferator-activated receptor; UPS, ubiquitin-proteasome system.

The Forkhead box O (FOXO) transcription factor family has received considerable attention as elemental regulators of metabolism. FOXOs play a crucial role in transcriptional upregulation of key enzymes of hepatic gluconeogenesis in early starvation (G6Pase, PEPCK), blockage of adipocyte differentiation, decrease in protein synthesis, and increase in protein degradation (through UPS and autophagy; see above). Its activity and cellular localization are controlled and increased by its acetylation (through SIRT1) and phosphorylation (through AMPK) states. The peroxisomal proliferator-activated receptors (PPARs) are another family of important ligand-activated transcription factors. PPAR α , for example, is one of the master regulators of cellular fatty acid burning capacity and acts as an intracellular fatty acid sensor. The estrogen receptor-related receptor (ERR) family (α , β , γ) represents a further important downstream target of AMPK. Activation is associated with increased expression of fatty acid oxidation genes and enzymes of the oxidative phosphorylation cascade (OXPHOS). It also enhances pyruvate dehydrogenase kinase 4 (PDK4), the crucial enzyme involved in the starvation-induced shutdown of the pyruvate dehydrogenase complex. Although these nuclear transcription factors confer a first level of specificity to transcriptional adaptive processes during times of energy deficit, coregulators are required for the transcriptional machinery to become fully activated, significantly increasing the diversity of interacting partners and thus the complexity of this regulatory process. The PPAR γ coactivator-1 α (PGC-1 α) is generally accepted as the central node in starvation-induced transcriptional coactivation and is involved in the regulation of large clusters of genes controlling

oxidative phosphorylation and fatty acid metabolism. It drives, for example, the switch from glycolytic to oxidative muscle fibers. It exerts its effect in conjunction with the transcription factors FOXO, PPAR, and ERR. PGC-1 α is controlled by the AMPK-SIRT1 axis and activated – similar to FOXO – by deacetylation and phosphorylation (Figure 6).

Postabsorptive State

The postabsorptive state commences when the last nutrient is absorbed from the previous meal and continues until the next meal or for approximately 12 h during a normal overnight fast. Metabolically, there is transition from exogenous energy consumption to reliance on endogenous energy sources. Proceeded by a decrease in insulin/glucagon ratio, a significant proportion of body energy requirement is met by the breakdown of liver glycogen. It is, therefore, called the glycogenolytic phase of starvation. The hepatic release of approximately 200–250 g of glucose per day or 8–10 g h⁻¹ balances the rate of glucose utilization of the brain and other tissues. A minor part of glucose formation derives from non-carbohydrate sources including glycerol (from triacylglycerols) and pyruvate and lactate (from muscle).

Prolonged Fasting

Fasting beyond 12 h will lead to the gluconeogenic phase of starvation represented by the transition from glycogen to metabolism of glucogenic amino acids as the main

source of energy. This is mediated by a further decrease in the insulin/glucagon ratio. As a result, blood levels of the branched-chain amino acids, alanine, and glutamine double after 3–5 days of fasting. The glucose–alanine cycle provides glucose to the muscle in exchange for alanine provided to the liver as a precursor for gluconeogenesis (see Figure 3). The intestine preferentially takes up glutamine released from the muscle during fasting where it is used as an energy source, and by the kidney where it is also used for renal ammonia production. Although the metabolism of amino acids to glucose is a very important step of metabolic adaptation to fasting, it only provides approximately 45 g glucose per day. This amount alone is insufficient to meet the glucose requirements of the brain and must be supplemented by energy produced from fat metabolism. Gluconeogenesis occurs at the expense of the functional protein compartment and provides energy substrates until the lipolytic and ketogenic machinery has fully adapted. Increased efficiency of the adaptive metabolic switch to fat and ketone body utilization is reflected by a fall in plasma amino acids if fasting is prolonged.

The mobilization of triacylglycerol stores to provide energy is regulated by a number of factors. Lipolysis is stimulated by glucagon and adrenocorticotrophic hormone (ACTH) during starvation. This effect is mediated by cyclic AMP-dependent protein kinase (AMPK), which stimulates hormone-sensitive lipase and inhibits acetyl-CoA carboxylase (Figure 7). In prolonged starvation, cortisol increases hormone-sensitive lipase synthesis. Insulin levels fall by 35% within 24 h of fasting. This is associated with a 50–80% increase in the rate of lipolysis. Low-circulating insulin levels cause a reduction in the uptake of glucose into adipocytes by altering the function of the GLUT4 glucose transporter (Figure 4). Adequate amounts of glycerol-3-phosphate are therefore unavailable for the reesterification of fatty acids produced from triacylglycerol breakdown. Nonesterified fatty acids are released into the circulation and free fatty acid concentrations increase from

0.5–0.8 to 1.2–1.6 mmol l⁻¹ within the first few days of fasting. Fatty acids circulate bound to albumin and can be oxidized in the liver or other tissues to produce energy. The switch to using ketone bodies as an energy source by the brain appears to be primarily controlled by the blood concentration of ketone bodies rather than a hormonal effect. Ketone body production by the liver peaks after 3–4 days of fasting. However, blood ketone body levels continue to rise rapidly for the first 7–10 days before stabilizing at approximately 6–8 mM at 2–3 weeks. The continued rise in blood ketone body levels despite achieving maximal liver production early in fasting is due to decreased renal excretion of ketone bodies and increasing muscle fatty acid oxidation.

As fatty acid oxidation and ketone body formation increase, there is a reduction in glucose production and oxidation mediated by downregulation of the pyruvate dehydrogenase complex activity. After a 3-week fast, a marked reduction in glucose metabolism throughout the brain is observed using positron emission tomography. Glucose uptake of the brain is more than halved after a fast of 5 weeks.

After a period of fasting longer than 3 weeks, the process of metabolic adaptation to starvation is complete. Gluconeogenesis and glycolysis have been minimized paralleled by increase in hepatic ketone body production. The kidney becomes the major gluconeogenic organ and produces half of the body's glucose requirements. Glutamine is the predominant substrate for kidney gluconeogenesis, and the nitrogen product of this process provides the ammonia needed to buffer ketoacids in the urine. This saves energy compared to the energy-intensive ammonia disposal through the hepatic urea cycle. As a result, urinary nitrogen losses decrease to 4–6 g day⁻¹. Two-thirds of brain fuel consumption consists of ketone bodies, thereby markedly diminishing the need for muscle proteolysis to provide gluconeogenic precursors. With prolonged fasting, muscles change from ketone body production to fatty acid oxidation.

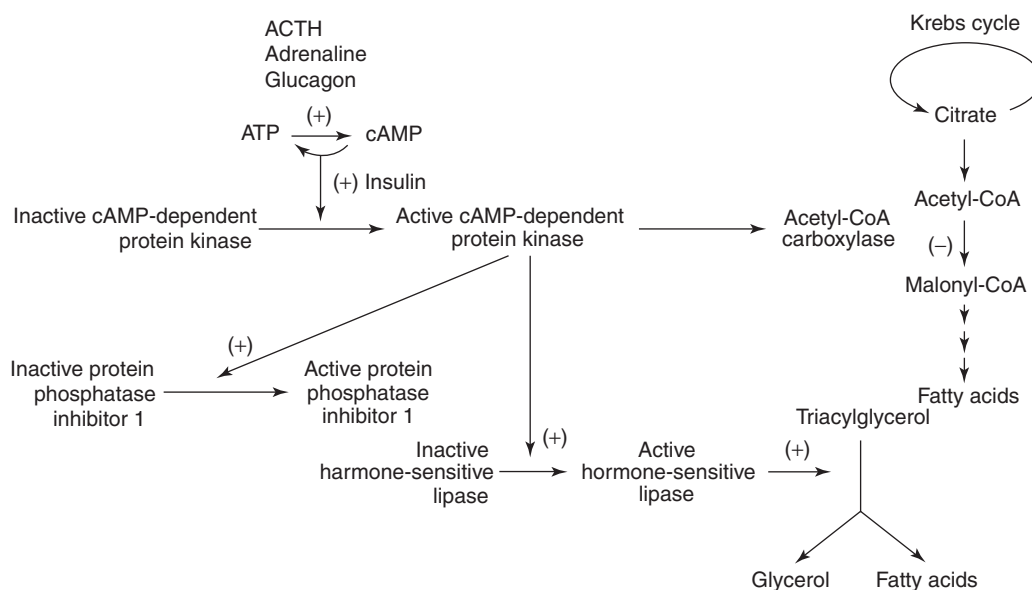


Figure 7 Lipolysis is stimulated by the action of glucagon, ACTH, and adrenaline. This effect is mediated by cyclic AMP-dependent protein kinase.

At completion of adaption, there is slow and ongoing depletion of the protein compartment and breakdown of the adipose tissue. Death will occur when there is a failure to replenish fuel stores through refeeding and insufficient available energy to maintain essential bodily functions. As fat is the predominant source of energy, the time until death in uncomplicated fasting will depend on the size of the prefasting fat stores. In a normal adult, fat stores will be sufficient to sustain life for approximately 60–70 days. The extent of protein loss is also linked to survival, and a loss of more than half of the lean body mass compartment (approximately half of total body protein) is predictive of death.

See also: Energy: Adaptation; Metabolism. Energy Requirements. Fatty Acids: Metabolism. Protein: Synthesis and Turnover

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STROKE NUTRITIONAL MANAGEMENT

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Glossary

Aphasia Acquired language disorder; may include difficulty in producing or comprehending spoken or written language. Originally aphasia referred to total inability to communicate, dysphasia to partial impairment; over time, aphasia has come to mean either both partial and total language impairment in common use.

Apraxia Loss of the ability to execute or carry out learned purposeful movements, despite the desire and physical ability to do so. It is a disorder of motor planning, not caused by inco-ordination, sensory loss, or failure to comprehend. The activity may be performed as a conditioned response (e.g., if handed a cup, the patient may take it and drink, although may not be able to do this to command).

Dysphagia Difficulty in swallowing, can be neurological, structural, or functional in origin. This article refers only to neurological dysphagia. Although classified under 'signs and symptoms' of the ICD-10 classification, it is often discussed as a disease-state *per se*.

Naso-Gastric Tube A means to deliver liquid feed directly into the stomach for people who are unable or unsafe to

take food orally. A fine-bore, usually silicone or polyurethane feeding tube with a guidewire to aid placement through the nose, the esophagus, and into the stomach. After checking its position, the guidewire is removed. The tube may be retained in place using adhesive tape applied to the nose, a clip attached to the tube may be secured to the face, or a nasal bridle may be used: a device that secures the tube in place by tapes that hold the tube in the nasopharynx and exit through both nostrils. Most often used for short-term feeding, but may be used longer-term.

Percutaneous Endoscopic Gastrostomy (PEG) A means to deliver liquid feed directly into the stomach for people who are unable or unsafe to take food orally. A feeding tube placed into the stomach through the abdominal wall, usually using a combination of local anesthetic infiltration into the abdominal wall and intravenous sedation. During the procedure an endoscope is passed through the mouth to help locate the trans-abdominal puncture and placement of the feeding tube. Once the tract is established, most PEG tubes need only their retaining devices (flanges or balloons that are part of the tubing) to maintain position. May be retained in place for long periods.

Introduction

Stroke is a common and devastating event, the incidence rising with age. Approximately 125 000 and 500 000 new or recurrent strokes affect individuals each year in the UK and the US, respectively, creating a significant burden of long-term disability in survivors. Stroke is a syndrome that is sudden in onset, featuring signs of cerebral dysfunction that are vascular in origin. In first strokes, thrombo-embolic infarction is the underlying pathophysiological event in approximately 80% of cases, with 10% attributed to primary intracerebral hemorrhage and 10% to other/uncertain types. Resulting neurological and functional impairments vary in range, combination, and severity. These can include altered consciousness, motor and somato-sensory, auditory, visual, smell and taste disturbance, and loss. Impairments of memory, speech, language, continence, and higher-order cognitive functioning such as planning and decision-making also occur. Overall, the impairments and disabilities that result from stroke can exert a variable, negative impact on eating, mobility, mood, ability to selfmanage and selfcare, social role-function, and quality of life.

The challenge of nutritional management is apparent in the scope and complexity of issues presented. These include the physical, psychological, and social impact of stroke on appetite and eating, as well as prestroke nutritional status. Potential effects of metabolic injury responses, immobilization, and infective complications on energy, nitrogen, and micronutrient requirements must be considered. In individuals in whom oral feeding cannot be established, parenteral hydration and artificial nutritional support may be necessary, by the enteral route if possible, utilizing nasogastric tubes (NGT) or percutaneous endoscopic gastrostomy (PEG).

Effective nutritional management of stroke requires the integrated skills and knowledge of a multidisciplinary team. Complex situations entailing ethical and legal issues relating to hydration and nutritional support can arise, and involvement of patient and carers in all aspects of planning is vital.

Risks of Protein-Energy Malnutrition

The reported frequency of malnutrition after acute stroke has ranged from 6% to 62%, measured via multiple assessment

methods, some of which have been previously validated (e.g., Subjective Global Assessment and Mini Nutritional Assessment). This is important because the presence of undernutrition shortly after admission has been independently associated with significantly greater mortality and likelihood of developing pneumonia, infections, pressure sores and gastrointestinal bleeds in hospital, with increased risk of death or dependency poststroke.

A number of factors may interact to impair the nutritional status of stroke patients. These include factors that led to deterioration in prestroke nutritional status, direct physical and psychosocial effects of stroke on the consumption of food and fluids following hospital admission, and organizational factors that can hinder efficient, effective meal delivery and consumption in institutional and residential settings.

Prestroke Nutritional Status

Risk factors for malnutrition identified at the time of hospital admission have included increasing age, living alone, dementia, and inadequate dental status. Findings are consistent with those from surveys of elderly populations, where a wide range of personal characteristics and social and environmental features have been linked with poorer appetite and nutritional status (see related articles). The presence of malnutrition in older adults may be associated with impaired immune responses, increasing vulnerability to pneumonia and sepsis. The significance of a low serum albumin concentration in predicting clinical outcomes has been noted in stroke and older populations, where it may be more a reflection of disease severity than an indicator of nutritional status.

Poststroke Eating Problems

Neurological and functional impairments can result in eating problems following stroke, which can lead to an increased risk of protein-energy malnutrition. During the acute phase of recovery, eating disability has been associated with inadequate consumption of food and fluids and deterioration in body mass index, triceps skin fold thickness, mid-arm muscle circumference, and serum protein concentrations. Four months poststroke weight loss > 3 kg (mean 6.6 kg) was found in 24% patients, and in 26% (mean loss 8.3 kg) at 1 year. Eating difficulties and low prealbumin values were associated with weight loss at both time points; at six months poststroke 66% of survivors were found with some degree of enduring disablement that affected eating. Specific eating problems include impaired lip closure leading to oral leakage of food and fluids, dysphagia, inability to manipulate utensils linked to loss of motor skills in eating, and difficulties in maintaining an upright posture at mealtimes. Visual field or perceptual deficits can result in inability to see or perceive the contents of a meal tray, whereas aphasia and dysarthria can hinder or prevent expression of dietary needs and preferences. Loss of concentration, short-term memory and cognitive impairments such as apraxias are common sequelae to stroke and can make it difficult for individuals to sustain the sequence of activities necessary to complete a meal,

Table 1 Eating disabilities of a cohort of acute stroke admissions to a South London hospital, March 1998–December 1999

<i>Eating disabilities^a at hospital</i>	<i>3–5 days after admission to hospital</i>	<i>Number (%)</i>
Posture control	No functional impairment	325 (56)
	Mild–moderate impairment	219 (37)
	Severe impairment	43 (7)
Arm movement	No functional impairment	159 (27)
	Mild–moderate impairment	295 (50)
	Severe	133 (23)
Lip closure	No functional impairment	449 (76)
	Partial impairment	109 (19)
	Severe impairment	29 (5)
Chewing	No functional impairment	369 (63)
	Partial impairment	172 (29)
	Severe impairment	46 (8)
Swallowing	No functional impairment	341 (58)
	Partial: Cannot tolerate 1 of 3 textures	66 (11)
	Severe: Cannot tolerate 2 of 3 textures	64 (11)
	Aspiration/high risk; nil orally	116 (20)
Communication	No functional impairment	276 (47)
	Partial impairment	149 (25)
	Severe impairment	162 (28)
Attention and praxis	No functional impairment	417 (71)
	Partial impairment	130 (22)
	Severe impairment	39 (7)
Visual field/perceptual loss/neglect	No functional impairment	428 (73)
	Partial impairment	132 (23)
	Severe impairment	25 (4)

^aTotal 670 stroke patients, of whom 587 (586 for attention and praxis; 585 for visual fields) were able to be assessed.

or even to remember to eat and drink (Table 1). Taste and smell dysfunctions have been reported but impact on eating has not been investigated. A number of assessment instruments have been developed to enable health professionals to identify the extent of eating disability and the nature of support needed.

Organizational Factors

Organizational factors can hinder dietary provision and consumption in institutional settings. These include an inadequate mealtime environment, marked by poor lighting, noise, distractions (e.g., television, consultant ward rounds), unpleasant smells, temperature extremes, and lack of facilities or facilities that deter social eating. Lack of adequate assistance for dietary selection, meal delivery, and supervision may result in a meal is that inappropriate in relation to texture, portion size, patient preference and swallowing ability, or, with around

half needing help to eat in the early stages poststroke, which the patient is unable to eat without help, which may not be available.

Management of Stroke-related Psychosocial and Physical Problems Impairing Food Consumption

Evidence-based Guideline Recommendations

The provision of effective nutritional care requires a concerted approach by health professionals in developing locally tailored evidence-based standards and guidelines for nutritional screening, assessment, and dietary support. These should be linked to national standards and guidelines (where they exist), clinical audit and processes for practice development. Recognition of the need for such guidance has led to the development and dissemination of evidence-based guidelines by interprofessional expert groups, designed to inform professional judgment and bench-mark care within wider stroke management. Implementation of guidelines can best be achieved by multifaceted strategies, for example, combining education of health professionals with leadership and sensitive change management approaches.

Following acute stroke, guideline recommendations emphasize the need for screening and assessment for nutritional risk to be undertaken on admission to hospital and repeated at regular intervals (e.g., weekly for hospital inpatients) using a valid, reliable instrument by appropriately trained personnel. Individuals who are malnourished at the point of admission, or likely to become so, can then be referred to dietitians for further assessment and prompt initiation of nutritional support. Early identification of individuals whose swallow function may be unsafe with oral food and fluids is vital, as well as referral for specialist swallow assessment, usually by a speech pathologist. Screening of swallowing function should be undertaken using a validated and reliable method by appropriately trained personnel, initially as soon as the patient is able to be assessed poststroke or on admission to hospital, before patients are given food or drink. Following detailed assessment, modification of dietary textures may be advised to ensure safe eating in those with some degree of swallowing impairment. In others, artificial nutritional support using enteral routes may be necessary, owing to the severity of dysphagia or cognitive impairments. In individuals who are capable of taking food orally, the provision of support for physical, functional, and psychosocial problems is an essential aspect of nutritional management. To achieve this, appropriate specialist assessment should be requested, e.g., occupational therapy, physiotherapy, and psychology.

Psychosocial Problems

In the acute phase following stroke, approximately 30% of patients develop clinical signs of depression, 30% are anxious, and a similar proportion report loss of confidence as a major psychological problem. However, mood dysfunction may occur at any time, from immediately after stroke onset to many months later. Depression may result from an interaction of factors, including a direct result of the stroke lesion. Despite

extensive investigation, there is no robust evidence that depressed mood after stroke is caused by a lesion in any particular area or side of the brain. Depression is more common amongst people with chronic disease than matched otherwise healthy groups, and associated with reactions to physical loss, altered lives and sense of identity. Comparatively little is known of interactions between depression, anorexia, and nutritional status in the early stages of recovery following stroke in individuals with and without physical eating problems. However, at 6 months patterns of behavioral disturbance characterized by depressed mood, anorexia, and insomnia have been identified and associated with weight loss. Anxiety-evoking experiences relating to being fed, or choking in the presence of dysphagia, may also result in avoidance or withdrawal from eating. General approaches to the treatment of poststroke depression are not different to the nonstroke population, and include antidepressant drugs, and behavioral and psychotherapeutic techniques. Use of therapeutic skills in communication, eating assistance, and provision of emotional support are vital in alleviating mealtime anxiety and increasing interest in food.

The enjoyment of eating as a social activity can be affected adversely by impairments of speech, lip closure, chewing and swallowing, and manual dexterity. Severely disabled individuals who are relearning eating and swallowing skills initially require privacy and a quiet environment. As rehabilitation progresses, social integration at mealtimes can be achieved.

Communication Problems

Whilst dysphasia refers to difficulty, and aphasia to inability to communicate verbally, aphasia is often used as a relative term. It affects 20–38% of acute stroke patients with possibly as many as 40% affected by dysarthria (speech difficulties due to oromotor dysfunction), resulting in variable difficulty or inability to express thoughts in language (expressive aphasia) or to comprehend language (receptive aphasia). Expressive aphasia, also known as Broca's aphasia, results from strokes affecting the prefrontal gyrus, whereas Wernicke's receptive aphasia results from lesions of the central sulcus. Dysarthria results from neurological damage affecting neuromuscular systems that control speech production; because these systems are also concerned with swallowing, dysarthria often coexists with difficulty swallowing (dysphagia).

Communication problems can result in inabilities/difficulties in expressing hunger, thirst, meal preferences (aphasia), reading a menu, or writing preferences (aphasia can occur alongside dyslexia and dysgraphia). Receptive aphasia can impair comprehension of instructions at mealtimes and thus affects response to information and rehabilitative advice. If paralysis and visual field and perceptual deficits are combined with expressive communication deficits, nonverbal communication can also be limited, affecting ability to signal assent or dissent by nodding the head or to use gestures or point to food items/utensils. Early involvement of speech pathologists is vital to enable individuals to regain lost functions in speech and language. In selected patients, visual material, e.g., pictures and symbols, can be helpful. Use of short sentences

with single topics, simple terms, no jargon, normal volume speech, a clear light with a good view of the speaker's face and appropriate gestures, and patience in allowing individuals time to respond are helpful in general communication.

Impairments of Arm Movement and Posture

Stroke can affect any of the neural mechanisms controlling voluntary movement and posture. These include the motor and sensory cortex and associated pathways, cerebellum, basal ganglia, and brain stem. The impact on eating skills can be considerable, because weakness or paralysis affecting the arm occurs in 80% of strokes. Loss of coordination, spatial awareness, abnormal muscle tone, and sensory loss may also occur. Common problems resulting from this are difficulties manipulating cutlery, lifting/loading food onto utensils, cutting food, inserting food in the mouth, drinking from a cup, or discerning the spatial relationships between objects.

If one arm is unaffected, then some degree of compensation is possible, particularly if this is dominant (unless this has been intentionally immobilized: Constraint Induced Therapy). Use of the unaffected hand is important in detecting temperatures of food and liquids where sensation is impaired. An occupational therapy assessment is necessary to identify specific deficits, rehabilitation techniques, and appropriate aids to eating. Lightweight plastic cups and cutlery with molded or built-up handles, plate-guards and nonslip mats can be provided. Where upper limb impairment is severe, individuals may require assistance or to be fed.

Postural impairment following stroke can result in inability to maintain an upright sitting position, required for safe and effective food preparation, eating and swallowing. A physiotherapy assessment can identify the most effective techniques for rehabilitation of muscle weakness and to counteract abnormal muscle tone (spasticity). Appropriate aids to seated balance can include molded seating and supports.

Visual Field Loss and Visual Neglect

Between 30% and 60% of individuals who sustain an acute stroke suffer from visual field loss due to partial or complete hemianopia. Neurological damage affecting the parietal or temporal lobes and involving the sensory pathway between the optic chiasma and visual cortex underlies this problem. The impact of loss in up to half the visual field is that food items on a meal tray may not be seen and therefore may remain uneaten. Compensatory interventions include instruction in scanning the visual field, or placing items within it for those who are unable to do this. Consistent placement of items on a meal tray and verbal identification of contents using a clock system is also helpful.

Neurological damage affecting the visual cortex of the occipital lobe, often following right hemisphere strokes, can result in neglect of half the visual space. A classic feature is failure to eat food on the left side of a plate. Affected individuals need reminding to focus on food items in the neglected space; placing a colored marker on one side of the plate can be helpful. This problem may occur in conjunction with visual field loss.

Attention Span, Short-Term Memory

Impairment of attention span and short-term memory of a few minutes duration are common following acute stroke. Attention deficits result in an inability either to focus on immediate events or to establish a new focus unless a current stimulus is removed. As a consequence, an activity that requires a sequence of steps, such as eating a meal with two or three courses, may not be completed unaided. Lack of concentration also deters relearning eating patterns. Removing or minimizing distractions at mealtimes, simplifying the complexity of information necessary to regain eating skills, and providing verbal, written, or auditory alarms as reminders to eat are important in overcoming this problem.

Swallowing Difficulties

Screening and Assessment

Dysphagia affects approximately 45–60% of people with acute stroke. Variable in severity, it is characterized by sensory or motor loss affecting one or more of the stages of swallowing, i.e., oral preparation, oral transport, pharyngeal transport, and reflex swallowing (Table 2). The effects of stroke on esophageal peristalsis have been little examined. It has been estimated that approximately 50% of dysphagic patients either die or recover their swallow spontaneously within the first 2 weeks of stroke onset. For most of the others, at least some degree of functional swallowing can be restored with time. Approximately 10–20% will still be affected by 6 months; a small number never recover swallow function, although for some, recovery may take years. Swallowing impairment that prohibits eating normal texture food can exert a negative impact on functional recovery and quality of life.

Typical clinical features of dysphagia include delayed oral and pharyngeal transit times, retention of food in the cheek cavities, uncontrolled leakage of food/fluids out through the lips or onwards into the pharynx, causing choking and regurgitation of food/fluids through the nose and mouth. Tongue coordination may be poor, triggering of swallow and laryngeal cartilage elevation may be delayed or absent, gag may be abnormal. After swallow there may be a wet, 'gurgly' voice or coughing. Alternatively, the patient may aspirate silently, with no overt signs. Complications resulting from dysphagia can be life threatening, i.e., aspiration of food/fluid into the respiratory tract resulting in pulmonary infection, and dehydration. Longer hospital stay, strong inverse correlations with functional capacity, and an increased mortality have been associated with dysphagia. Early identification of the problem is vital, encompassing screening as soon as possible after admission, clinical bedside assessment (CBA), and, if necessary, instrumental assessment using, for example, videofluoroscopic swallowing studies (VSS) or fiberoptic endoscopic examination of swallowing (FEES).

Screening is a procedure intended to identify patients with potential swallowing problems, who can then be referred for more detailed assessment of phases of swallowing, together with judgment of extent of dysfunction and risk of aspiration. Systematic reviews have identified a number of screening methods of varying validity and reliability, which combine identification of clinical features of dysphagia with or without

Table 2 Stages of swallowing: effects of stroke

Stage	Effects of stroke
(1) Oral preparation Duration variable Lip closure forms anterior seal Chewing of food by mandibular and maxillary teeth Salivation evoked by parasympathetic nervous system Bolus formation controlled by tongue Sensory feedback from oral mucosa on volume & consistency determine timing of bolus ejection	Inadequate lip seal causes leakage of food/fluid chewing slower, food impacts in oral sulci Hyposecretion of saliva Paralysis of tongue impairs bolus formation Sensory loss leads to impaired bolus lateralization
(2) Oral transport Duration 1 s Bolus of 5–15 cm ⁻¹ separated, moved to tongue midline Oral cavity sealed, mandible raised, pressure exerted by tongue against palate propels bolus to posterior oral cavity	Slowed transport Bolus localization, separation and formation impaired; can lead to food retention in oral cavity Lack of fine motor coordination may lead to loss of liquid bolus control; risk of aspiration Abnormal positioning of bolus; diminished tongue elevation; inadequate bolus propulsion
(3) Pharyngeal transport/reflex swallowing Duration 0.5–0.6 s Bolus impacts on sensory receptors in tissues of soft palate, pharynx, tongue, fauces Swallowing reflex stimulated; elevation/closure of velopharyngeal mechanism, elevation of larynx, closure of vocal cords, pharyngeal peristalsis, relaxation of esophageal sphincter Respiration transiently ceases as bolus enters esophagus; breathing resumed; soft palate returned to resting position	Events may occur in abnormal sequence/timing Impaired sensation, delay/absent swallowing reflex Velopharyngeal closure impaired; food regurgitated through nose/mouth Incomplete laryngeal elevation/vocal cord adduction Swallowing reflex delay/absence leading to coughing, aspiration
(4) Esophageal transport Duration 8–20 s Peristalsis moves bolus to stomach	Effects of stroke little investigated Aging results in slight impairment of peristaltic amplitude

swallowing water. Prescreen assessment of conscious level, oromotor and laryngeal function, signs of existing respiratory aspiration and the extent to which the patient can safely cooperate with the examination are necessary. Aspiration of food or fluid into the respiratory tract may be accompanied by choking, indicated by voice changes (wet, hoarse, gurgling) or breathlessness, or it may be silent. Loss of swallowing and protective gag reflexes or the presence of features of dysphagia or aspiration when attempting to swallow water at an initial screen are indications that nil should be given by mouth. Detailed investigation is then necessary. During the acute phase of stroke the patient's condition may not be stable, and even if there is no initial indication of problems, a high index of suspicion should be maintained and screening repeated if the patient's condition deteriorates.

There is no standardized format for clinical bedside assessment (CBA) but speech pathologists and other specially trained health professionals generally employ a combination of methods with demonstrated validity and reliability. These encompass the medical history relating to onset of swallowing problems; oral sensory and motor testing; laryngeal and pharyngeal assessment; presence/absence of swallowing, cough, and gag reflexes; cognitive and language function; alertness, attention span, and ability to follow instructions. Swallow function is usually assessed by observing the patient and feeling for swallow/laryngeal elevation with a graded sequence of foods and fluid consistencies. Adjunct assessments may be used, such as concurrent pulse oximetry to monitor

oxygen saturation during swallowing, cervical auscultation to assess swallow function from swallow sounds, or assessment of muscle activity by cervical surface electromyography.

CBA provides limited detail of swallowing and functions poorly in relation to subtle signs of dysphagia and silent aspiration of food, fluids, or saliva into the respiratory tract: more invasive assessment may be required. VSS, using a modified barium swallow procedure, provides a detailed radiological assessment of the oral, pharyngeal, and upper esophageal phases of swallowing; can detect functional impairments resulting in aspiration, evaluate optimal head/neck positioning during swallowing, and determine the impact of food textures on the process. Limitations include its labor-intensive nature, exposure to radiation, problems transporting disabled stroke patients to radiology departments, variable protocol standardization with respect to volumes, consistencies, or textures of food and fluids and screening positions adopted. Further, views represent a snapshot of swallowing under ideal, rather than normal, circumstances, and variability in the reliability of reporting has been identified between and within raters of VSS outcomes. FEES entails passage of a flexible endoscope nasally, over the velum and into the pharynx. Its advantages lie in observation of bolus transit through the hypopharynx and identification of laryngeal penetration and aspiration; being portable it can be performed in a range of settings, overcoming the transport problems of VSS. However, it cannot investigate the oral stage of swallowing. The clinical utility of other methods are being explored, such as

assessment of breathing patterns, to detect respiratory phases in relationship to swallows, and abnormal breathing patterns, such as swallow apnea followed by inspiration rather than expiration; impedance pharyngography, based on changes in the electrical impedance of the neck during swallowing.

Nutritional Management and Treatment

Use of modified food textures and fluid consistencies aims to reduce the level of challenge posed in terms of manipulation of the food bolus (i.e., by reducing the numbers of different textures presented and increasing the viscosity) and to alter the rate at which it passes through the pharynx, to maximize patient control of swallowing and reduce the risk of aspiration. National professional groups world-wide have developed classification systems for modification in the texture and consistency of foods and fluids. These usually cite approximately three grades, e.g., soft, minced, and pureed foods; mildly, moderately, and extremely thickened fluids (Tables 3–7).

It is commonly the responsibility of the speech pathologist to recommend the most appropriate food textures and maneuvers to promote safe eating for a dysphagic stroke patient (Tables 4 and 8). Dietitians ensure that texture-modified meals are adequate to meet nutrient requirements, offer choice, and are palatable. Skilled nursing assistance and interventions at mealtimes can also aid eating and mealtime processes. Impaired oral preparation may be compensated for by positioning food on, and tilting the head towards, the unaffected side; posterior positioning of food on the tongue may promote oral transport. If pharyngeal transport/reflex swallowing is impaired, it is important to ensure upright posture, head stable in the midline with a slight forward flexion to protect the airway; prompting of synchronization of the sequence of inspiration, breath-holding, swallowing, possibly repeatedly, expiration, and coughing on expiration to clear food debris may be helpful. Maintaining an upright posture for at least 30 min after meals minimizes risk of regurgitation/aspiration.

Many nondietary, therapeutic approaches to manage dysphagia have been identified including: oral electrical, thermal and chemical pharyngeal stimulation; high-intensity swallowing therapy; exercises to improve laryngeal closure, labial/mandibular closure, tongue elevation, and lateralization; use of palatal training devices and prostheses to assist triggering of the swallowing reflex or lower the palatal vault to improve bolus formation; drug therapy (nifedipine); use of bio-feedback involving mirrors and VSS. Although benefits have been described for many of these interventions, lack of randomized, controlled clinical trials with adequate power limits conclusions on effectiveness.

Nutrient Requirements

Diabetes mellitus, hypertension, and renal failure are common in the stroke population; their disease-related dietary management requirements need to be borne in mind for affected stroke patients. Many different approaches can be used to estimate energy requirements. Resting energy expenditure can be estimated on the basis of body weight, height, age, and sex, with modifications to accommodate activity and injury factors, and supplemental values to replenish malnourished

individuals. Estimation of resting energy expenditure by indirect calorimetry using a portable metabolic monitor provides more accurate estimates derived from the respiratory quotient. Values do not consider periods of activity, pyrexia, pain, or energy increments necessary for nutritional repletion, so further corrections are necessary.

Following stroke a number of factors may affect energy requirements. Inactivity caused by paralysis reduces energy expenditure, but muscular paresis increases the relative energy cost of activity. Infections, which commonly complicate stroke, will increase energy expenditure, with each 1 °C rise in core temperature raising it by 13%. The impact of the cerebral injury on poststroke metabolism, i.e., resting energy expenditure at different levels of stroke severity, has not been fully investigated. Evidence for metabolic injury responses based on hormonal profiles and changes in blood glucose concentration in the acute phase is limited. Hyperglycemia is common following stroke and has been associated with increased morbidity and mortality. Hyperglycemia can be attributed to overt or latent diabetes mellitus, stress responses, and effects of glucose intolerance in elderly subjects. Elevated plasma cortisol and catecholamine concentrations representing a transient stress response have been reported in the first 72 h following stroke. Nitrogen requirements following stroke can be estimated using reference ranges based on body weight, or using nitrogen balance studies.

Studies of nutritional supplementation of stroke patients have not conclusively demonstrated improved outcomes. One major international trial clearly showed no benefit from isocaloric supplements for unselected stroke populations, but early indications are that targeted protein-rich supplementation may be useful.

Fluid balance requirements require careful attention, because dehydration is a serious risk in individuals with dysphagia and physical disabilities that impair drinking, with potential to compromise already impaired cerebral circulation. Oral intake of fluid is contraindicated where the swallowing and gag reflex are lost or swallowing and level of consciousness are impaired. Parenteral (intravenous or other routes) fluid replacement therapy is then necessary, usually short term. Fluid requirements can be calculated on the basis of 35 ml per kg body weight daily in adults, but sepsis and fever can increase needs. Fluid intake and output in conjunction with insensible losses should be monitored on a daily basis together with the symptoms of dehydration, i.e., urine specific gravity, thirst, dry mucous membranes, and loss of skin turgor.

Artificial Nutritional Support

The presence of severe dysphagia and cognitive and complex physical impairments may render oral feeding unsafe or insufficient for nutritional requirements. If the gastrointestinal tract is functional, the options for delivering enteral nutritional support are either a fine-bore NGT or a PEG or radiologically inserted gastrostomy.

Decisions concerning choice of route are influenced by the anticipated duration of dysphagia and benefits versus risks. Impact on nutritional status, rehabilitation, quality of life, safety, tolerance, flexibility, ease of use, costs of insertion,

Table 3 Standardized classifications of texture modified foods and fluids

<i>Modified foods</i>	<i>Au texture A – Soft/UK Texture E</i>	<i>Au texture B – Minced and Moist/UK Texture D</i>	<i>Au texture C – Smooth Pureed/UK Texture C</i>
Description	May be naturally soft (e.g., ripe banana), or may be cooked or cut to alter its texture	Soft and moist, may have some variation in texture, should easily form into a ball	Smooth and lump-free; may have a grainy quality, but no lumps
Characteristics	Soft foods can be chewed but should not need to be bitten. Minimal cutting required – easily broken up with a fork. Food should be moist or served with a sauce or gravy to increase moisture content	Easily mashed with a fork. May be presented as a thick puree with obvious lumps, but lumps are soft and rounded, not hard or sharp. Small lumps can be broken by the tongue	Moist and cohesive enough to hold its shape on a spoon (i.e., when placed side by side on a plate these consistencies maintain their position without ‘bleeding’ into one another). Can be molded, layered, piped. Au Runny puree/UK Textures A/B: Smooth, uniform consistency, will ‘bleed’ into one another; may be poured (A only); cannot be molded, layered, piped
<i>Modified Fluids</i>	<i>Au Mildly thick/UK Stage 1</i>	<i>Au Moderately thick/UK Stage 2</i>	<i>Au Extremely thick/UK Stage 3</i>
Description	Thicker than naturally thick fluids such as fruit nectars and commercial sip feeds.	Similar to room temperature pouring honey or a thickshake	Similar to pudding or mousse
Flow rate	Steady, fast flow; runs fast through the prongs of a fork, but leaves a mild coating on the prongs.	Slow flow; slowly drips in dollops through the prongs of a fork.	No flow; sits on and does not flow through the prongs of a fork.
Characteristics	Pours quickly from a cup but slower than regular fluids. May leave a coating in the cup/on the back of a spoon after being poured. Can be drunk from a cup, or a standard-bore straw with effort.	Cohesive, pours slowly. Can be drunk from a cup although flows very slowly/difficult or impossible to drink using a straw, even a wide bore straw	Cohesive and holds its shape on a spoon. Cannot pour from a cup; needs to be taken by spoon. Too thick if the spoon is able to stand upright in it.

Table 4 Australian standardised terminology and definitions for texture modified fluids 2007

	<i>Mildly thick</i>	<i>Moderately thick</i>	<i>Extremely thick</i>
Description	Thicker than naturally thick fluids such as fruit nectars, but for example, not as thick as a thickshake	Similar to the thickness of room temperature honey or a thickshake	Similar to the thickness of pudding or mousse
Flow rate	Steady, fast flow	Slow flow	No flow
Characteristics	Pours quickly from a cup but slower than regular, unmodified fluids. May leave a coating film of residue in the cup after being poured. Drink this fluid thickness from a cup. Effort required to take this thickness via a standard bore straw	Cohesive and pours slowly. Possible to drink directly from a cup although fluid flows very slowly. Difficult to drink using a straw, even if using a wide-bore straw	Cohesive and holds its shape on a spoon. It is not possible to pour this type of fluid from a cup into the mouth. Spoon is the optimal method for taking this type of fluid. This fluid is too thick if the spoon is able to stand upright in it
Testing Information	Subjectively, fluids at this thickness run fast through the prongs of a fork, but leave a mild coating on the prongs ^a	Subjectively, fluids at this thickness slowly drip in dollops through the prongs of a fork	Subjectively, fluids at this thickness sit on and do not flow through the prongs of a fork

^aTesting scales for viscosity exist but are not formalised or standardised, and therefore are not included.

Table 5 Texture A—Soft: Australian standardised terminology and definitions for texture modified foods and fluids 2007

<i>Food type</i>	<i>Recommend</i>	<i>Avoid</i>
Bread, cereals, rice, pasta, noodles	<ul style="list-style-type: none"> ● Soft sandwiches(a) with very moist fillings, for example egg and mayonnaise, hummus (remove crusts and avoid breads with seeds and grains) ● Breakfast cereals well moistened with milk(b) ● Soft pasta(a) and noodles ● Rice (well cooked) ● Soft pastry, for example quiche with a pastry base ● Other, soft, cooked grains 	<ul style="list-style-type: none"> ● Dry or crusty breads, breads with hard seeds or grains, hard pasta, pizza ● Sandwiches that are not thoroughly moist ● Course or hard breakfast cereals that do not moisten easily, for example toasted muesli, bran cereals ● Cereals with nuts, seeds and dried fruit
Vegetables, legumes	<ul style="list-style-type: none"> ● Well cooked vegetables (a) served in small pieces or soft enough to be mashed or broken up with a fork ● Soft canned vegetables, for example peas ● Well cooked legumes (the outer skin must be soft), for example baked beans 	<ul style="list-style-type: none"> ● All raw vegetables (including chopped and shredded) ● Hard, fibrous or stringy vegetables and legumes, for example sweet corn, broccoli stalks
Fruit	<ul style="list-style-type: none"> ● Fresh fruit pieces that are naturally soft, for example banana, well-ripened pawpaw ● Stewed and canned fruits in small pieces ● Pureed fruit 	<ul style="list-style-type: none"> ● Large/round fruit pieces that pose a choking risk, for example whole grapes, cherries ● Dried fruit, seeds and fruit peel ● Fibrous fruits, for example pineapple
Milk, yoghurt, cheese	<ul style="list-style-type: none"> ● Milk, milkshakes, smoothies (b) ● Yoghurt (may contain soft fruit)(b) ● Soft cheeses, (a) for example Camembert, ricotta 	<ul style="list-style-type: none"> ● Yoghurt with seeds, nuts, muesli or hard pieces of fruit ● Hard cheeses, for example cheddar and hardened/crispy cooked cheese
Meat, fish, poultry, eggs, nuts, legumes	<ul style="list-style-type: none"> ● Casseroles with small pieces of tender meat(a) ● Moist fish (easily broken up with the edge of a fork) ● Eggs (a) (all types except fried) ● Well cooked legumes (the outer skin must be soft), for example baked beans ● Soft tofu, for example small pieces, crumbled 	<ul style="list-style-type: none"> ● Dry, tough, chewy, or crispy meats ● Meat with gristle ● Fried eggs ● Hard or fibrous legumes ● Pizza
Desserts	<ul style="list-style-type: none"> ● Puddings, dairy desserts, (b) custards, (b) yoghurt (b) and ice-cream (b) (may have pieces of soft fruit) ● Moist cakes (extra moisture, e.g., custard may be required) ● Soft fruit-based desserts without hard bases, crumbly or flaky pastry or coconut, for example apple crumble ● Creamed rice, moist bread and butter pudding 	<ul style="list-style-type: none"> ● Dry cakes, pastry, nuts, seeds, coconut, dried fruit, pineapple
Miscellaneous	<ul style="list-style-type: none"> ● Soup (b) – (may contain small soft lumps, e.g., pasta) ● Soft fruit jellies or nonchewy lollies(a) ● Soft, smooth, chocolate ● Jams and condiments without seeds or dried fruit 	<ul style="list-style-type: none"> ● Soups with large pieces of meats or vegetables, corn, or rice ● Sticky or chewy foods, for example toffee ● Popcorn, chips, biscuits, crackers, nuts, edible seeds

(a) These foods require case-by-case consideration.

(b) These foods may need modification for individuals requiring thickened fluids.

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removal, and maintenance are all important considerations. In the majority of cases dysphagia resolves within the acute phase of stroke, i.e., approximately 2–3 weeks. For relatively short time periods, enteral feeding by fine-bore NGT is

recommended, and a major international trial of early insertion of PEG has not shown benefit. Advantages of NGT are the technical simplicity of intubation, maintenance and removal, low cost and ease of use. Accidental displacement and

Table 6 Texture B—Minced and Moist: Australian standardised terminology and definitions for texture modified foods and fluids 2007

<i>Food type</i>	<i>Recommend</i>	<i>Avoid (in addition to the Foods to Avoid listed for Texture A – Soft)</i>
Bread, cereals, rice, pasta, noodles	<ul style="list-style-type: none"> ● Breakfast cereal with small moist lumps, for example porridge or wheat flake biscuits soaked in milk ● Gelled bread ● Small, moist pieces of soft pasta, for example moist macaroni cheese (some pasta dishes may require blending or mashing) 	<ul style="list-style-type: none"> ● All breads, sandwiches, pastries, crackers, and dry biscuits ● Gelled breads that are not soaked through the entire food portion ● Rice that does not hold together, for example parboiled, long-grain, basmati ● Crispy or dry pasta, for example edges of a pasta bake or lasagne
Vegetables, legumes	<ul style="list-style-type: none"> ● Tender cooked vegetables that are easily mashed with a fork ● Well cooked legumes (partially mashed or blended) 	<ul style="list-style-type: none"> ● Vegetable pieces larger than 0.5 cm or too hard to be mashed with a fork ● Fibrous vegetables that require chewing, for example peas
Fruit	<ul style="list-style-type: none"> ● Mashed soft fresh fruits, for example banana, mango ● Finely diced soft pieces of canned or stewed fruit ● Pureed fruit ● Fruit juice(a) 	<ul style="list-style-type: none"> ● Fruit pieces larger than 0.5 cm ● Fruit that is too hard to be mashed with a fork
Milk, yoghurt, cheese	<ul style="list-style-type: none"> ● Milk, milkshakes, smoothies(a) ● Yoghurt(a) (may have small soft fruit pieces) ● Very soft cheeses with small lumps, for example cottage cheese 	<ul style="list-style-type: none"> ● Soft cheese that is sticky or chewy, for example Camembert
Meat, fish, poultry, eggs, nuts, legumes	<ul style="list-style-type: none"> ● Coarsely minced, tender, meats with a sauce. ● Casseroles dishes may be blended to reduce the particle size ● Coarsely blended or mashed fish with a sauce ● Very soft and moist egg dishes, for example scrambled eggs, soft quiches ● Well cooked legumes (partially mashed or blended) ● Soft tofu, for example small soft pieces or crumbled 	<ul style="list-style-type: none"> ● Casserole or mince dishes with hard or fibrous particles, for example peas, onion ● Dry, tough, chewy, or crispy egg dishes or those that cannot be easily mashed
Desserts	<ul style="list-style-type: none"> ● Smooth puddings, dairy desserts,(a) custards,(a) yoghurt(a) and ice-cream(a) (may have small pieces of soft fruit) ● Soft moist sponge cake desserts with lots of custard, cream or ice-cream, for example trifle, tiramisu ● Soft fruit-based desserts without hard bases, crumbly or flaky pastry or coconut, for example apple crumble with custard ● Creamed rice 	<ul style="list-style-type: none"> ● Desserts with large, hard or fibrous fruit particles (e.g., sultanas), seeds or coconut ● Pastry and hard crumble ● Bread-based puddings
Miscellaneous	<ul style="list-style-type: none"> ● Soup(a) – (may contain small soft lumps, e.g., pasta) ● Plain biscuits dunked in hot tea or coffee and completely saturated ● Salsa's, sauces and dips with small soft lumps ● Very soft, smooth, chocolate ● Jams and condiments without seeds or dried fruit 	<ul style="list-style-type: none"> ● Soups with large pieces of meats or vegetables, corn, or rice ● Sweets and lollies including fruit jellies and marshmallow

(a) These foods may require modification for individuals requiring thickened fluids.

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repeated removal by confused or cognitively impaired patients are not uncommon; nasal bridles to maintain secure positioning are showing promise. Placement positioning difficulties and malposition, discomfort, and risks of aspiration

are potential complications, with aspiration occurring in up to 10% of patients.

For feeding over longer periods, a PEG inserted under local anesthetic and sedation offers greater comfort, toleration, ease

Table 7 Texture C—Smooth Pureed: Australian standardised terminology and definitions for texture modified foods and fluids 2007

<i>Food type</i>	<i>Recommend</i>	<i>Avoid (in addition to the Foods to Avoid listed for Texture B – Minced and moist)</i>
Bread, cereals, rice, pasta, noodles	<ul style="list-style-type: none"> ● Smooth lump-free breakfast cereals, for example semolina, pureed porridge ● Gelled bread ● Pureed pasta or noodles ● Pureed rice 	<ul style="list-style-type: none"> ● Cereals with coarse lumps or fibrous particles, for example all dry cereals, porridge ● Gelled breads that are not soaked through the entire food portion
Vegetables, legumes	<ul style="list-style-type: none"> ● Pureed vegetables ● Mashed potato ● Pureed legumes, for example baked beans (ensuring no husks in final puree) ● Vegetable soups that have been blended or strained to remove lumps(a) 	<ul style="list-style-type: none"> ● Coarsely mashed vegetables ● Particles of vegetable fiber or hard skin
Fruit	<ul style="list-style-type: none"> ● Pureed fruits, for example commercial pureed fruits, vitamised fresh fruits ● Well mashed banana ● Fruit Juice(a) without pulp 	<ul style="list-style-type: none"> ● Pureed fruit with visible lumps
Milk, yoghurt, cheese	<ul style="list-style-type: none"> ● Milk, milkshakes, smoothies(a) ● Yoghurt(a) (lump-free), for example plain or vanilla ● Smooth cheese pastes, for example smooth ricotta ● Cheese and milk-based sauces(a) 	<ul style="list-style-type: none"> ● All solid and semisolid cheese including cottage cheese
Meat, fish, poultry, eggs, nuts, legumes	<ul style="list-style-type: none"> ● Pureed meat/fish (pureed with sauce/gravy to achieve a thick moist texture) ● Soufflés and mousses, for example salmon mousse ● Pureed legumes, hummus ● Soft silken tofu ● Pureed scrambled eggs 	<ul style="list-style-type: none"> ● Minced or partially pureed meats ● Scrambled eggs that have not been pureed ● Sticky or very cohesive foods, for example peanut butter
Desserts	<ul style="list-style-type: none"> ● Smooth puddings, dairy desserts,(a) custards,(a) yoghurt(a) and ice-cream(a) ● Gelled cakes or cake slurry, for example fine sponge cake saturated with jelly ● Soft meringue ● Cream(a), sirup dessert toppings(a) 	<ul style="list-style-type: none"> ● Desserts with fruit pieces, seeds, nuts, crumble, pastry or nonpureed garnishes ● Gelled cakes or cake slurries that are not soaked through the entire food portion
Miscellaneous	<ul style="list-style-type: none"> ● Soup(a) – vitamised or strained to remove lumps ● Smooth jams, condiments and sauces 	<ul style="list-style-type: none"> ● Soup with lumps ● Jams and condiments with seeds, pulps or lumps

(a) These foods may require modification for individuals requiring thickened fluids.

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of use, and reported improvements in nutritional status. However, costs are greater and this more invasive procedure carries a 1–2% technique-related fatality, often more for delayed insertion in malnourished patients. Minor complications include stomal sepsis, leakage, and blockage. Peritonitis, perforation, gastrointestinal bleeding, and intestinal obstruction can rarely occur. Most enterostomy catheters are made from nonacid-hardening polyurethane or silicone and can be left *in situ* for 6 months and longer.

No consensus exists concerning the time period within which gastrostomy feeding should be initiated following stroke, but it should be considered where dysphagia persists beyond 14 days, or the patient cannot tolerate a NGT. Rarely,

enteral nutrition may be contraindicated following stroke owing to gastrointestinal bleeding resulting from severe stress ulceration; nonstroke-related contraindications may also be present, e.g., ascites, bleeding disorders. Parenteral nutrition should then be considered.

Issues relating to the optimum timing of commencement of artificial feeding can be challenging for a patient group that may experience sudden and major cognitive and physical impairments with uncertain recovery potential. Nutritional deterioration can be rapid in acutely ill patients unable to take food orally. Decision-making can be difficult and stressful, and require extensive and sensitive communication between healthcare team members, patient (where possible) and

Table 8 Compensatory strategies and restorative therapies for dysphagia

<i>Stage of swallow</i>	<i>Swallow disorder</i>	<i>Compensatory strategy</i>	<i>Restorative/rehabilitative exercises/therapy</i>
Oral preparatory	Poor lip seal	Supported lip and jaw closure	Lip exercises
Oral preparatory	Poor cheek tone	Intra-oral prosthesis, cheek hold technique (apply pressure to weak side), tilt head towards unaffected side	Cheek tone exercises
Oral preparatory	Poor sensation in oral cavity	Increase bolus taste, volume, density, temperature, carbonated drinks	Sensory awareness program
Oral preparatory	Poor tongue movement	Modify consistency of bolus, pace rate of bolus presentation, avoid mixed consistencies, remove residue from oral cavity post swallow	Tongue lateralization exercises
Oral preparatory	Poor chewing/ jaw closure	Jaw support, diet modification	Chewing exercises
Pharyngeal	Delayed swallow	Adapted cutlery and crockery to assist in self feeding, chin tuck posture, increase bolus taste, volume, density, temperature, fizzy drinks	Thermal stimulation, PNF to the fauceal arches
Pharyngeal	Reduced base of tongue movement	Chin tuck, clearing swallows, effortful swallow, decrease bolus size, increase bolus consistency	Tongue hold technique, gargle & yawn exercises, supersupraglottic swallow
Pharyngeal	Unilateral pharyngeal paresis	Head rotation to damaged side, head tilt to unaffected side, back or side lying, clearing swallows, liquid wash down	
Pharyngeal	Unilateral tongue and pharyngeal paresis	Head tilt to unaffected side, clearing swallows	
Pharyngeal	Reduced laryngeal closure	Chin tuck, head rotation to damaged side, supraglottic swallow, supersupraglottic swallow, alter bolus consistency	Supraglottic swallow, supersupraglottic swallow, breath hold maneuver, push–pull voicing
Pharyngeal	Reduced laryngeal elevation	Chin tuck & lie on side/back, supersupraglottic swallow, Mendelssohn maneuver, clearing swallows	Falsetto voicing, Mendelssohn maneuver, Shaker technique, surface electromyography
Pharyngeal	Cricopharyngeal dysfunction/ reduced anterior movement of hyolaryngeal structure	Head rotation, avoid mixed consistencies	Shaker technique
Fatigue		Nutritional supplements, decrease meal size, increase frequency of meals	

PNF: proprioceptive neuromuscular facilitation.

Source: Reproduced with permission from Perry L and Boaden E (2010) Nutritional aspects of stroke care. In: Williams J, Perry L, and Watkins (Eds) *Acute Stroke Nursing*. London: Wiley–Blackwell.

families (where available). Failure to establish timely agreed nutritional care planning for patients who have eaten little or nothing for more than 5 days and are likely to eat little or nothing for the next 5 days or longer, who are unable to take in nutrients properly, or who have increased nutritional needs is unacceptable.

Evaluation of Nutritional Support

It is vital that nutritional status is monitored in the acute phase of recovery and that dietary intakes are readjusted accordingly. Appropriate dietary, anthropometric, and clinical

assessments, which can be performed on a weekly basis, are discussed. Other important components of monitoring include recovery of physical functions related to independence in eating, including swallowing capacity, and observing for complications of enteral support techniques. Effective nutritional management following stroke requires coordination of the professional skills of doctor, nurse, speech pathologist, dietitian, occupational therapist, and physiotherapist, ideally within the context of a nutrition support team. Dynamic leadership, referral policies, clear accountability and lines of communication are essential for the team to deliver effective support. Follow-up services in the community are also

necessary to prevent deterioration in nutritional status in the later stages of rehabilitation.

See also: Biochemical Indices. Diabetes Mellitus: Dietary Management. Energy Expenditure: Indirect Calorimetry. Malnutrition: Secondary, Diagnosis and Management. Nutritional Assessment: Anthropometry. Nutritional Considerations for the Management of Hypertension. Nutritional Support: In the Home Setting; Infants and Children, Parenteral. Older People: Nutritional Management of; Nutritional Requirements. Protein: Requirements and Role in Diet

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SUCROSE

Dietary Sucrose and Disease

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Glossary

Added sugars Simple carbohydrates added to food items during industrial processing.

HFCS A sweetener made from corn syrup, enriched with fructose. In many countries it has replaced sucrose in sweetened beverages.

Nutrient dilution When caloric compounds with no nutrient content (such as sugars) fulfill part of the daily caloric needs of an individual, in the place of foods that, besides calories, would provide essential nutrients.

Introduction

For decades, sucrose (the disaccharide of glucose and fructose) has been the main sweetener added to human diets, particularly for sweetened beverages. But in the past 15 years, the use of sucrose has been progressively displaced by high-fructose corn syrup (HFCS). Still, sucrose continues to be an important dietary energy source in many parts of the world.

The term ‘sugars’ includes a variety of refined carbohydrates, and its use in literature is not always consistent, and it may refer to all refined carbohydrates present in a food item, or only sucrose, or only added sugars. The latter is particularly important for assessing health outcomes, because it is the fraction of dietary refined carbohydrates that can obviously be manipulated over a wide range, similar to the case of salt. These added sugars, contrary to natural sugars present in fruits or milk, contribute no essential nutrients to the diet, but add to energy intake, and are thus ‘empty’ calories.

Several mechanisms have been suggested for the possible health effects of refined sugars: (1) facilitating an increase in total energy intake, possibly mediated by changes in ingestive behavior or by metabolic effects on energy regulation; (2) displacement of nutrient-rich foods, with the consequent decrease in the intake of essential nutrients; and (3) metabolic effects adversely affecting glucose homeostasis. It should be noted that few studies have evaluated sucrose’s effects separate from that of other refined sugars that may also be present in the diet. Furthermore, some studies do not separate added sugars from other oligosaccharides naturally present in foods.

Effects on Energy Intake and Body Weight

The preponderance of evidence shows that increased consumption of added sugars is associated with increased total

energy intake. A study by the US Department of Agriculture using national survey data found that 60% of adults in the upper quartile of sugar intake exceeded their recommended energy intake, compared with 22% in the other quartiles. Individuals who did not exceed their energy allowance did so by reducing intake of fruits, vegetables, and milk, evidence of an undesirable displacement of nutrient-rich foods by added sugars. A similar displacement effect of sugars has been shown in 6–13-year-old children. It should be noted that several cross-sectional studies failed to identify a correlation between added sugars intake and total energy intake, but it is well recognized that studies of cross-sectional nature are difficult to interpret and may yield misleading associations. Many studies also fail to control physical activity level, which is an important factor determining total energy intake, and possibly fluid intake as well.

Sweetened Beverages

This issue is of interest given the dramatic increase in the consumption of sweetened beverages over the past decade, which have become the main contributors to added sugars intake in the US and elsewhere. This increase is particularly evident in adolescents and young adults. Nationally representative data from the US show that almost 20% of daily calories are obtained from caloric beverages. There is evidence that sweetened beverages may be the main source through which added sugars result in higher total energy intake. For example, adolescents who consumed at least two cans (12 oz) of soda per day had a total energy intake of 2600 kcals, compared to those in 1980 who did not consume sodas. Similarly, a randomized trial comparing sweetened and artificially sweetened beverages in overweight individuals showed

a significantly higher ad libitum dietary energy intake in those consuming sweetened beverages.

Longitudinal observational studies report relatively modest but significant effects on body weight, specifically for sweetened beverages. An observational study in 7-year-old children reported an increase of ~ 0.20 in Body Mass Index (BMI) units for each additional serving of sweetened drink over a 19-month period. Another 9-year follow-up of adolescents found a significant correlation of sweetened beverage intake with BMI, but not with body fat. A randomized trial aimed at reducing body weight in overweight adults found that reducing consumption of caloric beverages was associated with significant weight loss, independent of any other factor, such as physical activity or a reduction in caloric intake from solid foods. A reduction of one serving per day resulted in a loss of 0.5 kg at 6 months and of 0.65 kg at 18 months.

Nutrient Dilution

Adding sugars to food will increase their energy density (kcal per unit weight), and because they do not add nutrients, this will result in a net reduction in nutrient content per kcal. This dilutional effect is evidenced in the increasing percentage of individuals who do not meet their RDA for one or more micronutrients as their consumption of added sugars increase. In adolescents, soft drinks tend to displace nutrient-rich milk and juices. Data from the US population indicate that this dilutional effect starts at around 20% of sugars in the diet, and becomes significant at around 25%. Thus, this level has been defined as the 'maximum' acceptable intake.

Some investigators have suggested that HFCS, the most widely used sweetener for caloric beverages, may have specific adverse effects on health. There is evidence from animal studies that high fructose intake may result in excess total energy intake and eventually liver damage. However, it is unclear whether this evidence is relevant for the US population. Although undoubtedly fructose consumption has increased substantially over the past two decades, this increase is dwarfed by the large increase in total carbohydrate intake. A recent evaluation of national dietary data concluded that fructose represents around 1.2% of total carbohydrate intake.

Dental Caries

Sucrose, glucose, lactose, and fructose are excellent substrates for the first step of caries formation, which involves bacterial fermentation. This process results in acidification and subsequent demineralization of the tooth surface, allowing bacterial invasion. The more substrate available, the more fermentation and subsequent enamel invasion.

In spite of this clear relational pathway, the precise contribution of sucrose intake to dental caries is not simple. Several experts consider that dental hygiene is a more powerful determinant of cavity prevalence than sucrose intake. For example, the National Health and Nutrition Examination Survey III (NHANES III) from the US showed no correlation between sucrose intake and dental caries in people under the age of 25 years, who were born after widespread use of fluoride.

Conversely, the association is found in older people, before fluoridation was common. In studies in the UK, the correlation between socioeconomic status and caries was found to be three times that between sugar intake and caries, indicating a strong effect of dental hygiene and health practices in general. Thus, although the role of sucrose in the causative pathway of dental caries is unquestionable, it seems clear that there are other modulating factors that have come to the forefront in contemporary society, namely the use of fluoride and better oral hygiene practices. Nevertheless, sucrose continues to be one of the factors involved in caries formation, perhaps with more relevance to younger children. This is also affected by the food source, for example, hard candies that remain in the oral cavity for some time may allow longer periods of exposure than other sources of sucrose.

Type 2 Diabetes

The temporal association between the increase in consumption of refined carbohydrates and in the prevalence of type 2 diabetes (T2D) in the US has prompted interest in demonstrating this association as a causative factor. Although many cross-sectional studies have found no association, it is clear that in order to detect these types of diet-disease interrelationships large, long-term longitudinal studies are needed, and there are only a few of these. One such study, based on the Nurses' Health cohort, found a positive association between consumption of sugar-sweetened beverages and incidence of T2D. Women consuming > 1 caloric beverage per day had an 80% higher risk of developing T2D. About half of this effect is mediated by differences in BMI. A similar study in African-American women found comparable correlations.

Cardiovascular Disease

Consumption of refined sugars may affect cardiovascular risk by adversely altering the blood lipid profile, particularly increasing triacylglycerol concentrations. In addition, animal studies have shown that high fructose intake increases systemic blood pressure. In US national health surveys, there is a positive association between level of sugar beverage intake and blood pressure. In the Nurses' Health cohort, researchers reported that those consuming > 2 sugar-sweetened drinks per day had a 35% higher risk of developing coronary heart disease (fatal or non-fat myocardial infarction) than those consuming < 1 per month. Data from the PREMIER trial showed that reducing intake of sugar-sweetened beverages in overweight adults result in significant and consistent reductions in systolic and diastolic blood pressure.

Summary

Although some of the putative adverse effects of sucrose require further study, there is consistent evidence that added sugars facilitate excess energy intake, resulting in excess body weight, particularly when consumed as sweetened beverages. Sugar-sweetened beverages also appear to be another

independent risk factor for T2D, high blood pressure, and cardiovascular disease.

It is also alarming that a food item of very low nutrient value such as caloric drinks is providing almost 1 of every 5 calories consumed daily. Given that obesity is a major global public health problem, most experts advise to reduce consumption of added sugars as a means to avoid excess weight gain and other health risks. A recent WHO report has recommended that added sugars should not exceed 10% of total calories. Furthermore, there is ample consensus recommending that most of the carbohydrates in a healthy diet should be of the complex type, for which there is solid evidence of benefits to health maintenance and disease prevention.

See also: Adolescents: Requirements for Growth and Optimal Health. Dental Disease: Etiology and Epidemiology. Diabetes Mellitus: Classification and Chemical Pathology; Dietary Management; Etiology and Epidemiology. Fructose: Absorption and Metabolism. Glucose: Chemistry and Dietary Sources

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SUPPLEMENTATION

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Developed Countries

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Prevalence of Micronutrient Supplement Use

In many affluent countries, vitamins and minerals are the most widely used dietary supplements. In the USA, sales of all dietary supplements in the United States totaled an estimated \$26.9 billion in 2009. This amount included \$11.3 billion for all vitamin- and mineral-containing supplements, of which \$4.8 billion was for multivitamin/mineral supplements.

Findings by several research groups show that micronutrient supplement use is generally more common among individuals with higher education levels, higher incomes, and better diets. Survey results in the Netherlands indicate that micronutrient supplements are used by approximately 20% of adults in that country, fewer than in the US. Data collected in the 1988–1994 National Health and Nutrition Examination Survey (NHANES III) showed that approximately 40% of the US population 2 months of age and older (44% females vs 35% males) were taking a vitamin, mineral, or other type of dietary supplement during the month before the NHANES III interview. Data from NHANES 1999–2000 indicate that 52% of US adults were taking at least one dietary supplement. Supplement users were more likely to be toddlers and preschool-aged children and middle-aged and older adults. Across all age groups, vitamin/mineral combinations and multivitamins were the most common types of supplements used by individuals who took only one supplement. Collection of these types of data is important to monitor use, identify usage trends, and help understand the popularity of micronutrient supplement use.

[†]Deceased.

Motivation for Micronutrient Supplement Use

People choose to use micronutrient supplements for various reasons. Survey data indicate that many individuals decide to take micronutrient supplements based on advice from health professionals, family, and friends. A majority of supplement users regard micronutrient supplements as insurance against general poor health or becoming ill, even though they recognize that scientific evidence for this belief may be lacking. Generally, individuals report that they use supplements either because they think that it is difficult to consume a balanced diet or because they believe that even consuming a balanced diet cannot supply the quantity of micronutrients they need for optimal good health.

Major health reasons given for taking supplements include a sense of well-being and feeling better (especially multivitamins/minerals), preventing colds and flu (especially vitamin C), preventing chronic disease (especially vitamin E and calcium), increasing energy, coping with stress, and improving the immune system. Many vitamin E users believe that it helps prevent heart disease, and most calcium users know that calcium use helps prevent osteoporosis. Using micronutrient supplements is one way by which persons who may be at a high risk for certain diseases try to gain some degree of personal control over their health outcomes. Ironically, many individuals who take supplements regularly report that they do not discuss the supplement use with their physicians because they believe that physicians are biased against supplements and are not knowledgeable about the products.

Research Approach for Determining the Health Impact of Micronutrient Supplements

A micronutrient supplement will be beneficial to a person's health only when the person's normal dietary micronutrient intake is lower than the amount required for maximum biological benefit. Every person does not have the same micronutrient requirements. The amount of micronutrients required by any person is determined by metabolic, genetic, and environmental factors unique to that person. It might not be readily apparent when micronutrient supplements are needed by certain groups of individuals. Therefore, as new information becomes available, recommendations for supplementation must be revised. For example, it was observed that pregnant women with periconceptual folate intake at the low end of the range of recommended intake, which was still considered adequate, had an increased risk of giving birth to an infant with neural tube defects (NTDs) such as *spina bifida*. NTDs originate during the first 4 weeks of pregnancy, before a woman may even realize that she is pregnant. US survey data (1988–1994) indicated that typical dietary folate intake by women of reproductive age was less than the $400 \mu\text{g day}^{-1}$, believed to be required to reduce the risk of NTDs. Therefore, in 1992, the Centers for Disease Control and Prevention recommended that all women who could become pregnant should take a daily $400 \mu\text{g}$ folic acid supplement as a preventive measure. In addition, the FDA mandated that, as of January 1998, enriched grain products must be fortified with folic acid, adding an estimated $100 \mu\text{g}$ folic acid per day to the average diet of US women. Fortification refers to adding nutrients to commonly consumed foods at levels greater than those that are part of the standards of identity for the foods; other examples of fortification are vitamin D in milk and calcium in orange juice.

Any recommendations for supplementation must be based on scientific evidence that the supplements are both effective and safe. Ideally, a rigorous systematic research approach (Table 1) is carried out and the results are evaluated to assess whether a micronutrient supplement is beneficial to health and whether its recommendation is warranted. All available evidence, including epidemiologic and survey data, as well as preclinical evidence from *in vitro* laboratory research and *in vivo* animal studies, is reviewed thoroughly and objectively to determine whether the evidence regarding effectiveness and safety justifies proceeding to clinical trials. If so, the trials are normally conducted in three phases: (1) human safety trials; (2) small efficacy trials, usually in defined target groups; and (3) large-scale trials that are essential in moving from the basic science to evidence-based recommendations that have human health benefits. In fact, the large-scale, double-blind, randomized, placebo-controlled clinical trial, which is designed to eliminate all possible bias, is considered to be the gold standard of scientific intervention research. In such trials, some individuals receive the substance being tested (e.g., drug, micronutrient, or other dietary constituent) and some receive an inactive placebo. These trials may not be possible under all circumstances however, because of ethical issues that make it inappropriate to withhold the substance being tested from any trial participant. For example, now that it is established that low periconceptual folate intake by women is linked to NTDs,

Table 1 Components of a research approach to evaluate dietary micronutrient supplements

Basic biomedical laboratory research
<i>In vitro</i> experiments (e.g., in cell culture and tissue culture)
<i>In vivo</i> animal experiments (e.g., in mice and rats)
Human observational epidemiologic studies to identify possible links between micronutrients and nutrition/health status (includes surveys of micronutrient intake)
Hypothesis development: Evaluation of existing laboratory and epidemiologic evidence on micronutrient safety and effectiveness as related to human health benefits (decision point: proceed or do not proceed)
If proceeding
Human safety trials to identify adverse side effects and determine safe doses
Small trials in defined populations to measure micronutrient effectiveness at various safe doses (e.g., vitamin D supplementation in elderly Scandinavians with low serum 25-hydroxy-vitamin D)
Large-scale, double-blind, placebo-controlled, randomized clinical intervention trials to test whether micronutrient supplementation has the hypothesized human health benefit
After health benefits are confirmed, develop recommendation for supplementation

a placebo-controlled intervention trial to test the minimum effective supplemental amount would be unethical. In such cases, all available evidence from *in vitro* laboratory research and *in vivo* animal studies, as well as epidemiologic studies and surveys, must be reviewed systematically and objectively to draw conclusions about the possible effectiveness and safety of the substance of interest and to make recommendations for supplementation. However, convincing evidence is currently unavailable to indicate that lowering homocysteine through folate and other vitamin (vitamin B₆ and B₁₂) supplementation will reduce the risk of cardiovascular disease (CVD). A number of randomized, placebo-controlled clinical trials are ongoing to test the effects of vitamin supplementation on the primary and secondary prevention of CVD and stroke.

Research to determine a possible impact of micronutrient supplements on the nutritional status and health status of individuals has been under way for many years. Considerable preclinical evidence related to human health effects from *in vitro* laboratory research and *in vivo* animal studies exists for many micronutrients. In addition, many epidemiologic studies throughout the world have focused on the possible relationship between specific micronutrients and chronic disease. Small clinical studies related to chronic disease also have been carried out for many micronutrients, and human safety data are available for most micronutrients. A comprehensive review of epidemiologic studies and randomized controlled trials of vitamin supplementation to prevent either cancer or CVD was conducted by the US Preventive Services Task Force. The Task Force concluded that the findings did not demonstrate a consistent or a significant effect of any single vitamin or combination of vitamins on either the incidence of CVD or death from this disease. Also, the Task Force concluded that β -carotene supplements and combinations including

β -carotene appeared to be harmful to those at risk for lung cancer but not to the general population.

Important issues to be addressed in research aimed at determining the effects of micronutrient supplements on health include developing better methods to measure the contribution of micronutrient supplements to total micronutrient intake for various population groups and to monitor these contributions over time to identify usage trends. Having accurate data for micronutrient supplement intake and intake trends is essential to help identify the possible associations between supplements and health outcomes; such associations can then be tested for validity in future randomized, controlled trials. Collecting data to measure and ultimately monitor consumer use of micronutrient supplements can be expensive and time-consuming however, particularly if detailed data are required. Currently, in the US, NHANES interviewers collect the most detailed information about micronutrient supplement intake, including data on supplement brand, labeled ingredients, dose, and frequency of dose. Available dietary supplement databases are based on values declared on product labels rather than direct analysis. Evidence suggests, however, that supplement labels may not always give the true supplement content; this can decrease the accuracy of survey results.

A major concern associated with clinical trials designed to evaluate the health effects of micronutrients (as well as other dietary supplements and drugs) is that participants might take additional micronutrient supplements, which could influence trial outcomes. In the Prostate Cancer Prevention Trial (PCPT) of the drug finasteride, for example, almost half of the participants reported using a multivitamin/mineral supplement, approximately one-third used single supplements of either vitamin C or E, and one in five used calcium supplements. Very little evidence is available on how individual micronutrient substances may interact with one another to influence health outcomes. For minerals, particularly, supplementation with one mineral may compromise the bioavailability of another. Also, much remains to be learned about how individual genetic susceptibilities may influence the health-related effects of micronutrient supplements. This issue also must be addressed when designing clinical trials.

Evidence Supporting Recommendations for Micronutrient Supplement Use

Importance of Life Cycle

Evaluation of existing evidence related to the effects of micronutrient supplements on nutrition and health, aimed at formulating recommendations for supplementation, must take into account the influence of a person's stage of life and general health status on the absorption, usefulness, and need for any particular micronutrient. Physiological needs for specific micronutrients and, consequently, for micronutrient supplements differ at various stages in the life cycle. For example, infants require additional iron after 6 months of age, women who may become pregnant benefit from additional folate, and elderly persons who lose their ability to absorb naturally occurring vitamin B₁₂ in food require an alternative source of the vitamin. When studies are designed to investigate the relationship between micronutrient supplements and specific health outcomes, the outcomes that are chosen to be measured usually depend on the specific life cycle stage of the study participants. For any life cycle stage, a person's genetic makeup and lifestyle behaviors will also influence his or her individual micronutrient requirements (Figure 1).

Infants

Iron

Iron is a component of a number of proteins including hemoglobin, which is essential for transporting oxygen to tissues throughout the body for use in metabolic processes. The most well-known consequence of iron deficiency is anemia. A full-term infant normally has a high hemoglobin concentration and a large amount of stored iron. Based on research evidence, this stored iron plus the iron provided in human milk is assumed to be adequate for solely breast-fed infants during the first 6 months after birth. Even though the amount of iron in human milk is low, its bioavailability is greater (>50%) than that of the iron in infant formula (<12%). The body stores of iron in infants decrease during

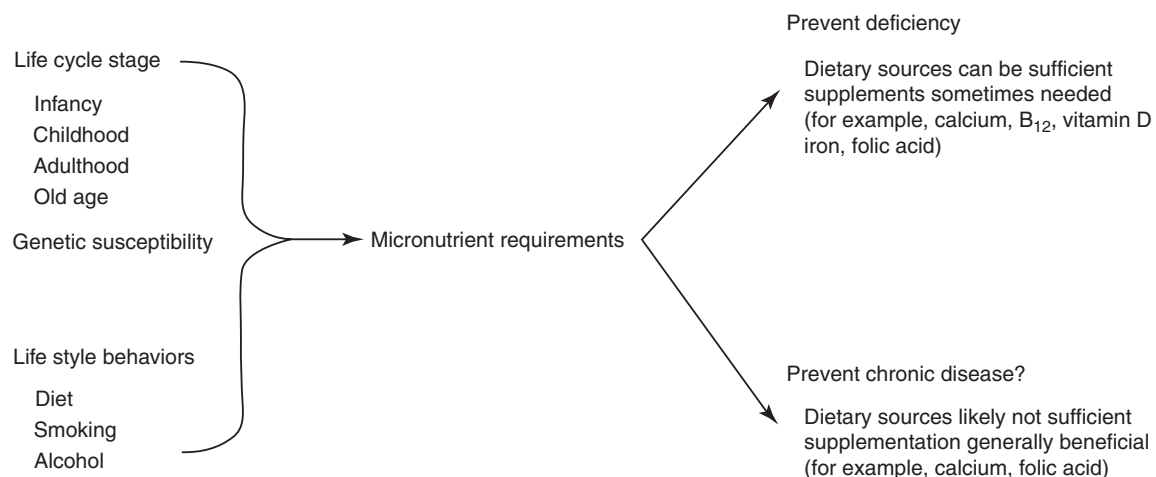


Figure 1 Factors that influence micronutrient requirements.

the fourth through sixth months after birth. After 6 months of age, most of the infant's iron needs must be met from food intake. In Western countries, the primary food introduced after 6 months is infant cereal, usually fortified with iron that has low bioavailability. Evidence suggests that infants benefit from iron supplementation after 6 months, and that administration of iron drops between 6 and 9 months has a significant influence on iron status. The American Academy of Pediatrics (AAP) discourages using low-iron infant formulas. AAP recommends that infants who are not breast-fed or who are only partially breast-fed should receive an iron-fortified formula from birth to 12 months of age.

Vitamin D

Vitamin D enhances the efficiency of the small intestine to absorb calcium and phosphorus from the diet and thus helps to maintain normal serum levels of these minerals. Vitamin D deficiency in infants and children results in inadequate mineralization of the skeleton, causing rickets, which is characterized by various bone deformations. The major source of vitamin D is its formation in the skin as a result of exposure to sunlight. Dietary sources include fortified foods, such as milk and cereals, and certain fish. Infant formula is fortified with vitamin D in many countries. Because human milk contains only low amounts of vitamin D, breast-fed infants who do not receive either supplemental vitamin D or adequate exposure to sunlight are at risk for developing vitamin D deficiency. Subclinical vitamin D deficiency can be assessed by measuring serum 25-hydroxyl-vitamin D; deficiency occurs months before rickets is obvious on physical examination. Rickets in infants continues to be reported in the US as well as in other countries. Epidemiologic evidence indicates that African-American infants and children are more likely to develop nutritional rickets than Caucasian infants and children. In the US, the AAP recommends that all breast-fed infants receive a daily supplement of 200 IU vitamin D per day⁻¹, beginning within the first 2 months of life, unless they are weaned to at least 500 ml day⁻¹ of vitamin D-fortified formula (<1 year old) or milk (>1 year old).

Children

Calcium

Bone is a dynamic tissue that is constantly being formed and resorbed; in children, bone formation is greater than

resorption. Adequate calcium intake during childhood is essential for bone mass development. Data for calcium intake, presented in **Table 2**, indicate that for children in the US, only those younger than 8 years of age are meeting their recommended intake. Factors that may contribute to low-calcium intake are restriction of dairy products, low vegetable consumption, and high intake of low-calcium beverages such as juices and sodas. The highest calcium intake levels are required during the preteen and adolescent years to support the rapid growth and bone mineralization associated with pubertal development. In girls, peak calcium absorption and deposition takes place at or near menarche; at this life cycle stage, the bone calcium deposition rate is five-times greater than that in adults. During peak bone mass development, calcium intakes of less than 1000 mg day⁻¹ are associated with lower bone mineral density. Epidemiologic studies have found a direct correlation between calcium intake and bone density in children. Evidence suggests that low intake of dairy products during childhood and adolescence may result in less bone mass and greater risk of fracture as an adult. In addition, evidence from randomized trials suggests that increasing the calcium intake of girls is associated with increased bone mineral deposition, especially during prepuberty. Although it is best to obtain as much calcium as possible from foods, because calcium-rich foods also provide nutrients involved in calcium utilization, calcium supplements may be necessary for children who do not eat calcium-rich foods.

Adults

Vitamin E

Vitamin E (α -tocopherol) functions as an antioxidant that promotes normal formation of red blood cells and normal function of the nervous and immune systems. The main dietary sources of vitamin E are vegetable oils; normally, it is possible, unless individuals consume a very low-fat diet, to obtain amounts of vitamin E intake from foods that are sufficient to prevent signs of deficiency. However, vitamin E is a commonly consumed supplement, likely because of its hypothesized role in decreasing the risk of CVD, prostate cancer, and various other chronic diseases.

Evidence from epidemiologic studies suggests that vitamin E supplementation is beneficial for reducing CVD risk. Nevertheless, data from randomized clinical trials, in populations both with and without a history of CVD, generally do not support the epidemiologic findings. The review of

Table 2 Average calcium intake and recommended adequate intake levels for US children

	Age/gender					
	1–3 years/M and F	4–8 years/M and F	9–13 years/F	9–13 years/M	14–18 years/F	14–18 years/M
Estimated intake (mg)	793	838	918	1025	753	1169
Estimated average requirements (mg)	500	800	1300	1300	1300	1300

F, female; M, male.

Source: Intakes from Institute of Medicine (1997) *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium, Vitamin D, and Fluoride*. Washington, DC: National Academy Press. Requirements from IoM 2010.

evidence by the US Preventive Services Task Force included five well-designed, large cohort studies that investigated the association between vitamin supplementation and CVD mortality, three clinical trials of primary prevention of CVD, and seven clinical trials of secondary prevention of cardiac events. As stated earlier, the Task Force concluded that the findings did not demonstrate a consistent or a significant effect of vitamin E on either the incidence of CVD or death from this disease. Four large clinical trials are currently in progress in the US to study the effect on CVD of vitamin E supplements alone or combined with other antioxidants: the Women's Health Study, the Women's Antioxidant and Cardiovascular Study, the Physicians' Health Study II, and the Heart Protection study.

Laboratory studies suggest that vitamin E can inhibit the growth of human prostate cancer cell lines. Results of epidemiologic studies, however, do not consistently support a beneficial effect of vitamin E on the risk for prostate cancer. Findings from the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) study, a large, randomized clinical trial conducted in Finland, suggest a substantial benefit of vitamin E in decreasing prostate cancer risk. This study reported a decrease of 32% in prostate cancer incidence and a decrease of 41% in deaths from prostate cancer among current and former male smokers who received supplemental vitamin E (50 mg day⁻¹). Additional information on the relationship between vitamin E supplementation and prostate cancer likely will be available from the PCPT, which was stopped in June 2003 when analysis showed that the test drug, finasteride, reduced the risk of developing prostate cancer by 25%. In PCPT, 35% of the study population took vitamin E supplements, and study analyses will include interactions between vitamin E and other supplements and between vitamin E and finasteride. The Selenium and Vitamin E Prevention Trial (SELECT) is also expected to help clarify the association between vitamin E and prostate cancer.

Selenium

Selenium, a strong antioxidant, also shows other biological activity, such as enhancing the immune response and inhibiting cell growth. Laboratory and epidemiologic studies support a beneficial effect of selenium on the risk of cancer. In a large clinical trial, selenium supplementation did not prevent the recurrence of nonmelanoma skin cancer, but it did significantly decrease the total number of deaths and deaths from cancer. In addition, the incidences of prostate, colorectal, and lung cancers all were significantly decreased in the group that received selenium supplements. These findings and the results of the ATBC study linking vitamin E supplementation with decreased prostate cancer risk led to the development of SELECT. Started in 2001, SELECT is a randomized, double-blind trial designed to test whether selenium (200 µg day⁻¹) alone, vitamin E (400 mg day⁻¹) alone, or selenium and vitamin E combined reduce the risk of prostate cancer among healthy men. Men who join SELECT are required to stop taking any purchased vitamin supplements that contain either selenium or vitamin E. An ongoing intervention trial in France, the Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) study, is testing the nutritional levels of both selenium and vitamin E, as

well as vitamin C, β -carotene, and zinc, for reducing the incidence of cancer and CVD. In addition to cancer and CVD, French researchers are investigating a possible beneficial role for selenium in arthritis and HIV/AIDS.

Folate

Folate, a B-complex vitamin, includes the naturally occurring form found in foods as well as the synthetic form (folic acid) found in fortified foods and supplements. The rationale for the recommendation that all women who may become pregnant should take daily 400 µg folic acid supplement, a preventive measure to reduce the risk of NTDs, has already been discussed in the section Research Approach for Determining the Health Impact of Micronutrient Supplements. Folate intake is important throughout pregnancy because of its key role in nucleic acid synthesis, which is essential for cell growth and replication.

A deficiency of folate, vitamin B₁₂, or vitamin B₆ may increase the level of homocysteine, an amino acid normally found in the blood. Evidence indicates that a high homocysteine level increases the risk for CVD and stroke, possibly by either damaging coronary arteries or making it easier for blood platelets to clump together and form a clot. However, no evidence is available to suggest that lowering homocysteine through vitamin supplementation will reduce the risk of CVD. Clinical intervention trials to test the effects of vitamin supplementation on CVD and stroke are needed.

Because folate is involved in the synthesis, repair, and functioning of DNA, some have hypothesized that a deficiency of folate may result in DNA damage that can lead to cancer. A comprehensive review of epidemiologic, preclinical, and clinical evidence linking folate deficiency with increased cancer risk concluded that the evidence is strongest for colorectal cancer. Also, it has been suggested that folate deficiency may increase the effects of other cancer risk factors. Researchers are continuing to investigate whether increasing folate intake from foods or folic acid supplements may reduce cancer risk.

Folate is important for cells and tissues that divide rapidly; therefore, high-dose methotrexate is often used to treat cancer because this compound interferes with folate metabolism. Methotrexate, however, has undesirable side effects, including inflammation in the digestive tract. It is not known whether folic acid supplementation can help control these side effects without decreasing the effectiveness of methotrexate. Low-dose methotrexate is used to treat a variety of diseases, such as rheumatoid arthritis, lupus, psoriasis, asthma, and inflammatory bowel disease. Low-dose treatment can deplete folate stores and cause side effects similar to folate deficiency. In this case, supplemental folic acid may help reduce the undesirable effects of low-dose methotrexate without decreasing treatment effectiveness.

Calcium

Bone formation and resorption are balanced in healthy adults, but formation becomes slower than resorption after menopause and also with aging in both men and women. In menopausal women, decreased estrogen production is

associated with accelerated bone loss in the first 5 years after menopause, particularly from the lumbar spine. Evidence indicates that although increasing calcium intake at menopause does not prevent this bone loss, it is beneficial for reducing bone loss in compact bones (e.g., hips, legs, and arms). Furthermore, data suggest that calcium supplementation also reduces lumbar spine bone loss in women who are more than 5 years beyond menopause. In the US, the recommended calcium intake is 1000 mg day⁻¹ for men and women aged 19–50 years and 1200 mg day⁻¹ for men and women aged 51–70 years. Individuals who are not able to obtain this amount of calcium from foods should consider taking calcium supplements to help decrease the risk of reduced bone mass and osteoporosis.

Elderly

Physiological changes that may occur during the natural course of aging can affect micronutrient requirements. Given the same amount of sun exposure, the skin of young adults synthesizes much more vitamin D than the skin of the elderly; thus, choosing good dietary sources of vitamin D becomes essential. Vitamin D deficiency can be a factor in reduced calcium absorption in the elderly. Furthermore, it is estimated that atrophic gastritis, a change in gastrointestinal physiology that results in low-acid conditions in the stomach, is present in approximately 20% of elderly individuals. Atrophic gastritis has been related to infection with the bacterium *Helicobacter pylori* and is not necessarily a result of normal aging. The low-acid conditions, however, can decrease the absorption of vitamin B₁₂ from food and of folate and calcium in general.

Vitamin D

Vitamin D is important in the elderly for enhancing calcium absorption, inhibiting cellular growth, and activating lymphocyte function. Vitamin D deficiency may lead to osteoporosis and osteomalacia and possibly increase the risk for some cancers; it has been associated with increased incidence of hip fractures. More than 50% of elderly persons have been reported to be vitamin D deficient in some studies. In addition to the skin's decreased ability to synthesize vitamin D as individual's age, the kidneys, which help to convert vitamin D to its active form, sometimes do not function as well when people age. All elderly persons, particularly those with limited sun exposure, such as those who either are homebound or live in northern latitudes, should include vitamin D-fortified foods and fish in their diets. If elderly persons are unable to meet their vitamin D needs using dietary sources, they may require a supplement. Evidence suggests that vitamin D supplementation may reduce the risk of osteoporotic fractures in elderly persons with low serum levels of vitamin D.

Vitamin B₁₂

Vitamin B₁₂ is essential for proper brain and nerve development and for DNA synthesis; also, it improves learning and

supports methylation metabolism. Dietary vitamin B₁₂ must be separated from food proteins before the vitamin can be bound to intrinsic factor and then be absorbed by the body. Under low-acid conditions in the stomach, neither the separation from protein nor the binding to intrinsic factor can take place, significantly decreasing the bioavailability of vitamin B₁₂. Elderly adults with atrophic gastritis and low stomach acid should consume a source of unbound vitamin B₁₂ such as that found in supplements or food that has been fortified with the vitamin to ensure adequate intake. In addition, evidence suggests that the use of antibiotics can improve vitamin B₁₂ absorption in these elderly adults.

Folate

Atrophic gastritis greatly reduces the ability of elderly persons to absorb folate. This problem can be corrected by administering folic acid with dilute hydrochloric acid to increase stomach acidity and thus increase absorption. There is concern, however, about the possibility that supplemental folic acid could mask the signs of vitamin B₁₂ deficiency. Folic acid can remedy the anemia that results from vitamin B₁₂ deficiency, its key diagnostic sign. It cannot, however, correct the permanent nerve damage that is possible if vitamin B₁₂ deficiency is not treated. Intake of supplemental folic acid should not be greater than 1000 µg day⁻¹ to prevent the masking of signs of vitamin B₁₂ deficiency.

Calcium

Adequate calcium intake is required to maintain bone mineral density and reduce the risk of osteoporosis in the elderly. In addition to the reduced absorption of calcium that results from age-related changes in vitamin D metabolism, the elderly also show a reduced ability to increase the efficiency of calcium absorption as an adaptive response to low-calcium diets. Also, as noted earlier, the low-acid conditions resulting from atrophic gastritis can reduce calcium absorption. Dietary calcium reacts with hydrochloric acid in the stomach to form soluble calcium chloride, which is absorbed in the small intestine. In the US, the recommended calcium intake is 1200 mg day⁻¹ for men and women older than 70 years. Many elderly persons may benefit from calcium supplements.

See also: Adolescents: Requirements for Growth and Optimal Health. Aging. Children: Nutritional Requirements. Folic Acid. Nutritional Requirements of Infants. Older People: Nutritional Requirements. Supplementation: Developing Countries; Dietary Supplements

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Developing Countries

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Glossary

Anemia Low blood hemoglobin levels, mostly usually caused by iron deficiency.

Child Health Days Outreach strategy allowing high coverage of interventions such as vaccines and supplements during quarterly or six monthly extension of specific services into villages on set days.

Micronutrient supplementation The provision of micronutrients as pills, capsules, or syrups, for periodic consumption.

Randomized controlled trials Experiments carried out with a control or placebo group that is randomly and blindly assigned.

Therapeutic supplements Supplements with a dose larger than the daily requirement, used to treat micronutrient deficiency.

Introduction

Micronutrient supplementation is the distribution of specially formulated preparations of one or more micronutrients, usually in the form of a pill, a capsule, or sirup. It is often described as a 'short-term' option and a 'medical' approach and considered more appropriate for the treatment of severe micronutrient deficiencies in those most affected than to prevent deficiencies in whole populations. However, for the half of humanity affected by micronutrient deficiencies, especially of iodine, vitamin A, iron and zinc, the overwhelming majority of whom are the poor concentrated in the developing world, solving these problems through food-based approaches will take some time.

Vitamin A deficiency affects 30% of children younger than 5 years old in the developing world, compromising their immune systems and potentially contributing to some 1 million deaths each year. In the 6- to 24-month-old age group, mental development is impaired due to iron deficiency in 60% of the developing world's children. Severe iron deficiency also causes more than 60 000 deaths of women during pregnancy and childbirth every year, and 40% of pregnant women suffer from anemia, which is largely caused by iron deficiency. Approximately 18 million infants per year are born mentally impaired as a result of iodine deficiency during pregnancy despite the enormous advances in salt iodization programs. Providing vulnerable groups, such as children and women of child-bearing age, with low-cost vitamin and mineral supplements is the least that governments can do to protect the survival, growth, and development of the next generation as a first step toward realizing the right of every individual to be adequately nourished.

Experience in achieving high coverage of those most at risk with micronutrient supplements is quite varied, with both successes and failures. A good communication strategy is an essential part of achieving high levels of adherence in micronutrient supplementation programs, but these aspects are not particular to nutrition programs and are not considered here. Deficiencies of iodine, iron, vitamin A, and folate are the most commonly recognized deficiencies for which there are programs, but in practice most of those affected have multiple vitamin and mineral deficiencies that overlap and interact at

great cost. This article reviews the policy dimensions of the efforts to establish programs aimed at eliminating iodine deficiency, iron deficiency anemia, and vitamin A deficiency through supplementation, and it provides a perspective on zinc supplementation and multiple micronutrient supplementation as future components of nutrition programs in developing countries.

Iodine Supplementation

Today, approximately 70% of the world's salt is iodized, compared to just 10% in 1990, and therefore the need for iodine supplementation programs is greatly reduced. Despite this progress almost 2 billion people still have low urinary iodine levels, and in these populations iodine supplements should be used during pregnancy and early childhood. In the past, it was common to provide annual intramuscular injections of iodized oil to women of reproductive age in order to ensure iodine status during the first months of pregnancy when the risk of cretinism is greatest. In more recent years, oral iodized oil capsules have proven to be as efficacious and more effective in controlling iodine deficiency in both women of reproductive age and schoolchildren. Oral iodine supplements initially based on expensive poppy seed oil have since been replaced by cheaper rapeseed and peanut oil preparations, which are equally effective.

Vitamin A Supplementation

The use of supplements to help control vitamin A deficiency has grown enormously during the past two decades. Although the elimination of vitamin A deficiency by the year 2000 was one of the goals set at the World Summit for Children in 1990, little progress was evident at mid-decade. Clinical vitamin A deficiency was estimated to affect approximately 3.3 million children younger than the age of 5 years in 1995, with an additional 100 million subject to subclinical deficiency. The periodic distribution of high-dose vitamin A supplements, originally employed in Indonesia during the 1970s for the prevention of blindness in children, was shown in the 1980s

to also impact young child mortality. However, the lack of perception of vitamin A deficiency as a problem was a substantial barrier to establishing large-scale preventive supplementation programs. The prevalence of clinical signs of frank vitamin A deficiency, such as Bitot's spot and corneal lesions, which make it a 'public health problem', is very small at just 0.5%. Because clinical signs are often more common in rural populations, a significant vitamin A deficiency problem can easily go undetected. National representative surveys were thus a prerequisite for taking action.

Convincing proof of the efficacy of vitamin A capsules for child mortality reduction in the early 1990s helped to create increased momentum for population wide preventive supplementation programs. The turning point for increasing the coverage of vitamin A supplements was the meta-analysis of eight efficacy trials, which indicated that improving the vitamin A status of children aged 6 months to 5 years by massive-dose capsule distribution reduced child mortality rates by approximately 23%. The important conclusion of these meta-analyses was that increased risk of mortality from vitamin A deficiency was not just limited to those portions of the population with severe vitamin A deficiency problems but was present across the whole population distribution. Further subsequent meta-analysis carried out over the past two decades have all confirmed these findings.

What consisted of 'the justification' for carrying out vitamin A supplementation programs evolved rapidly during the latter half of the 1990s. Many of these discussions were held at the meetings of the International Vitamin A Consultative Group and the working group on vitamin A of the Standing Committee on Nutrition of the United Nations. A broad technical consensus was finally accepted that even in the absence of survey data, it was highly likely that the benefits of vitamin A supplements would be evident in populations in which the mortality rates for those younger than 5 years old were higher than 70 per 1000. Before this, vitamin A supplements were targeted to those children with illnesses such as measles and diarrhea. Subsequent to this consensus, a global policy to integrate vitamin A capsule distribution into regular immunization schedules, and also to incorporate vitamin A capsules into the national immunization programs was rapidly adopted.

Programmatic vitamin A interventions received considerable impetus from the Vitamin A Global Initiative, an informal interagency advocacy group that worked to promote the adoption of vitamin A supplementation programs. The initiative included World Health Organization (WHO) and United Nations International Children's Emergency Fund (UNICEF), together with Canadian International Development Agency (CIDA) from Canada, DIFID from UK, United States Agency for International Development (USAID) from USA, and the Micronutrient Initiative (MI). Through their networks, these various organizations worked together to convince governments with high mortality rates for children younger than age 5 years to introduce periodic vitamin A capsule distribution programs.

By the end of the 1990s, vitamin A supplementation programs had seen a remarkable expansion, which has been maintained during the first decade of the new millennium. The number of countries with vitamin A programs increased

from 10 in 1995 to 72 in 2000, and 103 in 2004. These are mostly countries with high mortality rates for children younger than 5 years old and/or where vitamin A deficiency is a public health problem. The ways in which the vitamin A capsule programs were developed and implemented have varied by country and over time. The most common strategy was to use national immunization days for polio eradication to piggyback vitamin A supplements, but because the polio eradication strategy required two nationwide campaigns not more than 2 months apart, some countries also promoted separate micronutrient days, or child health days, so that children would get at least two capsules during the course of a year, 6 months apart. For example, as polio eradication has progressed and vaccination ceased, capsules have increasingly been delivered through child health days together with other interventions such as deworming and malaria bed net distribution.

The coverage of vitamin A capsules, which was already high by the turn of the century, has continued to climb during the first decade of the new millennium. Although 50% of children in 103 countries had received one dose in 1999, just 16 percent had received two doses. By 2004 those receiving two doses had climbed to 58% and by 2008 had reached 71% coverage. Extrapolation of the protective effect of a 23% reduction in child mortality shown by the meta-analysis to the increased coverage of capsules in the decade after 1998 suggests that many millions of lives would have been saved. Others have estimated child deaths saved as far fewer than this however, and many have lamented the lack of proof of any such impact. Furthermore despite this high coverage of capsules, the rates of vitamin A deficiency based on serum retinol levels remains high and stable during this period, still affecting some 163 million children or approximately 30%.

The challenge that remains for vitamin A supplementation is one of sustainability. Although supplements have always been viewed as a short-term solution, in reality they need to be maintained as long as mortality rates remain high and no dietary solutions are put in place. Sustaining the provision of the vitamin A capsules is likely to become a problem, as until now, supplements have been provided predominantly by the Canadian government and supplied through UNICEF. Whether governments will eventually pick up these costs remains to be seen. The costs for individual governments to take on are small, however, especially compared to the potential benefits in terms of mortality reduction. However, in most places more effort is also needed to increase access to other sources of vitamin A, be it through diet and/or fortification, so that capsules can be phased out.

Iron/Folate Supplementation

Although iron deficiency is the most widespread of nutritional problems, and despite the existence of policy and programs in most countries, supplementation with iron has not proven to be a very successful intervention. Global policy recommendations to routinely provide iron/folate supplements for women during pregnancy and lactation have changed little in almost three decades. These are that all anemic pregnant women should receive such supplements in almost all

contexts, i.e., there is no alternative to supplements for treating anemia and the recommendation is that at least ninety tablets should be taken during pregnancy to treat anemia. Despite most developing countries having national iron supplementation policies, the World Summit for Children's goal to reduce anemia in women by one-third was given little or no priority by the principal actors involved such that no progress was made during the past decade. In 2007 anemia was estimated to affect 40% of nonpregnant women and 41% of pregnant women in developing countries, virtually the same rates that existed in the nineties.

Although there is ample evidence that iron deficiency and the anemia associated with it are a great burden on society, especially the poor, the advocacy base for pushing for program implementation has remained weak. This is largely because the link of iron deficiency to maternal and child survival has not been concretely proven. The ethical difficulty of doing randomized controlled trials with a placebo group, when all countries have a policy to give iron supplements during pregnancy has contributed to this. However, new evidence from trials in nonanemic women in developed countries find that iron supplements increase birth weight by upward of 100 g compared to placebo, suggesting that iron supplements may well have unsuspected benefits for child survival, growth, and development. The effect of iron deficiency on cognitive deficits in children and on adults later in the life course have long been established. The absolute losses in Southeast Asia are estimated to be approximately \$5 billion annually, and for India the median value of productivity losses due to iron deficiency alone is approximately \$4 per capita or 0.9% of gross domestic product.

Despite high cost effectiveness, little or no priority has been given to iron deficiency anemia reduction programs. At \$0.002 per tablet, the iron supplement is relatively cheap, and the cost per disability adjusted life year of \$13 makes the supplementation of pregnant women with iron a very cost-effective intervention. At the national level, despite the existence of national policies, rarely is there a budget for the provision of supplements and/or supervision of iron deficiency anemia programs. Although UNICEF is a major supplier of iron/folate supplements to the developing world, the level of supply is far lower than that believed to be needed. In the period 1993–1996, 2.7 billion tablets were shipped to 122 countries at a cost of \$7.5 million as part of UNICEF assistance to programs aimed at eliminating maternal anemia. This was less than 5% of that needed to cover all pregnancies in developing countries. There have been few, if any, attempts to gauge the coverage of iron/folate supplements at any level, be it district, national, or international. Neither has there been any effort put into creating political accountability to ensure high coverage.

Many meetings and publications during the past few decades that have examined the causes and solutions of iron deficiency anemia conclude that lack of effectiveness of iron supplementation programs for anemia control is largely related to problems with supply of the supplement. Although the side effects of iron pills are often cited as the reason why iron supplementation programs do not work, this rarely seems to be the case. One of the major causes of nonadherence seems to be lack of understanding of the benefits the

supplements can bring among health staff that deliver the tablets. Most of the program reviews have concluded that where supportive community-level delivery mechanisms are put in place that encourage adherence, and the supply of supplements is ensured, high levels of coverage can be achieved and sustained and anemia controlled. It is often the case, however, that in health systems in developing countries, nutrition is everybody's business and nobody's responsibility, and iron supplements have ended up low on the list of things to do.

Despite an international consensus that supplementation has a key role to play in the control of iron deficiency anemia, and that this will contribute to reducing maternal mortality, there is still little traction in this area. Demographic Health surveys include questions concerning how many mothers took at least 90 iron tablets during their last pregnancy, and very few countries achieve high coverage. In 1998, a technical consensus meeting on what was needed to solve the problem of iron deficiency made the recommendation that although the interventions already existed for reducing both iron deficiency and iron deficiency anemia, more work was needed to develop large-scale programs with packages of interventions delivered through multiple sectors, including hygiene and sanitation, because iron supplements alone will not ensure anemia control in many settings. Infections such as malaria and gastrointestinal parasites are also important causes. Furthermore, a global review of anemia causality revealed that perhaps only half of anemia is solely due to iron deficiency, with other micronutrient deficiencies such as vitamin A contributing as well. To be effective, maternal anemia control programs must include infection control, together with the community delivery of iron supplements.

It is much easier to treat anemia before pregnancy, when supplements can be taken once a week instead of daily. Such approaches have been shown to be very effective among adolescent school girls, for example, as well as in family planning programs. To be effective the supplementation has to be supervised and accompanied by infection control, such as periodic deworming.

Zinc Supplementation

The WHO/UNICEF recommendation is to give supplemental zinc for 10 days as part of the treatment of diarrhea. This is based on strong evidence for the efficacy of therapeutic zinc in improving the prognosis of children being treated for diarrheal disease. A pooled analysis of randomized controlled therapeutic zinc trials in children with diarrhea showed that zinc-supplemented children with acute diarrhea had a 15% lower probability of continuing diarrhea on a given day, and in those with persistent diarrhea there was a 24% lower probability. In addition, children with persistent diarrhea had a 42% lower rate of treatment failure or death if zinc supplemented. Given that even the current interventions included in child health programs for diarrheal disease treatment, such as oral rehydration therapy, face enormous barriers to achieving and maintaining high levels of coverage, the challenge for achieving high levels of coverage of zinc supplements in the treatment of diarrhea is likely to be considerable.

If these efforts are successful however, then the impact is likely to be great. The most effective way to give preventive zinc supplements is an ongoing research question.

Multiple Micronutrient Supplementation

In recent years, the case has increasingly been made for providing multiple micronutrient supplements instead of iron supplements for young children and women of reproductive age in developing countries. A woman's or an infant's diet that is deficient in iron is likely to be deficient in many other micronutrients. Outside of emergency situations, such as natural catastrophes, famine, and civil strife, poor dietary quality rather than quantity is the determinant of inadequate micronutrient status among infants and women. The nutrient-to-energy ratios of iron, zinc, folate, vitamins B₆ and B₁₂, vitamin A, riboflavin, and calcium are commonly below the recommended levels needed, assuming energy needs are met.

The UN agencies agreed the composition of a multiple micronutrient supplement for use among pregnant and lactating women in developing countries a decade ago. The formulation includes 15 micronutrients (vitamins A, D, E, B₁, B₂, B₆, B₁₂, and C, niacin, and folic acid and minerals Fe, Zn, Cu, I, and Se), all at the RDA level, except for folic acid, which was included at the 400-μg level – considered sufficient to prevent neural tube defects if taken periconceptually. The main cost of the delivery of a nutrient supplement for women of reproductive age is not the cost of the supplement but the cost of the delivery system. Although it may not be working very well, a delivery system already exists for the iron/folate supplements that could be used to provide these other micronutrients. Adding the extra nutrients to the iron/folate tablets will not add more than 20% to the cost of the tablet, and although the incremental cost of distributing a multiple micronutrient supplement is likely to be small, the increased benefits may be large. Although the need for micronutrient supplementation in pregnancy is likely to be great because of widespread maternal undernutrition, and the supplements have been recommended for use in populations affected by emergencies, it was recognized that regular public health resources are always limited and priority is given to interventions that are both efficacious and effective. Proving the efficacy of multiple micronutrient supplements is thus essential for being able to advocate for their wide-scale use in nonemergency settings. Tablets of similar composition are regularly prescribed by physicians and/or purchased by mothers in developed countries, and they can be found in the pharmacies of the capitals of most developing countries and are widely consumed by the richer segments of the population.

This multiple micronutrient supplement was then tested in a series of 12 efficacy and six effectiveness trials covering 12 countries and spanning three continents – Asia, Latin America, and sub-Saharan Africa. A meta-analysis of these trials found that both supplements were equally effective in tackling maternal anemia, even though the iron content was often lower in the multiple micronutrient supplement than in the iron-folic acid supplement. There were no significant differences in the rates of stillbirth, early neonatal death, or

neonatal death between the supplemented groups. The small, significant increase in mean birth weight (24 g) among infants of mothers receiving multiple micronutrients compared with infants of mothers receiving iron-folic acid is of similar magnitude to that often produced by food supplementation during pregnancy, and larger micronutrient doses seemed to produce greater impact. Meaningful improvements have also been observed in height and cognitive development by 2 years of age in the children of the mothers from the multiple micronutrient group.

WHO and UNICEF recommend the use of sirup and/or tablets containing iron for the treatment of anemia in young children, and such products are available through UNICEF supply division in Copenhagen. These products have very little penetration considering the size of the infant anemia problem in most developing countries, where half of all children are commonly affected. Despite the recognition that iron deficiency often coexists with zinc deficiency, together with inadequate intakes of other B vitamins (B₆, riboflavin, and niacin) in infant diets, there is no multiple micronutrient supplement available for infants. UNICEF has also been testing the efficacy of a foodlet (a large crumbly pastille that is a cross between a tablet and a food) containing multiple micronutrients during infancy through the Infant Research on Infant Supplementation trials. Trials of multiple micronutrients as preventive supplements have also been carried out by many different groups using supplements provided in the form of sprinkles, tablets, and even as a beverage. Preliminary results of these trials point to a greater impact on anemia and enhancement of multiple micronutrient status by the multiple micronutrient supplements than that of iron supplements, as well as small improvements in growth. There is a need to bring all of this broad spectrum of experimental and programmatic work together to reach conclusions and achieve consensus before policy and program recommendations can be made on how best to include multiple micronutrient supplements in programs to improve maternal and child health in developing countries.

See also: Folic Acid. Iodine: Deficiency Disorders and Prevention Programs; Physiology, Dietary Sources, and Requirements. Iron: Physiology, Dietary Sources, and Requirements. Pregnancy: Nutrient Requirements. Supplementation: Developed Countries. Zinc: Physiology, Dietary Sources, and Requirements

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Dietary Supplements

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Introduction

In 2004, global sales of dietary supplements represented a significant business. Worldwide sales have been estimated at \$70–250 billion. The demand for herbal products worldwide increased at an annual rate of 8% from 1994 to 2001, although this growth has slowed in recent years.

Issues and controversies in the dietary supplement market are related to defining exactly what is a dietary supplement, understanding how sales and marketing data are derived, defining the regulatory environment, safety issues, product quality issues, labeling and health claim issues, and scientific evidence for benefit. This article describes some of these controversies and provides examples to illustrate these issues.

How is the Sales Data Derived?

Global sales have been estimated to be between \$70 billion and \$250 billion. This approximately three-fold difference in estimates is due to the variation in what products are actually included in product sales results. As will be discussed, the definition of dietary supplements varies greatly from country to country; therefore, deriving sales data is complex.

Another difficulty in assessing sales of dietary supplements is the source from which sales data are gathered. Many business surveys rely on only one or two of the following sales outlets to derive their results:

- Supermarkets and mass merchandisers
- Natural food and health food stores
- Direct sales from Internet, mail order, practitioners, and multilevel marketing
- Pharmacies and drugstore chains

What is a Dietary Supplement? How are they Regulated in Different Countries?

Each country has developed regulatory definitions and systems that place dietary supplements, particularly botanicals, into categories of drugs, traditional medicines, or foods. However, in the late 1980s, many countries launched major changes in regulations that may or may not have been approved at the time of this writing. Many regulations are still in draft form.

The US Congress defined the term 'dietary supplement' in the Dietary Supplement Health and Education Act (DSHEA) of 1994. A dietary supplement is a product, taken orally, that contains a 'dietary ingredient' that is intended to supplement the diet. The dietary ingredient includes vitamins, minerals,

herbs or other botanicals, amino acids, a dietary substance for use by man to supplement the diet by increasing the total dietary intake (e.g., enzymes or tissues from organs or glands), or a concentrate, metabolite, constituent, or extract. Dietary supplements may be found in many forms, such as tablets, capsules, softgels, gelcaps, liquids, or powders. They may also be produced in other forms, such as a beverage, spread, or bar, in which case information on the label must clearly state that the product is a dietary supplement and it is not represented as a conventional food or a sole item of a meal or diet.

Whatever their form, DSHEA places dietary supplements in a special category under the general umbrella of 'foods,' not drugs, and requires that every supplement be labeled a dietary supplement and carry a Supplement Facts Label.

In UK, there is a distinct separation of food supplements and herbal medicines. The Food Standards Agency developed the Food Standard Act of 1999 and is responsible for protection of public health. The Food Supplement Directive 2002/46/EC, which harmonizes European Community legislation on food supplements, was published in 2002. This directive is stricter than existing UK standards and regulations but is relatively more liberal than that which exists in other European countries. The directive defines the term 'food supplements,' contains a list of vitamin and mineral sources that may be used in the manufacture of food supplements, states labeling requirements, and, in the future, will provide a framework for maximum and minimum levels for vitamins and minerals in food supplements. Herbals and botanicals are not discussed in this directive.

The Foods Supplement Directive defines a food supplement as any food the purpose of which is to supplement the normal diet and which is a concentrated source of a vitamin or mineral or other substance with a nutritional or physiological effect, alone or in combination, and is sold in dose form. Dose form means capsules, pastilles, tablets, pills, and other similar forms, and also powders, ampoules, drops, or other similar forms of liquids or powders, designed to be taken in small measured quantities. Because the directive defines a food supplement as something to supplement the diet, products that are not meant to supplement the diet (e.g., a weight loss product) are outside the scope of the regulations. There remains a complex legal area between food supplements and medicinal products, although the directive indicates that if a product is used for treating or preventing disease, or restoring, correcting, or modifying a physiological function, then it falls under the Medicines Directive 2001/83/EEC, Medicines Act 1968, or Medicines for Human Use Regulations 1994.

The Transatlantic Business Dialogue (TABD) approved a position statement regarding dietary supplements in 2002. The TABD is a group of corporations that promote closer

commercial ties between the European Union and the United States. This position statement established industrywide consensus on standards and definition of permissible claims, as well as defining what is necessary for substantiation of those claims. In keeping with the Foods Supplement Directive, the TABD dealt only with vitamins and minerals, with the understanding that some of the conclusions may be revisited when warranted for herbals, botanicals, or other dietary supplements.

Herbal medicines, however, are regulated by the Medicine and HealthCare Products Regulatory Agency based in London. A herbal remedy is defined as

a medicinal product consisting of a substance produced by subjecting a plant or plants to drying, crushing or any other process, or of a mixture whose sole ingredients are two or more substances so produced, or of a mixture whose sole ingredients are one or more substances so produced and water or some other inert substance.

There are two alternative regulatory routes in UK for herbal medicines: Licensing and exemption from licensing requirements:

- Licensed herbal medicines: To receive a product license before marketing, herbal medicines are required to meet safety, quality, and efficacy criteria in a similar manner to any other licensed medicine.
- Herbal remedies exempt from licensing requirements: The exemption applies to herbal remedies meeting certain conditions set out in Section 12 of the Medicines Act 1968. Section 12 allows a person to make, sell, and supply a herbal remedy during the course of his or her business provided the remedy is manufactured or assembled on the premises and that it is supplied as a consequence of a consultation between the person and his or her patient. Section 12 also allows the manufacture, sale, or supply of herbal remedies where the processing of the plant consists only of drying, crushing, or comminuting; the remedy is sold without any written specification as to its use; and the remedy is sold under a designation that only specifies the plant and the process and does not apply any other name to the remedy.

Canada has been estimated to have approximately 3% of the market share of the global nutritional market. Health Canada established the Office of Natural Health Products. Premarket assessment, labeling, licensing, and monitoring of herbal supplements are items in its mandate. The definition of a natural health product includes products for the use in 'diagnosis, treatment, mitigation, or prevention of a disease, disorder, or abnormal physical state or its symptoms in humans; restoring or correcting organic function in humans; or modifying organic functions in humans, such as modifying those functions in a manner that maintains or promotes health.' These products include homeopathic preparations, substances used in traditional medicine, a mineral or trace element, a vitamin, an amino acid, an essential fatty acid or other botanical-, animal-, or microorganism-derived substance. Foods are not included in this product category called natural health products. Canada's Food and Drugs Act of 1953 regulates foods and drugs but does not specifically deal with natural health products. Therefore, these types of products are regulated as either a food or a drug depending on the type and

concentration of active ingredient and whether claims are made on the products.

Germany regulates vitamins and minerals as food if they are sold to complement the nutritive value of the diet and do not exceed safe levels. However, if the vitamin or mineral is used for disease treatment or prevention and is used at pharmacological levels, then it is considered a drug. Safety and efficacy of drugs must be established by clinical research. Medicinal plants are regulated differently depending on what plant and in what form it is sold. In general, extracts of plants are considered drugs and must be prescribed. Teas, however, are sold over-the-counter in pharmacies. Other teas, such as those that contain alkaloids, must be sold by prescription only. Beginning in 1980, an extensive analysis of the literature on more than 300 herbal remedies was undertaken by the German Kommission E. Approximately two-thirds of the herbals were listed as safe and at least minimally effective. The results were published as a series of monographs by the German Kommission E, and this body of work was summarized and translated into English by the American Botanical Council. These substances are generally purchased at the pharmacy and are reimbursable through health insurance. One caveat regarding the German herbal preparations is that they are not likely to be the same preparations that are produced by other countries; thus, the safety and efficacy statements in the Kommission E are only for the preparations that are prepared in German pharmacies.

Australia regulates therapeutic goods under the Therapeutic Goods Act of 1989. Therapeutic goods include vitamins, minerals, plants and herbals, nutritional food supplements, naturopathic and homeopathic preparations, and some aromatherapy. The Therapeutic Goods Administration (TGA) developed the Office of Complementary Medicine to evaluate new substances and products. Basically, the TGA regulates these therapeutic goods as they do pharmaceutical products, and thus their criteria are more rigorous than the criteria of other countries. Most of the therapeutic goods are 'generally listed' rather than regulated. Listed medicines are considered to be relatively harmless, so the regulations allow for manufacturers to 'self-assess' their products in some situations. The majority of listed medicines are self-selected by consumers and used for self-treatment, and they are all manufactured with well-known established ingredients, such as vitamin and mineral products or sunscreens. These are assessed by the TGA for quality and safety but not efficacy. This does not mean that they do not work; rather, it means that the TGA has not evaluated them individually to determine if they work. It is a requirement under the act that sponsors have information to substantiate all of their product's claims.

The Japanese Ministry of Health and Welfare does not define or recognize a distinct category known as dietary supplements. Instead, there are only two classifications, food and drugs. In 1993, Japan defined a group of foods known as Foods for Specific Health Use (FOSHU). As of 2004, approximately 342 foods had been approved as FOSHU. The dietary ingredients are sold in the form of foods, not in the form of capsules, tablets, or powders.

The herbal supplements market in Japan has been strongly influenced by the practice of *Kampo*. *Kampo* (or *Kanpo*) is the adaptation of Chinese herb formulas to Japanese medicine.

Table 1 Regulatory categories of different countries

Country, act	Definition
United States, DSHEA	Vitamins, minerals, herbal, other botanical, amino acid, enzymes, organs, glands
Europe, Food Supplement Act	Vitamin and minerals
United Kingdom, Medicine and Health Care	Medicinal plants
Canada, Office of Natural Products	Mineral; trace element; vitamin; amino acid; essential fatty acid; botanical-, animal-, or microorganism-derived substances; homeopathic preparation; traditional preparations
Germany, Kommission E	Vitamin and mineral as both foods and drugs, botanicals (approved and not approved), teas as prescription and as over-the-counter
Australia, Therapeutic Goods Administration	Vitamin and mineral, plants, herbs, nutritional food supplements, naturopaths and homeopathic preparations, aromatherapies
Japan, Ministry of Health and Welfare	No definition of dietary supplements, regulations for foods, drugs, and Kampo

Approximately 25 years ago, the Japanese Ministry of Health formally recognized that certain traditional Chinese herb formulas (and a few formulas of similar nature developed in Japan) were suitable for coverage by national health insurance. These formulas are prepared in factories under strict conditions.

In summary, developing global data on dietary supplement sales depend on how they are defined. (Table 1) summarizes the differences in regulatory categories of different countries.

Product Quality and Safety Issues

Product quality is an issue derived from the explosive growth of the industry in the post-DSHEA world. Quality issues revolve around products that contain wrong ingredients, incorrect claims, contamination, or incorrect amounts – either too much or not enough.

An example plant misidentification was published in 1998 by Slifman *et al.* Two patients were admitted to hospital emergency rooms with palpitations, vomiting, nausea, and chest pressure, among other symptoms. Both individuals, having been admitted 1 month apart, had each consumed a program of dietary supplements, one containing 14 herbs, a tablet containing 11 herbs, liquid clay, a bulking powder, and capsules containing microorganisms. Of the five supplements, the one made up of 14 herbs tested positive for cardiac glycosides. The investigators determined that *Digitalis lanata* was present in the supplement. *D. lanata* contains cardiac glycosides, which resulted in the cardiac symptoms. Further investigation revealed that raw material labeled as plantain (genus *Plantago*) had been contaminated with *D. lanata* due to misidentification in the field.

Another quality issue that has safety manifestations was an incorrect claim on a product. PC-SPES, a combination of eight herbs, is claimed to be a nonestrogenic treatment for prostate cancer. However, several of the herbs used in this preparation do in fact have estrogenic activity. In 1998, DiPaola *et al.* showed a significant amount of estrogenic activity in both *in vitro* (yeast) and *in vivo* studies (mice and humans) with PC-SPES. Use of the supplement by men with prostate cancer resulted in similar side effects as would develop with estrogen therapy and theoretically could confound the results of standard therapy.

By law (DSHEA), the manufacturer is responsible for ensuring that its dietary supplement products are safe before they are marketed. Unlike drug products that must be proven safe and effective for their intended use before marketing, there are no provisions in the law for the US Food and Drug Administration (FDA) to ‘approve’ dietary supplements for safety or effectiveness before they reach the consumer. Also unlike drug products, manufacturers and distributors of dietary supplements are not required by law to record, investigate, or forward to the FDA any reports they receive of injuries or illnesses that may be related to the use of their products. Under DSHEA, once the product is marketed, the FDA has the responsibility to show that a dietary supplement is ‘unsafe’ before it can take action to restrict the product’s use or remove it from the marketplace.

In 2003, the FDA banned all products containing ephedra alkaloids. Ephedra-containing products were, until the ban, marketed in conjunction with enhancing athletic performance and/or promoting weight loss. Recent studies provided enough additional evidence that ephedra presents a significant and unreasonable risk of illness and injury that the FDA banned all ephedra-containing products from the market and advised consumers to stop taking such supplements. Strong statements were issued cautioning about the use of ephedra-containing products, especially when strenuously exercising or in combination with other stimulants, such as caffeine.

Interactions

An issue that has become of concern is the interaction of dietary supplements with herbs and other dietary supplements, drugs, foods, lab tests, and diseases or other conditions. There are literally hundreds of potential interactions that have not yet been recognized. Both practitioners and consumers must be aware of the possibilities. In some cases, knowledge about interactions comes from documented reports. However, in other cases, the knowledge is theoretical, based on the pharmacological profile or mechanism of action of the supplement and the drug, food, test, or condition. For example, ginkgo biloba contains ginkgolides in the leaf that competitively inhibit platelet-activating factor (PAF). PAF inhibition decreases platelet aggregation among other many

other physiological effects. Inhibition of PAF may increase cardiac contractility and coronary blood flow. Concomitant use of herbs and supplements that affect platelet aggregation could theoretically increase the risk of bleeding in some people due to ginkgo's effects on platelet aggregation. Spontaneous hematomas (broken blood vessels) and hemorrhaging in the anterior chamber of the eye have been reported in ginkgo users, although it is not known what other drugs or supplements these individuals were taking.

Herbs and supplements that promote platelet inhibition include angelica, anise, capsicum, celery, chamomile, clove, fenugreek, feverfew, fish oil, garlic, ginger, horse chestnut, horseradish, licorice, meadowsweet, onion, Panax ginseng, red clover, vitamin E, and willow. Similarly, concomitant administration of drugs, including aspirin, clopidogrel (Plavix), dalteparin (Fragmin), enoxaparin (Lovenox), heparin, indomethacin (Indocin), ticlopidine (Ticlid), and warfarin (Coumadin), may increase the risk of bleeding in some people. This is just one example of the interactions between drugs with herbals and herbals with other herbals. There may be an infinite number of interactions.

Currently, there are no mandated US federal guidelines to report adverse events or consumer health complaints associated with the use of dietary supplements. MedWatch reporting is voluntary. In 2004, the Life Sciences Research Office published a report, *Recommendations for Adverse Event Monitoring Programs for Dietary Supplements*.

Label Claims

Label claims regarding dietary supplements are a complex issue that varies from country to country. Yet no matter what specific claims are allowed or disallowed by a country, it is reasonable to assume that any global regulation requires that the claim be true, not misleading, and be clear to the consumer. A summary of US label claims follows.

The Nutrition Labeling and Education Act (NLEA) was passed in 1990 as a result of a pre-1984 FDA position that prohibited making any therapeutic or disease-related claims on a food or dietary supplement label. The NLEA permits certain claims describing a positive relationship between a supplement and a health-related condition (or disease). These claims are considered 'health claims' in order to distinguish them from nutrient content claims. A health claim must be authorized by the FDA, and the FDA can only authorize a claim if there is 'significant scientific agreement among qualified experts' or by the 1997 amendment that permits a manufacturer to rely on a statement from an 'authoritative scientific body' of the US government or the National Academy of Sciences. This is a rigorous assessment and only 14 claims have been authorized to date.

In addition to health claims, dietary supplement labels are permitted to have qualified health claims or structure-function claims. The rationale behind the development of a qualified health claim was the idea that the First Amendment should allow disclaimers to be considered as solutions to making claims nonmisleading (Pearson vs Shalala). In other words, the First Amendment does not allow the FDA to reject health claims unless it shows that disclaimers would fail to remedy

harm from misleading statements. The criteria for a qualified health claim were released in 2003 and in this context the FDA will not take enforcement action against a manufacturer using the following specified qualifiers provided the FDA is satisfied that the qualifiers are not misleading:

- "Although there is scientific evidence supporting the claim, the evidence is not conclusive."
- "Some scientific evidence suggests.... However, FDA has determined that this evidence is limited and not conclusive."
- "Very limited and preliminary scientific research suggests.... FDA concludes that there is little scientific evidence supporting this claim."

Qualified health claims for dietary supplements recognized by the FDA as part of its enforcement discretion include such examples as the relationships between phosphatidylserine and cognitive function, B vitamins and cardiovascular disease, omega-3 fatty acids and cardiovascular disease, selenium and cancer, and antioxidant vitamins and cancer.

Dietary supplements are not permitted to carry labeling statements that imply such issues as 'cure,' 'mitigate,' 'treat,' or 'prevent disease' because these statements are considered within the definition of a drug and drugs are subjected to a rigorous premarket approval process. However, under DSHEA, structure-function claims are permitted on dietary supplements because dietary supplements may have effects on the structure or function of the body without the implication that they act as a drug and/or are related to disease. Structure-function claims include those that describe the role of the dietary supplement in affecting the structure or function in humans or the documented mechanism in which a dietary supplement acts to maintain such structure or function. In addition, dietary supplement label claims allow statements of benefits related to classical nutritional deficiency or statements regarding the general feeling of well-being derived from consumption.

Potential Benefits of Dietary Supplements

The 2000 *Dietary Guidelines for Americans* (new release due 2005) emphasizes choosing foods sensibly, maintaining a healthy weight, and exercising regularly. It acknowledges that some people may need a vitamin-mineral supplement to meet specific needs. Similarly, the Food and Nutrition Board and the American Dietetic Association also recognize that dietary supplements may be desirable for some nutrients and for some individuals. The following is a compilation of recommendations by these groups:

- Folic acid supplements for women of childbearing age due to the risk of neural tube defects
- Vitamin B₁₂ supplements for people older than age 50 years due to inefficient absorption
- Vitamin B₁₂ supplements for vegans who eat no animal products
- Calcium for people who seldom eat dairy products
- Vitamin D for elderly people who do not consume fortified dairy products and for others with little exposure to sunlight

- Iron supplementation for pregnant women
- Multivitamin-mineral supplement for people who are following a severely restricted weight-loss diet.

Specifically for athletes, the position of the American Dietetic Association, Dietitians of Canada, and the American College of Sports Medicine is that physical activity, athletic performance, and recovery from exercise are enhanced by optimal nutrition. These organizations recommend appropriate selection of food and fluids, timing of intake, and supplement choices for optimal health and exercise performance. In sports, athletes who are at greatest risk of micronutrient deficiencies are those who restrict energy intake or use severe weight-loss practices, eliminate one or more food groups from their diet, are sick or recovering from injury, or consume high-carbohydrate diets with low micronutrient density. In practice, athletes should consume diets that provide at least the Recommended Dietary Allowances/Dietary Reference Intakes for all micronutrients from food. It follows that, in general, no vitamin and mineral supplements are required if an athlete is consuming adequate energy from a variety of foods to maintain body weight. Supplementation may be necessary under conditions of inadequate food intake. Athletes, as for the general population, should follow supplementation recommendations unrelated to exercise, such as folic acid in women who may become pregnant.

Conclusions

One of the difficulties in assessing the nature of the worldwide dietary supplement industry and its regulations is largely in understanding what products are considered dietary supplements. In the United States, only pills, capsules, tablets, and the like are considered dietary supplements. Globally, it is sometimes difficult to discuss dietary supplements without discussing functional foods or nutraceuticals. Functional foods are similar in appearance to conventional foods but have demonstrated physiological benefits beyond the traditional nutritional value. Nutraceuticals may go so far as to declare not only health benefits, but also medical benefits that

reduce the risk of chronic disease beyond basic nutritional functions. Canada regulates functional foods, nutraceuticals, and dietary supplements under one regulatory agency. The United States clearly distinguishes between foods and dietary supplements, although both fall under the category of food, which is distinct from drugs. UK distinguishes between herbal medicines and dietary supplements containing vitamins and minerals. Japan regulates functional foods as FOSHU and has no regulatory definition for dietary supplements as defined in the United States. Moreover, these regulations are in a constant state of flux as the industry changes and develop over time. Issues that must be monitored regarding dietary supplements consumption are product quality and potential harmful interactions among supplements, foods, and drugs. Health claims that have been approved by regulatory agencies worldwide stress that the claims be truthful, clear, and not misleading to the ultimate consumer. Current scientific expertise acknowledges that dietary supplements, specifically some of the vitamins and minerals, have potential benefits in certain populations.

See also: Folic Acid. Supplementation: Developed Countries; Developing Countries

Further Reading

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Programmatic Issues

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Abbreviations

Hb Hemoglobin
IU International units

LBW Low birth weight
RDA Recommended daily allowance
WHO World Health Organization

Glossary

Anemia A decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood.

Ferritin A ubiquitous intracellular protein that stores iron and releases it in a controlled fashion. The amount of Ferritin stored reflects the amount of iron stored.

Food fortification The process of adding micronutrients (essential trace elements and vitamins) to food. It can be purely a commercial choice to provide extra nutrients in a food, or sometimes it is a public health policy which aims to reduce numbers of people with dietary deficiencies in a population.

Hemochromatosis An inherited blood disorder that causes the body to retain excessive amounts of iron.

Hemoglobin Iron-containing oxygen-transport metalloprotein in the red blood cells.

Micronutrients Nutrients required by humans and other living things throughout life in small quantities to orchestrate a whole range of physiological functions, but which the organism itself cannot produce. They include dietary minerals (e.g., iron, zinc, and iodine) and vitamins (e.g., vitamin A and B-complex) required as nutrients in tiny amounts by organisms.

Recommended dietary allowance A nutritional norm for planning and assessing dietary intake. The level of intake of essential nutrients considered to be adequate to meet the needs of practically all healthy people.

Teratogenesis The development of defects in an embryo.

Introduction

Globally almost two billion people (one third of the human race) are affected by vitamin A, iron, iodine, or zinc deficiencies that put them at an increased risk of poor growth, morbidity, intellectual impairment, and mortality. Since the mid-1980s micronutrient supplementation has been a major public health strategy in developing countries to prevent and control deficiencies of vitamin A, iron, folate and, to a lesser extent, iodine. More recently, zinc supplementation is now considered an efficacious adjunctive therapy for diarrhea in populations with an elevated risk for zinc deficiency. The past decade has seen renewed interest in vitamin D, the 'sunshine vitamin', because new data suggest that its benefits extend beyond healthy bones, and there is growing interest in calcium supplementation in pregnancy. The following discussion will define micronutrient supplementation, examine the role of supplementation as a strategy for the prevention and control of micronutrient deficiencies, and examine evidence for vitamin A, iron, iodine, zinc, calcium, and multiple micronutrient supplementation interventions with respect to efficacy, recommended dose, and frequency of administration, safety, and program effectiveness.

Micronutrient Supplementation

Supplementation refers to the provision of added nutrients in pharmaceutical form (such as capsules, tablets, or syrups)

rather than in food. Micronutrients are essential substances required by the body in small amounts for vital physiological functions. They cannot be synthesized by the body and therefore must be consumed in foods or in supplements.

Choice of Interventions

Micronutrient supplementation is one of three major categories of nutrition-focus intervention strategies – the other two being fortification and dietary change. The choice or mix of strategies will depend on multiple factors including (1) the magnitude, severity, and distribution of the micronutrient deficiency in the population, (2) relative intervention efficacy, (3) in-country resources available to effectively deliver the intervention to the target group, (4) the target groups' acceptance of the intervention, and (5) the ability to sustain the intervention.

The relative advantages of micronutrient supplementation over fortification and dietary improvement interventions include (1) rapid coverage of a high-risk population, (2) the ability to directly provide a controlled and concentrated dose of the micronutrient(s) to the target group, (3) immediate impact on micronutrient status and associated functional outcome(s), (4) relatively low cost of training workers compared with nutrition counseling for diet improvement, and (5) high coverage if supplements are delivered using existing services that already reach a high proportion of the target group. Most supplementation programs have been

shown to be cost-effective in achieving their nutritional goals and health impact, although sustaining large-scale programs over the long term may be more costly than either fortification or dietary improvement.

Generally, prophylactic micronutrient supplementation is intended as a short-term means of rapidly preventing the nutrient deficiency in high-risk individuals and populations until adequate and sustainable food-based programs become effective. However, in many cases, supplementation programs may be the only effective means of reaching specific vulnerable groups, particularly those who have limited or no access to processed fortified foods, or those who have high micronutrient requirements, such as young children and pregnant women, that may not be met even with fortification and dietary improvement interventions. In these situations and populations, supplementation should be sustained over a longer period until nutrient intake from fortified and non-fortified food is adequate.

Based on experiences from vitamin A, iron, and iodine supplementation programs, the key limitations of supplementation are (1) inadequate targeting or coverage where deficient individuals are missed or reached irregularly, (2) inability to sustain high coverage over long periods of time as financial, political, or other health priorities change, and (3) poor compliance by target individuals who are expected to take a daily supplement for extended periods of time (e.g., iron supplementation during pregnancy). As illustrated in **Figure 1**, in many countries, particularly those with high regional variability in socioeconomic status, food availability, and market access, a mix of strategies, rather than any single strategy, is more likely to reach a greater proportion of the at-risk population.

Cost of Micronutrient Interventions

The 2008 Copenhagen Consensus, a group of world-renowned economists, ranked micronutrient supplements (specifically high-dose vitamin A, and therapeutic zinc supplements for children with diarrhea) as the top international development priority. The criteria included the benefit:cost

ratio, as well as feasibility and sustainability of the interventions. Micronutrient programs had previously been considered to be among the most-effective of all health interventions in the World Bank's 1993 World Development Report. The cost of micronutrient supplementation needs to be balanced against the cost of other food based and public health interventions as well as the cost of not addressing the insidious effects of micronutrient deficiencies. Costs are likely to vary depending on the scope of the program, existing delivery mechanisms, the nutrient involved, and other factors. Based on World Bank estimates, the costs of vitamin A, iron, and iodine supplementation programs are relatively modest ranging from US\$0.20 to \$1.70 per beneficiary per year. These costs are slightly higher than the estimated costs for fortification (US\$0.05–0.15 per beneficiary per year), but are considerably lower than the unit costs of education programs (US\$5–\$10) and feeding programs (US\$70–\$100 per beneficiary per year).

Prophylactic Micronutrient Supplementation

Micronutrient supplementation has been the method of choice for the treatment of severe clinical nutrient deficiencies for several decades. Prophylactic supplementation, however, only gained wider acceptance in the late 1980s with the publication of results from a randomized trial in Aceh, Indonesia showing a 34% reduction in young child mortality among vitamin-A supplemented preschoolers. The introduction of routine vitamin A supplementation to preschool children in developing countries has encouraged this approach and the development of other micronutrient supplementation programs. Each single-nutrient or multiple micronutrient supplementation strategy should be evaluated separately for efficacy, feasibility, safety, cost, and appropriateness for the cultural and political context in which it will be implemented.

Vitamin A Supplementation

Periodic distribution of high-dose vitamin A supplements, either universally to all preschool children or targeted to high-risk groups, has been the most widely practiced intervention for the prevention and treatment of vitamin A deficiency throughout the world. Giving a high-dose of vitamin A on a 4–6 monthly basis is based on the assumption that vitamin A is stored in the liver and mobilized, as needed, to meet the demands of target tissues. This is in contrast to iron supplements which need to be given on a daily or weekly basis.

Recent critics of blanket approaches to preschool vitamin A supplementation programs have suggested that this approach has acted as a 'policy barrier' to other presumably more sustainable food-based interventions. It is true that semi-annual vitamin A supplementation does not correct the dietary causes of deficiency, and alone never normalizes the serum distribution of a deficiency population. However, as long as a substantial fraction of any population has deficiency serum retinol values, emphasis should be placed on finding ways to improve dietary intake in sustainable ways, while continuing to supplement children. Once serum retinol surveys

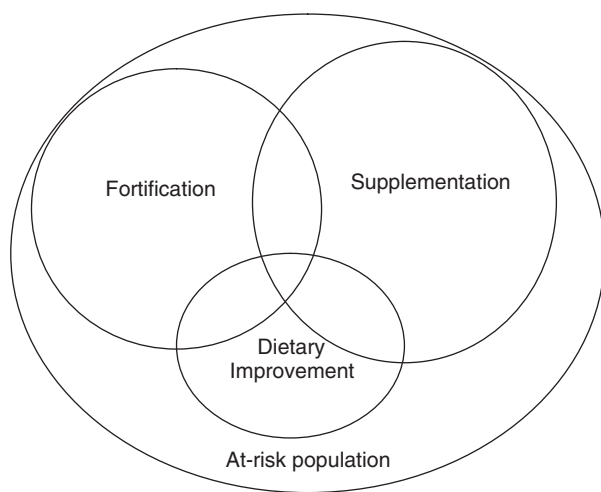


Figure 1 Improving micronutrient intake: complementary approaches.

repeatedly begin to indicate that the vitamin A status of children has improved and is adequate in the vast majority of young children in a sustained manner such that dietary adequacy and vitamin A sufficiency are assured, vitamin A supplementation can be safely withdrawn.

Efficacy of Prophylactic Supplementation

Preschool children: Giving children living in vitamin A deficient areas a large dose (i.e., 200 000 IU or 60 mg RE) of vitamin A every 4–6 months has been shown to reduce the risk of both noncorneal and corneal xerophthalmia by 90%, increase serum retinol levels for 1–2 months following supplementation, and reduce young child mortality by an average of 23% when coverage levels of $\geq 80\%$ are achieved in deficiency populations. In vitamin A deficient populations, prophylactic supplementation is one of the most cost-effective public health interventions to improve the survival of children from 6 months to 6 years of age.

Postpartum women: Postpartum vitamin A supplementation (i.e., giving women a large dose of vitamin A that is 200 000 IU or more within the first 6 weeks after delivery) is a strategy for improving maternal and infant vitamin A status and health outcomes in areas where vitamin A deficiency is endemic. Postpartum supplementation is designed to improve women's vitamin A status and to increase the vitamin A content of breast milk. This is meant to protect the mother's vitamin A reserves, while addressing one of the fundamental reasons that children become vitamin A deficient – low dietary vitamin A intake from breast milk. The supplement should be given within 6 weeks of delivery when the chance of pregnancy is remote, but ideally as soon as possible, because the physiologic demands of pregnancy and lactation deplete the mother's vitamin A stores.

Newborns and young infants: Newborn vitamin A supplementation (NVA) is a promising new intervention that involves supplementing infants shortly after birth with a single, large oral dose of VA (50 000 IU). The intervention was tested in three field trials in southern Asia (Indonesia, India, and Bangladesh), each of which reported significant reductions of $\geq 15\%$ in infant mortality in the first 6 months of life. When combined, the results suggest that infant mortality can be reduced by approximately 20% in southern Asia by giving newborns a single, oral dose of vitamin A. Given previous evidence of safety with respect to short- or long-term side effects, NVA appears to be a low-cost approach to reduce infant mortality in South and Southeast Asia.

In Africa, however, this intervention had no beneficial effect on early infant survival in an urban setting in Zimbabwe and a periurban setting in Guinea-Bissau. All three African studies (two in Guinea-Bissau) were done in populations with little, if any, vitamin A deficiency. Mortality in the Zimbabwean study was very low and in one study in Guinea-Bissau, investigators reduced mortality by excluding the highest risk infants (those with low birth weight (LBW)) and giving free care and drugs to sick infants. The World Health Organization (WHO) is currently supporting additional efficacy studies in Africa (Tanzania and Ghana) and South Asia (India), as well as studies investigating potential biological mechanisms through which NVA may decrease the

risk of early infant mortality to provide additional evidence to inform its decision about a global policy of supplementing newborns with vitamin A.

The current WHO recommendation is to provide 50 000 IU vitamin A to infants with each of the three doses of diphtheria, pertussis, and tetanus (DPT) at 6, 10, and 14 weeks of age to improve vitamin A status. Further trials, however, are needed to confirm the benefit of this recommendation on early infant vitamin A status, morbidity, and survival.

Pregnancy: Maternal vitamin A deficiency appears to be widespread in low-income countries. Currently, the WHO estimates nearly 20 million pregnant women to be vitamin A deficient, i.e., having a serum retinol concentration below $0.70 \mu\text{mol l}^{-1}$, of whom nearly 9 million have gestational night blindness, an ocular manifestation of deficiency. Where maternal night blindness or biochemical vitamin A deficiency is highly prevalent, prophylactic supplementation of up to 10 000 IU daily or 25 000 IU weekly has been given safely. The efficacy of low-dose maternal supplementation has been investigated in three large community-based randomized placebo-controlled studies. Evidence suggesting that preventing vitamin A deficiency in pregnancy could improve maternal survival was first reported in rural Nepal, where a randomized placebo-controlled trial observed a decrease of $\sim 44\%$ in mortality related to pregnancy following continuous, weekly receipt of vitamin A or beta-carotene during the reproductive years at dosages approximating a recommended dietary allowance (RDA). However, two recently completed large randomized controlled trials, in Bangladesh and Ghana, did not find a similar survival benefit, possibly because of much lower baseline maternal mortality and better vitamin A status of women in these study populations. Thus, the body of evidence does not support a general recommendation for maternal vitamin A supplementation as a means to reduce maternal mortality. The different results of these well-designed and implemented trials suggests that the impact of vitamin A on maternal survival seems to vary by severity of deficiency, mortality risk (by cause), general malnutrition, access to health services and likely other factors, as to be expected.

Form of Supplement

Vitamin A in a gelatinous capsule form is the overwhelming choice of delivery mode used in large public health programs, although there are reports of the successful use of liquid vitamin A in a bottle using a calibrated dispenser. Vitamin A has also been dispensed from an inhalation device among children with parasitic infections as an alternative delivery mode in the very small number of children in whom extreme intestinal malabsorption of vitamin A exists.

Safety

High-dose vitamin A is safe and well-accepted by preschool children, although evidence from program evaluations and a randomized trial in the Philippines suggests that up to $\sim 9\%$ of preschool children may experience acute, transient side effects including nausea, vomiting, headache, or fever after a

200 000 IU dose. Most episodes begin within 24 h of capsule receipt and resolve spontaneously within 12–24 h of onset.

Earlier animal experiments linked high doses of vitamin A to birth defects; however, experimental data proving the teratogenic effect of vitamin A in pregnant women are limited and, for ethical reasons, very difficult or even impossible to test rigorously. Nonetheless, high-dose vitamin A should be avoided during pregnancy because of the theoretical risk of teratogenesis.

Delivery Mechanisms

There are a variety of ways to deliver high-dose vitamin A supplements to at-risk populations including (1) restricting delivery to clinic-settings for treatment purposes, (2) integrating delivery with other existing services such as immunization contacts or routine growth monitoring, or (3) universal delivery, to attain the widest coverage of preschool children, through semiannual campaigns that specifically promote vitamin A capsule distribution or that are combined with other national programs such as child health weeks. Each delivery mode has advantages and disadvantages. Restricted delivery targets those most likely to be vitamin A deficient and requires few additional resources (apart from the supplements), however, it may result in poor coverage if those most at-risk do not regularly access health clinics. 'Piggy-backing' vitamin A distribution onto existing community services can be cost-effective, but may also miss children at greatest risk for vitamin A deficiency if their access to and use of these services is limited. Finally, universal distribution requires strong community mobilization and social marketing to attain coverage levels of $\geq 80\%$. Sustaining this coverage level every 4–6 months can be challenging but there are numerous examples of countries where such levels have been sustained for at least 10–15 years.

Iron Supplementation

Globally, supplementation with iron tablets is the most widely used strategy for the prevention and control of iron-deficiency/anemia in pregnancy. Pregnant women require nearly three times the amount of iron needed by nonpregnant women due to physiologic demands of pregnancy (expanded red blood cell volume, the needs of the fetus and placenta, and blood loss at delivery). This high requirement is unattainable for most pregnant women in developing countries, especially those who struggle to meet the 1.5 mg day^{-1} requirement during nonpregnancy, and therefore iron supplementation is recommended during pregnancy.

Efficacy

Pregnancy: A pooled analysis of data from eight studies of iron-folic supplementation during pregnancy suggested an increase of 12 g l^{-1} (95% CI 2.93, 21.07) in hemoglobin at term and a 73% reduction in the risk of anemia at term (95% CI 0.12, 0.56). A growing body of evidence links iron supplementation in pregnancy with improved pregnancy and

child outcomes. Anemia in pregnancy has also been linked to an increased risk of LBW, preterm birth, infant and child mortality, and an increased risk of iron deficiency in infants after 4 months of age, predisposing the young child to cognitive impairment and psychomotor and mental development. Two large recent clinical trials strongly suggest benefit of maternal iron supplementation for the fetus, neonate, and young child. A recent study in rural Chinese women, comparing iron and folic acid versus folic acid alone, on birth and neonatal outcomes found a significant 50% reduction in very preterm births and 54% reduction in neonatal mortality among women given iron and folic acid during pregnancy. A recent trial from Nepal found that women who received daily iron and folic acid beginning early in pregnancy experienced a significant but modest 16% reduction in LBW of their babies ($< 2500 \text{ g}$).

Also, the mortality impact of improving iron status during pregnancy appears to last longer than previously recognized. Based on 7 years of postnatal follow-up among surviving offspring of women enrolled in the above Nepal study, children of mothers who received iron and folic acid during pregnancy had childhood mortality rates that were 31% lower than children of mothers who had received placebo. These children also scored significantly higher on a battery of cognitive tests compared with children whose mothers received placebo.

Infancy: Iron supplementation in infants is sometimes advised to prevent iron deficiency, even in populations with a relatively low prevalence of iron deficiency anemia. There is accumulating evidence, mostly collected from 6–12 month old infants that children born to anemic mothers have lower iron stores, even when they are born at term and with normal birth weight. WHO recommends universal iron supplementation for LBW infants at a dose of 2 mg kg^{-1} body weight per day, and a similar dosage for children 6–23 months where the diet does not include foods fortified with iron or where the anemia prevalence is above 40%. The US Institute of Medicine recommends iron drops for exclusively breast-fed infants between 4 and 6 months of age. There is ample evidence from well-designed and controlled studies to show that iron supplementation in infancy significantly improves hemoglobin and ferritin levels, and studies are currently investigating the impact of iron supplementation on dimensions of cognitive development.

The benefits and risks of infant iron supplementation, however, remain controversial, particularly in iron-replete children. This is because, although iron is an essential nutrient needed for adequate infant growth, immune function, and development, it may also contribute to a greater risk of infection and infestation. In countries where malaria is prevalent, it has been suggested that iron supplementation increases the risk of malaria and deaths. The high dose of iron which is given as medicine may result in free iron circulating in the blood and available to the malaria parasite, which promotes its growth. A study conducted in Zanzibar, where malaria transmission is intense and occurs year round, showed that under certain conditions supplementation may be associated with adverse effects, specifically increased risk of hospitalization (primarily due to malaria and infectious disease), and mortality. The findings of the trial in Zanzibar suggest that caution should be exercised in settings where the prevalence of

malaria and other infectious diseases is high. Until the WHO recommendations are revised it is advised that iron and folic acid supplementation be targeted to those who are anemic and at risk of iron deficiency. They should receive concurrent protection from malaria and other infectious diseases through prevention and effective case management. In cases of severe undernutrition, iron supplementation should be delivered in accordance with the WHO guidelines, which state that supplementation be withheld until the acute problems related to infection have been effectively treated, and growth has resumed.

LBW infants: LBW infants are born with low iron stores and have higher iron requirements for growth. Their iron needs cannot be met from breast milk alone, and therefore, they are a priority target for iron supplementation.

Preschool and school-age children: Data pooled from 55 studies have shown a 7.4 g l^{-1} higher hemoglobin concentration in iron supplementation versus nonsupplemented children and anemia reductions ranging from 38% to 68% in nonmalarial regions, and 6–32% in malarial hyperendemic areas. The incidence of diarrhea appears to increase by $\sim 11\%$ in iron-supplemented children, and there is no overall effect on improved growth. There is a growing body of evidence confirming that iron supplementation of anemic children improves their school performance, verbal, and other skills.

In settings where the prevalence of anemia in preschool or school-age children is 20% or higher, the WHO recommends the intermittent use of iron as a public health intervention to prevent anemia and improve the iron status among these children. Supplementation with iron once, twice, or three times per week on nonconsecutive days has been proposed as an effective and safer way to increase children's iron intakes.

Dose

The WHO has published global guidelines for iron supplementation and recommends daily prophylactic iron supplementation with 60 mg iron for all pregnant women in developing countries in the second and third trimesters of pregnancy (Table 1). In other countries, iron supplementation is recommended only for anemic women with proven iron deficiency anemia (as in Great Britain) or for women with low prepregnancy iron stores (as in Canada). The efficacy of maternal iron supplementation increases with daily iron doses of up to 60 mg, although the amount of iron and the level of adherence that are necessary to produce maximum benefits depend on the iron and hematologic status of the target population for iron supplementation programs. The WHO also recommends providing LBW infants with supplemental iron drops beginning at 2 months of age.

Multiple Micronutrient Supplements with Iron

Currently, folic acid is added to most iron supplements for women of fertile age on the basis of reducing the risk of neural tube defects and because inadequate folic acid may limit the hemoglobin response to iron supplements. The lack of other nutrients such as vitamin A, vitamin B₁₂, and riboflavin may also limit the efficacy of iron supplements. Several studies

have assessed the effect of multiple micronutrient supplements on anemia, but have found that they are not more effective than giving iron alone, except when given in combination with vitamin A.

Safety

Iron supplements can cause unpleasant gastrointestinal symptoms (e.g., nausea, constipation, vomiting, and diarrhea), which may contribute to poor compliance, but these usually occur at higher doses. When iron tablets are taken with meals or if slow-release tablets are used, side effects may be mitigated. Complications of excessive iron storage, including hemochromatosis and hemosiderosis, are possible but uncommon in women consuming iron tablets. Another potential danger of iron supplements is accidental overdosage by children in the home, and therefore supplements should be kept out of the reach of children.

Frequency

Perceived concern about side effects, compliance, and potential toxicity of a daily regimen of iron supplementation has generated research to assess the relative efficacy of weekly versus daily supplementation in pregnant women, adolescents, and children. A review of these studies concluded that: (1) both daily and weekly iron supplementation reduced the prevalence of iron deficiency and anemia, (2) in pregnancy daily supplementation was more effective than weekly for increasing hemoglobin and ferritin because of the relatively short period during which supplements were taken, and (3) while daily supplementation produced only a slightly higher average hemoglobin response ($\sim 2 \text{ g l}^{-1}$) compared with weekly, its relative impact on reducing anemia risk in pregnancy was 34% largely because daily supplementation was more effective at increasing low hemoglobin concentrations.

Viewed in the context of two other studies, in Bangladesh and Indonesia, that carefully monitored the number of iron tablets consumed, the size of the hemoglobin response to iron appears to depend on the total amount of iron consumed. In these studies, most of the hemoglobin response was produced by the first 20–50 tablets consumed. But more research is needed before recommendations can be made about consuming a fixed number of tablets over a defined period of time while permitting flexibility about the consumption interval (i.e., daily, 2–3 times per week, weekly, etc.).

Taken together, the available evidence suggests that iron supplements should be taken daily to prevent and treat iron-deficiency anemia, especially in pregnant women. Weekly iron and folic acid supplementation or WIFS among nonpregnant women appears to be an approach that can be effective for ensuring adequate iron status of women, particularly before pregnancy and during the first trimester in communities where food-based strategies are not yet fully implemented or effective. This approach has shown short- and medium-term effectiveness in reducing the prevalence of anemia among women of reproductive age in several community settings where the necessary support, social marketing, and interpersonal advocacy ensured adequate compliance.

Table 1 Micronutrient supplementation: target groups and prevention schedules

<i>Micronutrient</i>	<i>Target group</i>	<i>Dosage</i>	<i>Frequency/Duration</i>
Vitamin A ^a	Children at risk for vitamin A deficiency <6 months	50 000 IU	One dose at 4, 10, and 14 weeks ^e
	6–11 months	100 000 IU	One dose every 4–6 months ^f
	1–5 years	200 000 IU	One dose every 4–6 months
	Postpartum women	200 000 IU ^g	One dose at ≤6 weeks postpartum
Iron (plus folate) ^b	Pregnant women (living in areas where anemia prevalence <40%)	60 mg iron + 400 µg folic acid ⁱ	Daily for 6 months ^h in pregnancy
	Pregnant women (living in areas where anemia prevalence ≥40%)	60 mg iron + 400 µg folic acid ⁱ	Daily for 6 months ^h in pregnancy, and continuing to 3 months postpartum
In nonmalaria areas and in areas with good health care including treatment for malaria, other parasitic infestations and infections	6–24 month old children of normal birth weight (living in areas where the prevalence of anemia in children is <40%)	12.5 mg iron + 50 µg folic acid	Daily for 6–12 months of age
	6–24 month old children of normal birth weight (living in areas where the prevalence of anemia in children is ≥40%)	12.5 mg iron + 50 µg folic acid	Daily for 6–24 months of age
	2–24 month old children of low birth weight (<2500 g)	12.5 mg iron + 50 µg folic acid	Daily for 2–24 months of age
In malaria-endemic areas where there is limited malaria prevention and clinical care	Iron therapy for 3 months starting at 2 months of age only to infants with clinical symptoms of severe anemia Iron therapy should only be given in conjunction with the measures to prevent and control malaria (including provision of insecticide-treated nets and vector control for prevention of malaria), and treatment of malaria illness with effective antimalarial drug therapy.		
Iodine ^c	Pregnant women in iodine deficiency endemic areas ^j	300–480 mg	One dose annually
	Nonpregnant fertile women ^j	400–960 mg	One dose annually
	Children in iodine deficiency endemic areas ^j	240 mg iodine	One dose annually
Zinc ^d	Children with persistent diarrhea ^k	10–20 mg	Daily for 14 days
	Children with an elevated risk for zinc deficiency; Children who are severely stunted, have low plasma zinc or both	Further research needed on relative efficacy of different frequencies and doses.	

^aAdapted from WHO (1997) *Vitamin A Supplements: A Guide to Their use in the Treatment and Prevention of Vitamin A Deficiency and Xerophthalmia*, 2nd edn. Prepared by a WHO/UNICEF/IVACG Task Force, Geneva: WHO

^bAdapted from WHO (1998) INACG/WHO/UNICEF Guidelines for the Use of Iron Supplements to Prevent and Treat Iron Deficiency Anemia.

^cAdapted from WHO (1996) Iodized oil during pregnancy – a statement by the WHO. *Bulletin of the World Health Organization* 74(1): 1–3.

^dAdapted from the recommendations of an expert group (2001) Effect of zinc supplementation on clinical course of acute diarrhea, *Journal of Health, Population and Nutrition* December 19(4):338–346.

^eGiven at the time of each of the three DPT vaccinations.

^fImmunization against measles provides a good opportunity to give one of these doses.

^gWHO/UNICEF/IVACG Task Force. Vitamin A supplements: A guide to their use in the treatment and prevention of vitamin A deficiency and xerophthalmia (1997) Geneva: WHO.

^hIf 6 months duration cannot be achieved in pregnancy, continue to supplement during the postpartum period for 6 months or increase the dose to 120 mg iron in pregnancy.

ⁱWhere iron supplements containing 400 µg of folic acid are not available, an iron supplement with less folic may be used. Supplementation with less folic acid should be used only if supplements containing 400 µg are not available.

^jWhere access to iodine-fortified salt is limited and immediate attention needed.

^kIn areas where there is an elevated risk of zinc deficiency in the population.

Form of Iron

In tablets, the most common form of iron is ferrous sulfate (containing 20% elemental iron), but ferrous fumarate (33% elemental iron) and ferrous gluconate (12% elemental iron) are also used. Infant supplements are usually in liquid form and more costly, but tablets, when crushed or mixed with food, can also be used.

Effective Iron Supplementation Programs

Reviews of large-scale iron supplementation programs in developing countries have reported limited effectiveness in reducing maternal anemia. The limited effectiveness is often attributed to one or combination of the following factors: (1) inadequate political support, (2) low priority for IFA within maternal health programs, (3) insufficient bundling of interventions to address causes of anemia, (4) inadequate supplies, low utilization, and weak demand, (5) the lack of community-based delivery platforms for iron supplementation to complement the antenatal care platform. For iron supplementation programs to achieve improved effectiveness careful attention must be given to ensuring (1) adequate supply of iron tablets at distribution points, (2) accessibility and access of pregnant women to the distribution points, (3) promotion of the benefits of iron supplementation, (4) counseling about managing possible side effects, and (5) communication strategies to encourage pregnant women to consume the supplement.

Iodine Supplementation

Universal salt iodization (USI), to complement the supply of iodine in the diet, is the recommended population-based intervention to prevent and control Iodine Deficiency Disorders (IDD) but, in isolated communities with an urgent need for iodine prophylaxis, direct supplementation of priority groups can be rapidly implemented as an interim measure while salt iodization is being established. A 2007 WHO statement recognized that pregnant and lactating women, and children less than 2 years of age, may not be adequately covered by iodization of salt where USI is not fully implemented, especially in emergencies, among displaced populations, and geographically remote areas. If iodized salt is not accessible in these specific situations, increasing iodine intake is required in the form of iodine supplements for pregnant and lactating women, and a supplement or complementary food fortified with iodine for children of 7–24 months of age.

Efficacy

Numerous studies have confirmed that iodine supplementation in pregnant women can prevent endemic congenital hypothyroidism in their child at age 4 years. Other documented benefits of maternal supplementation observed in controlled studies include reduced infant and young child mortality, improved birth weight, and better manual function in children born to iodine-supplemented mothers.

Oral iodized oil. Although efficacious, the use of iodized oil injections has largely been replaced by oral iodized oil due to the concern over the AIDS pandemic and use of needles, as well as the higher cost of supplies (syringes) and personnel (skilled injectors). Oral delivery of iodized oil appears as effective as intramuscular injection but is less costly, carries no infection risk (through a contaminated needle), is painless, and can be administered by untrained personnel. Oral iodized oil is considered to be safe for pregnant women and can be given any time during pregnancy; however, it appears only to protect against moderate and severe neurological abnormalities when given during the first two, but not the third, trimesters. The best outcomes are likely to occur when supplementation is given during the first trimester, but even if given in late pregnancy or to the infant after birth, slight improvements in brain growth and developmental quotients, but not neurological status, are evident.

Dose

Because damage to the developing brain is the most severe consequence of iodine deficiency, women of child-bearing age and children are the first priorities for receiving iodized oil, especially in countries and situations where the USI program is not fully implemented. In these situations, WHO recommends giving a daily dose of 250 µg or a single 400 mg dose each year to pregnant and lactating women; a daily dose of 150 µg or a single 400 mg dose each year to women of reproductive age (15–49 years), and a daily dose of 90 µg or a single 200 mg dose each year to children less than 2 years. Larger doses do not necessarily provide longer protection because of increased urinary loss after administration. It is possible that smaller, more frequent, doses may be more effective, although this issue requires further study. Evidence from studies using 200–500 mg of iodized oil suggests a protective effect of between 6 months and 2 years.

Safety

Oral iodine supplementation is safe, although side effects can include transient submandibular swelling and subclinical hypothyroidism.

Zinc Supplementation

During the past decade zinc supplementation has received increasing attention as results from research trials reveal the extent of zinc deficiency in developing countries and the role of zinc supplementation in improving outcomes such as intrauterine growth retardation, children's cognitive development, growth, disease incidence and severity, and child survival. However, most of what is known about zinc supplementation is based on experience from research trials and not from large-scale programs that deliver zinc supplements.

Efficacy of Preventive Zinc Supplementation

Although maternal zinc supplementation is associated with reduced prematurity rates particularly in low-income populations, there is no convincing evidence that zinc supplementation in pregnancy results in other important benefits. Preventive zinc supplementation in children reduces the incidence of diarrhea by ~20% among children in lower-income countries, although current evidence indicates that this beneficial effect of zinc is limited to children older than ~12 months. Zinc supplementation also lowers the incidence of acute lower respiratory tract infections (ALRI), reducing pneumonia and ALRI by ~15%. Evidence suggests that zinc supplementation has a marginal 6% impact on overall child mortality (but an 18% reduction in deaths among zinc-supplemented children older than 12 months of age) and a positive but small effect on length and weight gain.

Other outcomes. Evidence from a limited number of trials suggests a potential benefit from zinc supplementation on morbidity related to *Plasmodium falciparum* infections, child survival, weight gain in LBW infants, and severely malnourished children, length gain in stunted children, and a host of maternal health and pregnancy outcomes; however, more research is required to determine the benefits of large-scale introduction of zinc supplementation targeted to groups of infants, children, and pregnant women.

Therapeutic Efficacy of Zinc Treatment for Diarrhea

Based on therapeutic studies, giving zinc supplements in doses ranging between 1 and 4 RDAs per day (15 mg per day for children <1 year and 20–30 mg per day for children ≥1 year) for 14 days is efficacious in reducing the severity of prolonged, chronic diarrhea, and the duration of the episode significantly. A very recent study conducted in Bangladesh suggests that the therapeutic efficacy of a 5-day dose is equivalent to a 10-day dose with respect to duration of diarrhea and preventing diarrhea in the subsequent 3 months.

Dose

There is a need for systematic studies to determine the appropriate dose of supplemental zinc for the prevention of zinc deficiency in different age groups and clinical conditions. In hospitalized children, zinc can be given as two to three divided doses each day, although in community interventions a single dose of 20 mg per day appears both safe and efficacious.

Form of Zinc

Many zinc compounds can be used to produce zinc supplements. They differ in color (from colorless to white, gray, or yellowish white), taste (bitter, astringent, and slightly sour), odor (odorless, vanilla odor, or faint odor of acetic acid), solubility in water (insoluble, slightly soluble, or soluble), cost, side effects, and safety. Water soluble compounds (e.g., zinc acetate, zinc sulfate, and zinc gluconate) are more easily absorbed and therefore preferred. Water insoluble zinc oxide, which is the least costly zinc compound, also seems to be well absorbed because it is soluble in dilute acid, and likely

dissolves in the acidic secretions of the stomach. Zinc supplements have been prepared in flavored syrups, chewable tablets, single-dose 'sachets' to be added to food, and as a high-fat spread to be consumed alone or with other food for infants and young children, and as dry supplements (tablets, capsules, and powders) alone or with other nutrients. The choice of the supplement form will depend on the age of the target group, preference, and whether other nutrients will be included.

Effectiveness of Zinc Supplementation Programs

There is little information about the effectiveness of zinc supplementation programs implemented on a large scale. There is a need to conduct such studies to assess the best ways to deliver zinc supplements to children with diarrhea, paying particular attention to feasibility, sustainability, and cost-effectiveness of different zinc delivery mechanisms.

Multiple Micronutrient Supplementation in Pregnancy and Childhood

Maternal micronutrient requirements during pregnancy increase to meet the physiologic changes in gestation and fetal demands for growth and development. Maternal micronutrient deficiencies are high and coexist in many settings, likely influencing birth and newborn outcomes. The only recommendation for pregnancy currently exists for iron and folic acid use. A meta-analysis of 12 trials of multiple micronutrient compared with iron-folic acid supplementation reveals an overall 11% reduction in LBW but no effect on preterm birth and perinatal or neonatal survival. Currently, data are unconvincing for replacing supplementation of antenatal iron-folic acid with multiple micronutrients.

In recent years, from both biological and programmatic reasons, there has been a strong interest in moving from single to multiple micronutrient interventions to address the adverse effects of micronutrient deficiencies on the health, growth, and development of infants and young children. A 2009 meta-analysis by Allen *et al.* showed that providing multiple micronutrients rather than two or fewer micronutrients more effectively improved child length and weight; hemoglobin, zinc and serum retinol concentration; and young child motor development.

Calcium Supplementation in Pregnancy

Preeclampsia is a major cause of death in pregnant women and newborn babies worldwide. Preterm birth (birth before 37 weeks) is often caused by high blood pressure and is a leading cause of newborn deaths, particularly in low-income countries. A recent review of 13 trials, involving 15 730 women, found that calcium supplementation during pregnancy is a safe and relatively cheap means of reducing the risk of preeclampsia in women at increased risk, and women from communities with low dietary calcium. Women were also less likely to die or have serious problems due to

preeclampsia. Babies were less likely to be born preterm. No adverse effects have been found but further research is needed into the ideal dosage for supplementation. In 2011 WHO recommended calcium supplementation during pregnancy (at doses of 1.5–2.0 g elemental calcium per day) of all women where dietary calcium intake is low, but especially for those at high risk of developing preeclampsia, for the prevention of preeclampsia.

Vitamin D

Growing evidence suggests that chronically low levels of vitamin D raise a person's risk for certain major illnesses including breast, prostate, and colon cancers, as well as low bone calcium content.

Efficacy

A recent systematic review indicates that targeting children and adolescents with low serum vitamin D concentrations could result in clinically important improvements in bone density but that vitamin D supplementation in healthy children and adolescents to improve bone density is not justified. Vitamin D supplements are unlikely to be beneficial in children and adolescents with normal vitamin D levels.

Dose

Vitamin D supplements could address the high prevalence of vitamin D deficiency in temperate zones, but how much people should take is still a subject of debate. The American Academy of Pediatrics (AAP) recommends that exclusively and partially breast-fed infants receive supplements of 400 IU day⁻¹ of vitamin D shortly after birth and continue to receive these supplements until they are weaned and consume ≥ 1000 ml day⁻¹ of vitamin D-fortified formula or whole milk. Similarly, all nonbreastfed infants ingesting < 1000 ml day⁻¹ of vitamin D-fortified formula or milk should receive a vitamin D supplement of 400 IU day⁻¹. AAP also recommends that older children and adolescents who do not obtain 400 IU day⁻¹ through vitamin D-fortified milk and foods should take a 400 IU vitamin D supplement daily. However, this latter recommendation (issued November 2008) needs to be reevaluated in light of the US Food and Nutrition Board's vitamin D RDA of 600 IU day⁻¹ for children and adolescents (issued November 2010 and which previously was an AI of 200 IU day⁻¹).

Summary

The feasibility and degree to which micronutrient supplementation should be pursued in combination with other

strategies to prevent and control micronutrient deficiencies depends on the local needs, resources, capabilities, commitment, and evidence of benefit. The successful prevention and control of vitamin A, iron, and zinc deficiencies will likely rest on a combination of repetitive distribution of high-dose nutrient supplements, fortification of staple foods, and behavior change, whereas fortification of salt alone with iodine has already achieved much success in combating IDD. The adoption of supplementation approaches should also be guided by evidence of a need for targeting, impact potential, costs, and potential sustainability.

See also: Iodine: Deficiency Disorders and Prevention Programs. Iron: Physiology, Dietary Sources, and Requirements. Pregnancy: Nutrient Requirements. Vitamin A: Deficiency and Interventions

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TEA

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Description of Tea

All true teas come from a single species of plant, *Camellia sinensis*. Tea is one of the most widely consumed beverages in the world, second only to water. In some countries, such as the UK, *per capita* tea consumption is estimated to exceed 2 kg/d. The many varieties of tea include white, yellow, green, oolong, black, and postfermented teas (such as pu-er). The most common teas on the market are white, green, oolong, and black. Of these, green and black teas are the most popular, representing nearly 21–77% of world consumption, respectively. The term ‘herbal tea’ refers to an steeped beverage made from leaves, flowers, fruit, or herbs of plants other than *Camellia sinensis* (such as chamomile, yerba mate, or lemon verbena), but contains no material from the *Camellia sinensis* plant.

The types of tea differ in the processing they undergo. White tea is derived from buds and leaves from young plants, and those buds and leaves are wilted in natural sunlight, then are lightly processed to prevent any further oxidation. For green tea, the *Camellia sinensis* leaves are simply dried with minimal processing. Yellow tea refers to a type of tea processed similarly to green tea, but for yellow tea, the drying phase is slower and the damp leaves are allowed to sit and yellow. Oolong is a traditional Chinese tea in which the leaves are wilted under strong sunlight and then partially oxidized to varying degrees, from 8 to 85%. For black tea, the leaves are fully oxidized after wilting. Postfermented tea is a less common type of tea that has been aged in open air for periods ranging from months to years. This exposes the tea to microbiota, oxygen, and moisture, which facilitates fermentation. The oxidation of tea polyphenols is caused by the reaction of polyphenol oxidase with catechins and other phenolic compounds in tea leaves during the withering process. This process is often referred to as ‘fermentation’ even though no microorganisms are involved (except for postfermented teas).

Although the tea plant has its origins in southwestern China and eastern India, it is now cultivated in many other countries, including South America, Africa, the Middle East, and throughout Asia and consumed as a beverage throughout the world. Early references to tea date back as far as 5000 years. The origin of tea is attributed to the Emperor Shen Nung, who unintentionally made the first pot of tea when leaves from the tea tree under which he had camped fell into a pot of boiling water he was preparing. Although the true origins of tea drinking are unknown, it is clear that tea has been used for centuries as a means of improving health. In recent decades, the health effects of tea have been examined scientifically. The results of these studies indicate that tea is capable of modulating many physiologic processes associated with disease, but its true impact on human health is still a matter of debate.

Composition of Tea

The predominant phytochemicals of beverage tea are catechins, theaflavins, and thearubigins. Catechins are one form of a class of polyphenolic compounds termed ‘flavonoids,’ which are all 2-phenyl-benzopyran-based compounds. The flavonoids include six major structurally related subclasses of compounds: (1) flavones, (2) flavonols, (3) flavanones, (4) anthocyanidins, (5) isoflavones, and (6) flavanols. Catechins belong to the *flavanol* subclass, and are structurally defined as flavan-3-ols. The major catechins of the tea leaf are epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate. These four catechins comprise approximately 25% of the weight of a dry tea leaf. *Flavonols* (kaempferol, quercetin, and myricetin glycosides) are minor flavonoid components of the tea leaf (approximately 3% of dry weight). Polysaccharides and cellulose (approximately 20% of dry weight) and protein (approximately 15% of dry weight) are

other major components of the tea leaf, and other nutrients and compounds comprise less than 5% of dry weight each.

Green, oolong, and black are the predominantly consumed types of tea. The minimal processing of green tea results in high catechin content of 30–40% of the green tea solids. The oxidation process used to form black and oolong teas releases polyphenol oxidase that initiates polymerization and oxidation of the catechins to theaflavins and thearubigins. In contrast to the heterogeneous and unknown chemical structure of thearubigins, theaflavins are chemically well-defined. There are four primary theaflavins in black tea, which represent between 1 and 6% of the dry weight of the solids (theaflavin, theaflavin 3-gallate, theaflavin 3'-gallate, and theaflavin 3,3'-digallate). Thearubigins represent between 10 and 20% of the dry weight of black tea solids. Because oolong tea is processed and fermented to a lesser degree than black tea, oolong tea contains a higher concentration of the theaflavins and lower concentration of thearubigins than black tea. Black tea is popular in western countries, and oolong tea is sold commercially in the USA and is often served in Chinese restaurants. Theaflavins and thearubigins are responsible for the red-amber color of oolong and black tea and for its astringency.

Another important group of flavan-3-ol compounds found in tea is the procyanidin polymers. These compounds are catechin polymers linked by C4 to C6 and C4 to C8 bonds. The most predominant polymers are those ranging from dimers to decamers and are found in a variety of foods, including chocolate, cocoa, nuts, grains, fruits, and vegetables.

Antioxidant Properties of Tea

Polyphenols in tea convey notable antioxidative properties. Structural features of tea catechins that provide antioxidative activities include the di- and tri-hydroxyl groups on the B-ring and the meta-5,7-dihydroxyl groups on the A ring. The trihydroxyl structure in the D-ring (gallate) of the epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) molecules convey additional antioxidative properties. Generally, the polyphenolic structure results in electron delocalization, which conveys the ability to quench free radicals. Compounds in tea can react with reactive oxygen species, such as hydroxyl radical, peroxy radical, superoxide radical, singlet oxygen, nitric oxide, nitrogen dioxide, and peroxyxynitrite.

Tea compounds can also convey antioxidative activity through metal ion chelation through vicinal dihydroxyl and trihydroxyl structures, thereby preventing the generation of free radicals. For example, oxidation of lipoproteins induced by Cu^{2+} is inhibited *in vitro* by green tea.

These antioxidant compounds in tea appear to provide protection from oxidative stress *in vivo*. Animal studies have shown that EGCG can reduce levels of lipid peroxidation, malonyldialdehyde, and protein carbonylation in the liver. Mechanisms of antioxidative action include preservation or increased levels of other antioxidants (such as reduced glutathione) and induction of antioxidant enzymes (such as superoxide dismutase).

Human studies to date have shown at best modest increases in plasma antioxidant capacity after tea ingestion. One

study of tea consumption by healthy volunteers resulted in an 18% decrease in plasma oxidized low density lipoproteins. However, other studies have shown no improvement in oxidative status after tea consumption. It has been speculated that the low bioavailability of tea polyphenols limits *in vivo* activity.

Tea and Cardiovascular Disease (CVD)

CVD account for 13 million deaths annually worldwide, approximately 22% of all-cause mortality. CVD is the leading cause of death in the United States and most European countries. The relatively low CVD mortality in countries such as Japan has fueled interest into which lifestyle components may be responsible for these findings. In Japan, heart disease rates are less than one-third of those in the US, despite Japan's large population of smokers. One of the suggested reasons for low heart disease rates is the regular consumption of green tea by the Japanese population.

The effects of tea on heart disease remain unclear. Epidemiologic studies have reported potentially positive, negative, and null effects of tea on CVD risk. Two studies performed in the UK, where black tea is the primary type consumed, suggested a positive association between tea consumption and risk for CVD. Data from a large cohort conducted in Wales indicated that tea consumption was associated with an increased risk of heart disease. Ireland, which has the highest per capita black tea consumption rates in the world, is the European country with the highest heart disease mortality rates. Black tea has been reported to cause short-term increases in blood pressure, particularly in fasted subjects, and has also been reported to raise plasma homocysteine levels. In contrast, several cross-sectional studies of Asian populations have suggested a protective effect of tea with respect to heart disease, though the association was specifically for green tea. Other studies have indicated that long-term green, oolong, and black tea consumption is associated with reductions in blood pressure and reduced stroke risk.

A few studies have investigated the effect of tea or tea components on vascular function. Acute, but not chronic, administration of EGCG improved vascular function in clinically stable CVD patients. Daily consumption of green tea for a month did not lessen central arterial stiffness in diabetics. Two other studies, one with smokers and one with healthy women, showed improved vascular reactivity after consumption of green tea for 14 day or green tea extract for 5 weeks, respectively. Several studies conducted with green tea showed improvement in flow mediated dilation 2 h after consumption of green tea, compared to water or caffeine controls.

Studies of the impact of tea on blood lipids have also been equivocal, but generally suggest that tea consumption may improve blood lipid profile. One intervention study, in which volunteers consumed five servings of black tea daily, showed reduction in total and low density lipoprotein (LDL) cholesterol in mildly hypercholesterolemic adults. A crossover study of 33 healthy volunteers suggested that consumption of dried green tea extract daily for eight weeks reduced total cholesterol and LDL-cholesterol. Another large intervention study of 111 volunteers showed that consumption of a capsule containing a

mixture of *Camellia sinensis* compounds twice daily lowered total cholesterol and LDL-cholesterol, as well as systolic and diastolic blood pressure. A large intervention study of theaflavin-enriched green tea extract consumed daily resulted in a significant reduction of total cholesterol and LDL-cholesterol after 12 weeks. In contrast, another large intervention study with purified black tea theaflavins consumed daily for 11 weeks showed no change in total cholesterol or LDL-cholesterol. As with other outcome variables, the type of tea or tea compounds used for the interventions would have a major impact on the study outcome.

Tea and Cancer

Cancer is a leading cause of death worldwide, accounting for almost 8 million deaths annually, approximately 13% of all deaths. Tea has been shown to modulate a number of mechanisms associated with cancer. *In vitro* studies indicate that teas modulate inflammation, oxidative stress, deoxyribonucleic acid (DNA) damage, angiogenesis, and apoptosis. Epidemiologic and animal studies report that tea consumption is generally associated with a reduced risk of gastrointestinal and reproductive tract cancers. Studies of cancer with animal models have shown tea and tea components to inhibit both tumor formation and growth. These studies indicate that tea may inhibit DNA damage, decrease cell proliferation, increase apoptosis, suppress angiogenesis, and modulate P-450 detoxifying enzymes.

Epidemiological and case control studies, when taken as a whole, are inconclusive with respect to the effect of tea on cancer. These studies have included cancers of the colon, lung, stomach, breast, prostate, ovary, pancreas, kidney, bladder, and others. Slightly fewer than half of the case control studies and about one-fifth of the cohort studies showed an inverse relationship between tea consumption and cancer risk, whereas the others showed no such association. The influence of tea on cancer may in part be related to the type of tea, with green tea more likely to be effective than black tea. The effect of tea on cancer may also be dependent on type of cancer. Black and green teas have been reported to inhibit lung tumorigenesis in several rodent models, though large doses were required. Tea has also been effective against rodent models of chemically induced oral carcinogenesis. EGCG was shown to inhibit spontaneous development of small intestinal tumors in a dose-dependent manner and to slow growth of human prostate cancer xenografts. In contrast, oolong and black teas have also been reported to increase the risk of kidney and bladder cancer. Other workers reported that black tea, but not green tea, consumption increases circulating estrogen levels, a factor associated with increased breast cancer risk, in postmenopausal women.

One difficulty of assessing the role of tea in cancer development is that it is difficult to extend the effects of short-term interventions on diseases, such as cancer, that take decades to develop and for which few reliable biomarkers are available. In addition, although cancer is often considered a single disease, it is actually a group of many diseases. For this reason, it is entirely possible that tea consumption might reduce the risk of some types of cancers, have no effect on others, and increase

the risk for other cancers. Still, the general conclusion at this point is that the effect of tea on cancer is generally small.

Tea and Diabetes

Evidence surrounding the potential for tea to influence diabetes has been controversial. Epidemiological studies have yielded seemingly contradictory results for tea consumption and the development of diabetes. A study of tea consumption by adults in Japan suggested that green tea intake was associated with lower diabetes risk, whereas oolong and black teas were not. A large cohort study of US women showed reduced risk for diabetes among tea drinkers. Results from another large US cohort study, this time including both men and women, suggested that there was an inverse relationship between tea intake and diabetes risk, but only for adults under 60 years of age who had recently lost weight. The Dutch arm of the European Prospective Investigation into Cancer and Nutrition found that three cups of tea per day was correlated to a lower risk for diabetes. A survey of elderly adults in the Mediterranean showed lower blood glucose and lower risk of diabetes among tea drinkers. In contrast, other large survey studies, including the Health Professionals' Follow-up Study, the Nurses' Health Study, the Nurses' Health Study II, a cohort study in Singapore, and the large French E3N cohort study, revealed no association of tea and diabetes, though in some cases, many participants consumed fewer than three cups of tea per day.

Results from intervention studies also yield seemingly contradictory results. One study of diabetics consuming oolong tea showed lower fasting plasma glucose and fructosamine after 30 days. Another study with healthy adults showed that 1 g of black tea reduced blood glucose response after glucose intake, whereas 3 g of black tea had no effect. When overweight Japanese with borderline diabetes consumed green tea extract, there was a small reduction in hemoglobin A1C, whereas fasting glucose, insulin, and homeostasis model assessment-estimated insulin resistance (HOMA-IR) were not affected. Other studies of adults consuming green, oolong, or black tea showed no effects on fasting insulin, fasting glucose, glycosylated hemoglobin, HOMA-IR, or glucose and insulin response after ingestion of a glucose load. For example, recent crossover studies with oolong tea or green tea consumed with breakfast (vs a placebo) showed no improvement in glucose or insulin response after the meal. Another study of green and black tea extract delivered in capsules resulted in no improvement in fasting glucose and HbA1c. These intervention studies differed in subject population, type of tea administered, length of intervention, and control of background diet, as well as other design features, and these differences are likely responsible for the differing outcomes, making it at this point difficult to draw reliable conclusions about how to improve glucose management with tea.

Tea and Obesity

Obesity is a growing global epidemic. Between 1995 and 2000, the number of obese adults worldwide rose from 200

million to 300 million. In 2008, this number rose to an astounding 600 million. Given the magnitude of this health crisis, the potential for tea to influence the obesity problem has been investigated.

Epidemiological studies suggest that tea may play a role in preventing body weight gain. A cross-sectional study of Taiwanese adults found that habitual tea drinkers had lower body fatness and lower waist-to-hip ratio compared to those who did not drink tea. A longitudinal study of adults in the Netherlands reported an inverse relationship between catechin consumption and body mass index.

Animal and human intervention studies suggest that tea or tea components influence a variety of factors associated with body weight and fatness. Animal models have shown that tea or tea components administered to rodents can reduce body weight gain, lower adipose tissue weight, decrease plasma leptin, and increase fecal lipid content. Two Japanese clinical intervention studies have suggested that administration of low or high catechin Oolong tea or catechin-enriched green tea resulted in decreased body weight and fat mass. A third Japanese study of diabetics suggested tea consumption reduced waist circumference. Similarly, overweight and obese Thai subjects showed decreased body weight for participants consuming tea compared to placebo, but neither body mass index nor body fatness was affected. Further, a study of overweight women in Netherlands found no influence of green tea extract on body mass index, waist-to-hip ratio, or fat mass. Physiologic mechanisms affected by tea or tea components in animal and human studies include inhibition of *de novo* lipogenesis, increased fat oxidation, increased carbohydrate utilization, and decreased carbohydrate uptake in tissues such as small intestine, liver, adipose tissue, and skeletal muscle. A recent meta-analysis suggested that the effects of tea on fat oxidation are present only for catechin-caffeine mixtures. Although these many actions of tea suggest that tea may reduce tendencies for individuals to become overweight or obese, the most important factor in influencing weight gain is energy intake. However, animal models of obesity and tea have generally reported no influence of tea or tea components on total energy intake. Thus, the potential for tea to reduce incidence of obesity is likely limited at best.

Conclusion

Globally, tea is a widely consumed beverage. The polyphenols found in tea have strong *in vitro* antioxidant capacity. Tea consumption may reduce the risk of CVD through improved blood lipid profile and improved endothelial function. Epidemiologic studies provide weak evidence for an association between tea consumption and risk for CVD, diabetes, cancer, and obesity. Nevertheless, research with animals and cells suggest potential mechanisms by which tea could play a role in the etiology of these diseases.

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Contents

Beriberi

Physiology

Beriberi

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Glossary

Ataxic gait Inability to coordinate leg muscles.

Confabulation Description of events that have not happened.

Dyspnoe Labored or difficult breathing in sleep (paroxysmal nocturnal dyspnea is a respiratory distress that awakens patients from sleep).

Meteorismus Swelling of the abdominal cavity with gas usually in the large intestine or stomach.

Nystagmus Involuntary eye movements.

Ophthalmoplegia A paralysis or weakness of one of the muscles that control eye movement.

Opisthotonus A type of spasm in which the head and heels arch backward in extreme hyperextension and the body forms a reverse bow.

Paresthesia A sensation of numbness or tingling on the skin.

Rales (pronounced Ralz) Wet, crackly lung noises heard on inspiration which indicate fluid in the air sacs of the lungs.

Wernicke encephalopathy A syndrome characterized by ataxia, ophthalmoplegia, confusion, and impairment of short-term memory.

Beriberi is caused by a deficiency of thiamin (also called thiamine, aneurin(e), and vitamin B₁). Classic overt thiamin deficiency causes cardiovascular, cerebral, and peripheral neurological impairment and lactic acidosis. The disease emerged in epidemic proportions at the end of the nineteenth century in Asian and Southeast Asian countries. Its appearance coincided with the introduction of the roller mills that enabled white rice to be produced at a price that poor people could afford. Unfortunately, milled rice is particularly poor in thiamin; thus, for people for whom food was almost entirely rice, there was a high risk of deficiency and mortality from beriberi. Outbreaks of acute cardiac beriberi still occur, but usually among people who live under conditions where their freedom is restricted, food is of poor quality, and they have little access to supplements; mainly jails. The major concern today is subclinical deficiencies in patients with trauma or among the elderly. There is also a particular form of clinical beriberi that occurs in patients who abuse alcohol, known as the Wernicke–Korsakoff syndrome. Subclinical deficiency may be revealed by reduced blood and urinary thiamin levels, elevated blood pyruvate/lactate concentrations and α -ketoglutarate activity, and decreased erythrocyte transketolase (ETKL) activity. Currently, the *in vitro* stimulation of

ETKL activity by thiamin diphosphate (TDP) is the most useful functional test of thiamin status where an acute deficiency state may have occurred. The stimulation is measured as the TDP effect.

Epidemiology

Beriberi presents in several different clinical forms (**Table 1**). Beriberi became endemic following the introduction of steam-powered rice mills, which enabled milled rice to be produced cheaply enough so that almost everybody could afford it and consume it. It was particularly serious at the end of the nineteenth and the beginning of the twentieth centuries when seasonal epidemics of wet beriberi occurred with many deaths. The disease affected mainly the Chinese and Japanese populations, although outbreaks were reported in India and among settlers in the New World during the long cold winters, and the disease was not necessarily confined to rice-eating populations. Where acute cardiac beriberi occurred, dry beriberi was also present but usually in the older members of the community.

Milled rice has a thiamin concentration that is particularly poor (80 μ g per 100 g) and poor storage or cooking can reduce

Table 1 Forms of beriberi in man

Subclinical beriberi	Identified by transketolase activity or other biochemical tests of thiamin status. May be associated with early subjective symptoms such as anorexia, weakness, dysesthesia, and depression. Responds rapidly to treatment with thiamin.
Wet beriberi	Subacute or cardiac beriberi frequently having muscular pains, edema of feet and legs, enlarged heart, and tachycardia. Responds rapidly to treatment with thiamin. Major form and was typically seasonal in endemic areas. Acute fulminant type of beriberi in which the main feature is dominated by insufficiency of the heart and blood vessels. Responds rapidly to treatment with thiamin.
Dry beriberi	Chronic, atrophic type of polyneuropathy in which the main features are of a weak wasted person, with painful musculature making walking difficult, impaired sensory nerves and tendon reflexes, and flaccid paralysis of the motor nerves. Poor or no response to treatment with thiamin.
Infantile beriberi	Usually acute wet beriberi. Responds rapidly to treatment with thiamin.
Wernicke–Korsakoff syndrome	Predominantly neurological, affecting walking and vision in most and memory and cardiac function in over 50% of patients. Wernicke or ocular component responds rapidly to treatment but the Korsakoff psychosis responds slowly or not at all.

Source: Modified from Thurnham DI (1978) Thiamin. In: Rechcigl Jr M (ed.) *Nutrition Disorders*, pp. 3–14. West Palm Beach, FL: CRC Press.

this to negligible amounts. However, social conditions at the time of the large epidemics contributed to the problems. Bonded labor was common, with workers living on the work premises most of the time and paid mainly in the form of rice. In addition, reports at the time suggest that the rice was of uncertain freshness and quality, and that it could be so moldy, matted, and lumpy that it had to be remilled and washed, with a further loss of thiamin. The social conditions prevented natural eating practices because workers had little money to purchase additional food and they were dependent on what they were given. Likewise, badly stored cereals can lose up to 90% of the thiamin content, and toxins associated with mold growth have been implicated in causing sickness that may well precipitate clinical beriberi.

Reports suggest that the acuteness of the outbreak of beriberi and the interrelationship of thiamin deficiency with deficiencies of other nutrients probably had a major role in determining the nature of the pathological changes and lesions produced. For example, it is reported that protein energy malnutrition almost always accompanied subacute beriberi, reflecting the link between impoverishment and the disease. In contrast, it is also suggested that severe beriberi more often affected the more active, stronger, or supposedly better nourished members of the community. The younger, stronger rickshaw puller was most likely to suffer severe beriberi. This enigma may be due to thiamin intakes from a diet containing a high proportion of rice being insufficient to meet the thiamin requirement posed by the higher calorie intakes of the more active community members.

In older literature, it is reported that infantile beriberi appeared to affect the male infant who 'tended to be overfed.' Human milk is barely adequate in thiamin (0.23 mg per 4.2 MJ) but this may be reduced still further in marginally deficient mothers and the thiamin status of infants was further compromised if given supplements of thiamin-poor rice. It is a common habit even today for rural mothers in Northern Thailand and in Laos to give very young infants, even beginning at 1 week of age, a bolus of masticated rice to supplement the milk intake. In 2009, a study in Vientiane reported that 27% of infants were given water or formula milk before receiving breast milk and premasticated glutinous rice was the first food supplement in 20–48% of infants in the first

week of life. In the same study mothers underwent variable periods of dietary restriction in the first 3 months postpartum including exposure to hot beds of embers. The effects of these traditional practices on breast milk composition is not known but pyrexia is known to increase energy and thiamin requirements. It was widely observed that nonspecific pyrexia was a precipitating factor for beriberi. A 1 °C rise in body temperature is associated with a 10% increase in basal metabolic rate. It has been suggested that more than half the mild cases of beriberi were associated with a nonspecific bout of fever, and such cases responded less readily to treatment with thiamin.

Parboiled rice is partially cooked in the husk before milling, and this prevents beriberi because the thiamin is dispersed through the grain (190 µg per 100 g). The advantages of this were clearly seen in Malaya, where at the end of the nineteenth century there were large-scale immigrations of young, able-bodied Chinese to work in the tin mines and Indians to work on the rubber estates. In both cases, immigrants often lived in remote regions where there was little opportunity to purchase local food and they were dependent on imported rice. It was the Chinese who, because of their dietary preference for milled rice, died in enormous numbers.

Although ways of avoiding the disease were known to the Japanese navy at the end of the nineteenth century, because the director general of the medical department had demonstrated that the disease was almost eradicated if the traditional rice diet was supplemented with fish, vegetables, meat, and barley, this information was not widely available, and supplementation was not feasible by the vast majority of people. It was widely believed that the cause of beriberi was an infection or toxin resulting from bad food. In particular, Pasteur's work on the microbiological cause of infections led many to search for an infectious agent, but none could be consistently identified. The scale of the problem for the colonial powers in Southeast Asia in the latter part of the nineteenth and early twentieth centuries should not be underestimated. Labor was cheap but the death toll posed enormous problems. Extracts from reports at the time are illuminating: in 1887, there were 690 deaths out of 1931 native government officers in Sumatra, infant mortality was 445 per 1000 live births in the Philippines in 1910, and one

report stated that there were so many deaths that “there was insufficient earth to bury the corpses.”

The Dutch government sought to resolve the situation by appointing a medical bacteriologist, Christiaan Eijkman, to travel to Indonesia to investigate the problem. Working in Java, he showed within 6 years that beriberi was a nutritional problem and that a paralytic condition closely resembling the polyneuritic symptoms of beriberi could be produced in chickens by feeding them both stale and freshly cooked polished rice. However, it was Funk in 1911 who first reported the isolation of a ‘vital amine’ from rice polishings that had antiberiberi properties. Funk was the first person to coin the word ‘vitamine’ as an accessory food factor essential for life. The structure and synthesis of thiamin were reported in 1936.

Currently, clinical beriberi no longer occurs with the devastating effects of former years. Considerable improvements have occurred in nutrition worldwide, the diversity of foods available, the quality of food due to improved storage methods, and social and economic structures in many countries, especially in Southeast Asia. However, sporadic outbreaks do occur, which are usually of the acute, fulminating type of beriberi causing many deaths if not recognized. An outbreak of acute beriberi occurred in a Gambian village in 1988 at the start of the wet season and killed 22 young adults before it was recognized and treated. In Thailand, infantile beriberi was reported in Karen refugees in 2003 and in Laos, infantile beriberi is currently recognized as a public health problem. Usually, a combination of factors is responsible, but once the condition is identified, treatment is cheap and readily available and, if given rapidly, tragic circumstances can be averted.

Two iatrogenic causes of subclinical beriberi are known, namely that associated with diuretic treatment and one resulting from alcohol abuse. Both are of concern because the use of diuretics is introduced to manage cardiovascular disease, a condition that will deteriorate if thiamin status is impaired, and alcohol abuse can lead to Wernicke–Korsakoff syndrome, which can have many of the features of both wet and dry beriberi.

Severe multisystem trauma, endotoxemia, or situations in which there is a raised metabolic demand for thiamin,

such as pregnancy, thyrotoxicosis, and intercurrent illness or impaired absorption (e.g., alcohol abuse or gastrointestinal disease or resection), can produce subclinical evidence of thiamin deficiency or more severe life-threatening aspects of beriberi, such as renal or cardiovascular failure. The elderly may be particularly at risk of subclinical thiamin deficiency. One Belgian study on patients with a mean age of 83 years reported that 40% had a raised TDP effect (>15%), in whom there was a high proportion of Alzheimer disease, depression, cardiac failure, and falls. The diuretic furosemide was also more frequently taken by the thiamin-deficient patients.

Etiology

The factors associated with the various forms of beriberi are listed in [Table 2](#). Beriberi is caused by a lack of thiamin in the diet, but the onset of the disease and the symptoms associated with the disease are influenced by one or more etiological factors. Wet beriberi (i.e., cardiac beriberi) and Wernicke’s encephalopathy are conventionally described as acute manifestations of the disease and respond most rapidly to treatment. In contrast, dry beriberi is described as due to a chronic deficiency of thiamin and does not respond well to treatment. However, experimental acute deficiency studies, which very rapidly produced subjective feelings of malaise and weakness at the slightest exertion, very rarely produced evidence of edema and peripheral pain. These observations suggest that all forms of beriberi are probably preceded by an indeterminate period of chronic thiamin deficiency during which pathophysiological adaptations to the marginal nutritional state occur. Thus, physiological adaptations to the vascular system may well have occurred particularly in those who did heavy physical work and needed to overcome the weakness and malaise imposed by a low thiamin diet. The factor(s) that precipitated the clinical disease may not be thiamin at all. Platt, in his descriptions of beriberi in China, recounts how humid weather and infections such as malaria increased the number of cases of wet beriberi. The extra energy needed to cool the body in hot conditions or fuel the rise in temperature

Table 2 Etiological factors contributing to thiamin deficiency

Dietary thiamin deficiency	Commonly milled rice
High dietary carbohydrate to fat ratio	Metabolism of carbohydrate requires thiamin, whereas metabolism of fat spares thiamin requirements
Heavy physical activity	Predisposes to beriberi when accompanied by low intake of thiamine
Protein energy malnutrition	Older literature reports sometimes accompanies subacute beriberi indicating importance of impoverished diet
Poor storage conditions for food	Fall 6- to 10-fold in thiamin content of cereals. Molds may accelerate decay as well as increase risk of toxins
Thiaminases	Two known, but only of importance when uncooked foods are consumed
Antithiamin factors	Factors in food that chelate with thiamin and potentially reduce bioavailability
Alcohol abuse	Alcohol impairs the active absorption mechanism for thiamine
Infection and trauma	Increase requirements for thiamin to support increased carbohydrate metabolism and energy production
Diuretics, long-term use	Accelerate thiamin excretion and appear to block thiamin control mechanism
Seasonal factors	Combination of heavy work load, impoverished diet, and last season’s (badly stored) cereals
Male sex	Some evidence that men have higher thiamin requirements than women but more likely to be a combination of the first three factors listed here

during infection may have imposed a critical burden on energy production that the system could not meet, and beriberi ensued.

However, the increased number of cases associated with heat, humidity, and malaria may also be due to a seasonal decline in the quality of food. A 6- to 12-fold decline in thiamin content is reported for millet when stored under traditional thatched storage houses in The Gambia, and reports suggest that much of the rice consumed late in the season was not in the best condition. Some of the products introduced by mold growth may possess antithiamin properties that impair thiamin bioavailability. Thus, the ratio of thiamin to calories is likely to fall during the agricultural year and to be at its worst when calorie requirements are at their highest for land preparation and weeding. Land preparation also takes place just before or at the beginning of the rainy season, when the prevalence of malaria and diarrheal diseases increases.

The thiaminase enzymes destroy thiamin activity by breaking the thiamin molecule into two parts – the pyrimidine and thiazole moieties. Thiaminases are inactivated by cooking; thus, the enzymes are only a problem where certain foods are eaten raw. It has been suggested that in northern Thailand, where consumption of fermented and raw fish products is widely practiced, thiamin status may be impaired by these food habits. Even as recently as 2001, marginal thiamin status was reported in more than 50% of women 3 months postpartum despite thiamin supplements of 100 mg day⁻¹ from week 30 during pregnancy and 10 mg per week during lactation. The deficiencies were found in Karen refugee women living on the Thai–Burmese border and whose diet contained raw fish, fermented tea leaves, and betel nuts – substances suspected of containing thiaminases. Polyphenol compounds in tea and many vegetables may also possess antithiamin properties and impair bioavailability by complexing with thiamin, but their etiological importance in causing thiamin deficiency is difficult to assess.

Alcohol is an important factor in causing thiamin deficiency because it inhibits the active transport of thiamin across the gut and when abused it impairs the quality of the diet consumed. Diuretics accelerate the excretion of thiamin and appear to override the renal conservation mechanism. Their use is of potential concern in elderly people whose diet may be poor for other medical reasons and their physicians may be unaware of their need for supplemental nutrients.

Both sexes are vulnerable to the effects of thiamin deficiency, but in many of the sporadic outbreaks that have been reported, there appears to have been a male excess. This may be due to higher thiamin requirements in men than women because of their higher lean body mass or to hormonally driven sex differences. However, it is also possible that the cause is due to a higher risk of a thiamin:calorie imbalance in men compared to women. In many rural communities, men traditionally eat first and may satisfy their calorie requirements, whereas their womenfolk make do with the leftovers. Because of their greater physical strength, men frequently do heavier work than women, requiring more energy (i.e., more food to meet their requirements). Thus, men may consume more of the thiamin-depleted cereals in the diet to satisfy calorie needs and in doing so achieve a poorer thiamin: calorie ratio than women.

Experimental Thiamin Deficiency in Man and Measurement of Thiamin Status

In young and healthy nonalcoholic subjects, subjective symptoms appear after 2 or 3 weeks of deficient diet but urinary thiamin will already be falling (Table 3). Characteristic early symptoms include anorexia, weakness, dysesthesia, and depression. At this stage, urinary thiamin will be almost zero, ETKL activity depressed, and the TDP effect between 15% and 30%. After 6–8 weeks the only objective signs at rest may be a slight fall in blood pressure and moderate weight loss,

Table 3 Effects of thiamin deficiency on urinary thiamin, the ETKL TDP effect, and early clinical symptoms of thiamin deficiency in human volunteers

<i>Days of deficiency</i>	<i>Urinary thiamin ($\mu\text{g day}^{-1}$)^a</i>	<i>TDP effect (%)^a</i>	<i>Clinical signs of deficiency following diets containing 150–350 μg thiamin per day^b</i>
5	50	0–10	Mostly studies report no signs but in one study (360 $\mu\text{g day}^{-1}$) subjects developed chest pains, extreme lassitude, anorexia, palpitation, and burning feet within 1 week
10	25	~ 15	
21–28	<25	~ 30	Loss of body weight, anorexia, general malaise, insomnia, increased irritability, fatigue on slightest exertion
30–40	Negligible	≥ 40	Increased malaise, loss of body weight, intermittent claudication and polyneuritis, bradycardia, peripheral edema, cardiac enlargement, ophthalmoplegia
> 45	10–20	> 40	Additional signs of nausea and dizziness appeared
75	10–20		Additional signs of vomiting, low blood pressure, and tenderness of calves

^aBiochemical data and report of edema and cardiac enlargement from Brin (1964), in which healthy male medical students were fed 200 μg thiamin per day for 6 weeks. TDP effect is a measure of thiamin status obtained by measuring the activity of erythrocyte transketolase in the presence and absence of added thiamin diphosphate.

^bClinical signs adapted from several studies. Investigators were impressed by the rapid degree of debility induced by the specific withdrawal of thiamin from the diet. In one group (150 $\mu\text{g day}^{-1}$ for 75 days, four female mental patients), the authors reported that the condition more closely resembled ‘neurasthenia’ than beriberi and noted that edema, cardiac dilation, and peripheral pain characteristic of classic beriberi were all absent.

Source: Reported by Carpenter KJ (2002) Acute versus marginal deficiencies of nutrients. *Nutrition Reviews* 60: 277–280.

although urinary thiamin will now be negligible and the TDP effect $\geq 35\%$. After 2 or 3 months, apathy and weakness become extreme, calf muscle tenderness develops, and there is loss of recent memory, confusion, ataxia, and sometimes persistent vomiting. Urinary thiamin will be negligible and the TDP effect may be normal (because apo-ETKL is unstable even *in vivo*), but ETKL activity should be considerably depressed.

The clinical symptoms resulting from experimental thiamin deficiency in man have usually responded rapidly to treatment with thiamin. In one feeding study, however, two mental patients were kept for 110 days on a diet providing 200 μg thiamin daily and 1 mg of thiamin by injection 1 day each week; thus, their overall weekly average was 350 μg day⁻¹. They developed a polyneuropathy characterized by

defects in the sensory nervous pathways, loss of tendon reflexes, and paralysis of the legs, which took many weeks to respond to large doses of thiamin, and in one case response was still incomplete after 4 months of treatment. The slow cure suggested that degeneration of peripheral nerves had occurred, as is indicated in the dry form of beriberi, in which the neurological lesions are irreversible.

Clinical Features of Beriberi

Depletion and repletion studies suggest that intakes $> 300 \mu\text{g}$ per 4.2 MJ are compatible with normal biochemistry and good health, and clinical signs of thiamin deficiency occur at intakes

Table 4 Common features of wet beriberi

	<i>Subacute beriberi</i>	<i>Acute fulminating beriberi</i> ^a
Digestive system	Anorexia is common; constipation more frequent than diarrhea	Vomiting is common, often with intense thirst Liver enlarged and tender and the epigastric region spontaneously painful
Neurological	Aching pain, stiffness, tightness, or cramps in calf or associated muscles Increasing muscular tenderness and weakness with fatigue pains resembling muscular ischemia, especially at night Pain on squeezing calves Inability to rise from squatting position without use of hands Diminished reflexes of ankle and knees usually bilaterally Hypoesthesia or paresthesia presenting as 'pins and needles,' numbness particularly over the tibia, formication (like ants running on the skin) or itching	Pupils dilated with anxious expression on face Aphonia frequently present and patient moans with cries of a special kind as a result of hoarseness produced by paralysis of laryngeal muscles Reflexes of ankle or knee lost or diminished
Cardiac	Edema of feet and legs often appearing first on dorsa of feet and extending up legs but may also appear on back of hands and as puffiness in face Heart enlarged with tachycardia and bounding pulse Raised venous pressure (see Figure 2) with percussion sometimes revealing dilation of right auricle and ventricle Heart murmurs if present are usually systolic Apex beat is downward and outwardly displaced Neck vein possibly distended showing visible pulsations Dyspnea on exertion Palpitations, dizziness, and giddiness Extremities possibly cold and pale with peripheral cyanosis but where circulation is maintained, skin warm due to vasodilatation Electrocardiograms often undisturbed but QRS complex may show low voltage and inversion of T waves indicating disturbed conduction	Patients are severely dyspneic, have violent palpitations of the heart, are extremely restless, experience intense precordial agony but accessory muscles of respiration only slightly brought into action Widespread and powerful undulating pulsations visible in the region of the heart, epigastrium, and neck due to a tumultuous heart action Facial cyanosis more marked during inspiration Pulse is moderately full, regular, even with frequency of 120–150 per minute A wavelike motion may be felt over the heart On percussion, the heart is enlarged both to the left and right but mainly the latter, and the apex beat may reach the axilla Raised systolic pressure and low diastolic pressure give the 'pistol shot' sound on auscultation over the large arteries Rapidly increasing edema may extend from legs to trunk and face with associated pericardial, pleural, and other serious effusions
Urine	Nocturia; no albuminuria	Oliguria or anuria; no albuminuria or glycosuria

^aThe whole picture of acute fulminating beriberi is dominated by insufficiency of heart and blood vessels and this tends to mask all other features of the subacute form, although these are often present and accentuated. Death is accompanied by a systolic pressure falling to 70–80 mm, the pulse becomes thinner, and the veins dilate. The rough whistling respiration deteriorates and rales appear. The patient dies intensely dyspneic but usually fully conscious.

Source: Modified from Thurnham DI (1978) Thiamin. In: Rechcigl Jr M (ed.) *Nutrition Disorders*, pp. 3–14. West Palm Beach, FL: CRC Press.



Figure 1 Patient with dry beriberi showing evidence of motor nerve disturbances resulting in a flaccid paralysis of the extensor muscles and 'wrist drop' and 'foot drop'. Courtesy of the late Professor B.S. Platt, formerly head of the Department of Human Nutrition, London School of Hygiene and Tropical Medicine, UK.

of thiamin below 200 μg per 4.2 MJ (1000 kcal). The disease as studied from the 1880s onward in Asians subsisting on white rice began typically with weakness, 'wandering pains' in the legs, and lack of feeling in the feet. Some patients then developed edema (the presence of excessive amounts of fluid in the intercellular tissue spaces of the body) of the legs, trunk, and face. In severe cases, sufferers found it increasingly difficult to catch their breath and would die of heart failure. The clinical features of subacute and acute wet beriberi are summarized in [Table 4](#). The main form was subacute beriberi, which was typically seasonal in endemic areas. There are reports that the peripheral muscles most severely affected were those most frequently used; thus, in male laborers it was the legs. Aching pain, tightness, and cramps in the calf and associated muscles were usually a first cause of complaint, and pain on squeezing the calves was one of the most useful diagnostic tests for beriberi. In women who performed repetitive tasks involving hands and arms, a loss of sensation in the fingers was frequently a first cause of complaint.

Dry beriberi is essentially a chronic condition showing muscular atrophy and polyneuritis and frequently occurring in older adults. Walking is usually difficult because of the weak wasted and painful musculature, and in the later stages feeding and dressing may also become impossible. When bed-ridden and cachectic (extreme state of malnutrition and wasting), patients become very susceptible to infections. Sensory

nervous function is impaired (hypoesthesia) almost to the point of anesthesia. Hypoesthesia is particularly evident in the extremities and progressively extends over the outer aspects of the legs, thighs, and forearms. Motor nerve disturbances also begin in the extremities and ascend progressively. Flaccid paralysis of the extensor muscles precedes that affecting the flexors and results in 'wrist drop' and 'foot drop' ([Figure 1](#)). Loss of the Achilles tendon reflex usually precedes an impaired patellar reflex.

Mortality from infantile beriberi mainly affected breast-fed infants between the second and fifth months of life which coincides with when solid foods were usually introduced. The introduction of white rice porridges, poor in thiamin, to a rapidly growing child and the increased exposure to infections when solids were introduced may both have contributed to infantile beriberi. The onset of the disease was rare in the first month and early signs could be mild and somewhat subjective (e.g., vomiting, restlessness, anorexia, and insomnia). Early signs could progress to subacute infantile beriberi, the acute and usually fatal condition, or a chronic form. Features of acute infantile beriberi are presented in [Table 5](#). The subacute form was characterized by slight edema in the form of puffiness, vomiting, abdominal pain, oliguria, dysphagia, and convulsions. In addition, aphonia (soundless cry) was often a feature of subacute infantile beriberi and may have been due to nerve paralysis or edema of vocal cords. Vomiting was also a feature of chronic infantile beriberi and could be accompanied by inanition, anemia, aphonia, neck retraction, opisthotonus, edema, oliguria constipation, and meteorismus (swelling of the abdominal cavity from gas in the intestine). Opisthotonus is a characteristic of acute thiamin deficiency in birds and is described as due to a tetanic spasm in which the spine and extremities are bent backwards.

In a recent report from Laos, 43 breast-feeding infants (median age; range: 3; 1–9 months) were admitted with infantile beriberi which was defined as congestive heart failure or shock in the absence of fever or other signs of sepsis, hypovolemia or cardiac abnormalities and a rapid clinical improvement following parental thiamin.

In alcoholic and other malnourished subjects, one of the early signs of thiamin deficiency is anorexia. In alcohol abuse, the overwhelming desire for alcohol may outweigh all other interest in food, leading to generalized malnutrition. Alcohol specifically blocks the active absorption of thiamin and alcohol abuse can progress to the potentially fatal condition known as Wernicke–Korsakoff syndrome. The typical clinical features of Wernicke's encephalopathy comprise ophthalmoplegia, nystagmus (usually horizontal), ataxic gait, and an abnormal mental state that can range from mild delirium to global confusion. Liver disease and tachycardia occur in more than 50% of cases. Korsakoff's psychosis is characterized by a profound amnesia, disorientation, and often confabulation. The clinical features of Wernicke–Korsakoff syndrome are listed in [Table 6](#).

Management/Treatment

Patients in whom cardiac and renal signs of thiamin deficiency are identified usually respond well to treatment. The dose

Table 5 Features of acute infantile beriberi and frequency of occurrence

	Features	Frequency (%)
Appearance	Pale and cyanotic appearance, edematous, ill-tempered with abdominal distension	40
Voice	Hoarseness	80
	Sometimes groaning	50
Digestive system	Vomiting	80
	Dyspepsia	46
Cardiac	Tachycardia, <200 beats per minute	83
	Heart dilated	31
	Femoral sound on auscultation	5
Lungs	Rapid breathing	83
	Accentuation of the second pulmonary sound	
Neurological	Tendon reflex usually increased	74
	Less frequently decreased	26
	Convulsions	17
Urinary	Oliguria	65
Other	Slight fever	50
	Uneasiness	50

Source: Modified from Thurnham DI (1978) Thiamin. In: Rechcigl Jr M (ed.) *Nutrition Disorders*, pp. 3–14. West Palm Beach, FL: CRC Press.

Table 6 Clinical features of Wernicke–Korsakoff syndrome and frequency of occurrence

	Features	Frequency (%) ^a
Ocular disorders	Nystagmus (ocular ataxia – rhythmical oscillation of the eyeballs), almost always horizontal and in 50% of cases associated with vertical nystagmus on upward gaze	85
	Paralysis of one or more of the ocular muscles	50
	Sluggish reaction by pupils to light	19
Ataxia (inability to coordinate muscles)	Gait	87
	Legs	20
	Arms	12
	Speech	87
Polyneuropathy	Limbs only affected, mainly the legs only	82
	Of arms and legs	18
	Common symptoms include weakness, paresthesia, pain, loss of tendon reflexes, and of sensation and motor power	
Cerebral function	Some cases of foot drop or wrist drop or both	
	Global confusional state, profound disorientation, apathy, deranged perception and of memory, drowsiness, inattentiveness, indifference	56
	Disorder of memory: both retrograde and ante-retrograde amnesia, confabulation	57
	Alcohol abstinence syndrome	16
Cardiac	Tachycardia	51
General medical abnormalities	Disorders of skin and mucous membranes	36
	Redness or papillary atrophy of the tongue	29
	Liver disease	60

^aPercentages based on 188–245 cases.

Source: Modified from Thurnham DI (1978) Thiamin. In: Rechcigl Jr M (ed.) *Nutrition Disorders*, pp. 3–14. West Palm Beach, FL: CRC Press.

given and route used will vary with the seriousness of the deficiency. Intravenous doses as high as 250 mg day⁻¹ for 14 days and intramuscular doses of 25 mg followed by three daily oral doses of 10 mg have been reported for wet beriberi and are followed by a marked increase in urinary output

and improvement in cardiac function. Peripheral neuropathy (dry beriberi) is more resistant to treatment. Patients with the ocular signs of Wernicke disease usually respond to two or three daily injections of 50 mg thiamin. Long-term oral treatment of other manifestations of Wernicke–Korsakoff

syndrome with doses up to 50 mg day⁻¹ is reported, although benefit is variable and considerably influenced by the patients' ability to avoid further alcohol consumption. It is unlikely that patients receiving oral thiamin will absorb more than 5–7 mg day⁻¹, but in patients likely to abuse alcohol, absorption by passive diffusion of high thiamin doses is the only way to ensure that the patient will receive any thiamin. In addition, as in all patients who show evidence of nutritional deficiency, the likelihood of other coexisting deficiencies should not be overlooked and multinutrient treatment is probably desirable. Finally, it is important to realize that untreated thiamin deficiency can result in sudden death.

Lipid-Soluble Thiamin Derivatives

In recent years, several lipid-soluble derivatives of thiamin have been introduced, of which the best known is benfotiamine. The lipid solubility enables greater diffusion of these compounds across cell membranes than water-soluble forms of thiamin. In the cell benfotiamine undergoes catalytic reduction by intracellular sulphhydryl compounds to release active thiamin. Use of the supplement greatly increased transketolase activity and blood thiamin concentrations. Transketolase is the rate-limiting enzyme of the nonoxidative branch of the pentose phosphate pathway. Benfotiamine has

been shown to be useful for the management of rare genetic disorders in thiamin transport and may also prove useful to prevent damage from diabetic hyperglycemia. One study demonstrated that benfotiamine prevented experimental retinopathy. Diabetic hyperglycemia is accompanied by an increase in the potentially pathogenic glycolytic metabolites glyceraldehyde-3-phosphate and fructose-6-phosphate. Benfotiamine, by increasing transketolase activity, stimulates the pentose phosphate pathway to metabolize these glycolytic intermediates into pentose-5-phosphates and prevent the intracellular increase in potentially toxic products.

Case Study

A good example of the specific effect of thiamin in the treatment of beriberi is illustrated by the response of a 29-year-old male who was admitted with an unexplained acute renal failure and had been anuric for 24 h. The physicians' report on his symptoms was compared with the common clinical features of wet beriberi shown in Table 4. The patient's physical state and voice were extremely weak but speech was copious and confused. He complained intermittently of severe central chest and epigastric pain. A central cyanosis was present and he had a respiratory rate of 36 beats per minute. His temperature was normal and peripheries were lukewarm. He had gross generalized edema. The jugular venous pressure became

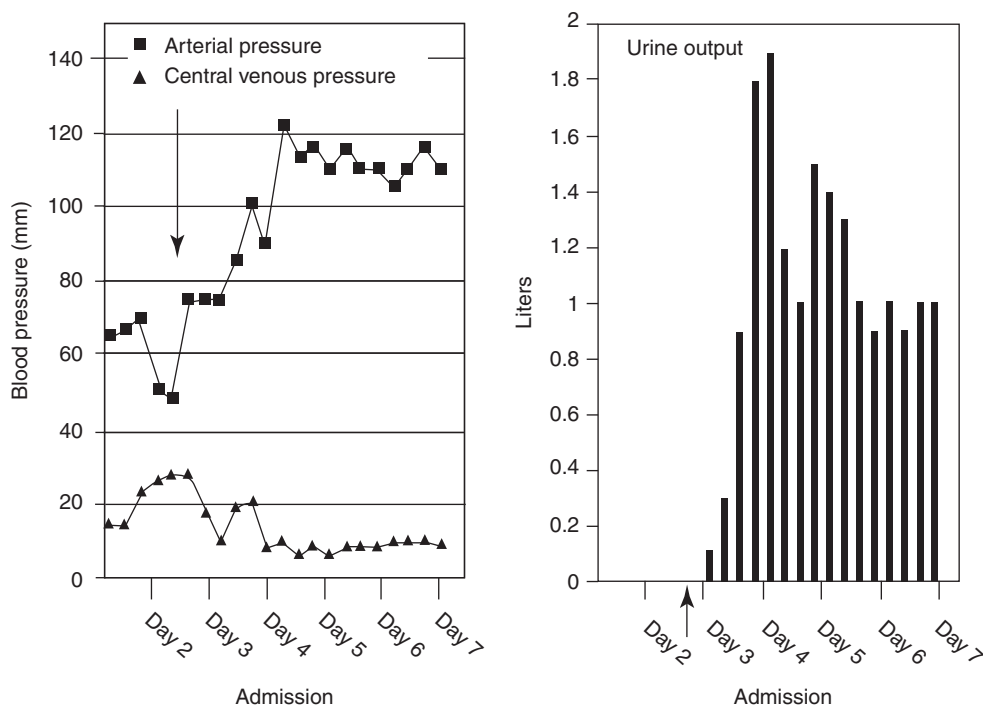


Figure 2 (Left) Arterial and central venous blood pressure and (right) urine output of a patient who was admitted with unexplained acute renal failure in a very weak physical state and whose speech (although very weak) was copious and confused. The patient was discovered to be a regular beer drinker consuming 6–12 pints daily, and his usual food intake amounted to no more than a sausage roll or pie. He had become progressively weaker over the past 8 weeks and had eaten nothing at all in the past 2 weeks. After excluding other diagnoses, it was suspected that the patient had fulminant beriberi and he was treated with thiamin after 36 h. The figures display the rapidly increasing arterial pressure, fall in venous pressure, and a rapid resumption in renal function following thiamin treatment. The patient lost ~20 l of urine during the first 7 days in the hospital. Modified from Anderson SH, Charles TJ, and Nicol AD (1985) Thiamine deficiency at a district general hospital: Report of five cases. *Quarterly Journal of Medicine* 55: 15–32.

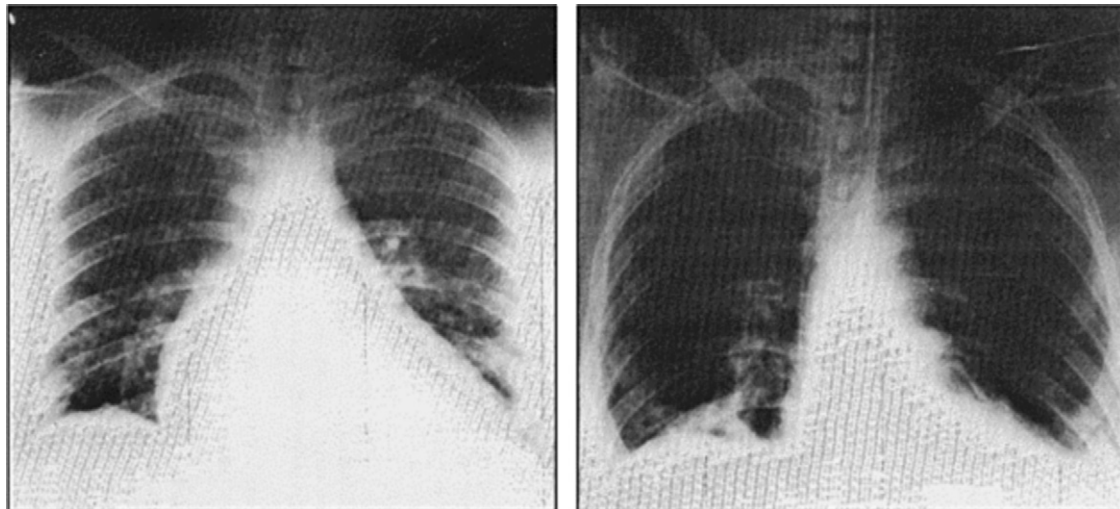


Figure 3 Chest radiographs of the patient described in **Figure 2** obtained on admission (left) and 14 days after high-dose, parenteral thiamin treatment (right). On admission, the heart was grossly enlarged, extending downward and to the right, with a cardiothoracic ratio of 0.63. After treatment for 14 days, the cardiothoracic ratio was 0.44. Modified from Anderson SH, Charles TJ, and Nicol AD (1985) Thiamine deficiency at a district general hospital: Report of five cases. *Quarterly Journal of Medicine* 55: 15–32.

grossly elevated (**Figure 2**). Pulse rate was 100 beats per minute, regular, and weak at the wrist, although the carotid pulses were visibly bounding. Blood pressure was 80/60. There was a marked parasternal heave present, with a loud pulmonary second heart sound. The chest was clear; the abdomen was obese.

The father reported that the patient's usual beer intake was 6–12 pints daily and his one regular meal was usually no more than a sausage roll or a pie. Before admission, for 6 weeks he had felt too tired to go out in the evening, and for 2 weeks he had suffered epigastric discomfort and had eaten nothing. Eight days before admission, he developed painful calf stiffness and he became too weak to go to work. He had a painful dry cough and dyspnoea on the slightest exertion. Finally, confusion, cyanosis, and intermittent vomiting led to admission.

The first diagnoses considered were myocardial infarction, pulmonary embolism, and overwhelming septicemia, and he was placed on dialysis and received appropriate treatments. His lack of response at 36 h, continuing low systolic pressure (70 beats per minute), increasingly gross hyperdynamic precordial signs, and moribund appearance led to a diagnosis of beriberi. Treatment with intravenous thiamin (250 mg for 14 days) brought about a dramatic response (**Figure 2**). Within 6 h peripheral pulses were strong, blood pressure had risen to 105 systolic, and central venous pressure had fallen by half. By 12 h the parasternal heave was less marked and diuresis of up to 6 l per day ensued. After 24 h, plasma urea concentration peaked at 50.4 mmol l^{-1} and creatinine at $832 \text{ } \mu\text{mol l}^{-1}$, and thereafter there was a steady fall over the next 2 weeks during which thiamin treatment continued and dialysis stopped. He lost a net 20 l of fluid over the first 7 days in the hospital and creatinine clearance 3 weeks after admission was 178 ml per minute, indicating a return to normal kidney function. Other biochemical abnormalities resolved over the 2 weeks on high-dose thiamin, including the chest radiograph (**Figure 3**). It is interesting to note, however, that when he was discharged

3 months after admission, he was walking with a calliper because of a right-sided foot drop (**Figure 1**). The persistence of the foot drop is a further indication of the greater difficulty in reversing neurological consequences, in contrast to the cardiac effects, of thiamin deficiency.

Toxicity

Chronic intakes in excess of 50 mg kg^{-1} , or more than 3 g day^{-1} , are toxic to adults with a wide variety of clinical signs, including headache, irritability, insomnia, rapid pulse, weakness, contact dermatitis, pruritis, and, in one case, death. Early researchers also indicated that regular administration or contact with thiamin occasionally led to allergic response, contact dermatitis, or hypersensitivity.

See also: Alcohol: Effects of Consumption on Diet and Nutritional Status. Cereal Grains. Fish and Seafood: Nutritional Value. Thiamin: Physiology

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Glossary

Achlorhydria Reduction in gastric acid content.

Acidosis Raised concentrations of pyruvic and lactic acids in the blood caused when pyruvate cannot be converted to acetyl CoA for onward metabolism by the tricarboxylic acid cycle and a fall in ATP production stimulates more glycolysis and more pyruvate production.

Aleurone layer Layer in the cereal grain occurring below the husk.

Beriberi The clinical condition resulting from a lack of dietary thiamine.

Cocarboxylase Alternative name for thiamin diphosphate or thiamin pyrophosphate.

Diuretic drug Increases production of urine to reduce edema in heart failure.

Gut neoplasia Cancer in the gut.

Parboiled rice Rice that has been boiled in the husk.

Polished rice Rice from which the outer husk and aleurone layers has been removed.

Thiamin Essential vitamin; also called thiamine, aneurin(e) and vitamin B1.

Thiaminase Enzymes that will inactivate thiamin. Found in a number of foods but are inactivated by cooking.

Ulcerative colitis Inflammation of the gut accelerating food transit and reducing absorption of thiamine.

Wernicke–Korsakoff syndrome A form of beriberi that occurs in patients who abuse ethyl alcohol.

Introduction

Thiamin is a water-soluble vitamin and the structure comprises a pyrimidine and a thiazole ring linked by a methylene bridge (**Figure 1**). In its metabolically active forms, the hydroxyl group on the thiazole moiety is replaced by one, two, or three phosphate groups to form three phosphorylated co-enzymes. A well-nourished human adult body contains approximately 30 mg of thiamin – approximately 80–90% as thiamin diphosphate (TDP), 10% as thiamin triphosphate (TTP), and a small amount of thiamin monophosphate (TMP) and thiamin. Like most water-soluble vitamins, there is no definable store in the body; the only reserves are thiamin co-enzymes that are present in most cells in combination with appropriate thiamin-requiring enzymes. The predominant need for thiamin is linked to energy production but there is increasing evidence that thiamin is also needed for additional neurological functions. Thiamin is found in the aleurone layer of cereal grains as well as in animal food products such as liver. Man's desire for high-extraction cereal products in situations wherein the diets contained little more than the cereal was a main contributory factor to the scourge of beriberi throughout much of Southeast Asia at the end of nineteenth and beginning of the twentieth century. Thiamin is relatively unstable and destroyed by poor cooking habits, and it is

susceptible to degradation in foods that are not stored properly. Thiamin turnover is also quite rapid, and the absence of stores means that a continuous supply of thiamin is required. So thiamin status can be fairly rapidly impaired by factors affecting intake (e.g., vomiting and alcohol abuse) or excessive excretion (e.g., induced by diuretics). Thus, thiamin deficiency is sometimes a problem in pregnancy, alcohol abuse, and the elderly. Seasonal outbreaks can also occur in poor developing countries when energy demand is high, cereals may have been badly stored for many months leading to loss of thiamin and food supplies are restricted.

Dietary Sources of Thiamin

Thiamin is present in most foods but cereal products provide most thiamin for most people in the world, although the source is fundamentally different in developing and more industrialized countries. In the developing world, unrefined cereal grains and starchy roots and tubers provide 60–85% of dietary thiamin, whereas most dietary thiamin in industrialized countries is obtained from fortified cereal products. In the UK, for example, wheat flour is fortified with 2.4 mg thiamin per kg and many breakfast cereals contain 30% or more of the daily thiamin requirement per portion. Thiamin is present in greatest

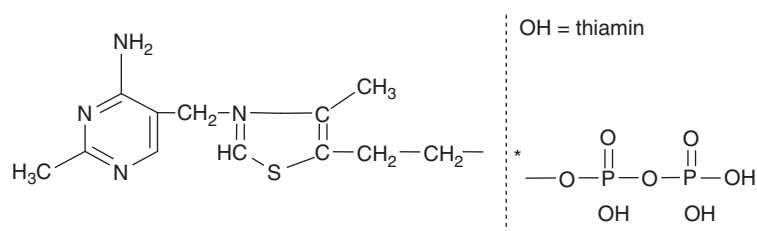


Figure 1 Thiamin and thiamin diphosphate (asterisk). Thiamin monophosphate and triphosphate are formed by the similar addition of one or three phosphate groups at the asterisk.

amounts in brewers yeast, the germ and aleurone layers of fresh wheat, egg yolk, and mammalian liver. It is also present in meat flesh, particularly pork, vegetables, nuts, and legumes (Table 1). Milk from both humans ($0.49\text{--}0.79\ \mu\text{mol L}^{-1}$; $0.23\ \text{mg}/4.2\ \text{MJ}$ ($1000\ \text{kcal}$)) and cows ($1.18\text{--}1.48\ \mu\text{mol L}^{-1}$) is a poor source of thiamin. Thiamin is actively secreted into milk by the lactating mother, and it is of interest that the amount of thiamin in human milk is not increased by supplements, but the concentration and of course the volume consumed increase during the first 6 weeks of lactation.

Refined foods in general, such as fat, sugar, and alcohol, are poor sources of thiamin. Polished rice is particularly low in thiamin ($80\ \mu\text{g}/100\ \text{g}$) and is especially important because of its widespread consumption and importance as a source of calories. Cereal grains lose thiamin during refining, but the process of parboiling rice before milling enables most of the thiamin to be retained ($190\ \mu\text{g}/100\ \text{g}$) since it migrates into the starchy endosperm during the procedure. Proper storage of cereal grains is also important to maintain thiamin activity.

Table 1 Thiamin content of common foods

Food group	Food item	Thiamin content (mg/100 g)
Bread	Wholemeal	0.26
	White	0.21
	Hovis	0.52
Breakfast cereals	Cornflakes (fortified)	1.2
	Rice Krispies	1.2
	Weetabix	1.2
Flour	Wholemeal (100% ^a)	0.46
	Brown (85%)	0.39
	White (fortified) (70%)	0.31
	Wheat germ	2.01
Milk, cheeses		0.03–0.06
Eggs	Whole raw	0.09
	Yolk raw	0.30
Vegetables (cooked)	Various leaf and root types	0.02–0.07
	Dahl, chick peas, green, beans, etc.	0.05–0.14
Pork products	Gammon rashers (lean)	1.16
	Bacon (various)	0.63–1.16
	Pork meat	0.5–0.88
Other meats	Beef steak (various)	0.05–0.15
	Lamb (average trimmed fat, raw)	0.07
	Lamb liver	0.38
	Lamb kidney	0.51
	Chicken (various)	0.04–0.11
	Chicken liver, roasted	0.61
	Game	~0.30
Yeast (dried)		2.33
Nuts	Peanuts (fresh)	1.14
	Peanuts (roasted and salted)	0.18

^aPercentages indicate the level of extraction in flour preparation.

Studies in The Gambia, West Africa, found that old season millet, which had been stored under thatch and in high humidity, when consumed in the middle of the rainy season had thiamin concentrations ($11\ \mu\text{g}/100\ \text{g}$) that were six- to 12-times lower than cooked samples obtained immediately postharvest. Imported rice used in the village likewise only contained $10\ \mu\text{g}/100\ \text{g}$ at the time of consumption.

Because of the water-soluble properties of thiamin, it can be leached from food during cooking. Thiamin is stable in slightly acidic water up to boiling point but is unstable in alkaline solution that oxidizes it quantitatively to thiochrome (Figure 2). In addition, antithiamin factors in food can accelerate thiamin losses. Paralysis in foxes fed with raw carp led to the discovery of the thiaminase enzymes. Two thiaminases are found in food. Thiaminase I is found in fish, shellfish, ferns, and some bacteria and catalyzes a base exchange reaction between thiazole and another base. Thiaminase II is a hydrolytic enzyme that cleaves the vitamin at the methylene bridge and is found mainly in bacteria. The thiaminases are heat labile, so only food that is eaten raw or fermented may lose thiamin during its preparation or in the gastrointestinal tract. There are also heat-stable antithiamin factors that are found in ferns, tea, betel nuts, large numbers of plants and vegetables, and some animal tissues. Antithiamin factors bind with varying degrees of attachment to thiamin and may or may not interfere with the bioavailability of thiamin. Diphenols, especially those with the hydroxyl groups in the ortho position, tend to react to give products that are both thiochrome negative and micro-biologically inactive (i.e., thiamin is deactivated). Thus, in areas of northern and northeastern Thailand where tea drinking, chewing fermented tea leaves, chewing betel nuts, and consuming raw/fermented fish are common practices, thiamin deficiency still occurs despite thiamin intakes of $0.44\text{--}0.50\ \text{mg}/4.2\ \text{MJ}$. Similar food habits are also found in Laos but there the mean thiamin intake of women during the postpartum period was recently reported to be $0.35\ \text{mg}/4.2\ \text{MJ}$ and infantile beriberi is a public health problem.

Absorption and Ethyl Alcohol

In food, thiamin occurs mainly as phosphate coenzymes and the predominant form is TDP (also called thiamin

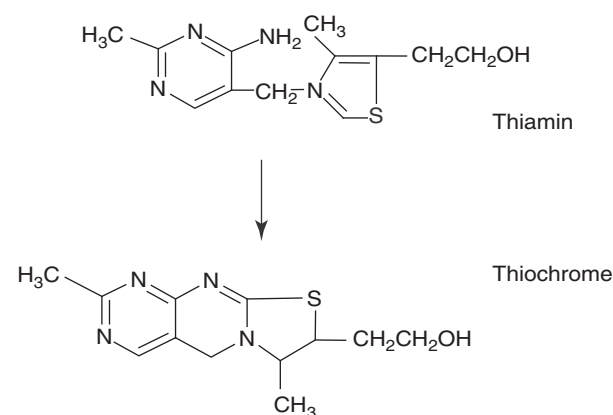


Figure 2 Structures of thiamin and thiochrome.

pyrophosphate and cocarboxylase). The phosphate coenzymes are broken down in the gut by phosphatases to give free thiamin for absorption. Thiamin is absorbed mainly from the upper intestine, and less thiamin is absorbed on an empty stomach than when taken with a meal. The latter could be due to the alkaline conditions in the duodenum, which are prevented by the presence of food. Absorption of up to 2 mg per meal occurs by an active saturable process involving a sodium-dependent adenosine triphosphatase and against a concentration gradient. During absorption, thiamin is phosphorylated to the monophosphate ester TMP. Thiamin is absorbed via the portal venous system. Further phosphorylation to TDP occurs on entry into all tissues. TDP can cross the blood-brain barrier, where a portion is converted to TTP, although even in the brain, TDP is the predominant form of thiamin. A second passive absorption process operates when intakes of thiamin are >5 mg but the maximum that can be absorbed from an oral dose is 2–5 mg.

The active process of absorption is impaired by ethyl alcohol. For example, 55% of a 5 mg dose of orally administered, labeled thiamin was recovered over 72 h in healthy adults, but this was reduced by 25–40% if they were previously given $1.5\text{--}2$ g alcohol kg^{-1} . In people with fatty livers who had previously been abusing alcohol, mean thiamin absorption was reduced by 60%. However, the passive absorption of thiamin is not inhibited by alcohol, nor does it block entry of thiamin into the liver or interfere with thiamin metabolism in the tissues. Absorption of thiamin may also be reduced by gastrointestinal disturbances, such as vomiting and diarrhea, ulcerative colitis, and neoplasia, and in patients with hepatic disease and achlorhydria.

Transport, Storage, and Excretion

Thiamin with some TMP ($19\text{--}75$ nmol l^{-1}) circulates in the blood bound to albumin. When the binding capacity of plasma albumin is exceeded, or thiamin is more than tissue needs, it is rapidly excreted in the urine. Most thiamin in erythrocytes is present as TDP principally bound to the enzyme transketolase. Likewise, in most other tissues, there is very little free thiamin and it is mostly present as TDP (90%) in coenzymes bound to respective enzymes and a smaller amount of TTP (10%) in nervous tissues. The concentration of thiamin in specific tissues is in the order of $2\text{--}3$ $\mu\text{g g}^{-1}$ for heart muscle; 1 $\mu\text{g g}^{-1}$ for brain, liver, and kidney; and 0.5 $\mu\text{g g}^{-1}$ in skeletal muscle. Thiamin supplements can increase these concentrations slightly and prolonged febrile illnesses are likely to reduce them. Thiamin is mainly excreted intact in the urine but there are small amounts of thiochrome (Figure 2) and other thiazole and pyrimidine metabolites. A linear relationship exists between intake and excretion of thiamin until intake falls to an amount approaching minimum requirements when excretion decreases rapidly indicating a renal conservation mechanism.

There is concern that the long-term use of diuretics in the management of chronic congestive heart failure (CHF) may impair thiamin status and, as a consequence, impair myocardial function. The diuretic drug furosemide has been the subject of much attention. In healthy volunteers, a dose-

dependent increase in urine flow accompanied by an increase in the urinary thiamin excretion rate have been demonstrated. In furosemide-treated patients, the concomitant presence of thiamin in the urine and biochemical deficiency of thiamin from measurements in blood have been shown. These results suggest that furosemide treatment can override the renal conservation mechanism. In one study, 23 patients with chronic CHF receiving 80–240 mg furosemide daily for 3–14 months were studied along with 16 age-matched controls without heart failure and not taking diuretics. No subjects in either group were identified as consuming inadequate thiamin intake or having increased thiamin requirements. However, biochemically, 21 of the 23 CHF patients and two of the controls were thiamin deficient. Furthermore, five of the CHF patients were treated with intravenous thiamin (100 mg thiamin HCl twice daily for 7 days). Biochemical thiamin status normalized and echocardiographic assessment of left ventricular ejection fraction increased in four of the five patients. Because no other changes were made in the patients' therapeutic regimen, the results suggest that the improvement in cardiac contractility was due to the correction of the thiamin deficiency.

Biological Functions

Thiamin functions as the coenzyme TDP in the metabolism of carbohydrates and branched-chain amino acids (α -ketoisocaproic, α -keto- β -methyl valeric, and α -keto-isovaleric acids). In association with Mg^{2+} ions, TDP is important (1) in various dehydrogenase complexes for the oxidation of α -keto acids (pyruvate, α -ketoglutarate, and the branched-chain α -keto acids) and (2) in the formation of α -ketols among the hexose and pentose phosphates catalyzed by transketolase (EC 2.2.1.1). Thus, a deficiency of thiamin has severe consequences for energy generation and amino acid interconnections, and these have important links with lipid metabolism, cell replication, and neural activity.

Two principal dehydrogenase complexes that require the participation of TDP are pyruvate dehydrogenase, which generates acetyl-CoA, and the oxidative decarboxylation of α -ketoglutarate to succinyl-CoA (Figure 3). Pyruvate dehydrogenase is situated at the junction of the glycolysis pathway, where it enters the tricarboxylic acid cycle. Acetyl-CoA is a key source of energy for mitochondrial oxidation and the production of adenosine triphosphate (ATP) as well as an important precursor in lipid metabolism. The impaired functioning of pyruvate dehydrogenase leads to a lactic acidosis, with increased concentrations of serum pyruvate and lactate especially as a result of exercise. The lactate acidosis can be explained by the fact that ATP depletion stimulates glycolysis, thus generating more pyruvate. As pyruvate concentrations increase, lactate dehydrogenase converts some of the pyruvate to lactate, producing the lactic acidosis. The increases in these compounds formed the basis of the earliest biochemical test for thiamin deficiency, which was later made more reproducible by taking blood soon after moderate exercise (e.g., climbing a measured number of steps).

Many features of beriberi indicate that thiamin plays an important role in neural tissues. TTP is specifically found in nervous tissues, but although this triphosphorylated meta-

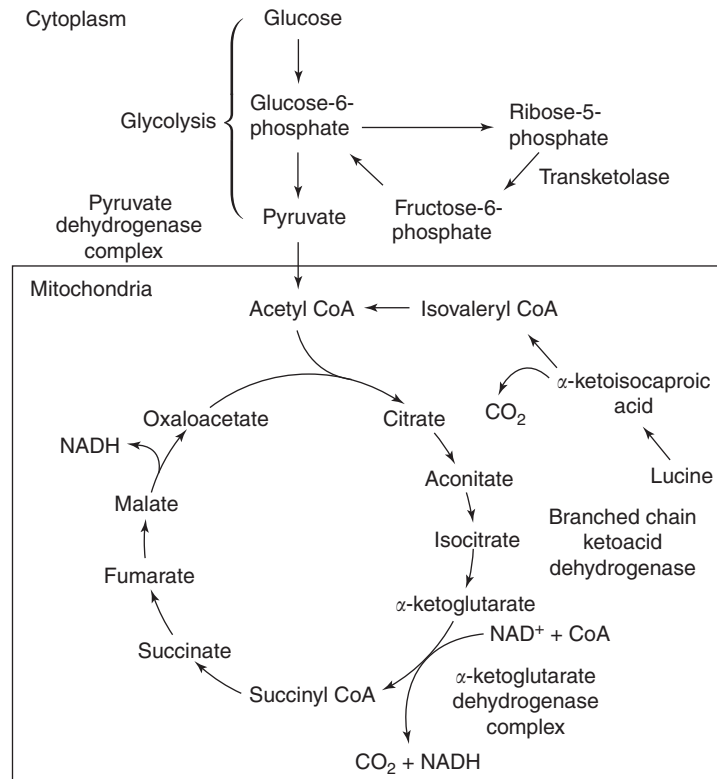


Figure 3 The four principal sites of action of thiamin diphosphate coenzyme in carbohydrate metabolism.

bolite of thiamin has been known for approximately 30 years, its precise role is still in doubt. TDP in the dehydrogenase complexes is undoubtedly also required for normal function. Some of the earliest biochemical studies on the brain documented abnormalities in the oxidative metabolism of glucose and a disruption in energy supply may underlie many of the neurochemical changes and structural lesions associated with thiamin deficiency. For example, acetyl-CoA produced by pyruvate dehydrogenase is a precursor of the parasympathetic transmitter molecule acetylcholine, but the obligatory requirement of glucose as an energy source for nervous tissue indicates the essentiality of TDP. Likewise, the cytosolic enzyme transketolase is also present in nervous tissue, and as a key enzyme in the HMS it may be important in minimizing oxidant stress. The HMS generates NADPH, which is required to maintain the antioxidant compound, glutathione, in the reduced state. Thus thiamin has an important role in maintaining redox homeostasis of tissues.

The cellular and subcellular localization of the enzymes responsible for metabolism of thiamin phosphates in nervous tissues may indicate possible sites of action of the specific metabolites. Thiamin that enters the brain is phosphorylated by thiamin pyrophosphokinase to form TDP. The concentration of thiamin phosphates is three or four times higher in neurons than in neuroglia, and the activity of thiamin diphosphatase (TDPase), which converts TDP to TMP, is 20 times higher in neurons than neuroglia. Thiamin monophosphatase is only detected in neuroglia. Within the neuron, TDPase is mostly localized in the microsomal fraction. Thiamin triphosphatase (TTPase), which converts TTP to TDP, is

particularly enriched in presynaptic terminals. Stimulation of nerves or treatment with certain neuroactive drugs results in decrease in TDP and particularly TTP in the nerve, with an increase in free TMP in the surrounding fluid. It is postulated that TTP plays an essential role in nerve transmission involving a gating mechanism for sodium and potassium ion transport via the specific ATPase. Some evidences for this comes from patients with Leigh's disease (pathologically similar to Wernicke-Korsakoff syndrome), in whom severe neurological disease is accompanied by a deficiency in TTP but normal TDP concentrations.

The well-documented role of mitochondria in programmed cell death and the importance of thiamin for oxidative stability have stimulated investigators to examine brain thiamin homeostasis in neurodegenerative diseases. Diminished thiamin-dependent processes, abnormal metabolism, and oxidative stress accompany the neurodegeneration of Alzheimer's disease (AD), Huntington's disease, Wernicke-Korsakoff syndrome, progressive supranuclear palsy, and the adult-onset neurodegenerative diseases that are caused by genes containing variable numbers of DNA-triplets, otherwise known as CAG repeats, within their coding regions. Abnormalities in the thiamin-dependent processes have also been linked with thiamin-responsive maple sirup urine disease, Leigh's disease (a subacute necrotizing encephalomyelopathy), sudden infant death syndrome, cerebellar degeneration, thiamin-responsive anemia, ataxia, and disorders of energy metabolism including pyruvate dehydrogenase deficiency. The extent to which disturbances in thiamin metabolism are a cause or a consequence of the disease process is still under examination.

Assessment of Thiamin Status

Thiamin status can be assessed using methods that measure thiamin or its metabolites in plasma, erythrocytes, and urine (Table 2). Samples are acidified to stabilize the thiamin and precipitate any protein. Usually, thiamin is oxidized to thiochrome (Figure 2) using cyanogen bromide in alkaline solution and is measured by fluorescence with or without chromatography. Concentrations of thiamin in urine and plasma tend to reflect dietary intake, being high when intake is adequate and low when dietary sources are poor. Erythrocyte thiamin is mainly in the form of the coenzyme TDP, which can be extracted from washed erythrocytes, derivatized as described previously, and quantified by high-performance liquid chromatography. The most popular test, however, is the erythrocyte transketolase (ETKL) stimulation test, which measures enzyme activity with and without added TDP. The reference range for ETKL activity in well-nourished, thiamin-adequate people is reported to be 570–830 mU g⁻¹ hemoglobin. The stimulation test measures the proportion of the apoenzyme in red cell homogenate (i.e., the proportion that is not bound to TDP and represents the degree of thiamin deficiency). Studies have shown that results from the urinary assay for thiamin agree reasonably well with those obtained by the ETKL stimulation test.

One of the reasons for the popularity of the ETKL stimulation test is that sensitivity is still good even in the presence of thiamin deficiency. In all other measurements of thiamin status, as deficiency approaches, the quantity of thiamin or its metabolites diminishes in the biological fluid. Low concentrations of a product are usually more difficult to measure and precision deteriorates, or the amount of sample has to be increased to provide sufficient material to detect. In contrast with the ETKL stimulation test, in an acute thiamin deficiency, ETKL activity is maintained and only the amount of TDP decreases, so the test becomes more sensitive. However, in chronic thiamin-deficient states, the apoenzyme of ETKL is reported to be unstable *in vivo*, and in absence of the

coenzyme, concentrations of the apoenzyme decrease with the result that *in vitro* stimulation may show normal thiamin status and basal ETKL activity may be a better indicator of status than the stimulation test. Thus, in situations in which chronic thiamin deficiency is suspected as a result of a long-term marginal thiamin intake, alcohol abuse, or use of diuretics for many months, one or more of the concentration tests may be useful as an adjunct to the stimulation test.

Certain precautions should be taken in handling samples for thiamin analysis. Urine should be acidified to avoid degradation and stored below –20 °C. Heparinized whole blood should be collected and immediately put on ice. For total erythrocyte TDP measurements, cells are separated from plasma within 2 h when possible, washed in saline, and diluted 1:1 with saline before acidification. Centrifugation of the acidified mixture provides a clear extract that can be stored for not more than 5 days at 4 °C or longer at ≤ –20 °C. Washed red cells are also used for the ETKL assay. Duplicate tubes of the red cells in saline suspension with and without added TDP are mixed and can be stored at –70 °C before enzymatic analysis of ETKL activity. Even at –70 °C, however, storage should be for no more than a few weeks. The ETKL apoenzyme is unstable and even in the tubes to which TDP has been added; if mixing did not thoroughly expose all apoenzyme to the added coenzyme, deterioration will occur and results will be unreliable.

Recommended Dietary Allowances

Quantifying thiamin requirements is based on a variety of biochemical data. Early results indicated that a thiamin intake of 0.4 mg day⁻¹ on a low-energy intake was close to the absolute minimum requirement. Epidemiological evidence suggested that beriberi occurred when the intake of thiamin was <0.2 mg thiamin/4.2 MJ (1000 kcal); however, when 0.188 mg/4.2 MJ was fed to sedentary elderly men for 2 years, no indisputable alteration in clinical state occurred. Thiamin

Table 2 Biochemical assessment of thiamin status

Test	Acceptable	Marginal risk	High risk
Urinary thiamin (μmol mol ⁻¹ creatinine) ^a			
	1–3 years	> 66	45–66
	4–6 years	> 45	32–45
	Adults	> 25	10–25
Erythrocyte transketolase activity ^c			
	Activity coefficient	< 1.11	1.11–1.25
	TDP effect (%)	< 11	11–25
Red cell thiamin concentrations (nmol l ⁻¹)		749 ± 196	~560 ^b
Whole blood thiamin concentrations (nmol l ⁻¹) ^{c,d}		166–266	< 133

^aConverted from μg g⁻¹ creatinine using the factor (× 0.376) (Saubertlich, *et al.* (1974) *Laboratory Tests for the Assessment of nutritional status*. CRC Press: Boca Raton).

^bBased on a decrease of 25% in red cell thiamin diphosphate (TDP).

^cReproduced from Thurnham (1985) Interpretation of biochemical measurements of vitamin status in the elderly. In: Kemm J (ed.) *Vitamin Deficiency in the Elderly: Prevalence, Clinical Significance, and Effects on Brain Function*, pp. 46–67. Pergamon Press: London.

^dReproduced from Gibson (ed.) (2005) Assessment of the status of thiamin, riboflavin and niacin. In *Principles of Nutritional Assessment*. Oxford: Oxford University Press, Chap. 20, pp. 545–574.

Source: Reproduced from Paul AA and Southgate DAT (eds.) *McCance & Widdowson's, The Composition of Food*. London: HMO. <http://www.food.gov.uk/science/dietarysurveys/dietarysurveys/>, consulted June 2011.

requirements are strongly influenced by physical activity and at higher energy intakes with liquid formula diets containing 11.76 and 15.12 MJ (2800 and 3600 kcal), there was good agreement between thiamin excretion and ETKL stimulation to interpret thiamin status at different levels of thiamin intake. Increasing intake from 0.2 to 0.23 mg/4.2 MJ moved first the urinary excretion and then ETKL activation out of the deficient range. Both measurements were normalized at intakes of 0.3 mg/4.2 MJ, and to allow for variance the recommended nutrient intake adopted by the Department of Health in the UK was 0.4 mg/4.2 MJ. This amount is recommended for all groups of the population since additional needs in pregnancy and lactation are met by increased energy intakes. It was recommended that formula feed should contain not less than 0.3 mg/4.2 MJ.

Women are less affected by beriberi than men even when they are consuming the same diet, but there is no consistent indication that men have greater needs than women. Differences between the sexes that may affect susceptibility to beriberi need further investigation (e.g., the amount of food eaten by the sexes when supplies are short or of poor quality, metabolic responses to infection during illness, and differences in energy requirements). The close association between thiamin metabolism and carbohydrate metabolism means that thiamin requirements are determined by basal metabolic rate (BMR) and physical activity. BMR of men is slightly higher than that of women of the same weight, but total energy expenditure can vary from 1.4 to 2.5 times BMR depending on physical activity.

Drug–Nutrient Interactions

Mention has already been made of the influence of alcohol and diuretics on thiamin status. Oral contraceptives are reported to have no effects on thiamin status.

Toxicity

High intakes of thiamin administered orally are nontoxic. The rapidly saturable thiamin absorption mechanism limits the amount taken up from a single dose to ~2.5 mg, and thiamin present greater than protein binding capacity is excreted.

However, there are reports of toxicity from chronic intakes greater than 50 mg kg⁻¹ or > 3 g day⁻¹ with a wide variety of clinical signs, including headache, irritability, insomnia, rapid pulse, weakness, rapid pulse contact dermatitis, pruritus, and, in one case, death.

See also: Carbohydrates: Regulation of Metabolism. Cereal Grains. Drug–Nutrient Interactions. Thiamin: Beriberi

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THIRST PHYSIOLOGY

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Role of Thirst in Water Balance

Approximately 70% of the lean body mass of an individual is composed of water, with approximately two-thirds of the total body water (TBW) volume being held within the cells of the body (intracellular pool), with the remaining one-third (extracellular pool) divided between the circulating blood plasma (intravascular pool) and the fluid-filled spaces between the cells (interstitial pool). The volume and distribution of the body fluids are mainly determined by the amounts of body water and sodium. In humans, the TBW content is regulated daily to within approximately 0.2% of the lean body mass under normal, temperate conditions by factors that control input and output. The kidneys regulate water excretion in excess of the evaporative loss and the fecal and obligatory urine losses. Water intake occurs in the form of food and drink, with the sensation of thirst underpinning drinking behavior.

The mechanisms that monitor the body's hydration status also interact with the thirst control centers in the brain to regulate the desire to drink. This article focuses on the physiological factors that govern the perception of thirst and how this is altered by drinking.

Perception of Thirst

Thirst is a sensation that is best described as the desire to drink. The reason for drinking may not be directly involved with a physiological need for water intake, but can be prompted by habit, ritual, taste, nutrients, craving for alcohol, caffeine, or other drugs in a beverage, or a desire to consume a fluid, which will provide a warming or a cooling sensation. Much of the perception of thirst is a learned or a conditioned process, with signals such as dryness of the mouth or throat initiating drinking, whereas a feeling of fullness of the stomach can stop ingestion before a fluid deficit has been restored.

Currently, the thirst response is thought to be regulated by neural modulators that operate as a reward mechanism, integrating the effective requirement for water intake with the sensations of taste and pleasantness of the fluid ingested. Thus, when the individual is hypohydrated multiple areas of the brain are activated, promoting the intensity of the thirst sensation. As the water deficit is restored, the feeling of thirst diminishes and this subjective sensation correlates well with a reduction in neural activation. However, areas of the brain associated with taste, which are activated by water when thirsty, remain active following drinking to satiety when water is ingested.

Although it is true that thirst in humans is a poor indicator of acute hydration status and that daily fluid intake is normally in excess of obligatory water loss, the preservation of the TBW volume under a variety of environmental and nutritional stresses is remarkably robust and is mainly due to the drive to drink that the sensation of thirst chronically induces.

Assessment of Thirst

In humans, two main techniques have been used to identify the perception of thirst and its alleviation by drinking. The first method is to monitor the volume of drink voluntarily ingested by an individual within an allotted time period and to compare the amount consumed with the volume of fluid required to restore a given water deficit or other imbalance of the body water pools. The other method is to assess the individual's perceived rating of thirst by asking them to record on a visual analog scale their responses to a series of questions that are thought to relate to the sensation of thirst (**Figure 1**). The questionnaire technique has the advantages that it allows a series of measurements to be made before, during, and following the period of drinking, and appears to provide an indication of the relative strength of a given stimulus. Although in many studies both methods are used to gauge the sensation of thirst and the responses have been correlated to a number of physiological parameters that are known to influence the drive to drink, it is widely recognized that at present there is no consistently reliable measure of the thirst sensation.

A more recently introduced technique has been the use of noninvasive methods of imaging to identify the specific regions of the brain that are activated during the genesis and satiation of the thirst response. Different techniques are used to image brain activation. Both Positron Emission Tomography (PET) and functional Magnetic Imaging (fMRI) are used to visualize brain activation by detecting either temporal changes in blood flow or in the chemical composition of regions in the brain that occur when individuals are exposed to specific stimuli. The number of brain regions, their specificity, and the intensity of activation have also been correlated with the subjective perception of thirst.

The Physiological Regulation of Thirst

As thirst is the major factor controlling water intake, the physiological regulation of thirst is associated with the need to maintain a relatively stable volume of TBW. Although water is lost from the body continually, albeit usually in relatively small amounts, and hence the body is almost always

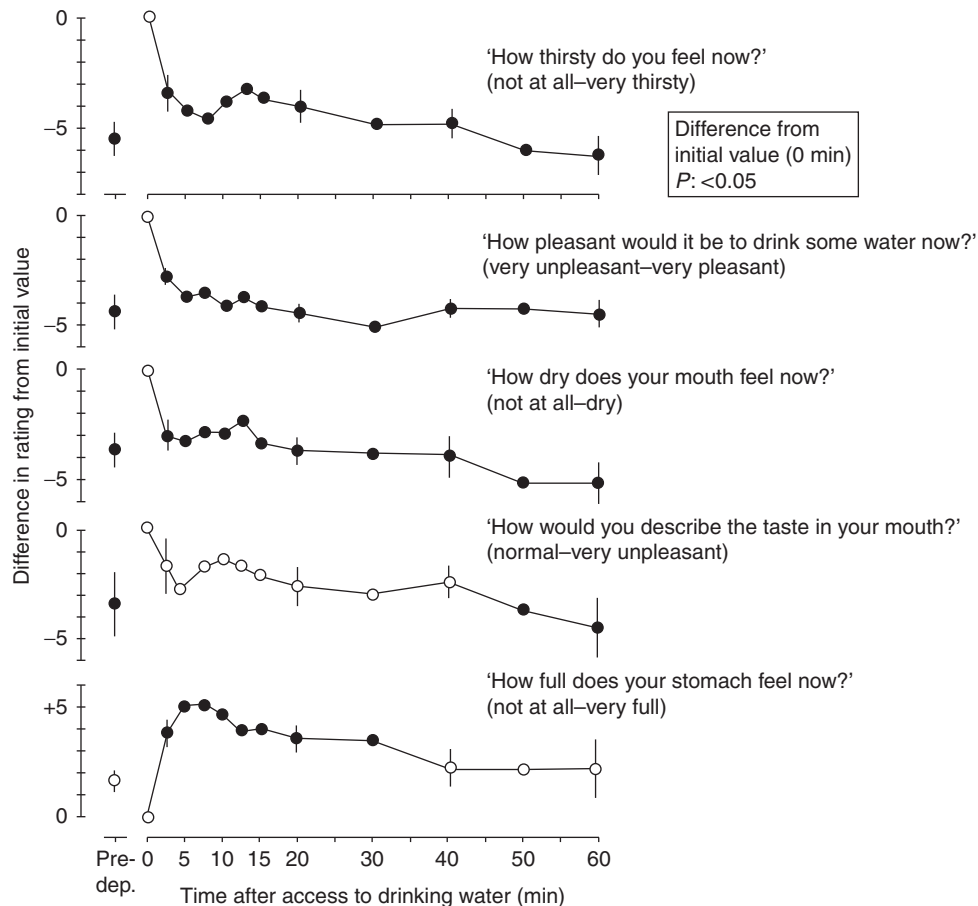


Figure 1 Subjective responses to a series of five questions assessed by visual analog scale ratings from 24-h water-deprived individuals before and during a 60-min rehydration period. The data are shown as differences (in centimeters) from the initial values and significant differences are indicated by filled symbols. Reproduced from Rolls BJ, Wood RJ, and Rolls ET (1980) Thirst: The initiation, maintenance, and termination of drinking. *Progress in Psychobiology and Physiological Psychology* 9: 263–321.

developing a water deficit, water intake is intermittent. The amount of fluid usually ingested is in excess of that required to replace the losses incurred since the last water intake. The factors that initiate, maintain, and end the drinking response are various and are not fully understood. However, as the regulation of the volume and composition of the various water pools of the body play an essential role in controlling the perception of thirst, an understanding of the homeostatic mechanisms involved has provided us with the best insight we have into the complexities of the perception of thirst.

The total volume, distribution, and composition of body fluids must be regulated within narrow limits for normal cellular function to be maintained. Body water is passively distributed between the extracellular and intracellular pools according to osmotic, oncotic, and hydrostatic forces as shown in **Figure 2**. The sodium and chloride contents of the extracellular fluid constitute the two greatest osmotically active components of this fluid and are therefore important in maintaining its volume. Potassium, phosphate, and protein play a similar role in regulating the intracellular fluid volume. The distribution of water between the intravascular and the extravascular pools is dependent on the balance of hydrostatic

and oncotic pressures across the capillaries and post capillary venules.

Variation in the water to solute ratio of a body fluid pool results in changes in the tonicity and hence effective osmolality of the fluid. As the various body water pools are in dynamic equilibrium with each other (**Figure 2**), there is a tendency for adjustments to occur throughout the body as water moves from regions of low solute concentration to those of higher solute concentration. Changes in plasma osmolality are relatively easy to monitor; therefore, there is a tendency to consider changes in the circulation as the effector of fluid balance control. It is, however, important to remember that any alteration in one body pool will affect the others and that receptors that initiate responses affecting water balance may reside at sites far removed from the circulation.

Loss of water from the body or an increase in the circulating solute concentration causes an increase in the osmolality of primarily the extracellular fluid; water then moves into the extracellular space from the cells, producing a reduction in cell volume. Changes in plasma osmolality are therefore thought to be signaled to the effector mechanisms by changes in the cell volume of specific specialized cells, collectively termed osmoreceptors. As the main solute determining the

tonicity of the extracellular fluid is sodium, there has been some debate as to whether the receptor cells detect changes in osmolality or changes in the sodium ion content. The evidence to date suggests that at least the majority of the receptors respond to osmolality rather than to sodium concentration. These osmoreceptors play a regulatory role not only in the perception of thirst but also in the maintenance of the circulating levels of hormones that regulate the excretion of water and solute by the kidneys (Figure 3). As increases in the extracellular osmolality effectively decrease the volume of the cells in the body, this form of dehydration is termed cellular dehydration.

Alteration in the volume of the extracellular fluid pool without change in its osmolality also affects the fluid balance hormone concentrations and the sensation of thirst. Changes in blood volume affect the blood and capillary pressures and atrial filling pressure. The effect on capillary pressure will tend to redistribute body water and help to adjust the circulating fluid volume, and the change in venous return to the heart will alter the cardiopulmonary and arterial stretch receptor (baroreceptor) activity. The level of afferent activity from these baroreceptors directly affects both the sensation of thirst and

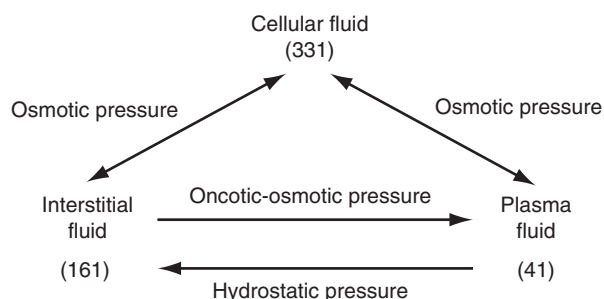


Figure 2 Diagrammatic representation of the forces that regulate the distribution of the body water pools. The volumes given are those determined in a single male subject with a lean body mass of 75.8 kg.

the secretion of some fluid balance hormones. Additionally, modifications to the arterial blood pressure can directly affect renal perfusion, which, together with baroreceptor activity to the kidneys, regulates the renin-angiotensin system (Figure 4). Although the effect on the kidneys can influence the perception of thirst, the main renal response is to regulate urine water and solute excretion. A decrease in the volume of the extracellular pool with no concomitant change in plasma osmolality is termed extracellular dehydration.

When humans are given access to fluids after the development of a water deficit, their drinking response usually follows a pattern of rapid ingestion of more than 50% of the total intake, followed by intermittent consumption of relatively small volumes of drink over a longer period. Although initiation of the response to drink is due mainly to osmotic or blood volume (volemic) changes, other mechanisms appear to be involved in the control of the continuation and satiety responses. Receptors in the mouth, esophagus, and gastrointestinal tract appear to be major factors in the acute regulation of thirst satiation, with the effects that the volume and solute content of the ingested drink have on restoring the fluid deficits controlling the chronic regulation of thirst (Figure 5). There is a close relationship between eating and drinking, with approximately 70% of daily fluid intake normally being associated with meals (Figure 6). The desire to drink while eating is probably produced by a series of responses including the mechano-chemical composition of the food before absorption, the neuroendocrine response to digestion, the movement of water into the intestine during digestion, and the osmotic solute load that occurs following absorption. The intake of minerals is essential to replace that lost from the body and for growth. The majority of mineral intake is supplied by the food ingested, and indications of a desire or appetite for ingesting specific minerals have been shown in animals and humans. Although sodium appetite has been linked to the sensation of thirst, anatomically and functionally, the controlling mechanisms are distinct and separate.

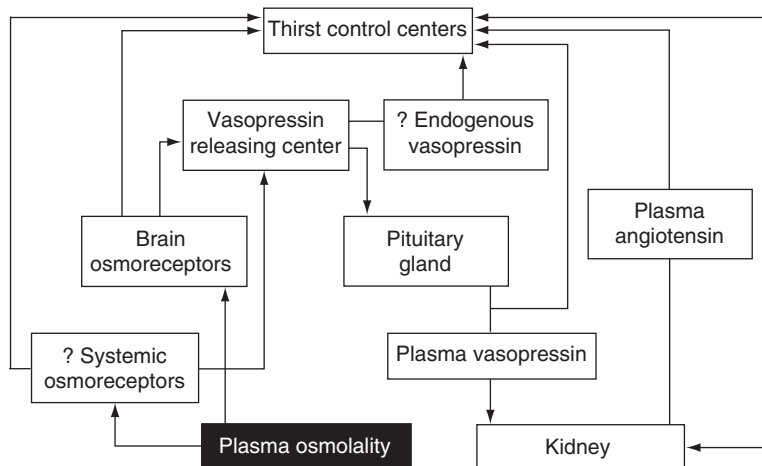


Figure 3 Schematic representation of the main factors proposed in osmotically induced regulation of the sensation of thirst and their interaction with the control of diuresis. An increase in plasma osmolality will tend to stimulate greater excitatory activity, whereas a decrease in osmolality will activate more inhibitory inputs. Neural pathways are indicated by \rightarrow and hormonal input by \rightarrow .

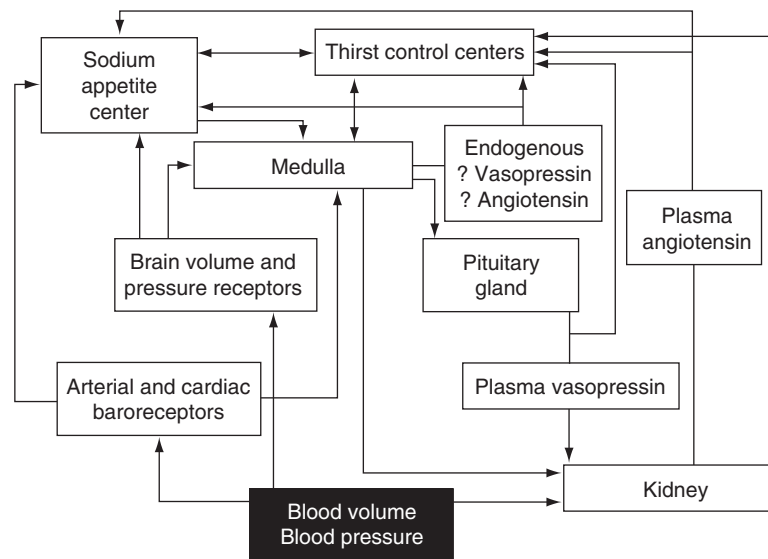


Figure 4 Schematic representation of the main factors proposed in volumic-induced regulation of the sensation of thirst and their interaction with the control of diuresis and sodium appetite. A decline in circulating blood volume will decrease baroreceptor activity, which will increase excitatory activity, whereas an increase in volume will have the opposite effect. Reduction in blood pressure will decrease renal perfusion, which will activate the renal renin-angiotensin system. Neural pathways are indicated by → and hormonal input by →.

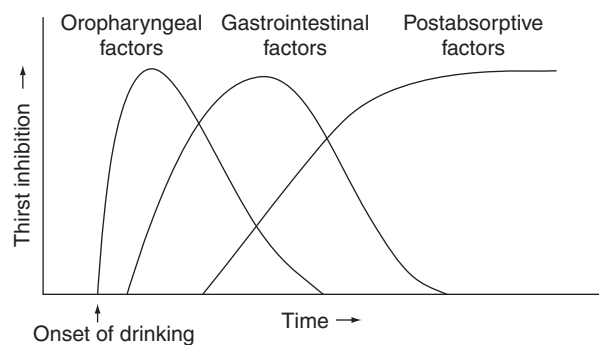


Figure 5 Schematic diagram depicting the proposed onset, duration, and overlap of various inhibitory signals to continue fluid ingestion following initiation of drinking in response to a fluid deficit. Reproduced from Verbalis JG (1990) Inhibitory controls of drinking: Satiation of thirst. In: Ramsay DJ and Booth DA (eds.) *Thirst: Physiological and Psychological Aspects*. ILSI Human Nutrition Reviews, pp. 313–330. London: Springer-Verlag.

of the brain have been termed the thirst control centers. Neurons that are responsive to changes in osmolality, intravascular volume (volemia), and blood pressure are found within these areas of the brain, as are other receptors that are responsive to many of the fluid balance hormones. Neural pathways from the thirst control centers and the kidneys may allow some direct integration between the control of thirst and excretion, whereas within the brain, all of the major fluid balance hormones are present as neuro-hormones. Afferent input from systemic receptors monitoring osmolality, circulating sodium concentration, and changes in intravascular volume and pressure also play roles in controlling the feeling of thirst. Therefore, there appears to be a complex integrated system for both monitoring the status of the body water pools and controlling intake and excretion (Figures 3 and 4). Much of the regulatory mechanisms controlling water balance appear to overlap, with several stimuli appearing to subserve the same response; however, it is assumed that this effect is required in order to ensure that the blocking of one type of stimulus will not prevent homeostatic control.

Mechanisms of Thirst Regulation

The sensation of thirst is regulated separately both by the osmotic pressure and by the volume of the body fluids and as such is closely related to the control mechanisms that are responsible for the secretion of the fluid balance hormones, which affect water and solute reabsorption in the kidneys and play a role in blood pressure control. These hormones, arginine vasopressin, atrial natriuretic peptide, oxytocin, and the renin-angiotensin-aldosterone system, are central to the regulation of thirst. Regions of the brain, in the hypothalamus and the forebrain, appear to be the main areas involved in the control of thirst and antidiuresis, and collectively, these parts

Osmotic Regulation of Thirst

The osmolality of circulating plasma is normally maintained within a very narrow limit of between 270 and 295 mosmol kg⁻¹, with the circulating levels of the antidiuretic hormone arginine vasopressin playing a major role in its homeostatic regulation. An increase of as little as 2–3% in plasma osmolality is sufficient to produce a strong sensation of thirst and a significant increase in circulating arginine vasopressin concentration (Figure 7). The osmoreceptors that monitor the tonicity of the body pools appear to reside mainly in the subfornical organ (SFO) and organum vasculosum of

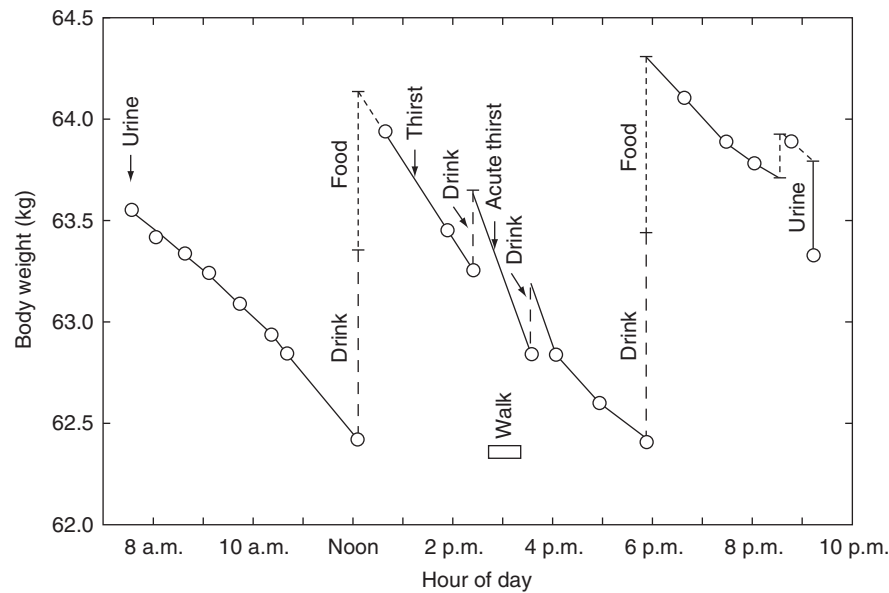


Figure 6 Changes in body weight during 13 h in the desert. The majority of the volume of drink ingested was associated with food intake. Sweat loss varied from 150 to 700 ml h^{-1} ; the total fluid intake was 3.05 l. At the end of the period, body mass was essentially the same at the beginning and end of the day; therefore, water intake and output were equal. Reproduced from Adolph ED (1947) *Physiology of Man in the Desert*. New York: Interscience.

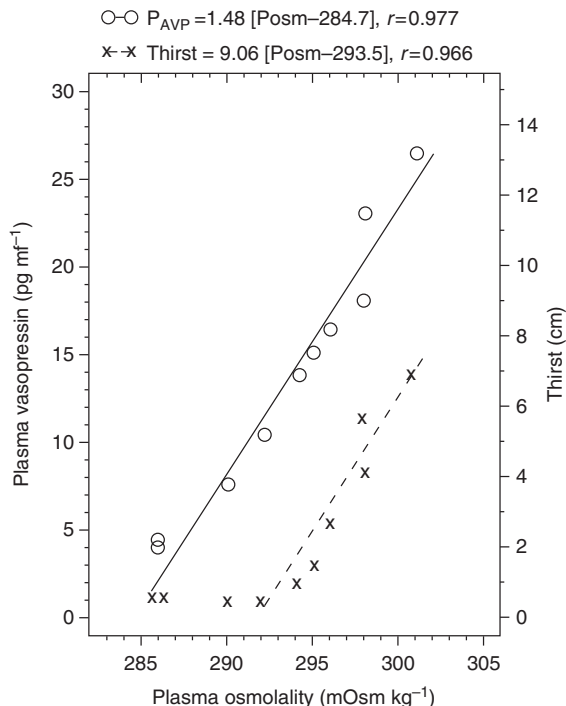


Figure 7 The relationship of plasma vasopressin (○) and thirst (x) with plasma osmolality in a volunteer during an infusion of 5% saline. Reproduced from Robertson GL (1984) Abnormalities of thirst regulation (Nephrology forum). *Kidney International* 25: 460–469.

the lamina terminalis (OVLT), an area of the brain that lacks a blood–brain barrier; therefore, they appear to respond mainly to the changes that occur in the osmolality of the blood rather than in the cerebral interstitium. Although the changes in the

circulating levels of arginine vasopressin and the perception of thirst appear to parallel one another, it is unlikely that the same receptors are responsible for both responses; it is likely that there are different neurons that react to the same stimulus. However, there may be some neuro-hormonal interaction between the osmotically activated thirst centers and the ‘vasopressin releasing center’ in the brain, and arginine vasopressin-responsive neurons have been detected within the thirst centers (Figure 3).

The current theory of the osmotic control of thirst suggests that there is a constant output of both inhibitory and excitatory neural activity from the respective osmoreceptors to the thirst centers and the arginine vasopressin-releasing centers. Within the normal range for plasma osmolality, the inhibitory and excitatory activities in the thirst centers effectively cancel out one another and there is neither a sensation of thirst nor satiety. However, at this level of activity, there is release of a basal level of arginine vasopressin, which is sufficient to maintain a state of half-maximum anti-diuresis. An increase in plasma osmolality above the normal level stimulates greater excitatory output, causing an increase in the feeling of thirst and higher levels of circulating arginine vasopressin. Elevated levels of arginine vasopressin increase the concentrating ability of the kidneys. A decrease in plasma osmolality below the normal range increases the inhibitory output, producing a feeling of satiety, and arginine vasopressin release is suppressed, allowing urinary dilution (Figure 3).

Cells and fibers within the brain have been shown to contain several hormones, including angiotensin and vasopressin, within the same cell. Although neurons associated with the thirst centers can be activated *in vitro* by vasopressin, it is not clear at present whether either peripheral- or neural-generated arginine vasopressin levels do influence the perception of thirst.

Volemic Regulation of Thirst

The receptors that initiate hypovolemic thirst are generally thought to be the cardiovascular baroreceptors, which respond to underfilling of the circulation by reducing their inhibitory nerve impulse activity to the thirst centers. However, in areas of the brain associated with the thirst centers, there are neurons that are separately responsive to volemic, pressure, and osmotic changes. This suggests that at least part of the response to changes in blood volume originates in the brain. It is thought that changes in blood pressure and osmolality are monitored mainly within the brain, whereas variations in blood volume are principally sensed by the peripheral baroreceptors, with a degree of overlap between the different receptors. The mechanisms that respond to changes in intravascular volume and pressure appear not to be as sensitive as those responsive to osmotic changes, for example, a decrease of approximately 10% of the plasma volume is required to initiate hypovolemic thirst. As fairly large variations in blood volume and pressure occur during normal daily activity, such as postural changes and physical activity, this apparent lack of sensitivity presumably prevents overactivity of the volemic control mechanisms. As with osmotic thirst, the control of volemic thirst is thought to be a balance between continuous inhibitory and excitatory neural activity, although in this system, the basal level appears to be essentially inhibitory. Another difference in the basic control mechanism between the two systems occurs due to the requirement for both solute, mainly sodium, and water to restore the extracellular volume. Therefore, extracellular dehydration causes an initial thirst and a delayed increase in sodium appetite.

Reduction in the intravascular volume sufficient to lower cardiac output and arterial blood pressure decreases afferent activity from the low- and high-pressure cardiovascular baroreceptors to the thirst centers and increases sympathetic activity to the kidneys. As afferent input from the baroreceptors to the thirst centers is inhibitory, a decrease in activity produces a reflex increase in the perception of thirst and also appears to directly stimulate arginine vasopressin release. The increase in sympathetic activity to the kidneys directly promotes greater renal renin release. In addition, reduction in blood pressure lowers the renal perfusion pressure, which stimulates renin release both as a direct pressure effect and by decreasing the delivery of sodium to the kidneys.

Increased activation of the renin-angiotensin-aldosterone system also helps regulate hypovolemic thirst. Although circulating levels of both vasopressin and aldosterone affect water and sodium reabsorption in the kidneys and thereby control water and solute loss, angiotensin acts directly on the thirst and sodium appetite centers to stimulate their respective responses. Neurons that are stimulated by angiotensin are found in areas of the brain that lack a blood-brain barrier; therefore, circulating angiotensin has direct access to both centers. In addition, the release of neurally generated angiotensin is promoted by suitable neuron activity responding to sensory stimuli (Figure 4).

There are therefore a variety of neural and hormonal responses that interact to modulate and control both thirst and urine excretion. A number of other hormones including oxytocin, atrial natriuretic peptide, tachykinins, neuropeptide Y,

thyroid hormones, corticotrophin releasing factor, and steroid hormones have also been shown experimentally to affect the drinking response. Under normal conditions of water and solute loss, both osmotic and volemic dehydration occur; therefore, stimuli from receptors for both systems are usually involved in the sensation of thirst. Increases in extracellular osmolality appear to be more effective than hypovolemia in promoting the thirst and hence the drinking response. Greater than 70% of the stimulus to drink appears to be generated by increased osmolality.

Sensory Regulation of Thirst

The sensations of a dry mouth or the desire for a specific taste or effect also generate the desire to drink while there may be no physiological requirement to drink. A dry mouth promotes changes in neural activity in the parahippocampus, amygdala, thalamus, cingulate, insula, allocortex, and transitional cortex of the brain. This finding has strengthened the hypothesis that the perception of thirst is a primitive vegetative function that appeared long before vertebrates evolved.

Drinking water activates areas of the anterior insular and frontal opercular cortex that are also involved in the perception of taste. Areas of the orbitofrontal cortex are also activated by the ingestion of water or sweet or salty tastes, but here, activation is greatest when the subjects are thirsty and it diminishes when the subjects have drunk water to satiety. This has been interpreted as functionally separated areas of the brain, one of which responds to taste stimuli that are not diminished following drinking to satiety, whereas the other is highly active during drinking when water is physiologically required but that reduces as the need for water is met.

Mechanisms for Terminating the Sensation of Thirst

Although undoubtedly decreasing osmolality and increasing extracellular volume promote a reduction in the perception of thirst by reactivating inhibitory neuron activity, usually, there is a decrease in the perception of thirst and termination of drinking before circulating osmolality, volume, and hormonal levels have returned to pre-dehydration levels. Although it could be argued that receptors in the brain may be responsible for the early cessation of the perception of thirst, the majority of the evidence suggests that it is receptors in the upper gastrointestinal tract that promote the early termination of drinking. Although the nature and neural connections of these proposed receptors have not been fully characterized, most appear to have an inhibitory response. It has been suggested that as much of the thirst and drinking response is behavioral, an individual learns what volume of drink is required to restore a given water deficit. Termination of drinking therefore could be a learned response, which anticipates a future fluid deficit or matches a known current level of dehydration. The stimuli for gauging the current level of dehydration may be the same as that which initiates the sensation of thirst.

The mere presence of a liquid, particularly cold liquid, in the mouth reduces the perception of thirst. Receptors in the mouth and esophagus responding to different chemical,

tactile, pressure, and temperature stimuli are thought to be part of the mechanism that influences the relative intensity of the perception of thirst. Although the neural activity involved in swallowing, and perhaps oropharyngeal and gastric receptors are thought to be effective in sensing or metering the volume of liquid ingested, distension of the stomach tends to inhibit drinking due to increased gastric stretch receptor activity, although this response does not always reduce the perception of thirst. Taste and other psychological factors can have a stimulatory effect on consumption of a drink that is considered to be palatable.

The continuation and termination of the acute sensation of thirst is regulated by a series of stimuli that operate before all of the drink consumed has been absorbed and before disturbances in the body water pools have been corrected. A variety of receptors located from the mouth to the upper part of the small intestine and probably neural control from the higher centers of the brain appear to monitor and regulate the initial volume consumed. After absorption, if restoration of body water pools does not occur, the sensation of thirst is once again initiated, presumably by the same homeostatic stimuli that initially induced the feeling of thirst, and drinking restarts. The integration of the pre- and post-absorptive stimuli modulates the sensation of thirst and finally the volume of drink consumed.

Fluid Requirements

Renal reabsorption can reduce the volume of water and solute loss, and hence slow the rate of progress of a fluid deficit, but it cannot stop its development. Intake of fluid either as food or drink is required to restore a fluid deficit. Daily fluid intake is highly variable between individuals and the rate of loss is dependent on factors such as environmental temperature, physical activity, sweating rate, antidiuretic function, and dietary solute load. A representative normal daily water turnover in a sedentary individual living in a temperate climate and eating a typical western diet is approximately 2–3 l, and a minimum daily fluid intake of approximately 1.7 l is necessary to conserve fluid balance. The water content of the typical western diet approximates to approximately 1 l and metabolically derived water produces in the order of approximately 300 ml, which together almost offsets the daily obligatory water loss. Therefore, in many situations, the requirement for fluid intake can actually be very low.

There are conditions in which water loss is greater than that indicated above and replacement obviously requires a compensatory increase in the daily fluid intake (Figure 6). Urine volume is related to the solute content of the diet, with a minimum volume of approximately 500 ml being necessary to eliminate the usual daily solute load. Diets rich in protein or foods with a high sodium content require a greater obligatory urine output for excretion. The renal concentrating ability at maximum antidiuresis determines the minimum urinary water loss for a given dietary solute load. Normally, there is a wide range of urinary osmolality such that the same solute load can be excreted in 500 ml of urine with an osmolality of 1400 mosmol kg⁻¹ or in 23 l of urine with an osmolality of 30 mosmol kg⁻¹. This feature of renal excretion allows body

water balance to be maintained while fluid intake volume is varied.

Prolonged relatively intense muscular activity, elevated ambient temperature, and fever all increase the rate of evaporative sweat loss. Individual sweat rates are highly variable, with average exercise-induced losses usually in the order of 1 l h⁻¹, but daily losses of between 10 and 15 l have been recorded. Daily fecal losses associated with a western diet are usually between 100 and 200 ml; however diarrhea, particularly infectious diarrhea, can produce prodigious fecal water losses that are potentially fatal.

Inappropriate fluid intake can be produced following lesions or development of tumors in regions of the brain associated with the thirst centers. Diabetes insipidus promotes an increase in the volume of fluid ingested, which is caused by a lowering of the basal threshold set point for osmotic thirst. A similar, although less pronounced, lowering of the osmotic thirst threshold occurs during pregnancy. In both the young and the elderly, the thirst response can be blunted and inappropriate drinking habits may occur. Psychogenic disturbances in the sensation of thirst and hence fluid intake have also been reported for a variety of clinical conditions.

See also: Appetite: Physiological and Neurobiological Aspects; Psychobiological and Behavioral Aspects. Behavior: Effects of Diet on Behavior. Body Composition. Brain and Nervous System: Biology, Metabolism, and Nutritional Requirements. Caffeine. Dehydration

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TRANS-FATTY ACIDS

Health Effects, Recommendations, and Regulations

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Glossary

C-reactive protein An acute-phase protein synthesized by the liver whose concentrations in blood increase during inflammation.

E-selectin Cell adhesion molecule that mediates adhesion of immune cells to cytokine-activated endothelial cells.

ICAM-1 Intercellular adhesion molecule-1; glycoprotein that is expressed on a variety of cells and involved in cytokine-stimulated cell adhesion.

Interesterification A process in which fatty acids are rearranged on a triacylglycerol molecule.

Interleukin-6 Cytokine derived from activated T lymphocytes involved with induction of hepatocyte secretion of acute-phase inflammatory proteins.

Tumor necrosis factor-alpha Cytokine that stimulates the acute-phase reaction and is involved in systemic inflammation.

VCAM-1 Vascular cell adhesion molecule-1; cytokine-induced cell adhesion molecule present on a variety of cells and important for the recruitment of leukocytes to the sites of inflammation.

Introduction

Trans-fatty acids are a class of fatty acids that contain one or more double bonds in the *trans* configuration. The two primary sources of *trans*-fatty acids are those that are produced through the partial hydrogenation of vegetable oils (industrially produced), and those that are produced by ruminant animals (naturally occurring). Historically, vegetable oils were partially hydrogenated for use in margarines, commercial cooking and frying, and food manufacturing. The presence of *trans*-fatty acids results in solid fats at room temperature, longer shelf-life of commercial products, and stability of oils at very high temperatures. The majority of *trans*-fatty acids in foods are C18:1 isomers. Elaidic acid, which is derived from oleic acid, typically is the major isomer in industrial sources of *trans*-fatty acids. Ruminant *trans*-fatty acids are synthesized via bacterial metabolism of C18 unsaturated fatty acids in ruminant animals, and found in ruminant-derived products, such as beef, lamb, and dairy. Vaccenic acid, the predominant *trans*-monoene isomer in ruminant fats, comprises 50–80% of the total *trans*-fatty acids in ruminant fat. Vaccenic acid is the precursor for the c9,t11 isomer of conjugated linoleic acid (CLA). CLA is a group of positional and geometric conjugated isomers of linoleic acid. The two predominant isomers of CLA with known bioactive properties are c9,t11 and t10,c12.

Dietary Intake

Owing to increased public awareness of the adverse health effects of *trans* fat, as well as government regulations worldwide, recent intake data suggest that *trans* fat consumption has significantly declined. Dietary intake of *trans* fat in the US and

Canada ranges from 3 to 4 g day⁻¹. In northern European countries, dietary intake ranges from 2 to 4 g day⁻¹, whereas in Mediterranean and eastern Asian countries intake ranges from 1 to 3 g day⁻¹ and less than 1 g day⁻¹, respectively.

The proportion of *trans* fat intake derived from ruminant sources varies across countries. In a study assessing the dietary intake of *trans*-fatty acids in 14 Western European countries, *trans* fat from milk and ruminant fat ranged from 28% to 79% of the total *trans*-fatty acid intake. In Mediterranean countries, > 50% of *trans* fat intake came from ruminant sources. In the US, intake of ruminant *trans*-fatty acids represents approximately 20% of the total *trans*-fatty acid intake, with more than 85% of ruminant *trans*-fatty acids coming from milk fat. In European and Australian populations, ruminant *trans*-fatty acids intake represents approximately 63–75% of the total *trans*-fatty intake.

Health Effects

There are distinct differences in the isomeric distribution of *trans*-fatty acids from industrial sources and ruminant animals, which may translate to differential effects on health outcomes. There is a vast literature that demonstrates the deleterious effects of industrially produced *trans*-fatty acids on the risk of chronic diseases, including cardiovascular disease, cancer, and diabetes; however, the health effects of ruminant *trans*-fatty acids in humans have not been well studied. More recently, epidemiological studies have begun to differentiate between industrially produced and ruminant *trans*-fatty acids. Clinical studies comparing the two sources of *trans*-fatty acids are limited.

Cardiovascular Disease

Coronary Heart Disease

Numerous epidemiological studies have demonstrated an association between intake of *trans*-fatty acids and risk of coronary heart disease. In the early 1990s, results from the Nurses' Health Study, a prospective study, demonstrated that intake of *trans*-fatty acid isomers was directly related to risk of coronary heart disease (RR = 1.5, 95% CI 1.12–2.00, p for trend = 0.001). In contrast, there was a nonsignificant inverse association with intake of *trans*-fatty acids from ruminant sources and risk of coronary heart disease. In a recent meta-analysis of six prospective cohort studies, the total *trans*-fatty acid intake was associated with an increased risk of coronary heart disease (RR = 1.22, 95% CI 1.08–1.38; p = 0.002); there was a trend for a positive association with intake of industrially produced *trans*-fatty acids and coronary heart disease (RR = 1.21, 95% CI 0.97–1.5; p = 0.09), whereas there was no association with intake of ruminant *trans*-fatty acids. One case-control study and three prospective studies reported no association with intake of ruminant *trans*-fatty acids and risk of coronary heart disease, whereas one study found similar associations between ruminant and industrial *trans*-fatty acids. Overall, data suggest that intake of ruminant *trans*-fatty acids may have differential effects compared with those of industrially produced *trans*-fatty acids; however, more research is needed to determine the effects of ruminant *trans*-fatty acids on cardiovascular disease risk factors.

Lipids and Lipoproteins

Clinical trials consistently demonstrate that consumption of *trans* fat adversely affects lipids and lipoproteins. Studies have demonstrated that these effects are greater than those of saturated fatty acids. Compared with saturated fatty acids, *trans*-fatty acids decrease high-density lipoprotein (HDL)-cholesterol and increase low-density lipoprotein (LDL)-cholesterol (specifically small dense LDL particles), triglycerides, and Lp(a).

There have been few clinical studies that have investigated the effects of ruminant *trans*-fatty acids on lipids and lipoproteins. In a study in healthy men and women, ruminant *trans*-fatty acids increased HDL-cholesterol, but this effect was only observed in women. In addition, LDL-cholesterol and triglycerides were also increased. Results from another study suggest that at lower doses ruminant *trans*-fatty acids may not affect lipids and lipoproteins, but at higher doses (i.e., higher amounts than typically consumed) they may have similar effects as industrially produced *trans*-fatty acids. Owing to the inconsistencies in the data, additional highly controlled clinical studies are warranted.

Inflammation

Some epidemiological studies have demonstrated an association with total or industrial *trans*-fatty acids and plasma markers of inflammation, including tumor necrosis factor- α , interleukin-6 (IL-6), and C-reactive protein (CRP). In a study in overweight women, CRP was significantly higher in individuals in the highest quintiles of intake versus the lowest quintiles of intake, after multivariate adjustment (p for trend = 0.009). In a study in individuals with established heart disease, membrane *trans*-fatty acids levels were associated with

higher concentrations of IL-6. Results from clinical studies have been mixed, with some demonstrating a proinflammatory effect of *trans*-fatty acids, and others reporting no effect. In a study in hypercholesterolemic individuals, the effects of *trans*-fatty acids (consumed as margarine) on markers of inflammation did not differ from those of saturated fatty acids (consumed as butter). In a study in men, consumption of a diet rich in *trans* fat (8% energy) for 5 weeks led to higher concentrations of IL-6 when compared with a carbohydrate diet (p < 0.05), and higher concentrations of CRP when compared with a carbohydrate diet or monounsaturated fat diet. In contrast, in two other studies, neither industrial *trans*-fatty acids nor ruminant *trans*-fatty acids had an effect on concentrations of CRP, following 4 weeks of intervention. Overall, results from these studies suggest that *trans*-fatty acids increase markers of inflammation when compared with carbohydrate and unsaturated fatty acids; their effects when compared with saturated fatty acids remain unclear.

The effects of ruminant *trans*-fatty acids on inflammation are not well studied. Observational studies have not reported an association with ruminant *trans*-fatty acids and markers of inflammation, whereas few clinical studies have evaluated their effects. In some studies, mixtures of c9,t11 and t10,c12-CLA caused an increase in CRP in both healthy individuals and those with metabolic syndrome. In contrast, other studies have found no effect of CLA on markers of inflammation in diabetics and individuals at risk for coronary heart disease. Owing to the discrepancies in results from current studies, more research is needed to clarify the effects of industrial and ruminant *trans*-fatty acids on markers of inflammation.

Endothelial Function

Some epidemiological studies have shown an association with *trans* fat intake and plasma markers of endothelial dysfunction, specifically adhesion molecules (intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and E-selectin). Results from randomized controlled trials indicate that long-term consumption of *trans*-fatty acids impairs endothelial function to a greater extent than saturated fatty acids. In a postprandial study, consumption of a meal rich in *trans* fat or saturated fat did not impair postprandial flow mediated dilation of the brachial artery in healthy men. However, in a chronic feeding study in healthy men and women (4 weeks), the *trans* fat diet (9.2% energy as *trans* fat) significantly impaired flow mediated dilation of the brachial artery (decreased by 29%) compared with a diet rich in saturated fat.

Cancer

Epidemiological Studies

The association of intake of *trans*-fatty acids and risk of cancer is not well understood. Results from epidemiological studies have been inconsistent, particularly with ruminant *trans*-fatty acids. Three studies have demonstrated a direct association with concentration of vaccenic acid in serum or erythrocytes and risk of breast or prostate cancer, whereas one study demonstrated an inverse association of serum concentration of vaccenic acid and breast cancer (among postmenopausal women). Studies

with CLA have been mixed; one case-control study reported an inverse association with dietary intake of CLA and risk of colorectal cancer, and one case-control study found significantly lower CLA (dietary intake and serum concentrations) in individuals with breast cancer compared to those without breast cancer (among postmenopausal women). In two other case-control studies, there was no significant association of CLA and breast cancer risk. Interestingly, in one of these studies, intake of CLA was associated with a reduced risk of having an estrogen receptor (ER)-negative tumor in premenopausal women (OR = 0.40, 95% CI 0.16–1.01). In a prospective cohort study, intake of CLA was weakly associated with breast cancer incidence (RR = 1.24, 95% CI 0.91–1.69; p for trend = 0.02). The relationship of CLA and metastasis has been investigated in one study; there was no significant association between the concentration of CLA in breast adipose tissue at the time of diagnosis and risk of metastasis or death.

Some epidemiological studies have shown a direct association between intakes of industrially produced *trans* fat or total *trans* fat and risk of colorectal, prostate, and breast cancers. In studies with prostate cancer, results have varied depending on the methodological approaches that have been utilized. Studies using food frequency questionnaires to assess dietary intake have not found associations with prostate cancer risk; in contrast, other studies have found direct associations with serum and adipose tissue concentrations of *trans*-fatty acids. Most studies with colon cancer suggest a direct association with intake of *trans*-fatty acids and colon cancer risk, although one prospective study found no association. In studies of breast cancer risk, direct associations have been reported with concentrations of *trans*-fatty acids in adipose tissue; however, studies using food frequency questionnaires to assess dietary intake or serum concentrations of *trans*-fatty acids have not found an association with *trans* fat intake and risk of breast cancer. Some studies have demonstrated that both genetic factors and hormone levels can influence the association of *trans* fat and cancer risk; thus, additional studies are needed that account for these types of variables. Overall, results of case-control studies suggest that intake of *trans*-fatty acids is associated with increased risk of cancer; however, results from prospective studies, which are stronger study designs, do not consistently support the results of case-control studies. Thus, further studies are warranted to investigate the relationship of *trans*-fatty acids, particularly specific isomers of *trans*-fatty acids, and risk of cancer.

Clinical Trials

Although some cell culture and animal studies have suggested an anticarcinogenic effect of CLA on various forms of cancer, including breast, colon, and prostate, human intervention studies are lacking. There are two ongoing studies designed to investigate the effects of CLA consumption in patients with newly diagnosed and advanced cancers. Results from these studies will provide valuable information regarding the effects of CLA and the progression of various cancers.

Diabetes

In the Nurses' Health Study, high intake of *trans*-fatty acids (3% of energy) was associated with an increased risk of type 2

diabetes. Results from this study suggest that replacement of 2% of energy from *trans*-fatty acids with polyunsaturated fatty acids would result in a 39% increase in risk of type 2 diabetes. However, in two other prospective studies, the Health Professionals Follow-Up Study and Iowa Women's Health Study, there was no association with *trans* fat intake and risk of diabetes.

Insulin Sensitivity

Studies in healthy individuals have found no effect of *trans* fats on insulin sensitivity. In a study in hypercholesterolemic overweight adults, both fasting insulin levels and insulin resistance (homeostatic model assessment) were higher in those following a partially hydrogenated soybean oil diet (4% of energy as *trans* fat), compared with a soybean oil or canola oil diet. In a study in insulin-resistant individuals with type 2 diabetes, a high *trans*-monounsaturated fat diet (20% of energy) resulted in a 59% higher postprandial insulin response ($p < 0.05$) when compared to a *cis*-monounsaturated fat diet (20% total energy); however, this response was not significantly different when compared to the saturated fat diet. In a recent study in healthy women with abdominal obesity, neither a diet rich in industrial *trans*-fatty acids (5.58 g day⁻¹; 4 weeks) nor a diet rich in ruminant *trans*-fatty acids (4.86 g day⁻¹; 4 weeks) had an effect on insulin sensitivity, as assessed by hyperinsulinemic-euglycemic clamp, when compared to a low *trans* fat diet (0.54 g day⁻¹; 4 weeks each). Results from these studies suggest that *trans*-fatty acids may have little or no effect on insulin sensitivity in healthy individuals, but may have adverse effects in individuals with diabetes or those who are overweight.

Studies with ruminant *trans*-fatty acids are limited, but suggest that vaccenic acid does not have an effect on fasting glucose and insulin. Some studies in obese, insulin-resistant men suggest that supplementation with CLA (purified t10,c12-CLA, purified c9,t11-CLA, or 50:50 mixture) increases insulin resistance and fasting glucose, compared to placebo. In a study in individuals with type 2 diabetes, CLA supplementation (50:50 mixture of c9,t11 and t10,c12 isomers) increased fasting glucose and reduced insulin sensitivity. However, other studies have found no effect of *trans* fat on measures of insulin sensitivity.

Dietary Recommendations and Regulations

In 2002, the panel on Macronutrients of the US National Academies, Institute of Medicine, recommended that *trans*-fatty acid consumption should be as low as possible in a nutritionally adequate diet. In 2003, the World Health Organization recommended that *trans*-fatty acid intake should be limited to less than 1% of overall energy consumption. Numerous other national and international organizations recommend that *trans* fat consumption should be as low as possible, including the Institute of Medicine, the US Dietary Guidelines, the National Cholesterol Education Program, and the American Dietetic Association.

Owing to the adverse health effects of *trans*-fatty acids, numerous countries and cities have started to regulate *trans* fat in the food supply. In 2003, Denmark became the first country to regulate *trans* fats by mandating that only 2% of fats and

oils could be *trans* fats. Switzerland also imposed federal regulations to restrict the amount of *trans* fats in the food supply, whereas Canada has requested that manufacturers voluntarily reduce *trans* fats. In 2008, the state of California implemented a state-wide ban on *trans* fats in food service establishments (excluding prepackaged goods). In addition, multiple US cities have banned *trans* fat in food service establishments, including Philadelphia and Boston. In New York City, restaurant food must contain less than 0.5 g of *trans* fat. Most bans were implemented in two phases: (1) elimination in frying, grilling, and spreads and (2) complete elimination including baking.

There are no federal regulations in the US limiting *trans* fats; however in 2003, the US Food and Drug Administration ruled that *trans* fat, defined as all unsaturated fatty acids that contain one or more isolated double bonds in the *trans* configuration, must be listed on the Nutrition Facts Panel of the food label (as of 2006). CLA, which contains conjugated double bonds in the *trans* configuration, is excluded from being listed on the food label, because the ruling specifies isolated double bonds in the definition of *trans* fat; however, other ruminant *trans*-fatty acids, such as vaccenic acid, are included under the definition and would be accounted for on the food label. In the US, the amount of *trans* fats in a product can be listed as 0 g if the product contains less than 0.5 g per serving. Many food manufacturers have responded by lowering the *trans* fat content of many of their products to less than 0.5 g per serving. A listing of 0 g of *trans* fats on a food label when the product does indeed contain *trans* fats may be misleading to consumers, particularly if multiple servings of that product are typically consumed. In other countries such as Canada *trans* fat content of a given product must be less than 0.2 g per serving to be listed as 0 g on the label.

Trans Fat Alternatives

Trans fats became a prevalent ingredient in food products as a means for replacing saturated fats in the diet, as the adverse effects of saturated fats were well known. It is now understood that the effects of industrial *trans*-fatty acids on numerous disease risk factors are more deleterious than those of saturated fatty acids. Increased pressure in the last decade to remove or significantly reduce *trans*-fatty acids in the food supply has prompted the question: What alternatives should be used as food manufacturers are reformulating products to remove partially hydrogenated oils? There are multiple considerations when choosing an alternative for *trans* fats, including taste and texture, as well as cost and availability. Moreover, the potential health effects of the alternative should be considered, so as to avoid introducing a harmful ingredient into the food supply. A common technique that is utilized for preparation of *trans* fats alternatives is interesterification – a process in which fatty acids are rearranged on a triacylglycerol molecule. This technique is useful because the position of fatty acids on the glycerol molecule is critical in creating a fat with the proper physical properties required for a *trans* fats alternative. Stearic acid and palmitic acid, both saturated fatty acids, typically are used in interesterification. Other alternatives are oils that require little or no hydrogenation, such as tropical oils (palm oil, palm

kernel oil, and coconut oil), as well as oils developed by manipulating the fatty acid composition of oil seeds (low-linoleic, midoleic, or high-oleic oils) to create products that exhibit increased oxidative stability during deep-frying, and increased shelf-life by increasing relatively stable fatty acids (i.e., oleic acid) and decreasing relatively unstable fatty acids (i.e., linolenic acid). The choice in alternatives for *trans* fats depends on the application. For frying applications, oils such as soybean, canola, cottonseed, and high-oleic sunflower oil can be used; however, for baking applications, where solid or semisolid fats are needed for consistency and feel, alternatives such as tropical oils, fully hydrogenated vegetable oil, and interesterified fats are more useful. Although these alternatives have been useful in manufacturing foods without *trans* fats, their health effects are not yet well understood; more research is needed to compare the health effects of different *trans* fats alternatives.

Conclusions

Overall, data from epidemiological and clinical studies consistently demonstrate the adverse effects of industrial *trans*-fatty acids on coronary heart disease risk and lipids and lipoproteins; their effects on other chronic disease risk factors, such as inflammation, endothelial function, and insulin resistance, as well as risk of type 2 diabetes, are less consistent. The effects of industrial and ruminant *trans*-fatty acids on risk of cancer remain unclear. Although some animal studies suggest that industrial and ruminant *trans*-fatty acids may differ in their biological effects, further research is needed to elucidate whether they exhibit differential effects on chronic disease risk factors. As various *trans* fats alternatives are used in the food supply, it is important to adequately study their effects on health outcomes, so as to avoid increasing the use of ingredients that are more harmful than the ingredient for which they replaced.

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TUBERCULOSIS

Nutritional Management

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Glossary

Antimicrobial drug resistance The inability of a drug to kill or slow the growth of a microbe due to genetic mutations in the microbe.

Bacillary load The amount of disease causing bacteria found within a medium (i.e., sputum), usually associated with severity of disease.

Multidrug-resistant (MDR) TB Drug resistant TB that is resistant to at least rifampin and isoniazid.

Protein–energy malnutrition (PEM) A potentially fatal body-depletion disorder of which there is inadequate protein and calorie intake.

Tuberculosis (TB) An infectious disease caused by *Mycobacterium tuberculosis*, which usually affects the lungs, but can be found elsewhere (extrapulmonary disease).

Introduction

Globally, an estimated 2 million tuberculosis (TB) deaths and 9 million new TB cases occur each year, whereas an estimated 16 million people are living with TB. One-third of the world's population may be latently infected with *Mycobacterium tuberculosis* and at risk for reactivation TB. Before the advent of specific anti-TB drugs, nutritional support was a mainstay of the treatment of TB. Highly effective anti-TB drugs were first developed in the 1940s and 1950s. In the 1970s, the combination of isoniazid, rifampin, and pyrazinamide enabled the duration of treatment to be shortened to 6–9 months. However, TB staged a dramatic comeback in the late twentieth century in both affluent and developing countries, especially in countries of the former Soviet Bloc and in countries with a high prevalence of HIV infection in sub-Saharan Africa and parts of Southeast Asia. Today, TB is the leading cause of death among persons with HIV infection, one of the top five leading infectious cause of death, and one of the leading causes of maternal mortality worldwide.

TB patients may be difficult to cure for many reasons, including antimicrobial drug resistance, drug toxicity and intolerance, advanced TB disease, and comorbidities such as HIV infection. Therefore, there is renewed interest in nutritional support in the management of TB.

Nutritional Status of Tuberculosis Patients

Undernutrition is an important risk factor for developing TB, and TB causes anorexia, weight loss, and cachexia. Weight loss can be severe. Compared to healthy individuals, TB patients have significantly lower body mass index (BMI), skin fold thickness, limb circumference, and overall proportion of body

fat. In the US, weight loss was present at diagnosis in 45% of patients. In Tanzania, among 200 consecutive adults with sputum smear-positive pulmonary TB, 77% of males and 58% of females had a BMI $<18.5 \text{ kg m}^{-2}$, whereas 20% had a BMI $<16 \text{ kg m}^{-2}$. In Malawi, TB patients were substantially weaker than controls as measured by hand-grip dynamometry, suggesting loss of skeletal muscle protein. In addition, these patients had 35% lower fat mass and 19% lower lean body mass than the control group. More extensive TB disease and longer duration of symptoms were associated with lower BMI. BMI among Asian TB patients living in the United Kingdom (UK; 19.3 kg m^{-2}) was lower than in controls (22.2 kg m^{-2}), skin fold thickness was 13% lower, and arm muscle circumference was 20% lower. Patients with concurrent HIV infection tend to be even more malnourished.

Weight loss and wasting in TB may result from the production of tumor necrosis factor- α (TNF- α) and other proinflammatory cytokines that play a critical role in protection against TB. In experimental mycobacterial infections, TNF- α is required for the control of bacillary growth and the protective granulomatous response. Patients receiving anti-TNF- α monoclonal antibodies for the treatment of rheumatoid arthritis and other autoimmune diseases develop reactivation TB at much higher rates than comparable patients not receiving TNF blockers.

Protein utilization may be altered by the cytokine milieu. Studies in the UK documented that TB patients had significantly lower serum albumin levels than controls (mean 37 g l^{-1} vs. 46 g l^{-1}), suggesting protein undernutrition or a systemic inflammatory response. Anabolic pathways may be functionally blocked due to preferential oxidation of ingested amino acids for energy rather than for protein synthesis, contributing to wasting despite nutritional support.

At the same time, protein–energy malnutrition (PEM) rarely occurs without micronutrient deficiencies as well. TB patients have been found to be deficient in vitamins A, B₆, and D as well as zinc, copper, iron, and selenium, although serum levels of fat soluble vitamins A, carotene, and D, and certain micronutrients like zinc, iron, and selenium also fall whereas serum copper levels rise with systemic inflammation. In Ecuador, Koyanagi *et al.* observed that TB patients had significantly lower serum concentrations of zinc, retinol, and selenium. More than 800 TB patients in Malawi demonstrated deficiencies in circulating selenium, carotenoids, and vitamin A. These deficiencies were exacerbated in the most severely wasted group (BMI < 16). There were no significant differences between the HIV-infected and HIV-uninfected TB patients. Low plasma selenium levels were associated with anemia.

In a study of 155 Ethiopian TB patients, HIV coinfection was associated with lower serum zinc and selenium concentrations and an elevated copper/zinc ratio compared with HIV-negative TB patients. After the intensive phase of antibiotic therapy, serum levels of both selenium and zinc improved in both patient groups. Another study in Ethiopia reported that serum concentrations of vitamins C, E, and A were significantly lower in TB patients than in healthy controls. High malondialdehyde concentrations, an indicator of overall oxidant stress, were associated with increased clinical severity of TB, and these parameters were exacerbated in HIV coinfecting individuals.

Wiid and coworkers observed significantly lower total antioxidant status (TAS) in TB patients compared with community controls, and TAS values increased during anti-mycobacterial chemotherapy. Similar results were seen with vitamin A and zinc levels, but not with vitamin E. The vitamin A status of 100 TB patients was studied in Tanzania before and after the intensive phase of anti-TB therapy. Vitamin A levels were low in TB patients and improved with therapy in HIV-negative, but not in HIV-infected patients. HIV infection was also associated with low vitamin A status in otherwise asymptomatic controls.

Ramachandran *et al.* observed low serum vitamin A levels in 47 newly diagnosed TB patients compared with household contacts and healthy controls. Their vitamin A status improved significantly following anti-TB therapy without the need for vitamin A supplementation. Pediatric TB patients in India had markedly reduced levels of plasma zinc, irrespective of their general nutritional status, and there was significant improvement after 6 months of anti-TB therapy. Turkish investigators also observed a significant improvement in serum zinc (which increased) and copper/zinc ratios (which decreased) after 2 months of anti-TB therapy in adult patients.

Vitamin D is linked with TB because of its importance as a macrophage-activating hormone. Vitamin D receptor genetic polymorphisms are associated with vitamin D deficiency and increased incidence of TB. TB patients of Asian and African origin in the UK were significantly vitamin D deficient. Studies in India and Africa also found vitamin D deficiency associated with receptor polymorphisms in TB patients. Recently, *in vitro* studies of human macrophages have revealed mechanisms that may explain the link between vitamin D deficiency and TB. Vitamin D is critical for induction of innate macrophage functions via Toll-like receptor ligation of mycobacterial

cell surface molecules, mycobacteria-specific activation of T lymphocytes by infected macrophages, and fusion of phagosomes containing mycobacteria with lysosomes within infected macrophages.

Effect of Nutritional Factors on the Course of TB

In one study of nearly 1200 TB patients followed prospectively, 10.9% of patients with moderate to severe malnutrition died in the first 4 weeks compared with 6.5% of the patients with normal nutritional status or mild malnutrition. Another study found that TB patients with a BMI < 17.0 kg m⁻² were at increased risk of early death. In children, weight for age is an important indicator of prognosis. In other words, malnutrition correlates strongly with disease severity. The severity of malnutrition is an important indicator of the progress of the disease, and normalization of body weight in response to treatment is a positive sign.

Among patients with multidrug-resistant (MDR) TB in Latvia, Leimane and colleagues reported worse outcomes of chemotherapy if patients had a BMI < 18.5 kg m⁻², independent of other factors. Although low BMI may be a sign of worse disease, these authors controlled for disease severity by multivariable logistic regression. Chemotherapy is less effective in MDR TB, and the impact of nutritional deficits may be more pronounced.

Low serum albumin, anemia, weight loss, and lack of weight gain were associated with the severity and clinical course of TB. Among 373 patients hospitalized for TB in Ecuador, hypoalbuminemia increased the odds of in-hospital death >3-fold, controlling for HIV infection and other comorbidities. In the US, Khan and coworkers demonstrated that TB patients who were underweight at diagnosis had a 4-fold increased risk of relapse within 2 years after completing treatment, and patients who did not gain weight had a 2-fold increase in risk of relapse.

Controlled Intervention Studies of Nutritional Supplements in the Management of TB

Historically, the use of cod-liver oil for the treatment of TB was the taproot, from which grew the broader field of nutritional management of TB. In eighteenth and nineteenth century Europe, TB was responsible for 25% of adult deaths. Survival after diagnosis was approximately 2 years. Treatment for TB was revolutionized by the use of cod-liver oil, which had been used for its medicinal properties. Treating TB patients with cod-liver oil in the eighteenth century resulted in weight gain and increased survival rates from 2 to 8 years. In the early twentieth century, one US study reported that TB patients treated with cod-liver oil gained weight and only 10% died in contrast with weight loss and 70% mortality in the comparison group during approximately 1 year of observation. Cod-liver oil contains vitamins A and D, which are important in host defense against TB.

With the advent of highly effective anti-TB drugs in the 1940s and 1950s, interest in cod-liver oil and other nutritional interventions waned. An influential clinical trial was carried

out in Madras, India, in the 1950s to compare sanatorium treatment with outpatient treatment with regard to nutritional influences on treatment outcome. Patients treated in the sanatorium had substantially better diets and gained more weight than home-treated patients. Improvement was slightly faster in the sanatorium treated group, but the outcomes were nearly the same in the two groups after 12 months after controlling for baseline differences in disease severity. Chemotherapy was so effective that any modest effects of nutritional support apparently were overshadowed.

Chemotherapy is less effective in MDR TB and HIV-associated TB, renewing interest in nutritional interventions. In the past decade, trials of nutritional intervention during chemotherapy of TB patients have shown modest to no benefits. Eighty Indonesian TB patients with low BMI, low plasma retinol, and low plasma zinc were treated with a retinol and zinc supplement versus placebo in addition to standard anti-TB drugs. Sputum conversion, radiographic improvement, and increased plasma retinol levels were observed in the treated group after 6 months of therapy. Two weeks post-therapy, the percentage of patients with negative sputum smears was significantly higher ($p < .01$) in the micronutrient-treated group (23%) compared with the placebo group (13%). Lesion area was significantly reduced in the treated group after 2 months of therapy ($p < .01$). Plasma retinol concentrations were correlated inversely with reduction in mean lesion size at 6 months ($r = -0.367$; $p = 0.02$).

A much larger randomized control trial (RCT) in Tanzania examined the effect on treatment of supplemental zinc alone, multiple micronutrients (MMN: vitamins A–E and minerals Se, Cu), MMN + zinc, or a placebo. Approximately 43% of each group was HIV-infected, and all received standard anti-TB chemotherapy. After 8 weeks of therapy, neither supplement had a significant effect on sputum culture positivity; however, patients receiving the MMN experienced a significant improvement in body weight. HIV status had no influence on the outcome. However, after 8 months of therapy, the MMN + zinc group had significantly reduced mortality (RR = 0.29; 95% confidence interval 0.10–0.80), but only in the TB-HIV coinfecting patients.

Three further RCTs have examined the impact of supplemental zinc, iron, or vitamin D on the outcome of chemotherapy in TB patients. Sixty-six HIV-infected TB patients in Singapore who were receiving antiretroviral and anti-TB therapies were assigned to 28 days of oral zinc sulfate supplements or placebo. Zinc supplements had no effects on PPD-stimulated IFN γ production; however, nearly all (94%) of the subjects had normal plasma zinc levels at baseline. In the second study, 117 anemic, adult male TB patients in India were enrolled in an RCT of iron supplementation during the first 2 months of conventional anti-TB therapy. During 6 months of followup, hematological status improved as the TB disease improved, but supplemental iron had no additional benefit as determined by the extent of radiographic abnormalities. In Egypt, 24 newly diagnosed pediatric TB patients were enrolled in an RCT to examine the effect of vitamin D supplementation (1000 IU/day for 8 weeks) on the outcome of anti-TB therapy. Most of the children were vitamin D deficient at baseline and serum levels of 1,25 (OH) $_2$ D $_3$ improved in both groups during chemotherapy,

but, supplementation did not affect this parameter. The supplemented group showed significant radiological and clinical improvement at followup with significantly increased body weights (+3.3 kg) compared with the placebo group (+2.2 kg; $p < .05$).

Hanekom *et al.* conducted an RCT of vitamin A supplementation in 85 South African children with TB who were not coinfecting with HIV. Children were given either 200 000 IU of retinyl palmitate or placebo on day 0 and day 1 and then followed up during 3 months of conventional anti-TB therapy. Nearly two-thirds of the patients were vitamin A deficient at the beginning of the study, and the deficiency was more pronounced in children with extrapulmonary disease. Vitamin A status improved in both groups, but supplementation had no significant effect on treatment outcome. Vitamin A supplementation was associated with significant decreases in a plasma protein biomarker of a Th2-type cytokine response, which may indicate that vitamin A supplementation promoted a protective Th1-type 1 cytokine profile.

Inducible nitric oxide (NO) is a critical proximal mediator of antimycobacterial resistance in rodent models of TB, although its role in human TB remains controversial. Arginine is the substrate for inducible nitric oxide synthase, the enzyme that produces NO in macrophages. An RCT of oral arginine supplementation (1 g per day) was conducted in 120 HIV-infected and HIV-uninfected Ethiopian TB patients for 4 weeks along with standard anti-TB therapy. At 8 weeks, arginine supplementation resulted in significant improvement in serum arginine levels, weight gain, sputum conversion, and reduction of symptoms compared with the placebo group, but these benefits were observed only in HIV-negative patients. No treatment effect was observed in HIV coinfecting patients.

In a small RCT in Mexico City, investigators compared the clinical responses of 10 TB patients who received a cholesterol-rich diet (800 mg per day) with 11 TB patients who consumed a control diet (250 mg per day) during the first 8 weeks of standard anti-TB chemotherapy. Respiratory symptoms improved in both groups; however, culture-negative sputum at 2 weeks was more frequent in patients consuming a high-cholesterol diet (91%) compared to the placebo group (20%; $p < 0.002$). The bacillary load in sputum was much lower in the cholesterol-supplemented patients (0.05 colony forming unit (cfu)) than in the placebo patients (3.4 cfu; $p < 0.0002$). The cholesterol content of macrophage vesicle membranes (i.e., phagosomes and lysosomes) has been shown to affect the ability of the phagocytes to suppress intracellular growth of mycobacteria.

Nutritional Management of TB

The goal of nutritional interventions is to (1) compensate for the elevated resting energy expenditure and catabolic state associated with TB, (2) support the extensive cellular proliferation and protein production associated with antimycobacterial immune responses, (3) allow repair of diseased tissues, and (4) replenish somatic reserves. Supplementation with specific nutrients (e.g., vitamin A, vitamin D, and zinc) may be required to correct specific deficiencies. The research reviewed above provides limited support for

the use of dietary supplementation with specific macro- and micronutrients for their beneficial impact on both nutritional status and treatment outcomes in TB.

Although this article is not intended to offer specific recommendations for medical practice, some general guidelines are discussed. The expertise of clinical nutritionists and dieticians should be sought in managing TB patients with complex nutritional requirements. In the absence of such expertise, reference works focusing on the nutritional management of patients with infectious diseases should be consulted.

Careful assessment of nutritional status is the starting point, including the medical history and physical examination, anthropometric data, and dietary information, as well as laboratory tests. Nutritional interventions to correct specific nutrient deficiencies can be based on this assessment.

The medical history and physical examination should include questions to identify unintentional weight loss, which is associated with poorer treatment outcomes, higher risk of relapse, and increased mortality. Anorexia, abdominal discomfort, nausea, vomiting, and diarrhea will affect nutritional status and nutritional support. Comorbidities that have nutritional implications such as diabetes mellitus, hepatitis, alcohol abuse, and HIV infection should be identified. Fever affects resting energy expenditure and caloric requirements. The expert clinician's subjective global assessment is one of the critical aspects of evaluating nutritional status. The clinical manifestations of specific nutrient deficiencies have been described in many other reference works, to which the interested reader is referred. Peripheral neuropathy deserves special mention because it is a common side effect of isoniazid, one of the primary anti-TB drugs, and vitamin B₆ supplements are routinely prescribed with anti-TB treatment to prevent peripheral neuropathy.

Anthropometric data should include weight and height in relation to age, sex, and reference standards. BMI can be calculated to determine the overall macronutrient deficit. Skin fold thicknesses, linear and circumferential measurements of specific body parts, and bioelectric impedance may help categorize the nutritional deficit as involving energy, protein, or essential fats.

A dietary survey should include questions regarding recent patterns and quantities of food consumption pre- and post-illness (e.g., food availability and intake, dietary restrictions, and preferences). Standardized tools include 24 h diet recall, 72 h food diaries, and food frequency questionnaires used in clinical and epidemiological research, but they may be useful also in patient care. The information is translated into nutrient intakes based on the known composition of foods and estimates of the quantities consumed. Dietary information should include specific requirements and restrictions based on age, coexisting medical conditions, cultural and religious practices, personal preferences, food allergies, and intolerance of certain foods. Dietary assessment will set specific boundaries around possible dietary recommendations, and the availability and cost of foods and nutritional supplements will affect these boundaries.

Basic hematology and biochemistry laboratory tests can be supplemented as indicated by the history, clinical examination, and abnormalities identified in these basic tests. Anemia

is common in inflammatory conditions such as TB. However, anemia may also result from deficiencies of iron, folate, or vitamin B12. Apart from anemia, serum albumin level may be the most important predictor of nutritional risk for poor outcomes associated with TB. Because albumin is synthesized in the liver with a half-life of 21 days, hypoalbuminemia may reflect inadequate protein intake over a period of weeks or longer, although hypoalbuminemia is also a marker of systemic inflammation that is minimally effected by nutritional support until the inflammatory response remits.

Nutritional support of the TB patients should include a varied diet containing ~50% of calories from carbohydrates, 20–30% from proteins (with an emphasis on high quality proteins), and 20–30% from fats. Energy requirements start at a basal level of approximately 30 kcal kg⁻¹ body mass, increasing to 40 kcal kg⁻¹ or more for persons with significant deficits or with increased resting energy expenditure (e.g., fever). Respiratory function may be modestly or severely compromised, including both oxygenation and ventilation, in TB. As an energy source, dietary carbohydrates generate 20% more carbon dioxide than proteins and 30% more than fats. Thus, for patients who are short of breath, hypercapnic, or lack adequate ventilation, fats and proteins may be preferred dietary sources of energy. This is generally a problem only when the carbohydrate content of the diet exceeds the energy expenditure, where the RQ can exceed 1.0 and substantially increase CO₂ production. Inflammation drives the utilization of both endogenous and exogenous proteins for energy due to hepatic gluconeogenesis and an anabolic block in protein synthesis created by the immune cell cytokines. Protein requirements may be as high as 1.5 gm kg⁻¹ body mass per day. TB patients who are acutely ill, have advanced disease, or substantially compromised nutritional status, and whose dietary intake has been inadequate for an extended period of time may benefit from a gradual increase in dietary macronutrients up to the recommended amounts. Overfeeding is not recommended. Omega-3 polyunsaturated fatty acids in the diet have anti-inflammatory and immunomodulatory effects, including increased susceptibility to TB in guinea pigs and mice, whereas dietary omega-6 polyunsaturated fatty acids have proinflammatory qualities, either of which may prove to be undesirable in the management of TB. The effects of different fatty acids on the nutritional management of TB remain to be established. A multivitamin and mineral supplement is recommended to ensure the availability of micronutrients. Higher doses of individual components to treat specific micronutrient deficiencies should follow guidelines established for the treatment of those conditions. Otherwise, doses of vitamins and minerals above those demonstrated to be safe and beneficial are not recommended.

Iron deficiency and iron replacement also merit special mention. Increased severity of TB has been observed in individuals with hemochromatosis, an iron overload syndrome, and in indigenous societies with high levels of iron intake from drinking a type of traditional beer fermented in iron vessels. Moreover, epidemics of malaria have been reported during refeeding programs in refugee and famine situations, related to the increased availability of iron for the parasite from supplements provided for the treatment of iron deficiency. *M. tuberculosis* acquires iron by means of highly

developed scavenging mechanisms that must outcompete the host's iron-binding proteins (e.g., lactoferrin and transferrin). Iron deficiency is not necessarily more deleterious to the host than to the pathogen, and iron replacement therapy may benefit the microbe as well as the patient.

Nutrient–Drug Interactions

The standard treatment for all newly diagnosed patients with active, drug-susceptible TB includes four drugs, isoniazid, rifampin, pyrazinamide, and ethambutol, taken for 2–3 months followed by two of these drugs, isoniazid and rifampicin, taken for an additional 4–6 months. Isoniazid (INH) interferes with the metabolism of vitamin B₆, which includes pyridoxine, pyridoxal, and pyridoxamine. Isoniazid combines with pyridoxal or pyridoxal phosphate to form potent inhibitors of pyridoxal kinase, thus, blocking formation of the coenzyme form of the vitamin. In the absence of vitamin B₆ supplements, TB patients treated with INH may experience peripheral neuritis, manifested as tingling, numbness, or a painful 'prickly' sensation in a stocking and glove distribution. Approximately 20% of patients treated with high doses of INH or patients who are otherwise predisposed to peripheral neuropathy (e.g., alcoholics, diabetes mellitus) will develop peripheral neuritis. Administration of 25 mg daily of vitamin B₆ prevents peripheral neuritis in nearly all TB patients treated with INH.

Patients treated with cycloserine, an important second-line drug used in the treatment of MDR TB, should also receive vitamin B₆ supplements in doses of 200–300 mg per day because of central nervous system side effects (psychosis, depression) also related to pyridoxine metabolism.

Other anti-TB drugs that have adverse consequences on nutrition include para-aminosalicylic acid (PAS) and ethionamide, because these drugs commonly cause nausea, abdominal pain, anorexia, or vomiting. Such side effects will have a significant negative impact on the patient's nutritional status and well-being.

Treatment of TB may induce other problems that affect nutritional status and nutrient intake. Three of the first line drugs, isoniazid, rifampicin, and pyrazinamide, all carry a small risk of chemical hepatitis characterized by anorexia, nausea, vomiting, and decreased nutrient intake. In its more severe forms, drug-induced hepatitis results in disturbances in carbohydrate, protein, and lipid homeostasis that clearly impact the patient's metabolic and nutritional status. Although nutritional factors do not contribute to the cause, TB drug-induced hepatitis has many important consequences affecting nutritional status and nutrient intake.

Conclusions

Nutritional support is an important component of comprehensive care for persons with TB, which must be adapted to each geographic region and socioeconomic context. Specific nutritional recommendations should be adapted to all patients depending on their clinical condition, nutritional status, and the practical possibilities of supplementation. Simplified,

more generic approaches may be most suitable in some circumstances. These considerations notwithstanding, all TB patients should be offered nutritional/dietary advice based upon their nutritional status and accompanying diseases. Although a specific, often costly diet that targets the individual nutritional needs of a TB patient may improve treatment outcome, it may be difficult to achieve this level of care in the absence of sufficient economic resources. However, we believe a deeper understanding of the essential role of nutrition in TB pathogenesis and treatment will help improve current TB treatment practices and improve outcomes of TB patients.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily represent an official position of the Centers for Disease Control and Prevention, the US government.

See also: Lung Diseases. Malnutrition: Secondary, Diagnosis and Management. Nutrition and Susceptibility to Tuberculosis. Vitamin A: Deficiency and Interventions. Vitamin B₆: Physiology. Vitamin D: Physiology, Dietary Sources, and Requirements

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ULTRATRACE ELEMENTS

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Definition

In the earlier part of this century, scientists could qualitatively detect small amounts of several mineral elements in living organisms. In reports, these elements were described as being present in ‘traces’ or ‘trace amounts.’ It is not surprising that these elements soon became known as trace elements. Today, sophisticated analytical techniques have permitted the accurate measurement of the amount of many mineral elements, some at very low concentrations, in biological material. The trace elements found in living organisms may be essential, that is, indispensable for growth and health, or they may be nonessential, fortuitous reminders of our geochemical origins or indicators of environmental exposure. Some of the nonessential trace elements can be beneficial to health through pharmacological action. All of the trace elements are toxic when intake is excessive.

Trace elements are those elements of the periodic table that occur in animals or humans in amounts measured in milligrams per kilogram of body weight or less. The trace elements essential for health are usually required by humans in amounts measured in milligrams per day; these elements include copper, iron, manganese, and zinc. The individual trace elements are discussed elsewhere in the encyclopedia. Since 1980, the term ‘ultratrace element’ has appeared in the nutritional literature. Ultratrace elements have been defined as those elements with estimated dietary requirements usually less than 1 mg kg^{-1} , and often less than $50 \text{ } \mu\text{g kg}^{-1}$ of diet for laboratory animals. For humans, the term often is used to indicate an element with an established, estimated, or suspected requirement of less than 1 mg day^{-1} or generally indicated by micrograms per day. At least 18 elements could be considered ultratrace elements: aluminum, arsenic, boron, bromine, cadmium, chromium, fluorine, germanium, iodine, lead, lithium, molybdenum, nickel, rubidium, selenium, silicon, tin, and vanadium. Emerging evidence indicates that silicon should be categorized as a trace element instead of an

ultratrace element. However, knowledge about the practical importance or beneficial actions of silicon is in a state similar to that for most of the ultratrace elements; thus, it is considered as one of them here. Cobalt perhaps also belongs in the ultratrace category; however, it is required only in the form of vitamin B₁₂ and thus is usually discussed as a vitamin.

The quality of the experimental evidence for nutritional essentiality varies widely for the ultratrace elements. The evidence for the essentiality of three elements, iodine, molybdenum, and selenium, is substantial and noncontroversial; specific biochemical functions have been defined for these elements. The nutritional importance of iodine and selenium are such that they have separate entries in this encyclopedia. Molybdenum, however, is given very little nutritional attention, apparently because a deficiency of this element has not been unequivocally identified in humans other than individuals nourished by total parenteral nutrition or with genetic defects causing disturbances in metabolic pathways involving this element. Specific biochemical functions have not been defined for the other 15 ultratrace elements listed above. Thus, their essentiality is based on circumstantial evidence, which most often is that a dietary deprivation in an animal model results in a suboptimal biological function that is preventable or reversible by an intake of physiological amounts of the element in question. Often the circumstantial evidence includes an identified essential function in a lower form of life, and biochemical actions consistent with a biological role or beneficial action in humans. The circumstantial evidence for essentiality is substantial for arsenic, boron, chromium, nickel, silicon, and vanadium. The evidence for essentiality for the other elements is generally limited to a few gross observations in one or two species by one or two research groups. However, it should be noted that two of these ultratrace elements have beneficial actions when ingested in high (pharmacological) amounts: they are fluorine, which prevents tooth caries, and lithium, which is used to treat manic-depressive disorders.

Although aluminum has a separate article, and the elements cadmium, lead, and nickel are discussed in the entry; the focus in those entries is toxicity; thus, these elements will be included in the following discussion. Chromium, however, which also has a separate entry, will not be included.

Absorption, Transport, and Storage

Homeostasis (maintenance of a steady optimal concentration of an element in the body) regulation involves the processes of absorption, storage, and excretion. The relative importance of these three processes varies among the ultratrace elements. The amount absorbed from the gastrointestinal tract is often the controlling mechanism for positively charged ultratrace elements such as aluminum, nickel, and tin. With these trace elements, if the body content is low, or if intake is low, the percentage of the element absorbed from the gastrointestinal tract is increased, and *vice versa*. Elements that exist mainly as negatively charged ions or oxyanions, such as arsenic, boron, and fluoride, are usually absorbed quite freely and completely from the gastrointestinal tract. Excretion through the urine, bile, sweat, and breath is, therefore, the major mechanism for controlling the amount of these ultratrace elements in an organism. By being stored at inactive sites or in an inactive form, some ultratrace elements are prevented from causing adverse reactions when present in high quantities. An example of this homeostatic process is the binding of cadmium by the cysteine-rich protein called metallothionein. Release of an ultratrace element from storage forms also can be important in preventing deficiency.

Absorption of ultratrace elements from the intestinal lumen can occur in three ways. These are described below.

1. Passive diffusion – passive transport driven by a difference in concentration of the element between the two sides of the luminal membrane and the mucosa. Transmembrane movement of ions occurs through pores or channels within the membrane and is an energy-independent process. A significant amount of passive transport across the intestinal mucosa may occur through a paracellular pathway, or the transport between cells across intercellular tight junctions.
2. Facilitated diffusion – the transfer of an element across the membrane by carrier proteins embedded in the membrane. Facilitated transport resembles simple diffusion because it is not energy dependent and is driven by a difference in the ion concentration between two sides of a membrane. Facilitated transport occurs much more rapidly than simple diffusion and is saturable because of a finite number of carrier proteins.
3. Active transport – the accumulation within, or the extrusion from, a cell of an element in opposition to a concentration gradient. Active transport is saturable, is energy dependent, and involves a carrier protein that usually is quite specific for an element. The mechanisms of absorption for the various ultratrace elements are given in [Table 1](#); this table also lists the known transport and storage vehicles for these elements.

Metabolism and Excretion

Knowledge about chemical changes that must occur before excretion for most of the ultratrace elements is quite limited. Perhaps the best characterized is inorganic arsenic, which is methylated into monomethylarsonic acid and dimethylarsinic acid, and organic arsenic, which is converted into, or remains mostly as, arsenobetaine before being excreted in the urine. Other ultratrace elements that are known to be incorporated into biochemical metabolites for transport and/or excretion include aluminum bound to transferrin, cadmium incorporated into metallo-thionein, nickel as the α -2-macroglobulin nickeloplasmin or bound to albumin and L-histidine, and vanadium converted into vanadyl-transferrin and vanadyl-ferritin (see [Table 1](#)). A known important metabolite of molybdenum is a small nonprotein cofactor containing a pterin nucleus that is present at the active site of molybdoenzymes. More than 40% of molybdenum not attached to an enzyme in liver also exists as this cofactor bound to the mitochondrial outer membrane. This form can be transferred to an apoenzyme of xanthine oxidase or sulfite oxidase, which transforms it into an active enzyme molecule. Molecules of biological importance for the ultratrace elements are shown in [Table 2](#). The ultratrace elements are excreted from the body mainly via the feces and urine. Fecal excretion of absorbed ultratrace elements usually results from biliary excretion, but may be of nonbiliary origin (e.g., through the pancreas or intestine). Ultratrace elements may also be excreted through sweat and breath and also are removed from the body through the loss of blood (e.g., menses), skin, hair, semen, saliva, and nails. [Table 2](#) gives the major routes of excretion for the ultratrace elements.

Requirements and High Intakes

As already mentioned, the ultratrace elements other than selenium and iodine are a disparate group in terms of their possible requirement or nutritional importance for human health and well-being. Although molybdenum has known essential functions, it has no unequivocally identified practical nutritional importance. The other 14 ultratrace elements discussed here have been suggested to be essential based on circumstantial evidence. This evidence is presented below along with some indication of possible requirement (extrapolated from the deficient animal intakes shown in [Table 3](#)), and some indication as to what constitutes a high intake.

Aluminum

A dietary deficiency of aluminum in goats reportedly results in increased abortions, depressed growth, incoordination and weakness in hind legs, and decreased life expectancy. Aluminum deficiency has also been reported to depress growth in chicks. Other biochemical actions that suggest aluminum could possibly act in an essential role include the *in vitro* findings that it activates the enzyme adenylate cyclase, enhances calmodulin activity, stimulates DNA synthesis in cell

Table 1 Absorption, transport, and storage characteristics of the ultratrace elements

Element	Major mechanism(s) for homeostasis	Means of absorption	Percentage of ingested absorbed	Transport and storage vehicles
Aluminum	Absorption	Uncertain; some evidence for passive diffusion through the paracellular pathway; also, evidence for active absorption through processes shared with active processes of calcium; probably occurs in proximal duodenum; citrate combined with aluminum enhances absorption	Less than 1%	Transferrin carries aluminum in plasma; bone a possible storage site
Arsenic	Urinary excretion: Inorganic arsenic as mostly dimethylarsinic acid and organic arsenic as mostly arsenobetaine	Inorganic arsenate becomes sequestered in or on mucosal tissue, then absorption involves a simple movement down a concentration gradient; organic arsenic absorbed mainly by simple diffusion through lipid regions of the intestinal boundary	Soluble inorganic forms, > 90%; slightly soluble inorganic forms, 20–30%; inorganic forms with foods, 60–75%; methylated forms, 45–90% Greater than 90%	Before excretion inorganic arsenic is converted into monomethyl arsonic acid and dimethylarsinic acid; arsenobetaine not biotransformed; arsenocholine transformed to arsenobetaine Boron transported through the body as undissociated $B(OH)_3$; bone a possible storage site None identified Incorporated into metallothionein, which probably is both a storage and transport vehicle Exists as fluoride ion in plasma; hydrogen fluoride is the form in diffusion equilibration across cell membranes. Stored in bone
Boron	Urinary excretion	Ingested boron is converted into $B(OH)_3$ and absorbed in this form, probably by passive diffusion	Greater than 90%	
Bromine	Urinary excretion	Probably passive diffusion because no apparent saturable component	75–90%	
Cadmium	Absorption	May share a common absorption mechanism with other metals (e.g., zinc) but mechanism is less efficient for cadmium	5%	
Fluorine	50% daily intake excreted in urine; approximately 50% daily intake stored in bone and developing teeth	Absorption by passive diffusion and inversely related to pH. Significant portion absorbed as hydrogen fluoride from stomach; absorption of fluoride also occurs throughout the small intestine	76–90%	
Germanium	Urinary excretion	Has not been conclusively determined but most likely is by passive diffusion	Greater than 90%	None identified
Lead	Absorption	Uncertain; thought to be by passive diffusion in small intestine, but evidence has been presented for an active transport, perhaps involving the system for calcium	Adults 5–15% Children 40–50%	Bone is a repository for lead
Lithium	Urinary excretion	Passive diffusion by paracellular transport via the tight junctions and pericellular spaces	Lithium chloride highly absorbed – greater than 90% 50–93%	Bone can serve as a store for lithium
Molybdenum	Urinary and biliary excretion	Uncertain, possible that molybdate is moved both by diffusion and by active transport, but at high concentrations active transport is a small portion of flux; absorption occurs rapidly in stomach and continues throughout the small intestine		Molybdate in blood loosely attached to erythrocytes and tends to bind α_2 -macroglobulin. Liver and kidney retain highest amount of molybdate (Continued)

Table 1 Continued

<i>Element</i>	<i>Major mechanism(s) for homeostasis</i>	<i>Means of absorption</i>	<i>Percentage of ingested absorbed</i>	<i>Transport and storage vehicles</i>
Nickel	Both absorption and urinary excretion	Uncertain, evidence both for passive diffusion (perhaps as an amino acid or other low molecular weight complex) and for energy driven transport; occurs in the small intestine	<10% with food	Transported in blood principally bound to serum albumin with small amounts bound to L-histidine and α_2 -macroglobulin; no organ accumulates physiological amounts of nickel None identified
Rubidium	Excretion through kidney and intestine	Resembles potassium in its pattern of absorption; rubidium and potassium thought to share a transport system	Highly absorbed	
Silicon	Both absorption and urinary excretion	Mechanisms involved in intestinal absorption have not been described	Food silicon near 50%; insoluble or poorly soluble silicates = 1% Approximately 3%. Percentage increases when very low amounts are ingested <10%	Silicon in plasma believed to exist as undissociated monomeric silicic acid None identified. Bone might be a repository
Tin	Absorption	Mechanisms involved in intestinal absorption have not been described		
Vanadium	Absorption	Vanadate has been suggested to be absorbed through phosphate or other anion transport systems; vanadyl has been suggested to use iron transport systems. Absorption occurs in the duodenum		Converted into vanadyl-transferrin and vanadyl-ferritin; whether transferrin is the transport vehicle and ferritin is the storage vehicle for vanadium remains to be determined. Bone is a repository for excess vanadium

Table 2 Excretion, retention, and possible biological roles of the ultratrace elements

Element	Organs of high content (typical concentration)	Major excretory route after ingestion	Molecules of biological importance	Possible biological role
Aluminum	Bone ($1\text{--}12\ \mu\text{g g}^{-1}$) Lung ($35\ \mu\text{g g}^{-1}$)	Urine; also significant amounts in bile	Aluminum binds to proteins, nucleotides, and phospholipids; aluminum-bound transferrin apparently is a transport molecule	Enzyme activator
Arsenic	Hair ($0.65\ \mu\text{g g}^{-1}$) Nails ($0.35\ \mu\text{g g}^{-1}$) Skin ($0.10\ \mu\text{g g}^{-1}$)	Urine	Methylation of inorganic oxyanionic anions occurs in organisms ranging from microbial to mammalian; methylated and products include arsenocholine, arsenobetaine, dimethylarsinic acid, and methylarsonic acid; arsenite methyltransferase and monomethylarsonic acid methyltransferase use S-adenosylmethionine for the methyl donor	Metabolism of methionine, or involved in labile methyl metabolism; regulation of gene expression
Boron	Bone ($1.6\ \mu\text{g g}^{-1}$) Fingernails ($15\ \mu\text{g g}^{-1}$) Hair ($1\ \mu\text{g g}^{-1}$) Teeth ($5\ \mu\text{g g}^{-1}$)	Urine	Boron biochemistry essentially that of boric acid, which forms ester complexes with hydroxyl groups, preferably those adjacent and <i>cis</i> , in organic compounds. Five naturally occurring boron esters (all antibiotics) synthesized by various bacteria have been characterized	Cell membrane function or stability such that it influences the response to hormone action, transmembrane signaling or transmembrane movement of regulatory cations or anions
Bromine	Hair ($3.0\ \mu\text{g g}^{-1}$) Liver ($4.0\ \mu\text{g g}^{-1}$) Lung ($6.0\ \mu\text{g g}^{-1}$) Testis ($5.0\ \mu\text{g g}^{-1}$) Kidney ($14\ \mu\text{g g}^{-1}$) Liver ($4\ \mu\text{g g}^{-1}$) Bones ($1\text{--}5\ \text{mg g}^{-1}$) Teeth ($500\ \mu\text{g g}^{-1}$) Bone ($9\ \mu\text{g g}^{-1}$) Liver ($0.3\ \mu\text{g g}^{-1}$) Pancreas ($0.2\ \mu\text{g g}^{-1}$) Testis ($0.5\ \mu\text{g g}^{-1}$) Aorta ($1\text{--}2\ \mu\text{g g}^{-1}$) Bone ($25\ \mu\text{g g}^{-1}$) Kidney ($1\text{--}2\ \mu\text{g g}^{-1}$) Liver ($1\text{--}2\ \mu\text{g g}^{-1}$) Adrenal gland ($60\ \text{ng g}^{-1}$)	Urine	Exists as Br ion <i>in vivo</i> , binds to proteins and amino acids	Electrolyte balance
Cadmium		Urine and gastrointestinal tract		
Fluorine		Urine	Metallothionein, a high sulfhydryl-containing protein involved in regulating cadmium distribution	Involved in metallothionein metabolism and utilization
Germanium		Urine	Exists as fluoride ion or hydrogen fluoride in body fluids; approximately 99% of body fluorine found in mineralized tissues as fluorapatite	Role in biological mineralization
Lead		Urine; also significant amounts in bile	Plasma lead mostly bound to albumin; blood lead binds mostly to hemoglobin but some binds a low molecular weight protein in erythrocytes	Role in immune function
Lithium		Urine	None identified	Facilitates iron absorption and/or utilization
				Regulation of some endocrine function
Molybdenum	Bone ($100\ \text{ng g}^{-1}$) Lymph nodes ($200\ \text{ng g}^{-1}$) Pituitary gland ($135\ \text{ng g}^{-1}$) Kidney ($0.4\ \mu\text{g g}^{-1}$) Liver ($0.6\ \mu\text{g g}^{-1}$)	Urine; also significant amounts in bile	Molybdoenzymes of aldehyde oxidase, xanthine oxidase/dehydrogenase and sulfite oxidase in which molybdenum exists as a small nonprotein factor containing a pterin nucleus; molybdate ion (MoO_4^{2-}), the form that exists in blood and urine	Molybdoenzymes oxidize and detoxify various pyrimidines, purines, and pteridines; catalyze the transformation of hypoxanthine to xanthine and xanthine to uric acid; and catalyze the conversion of sulfite to sulfate

(Continued)

Table 2 Continued

<i>Element</i>	<i>Organs of high content (typical concentration)</i>	<i>Major excretory route after ingestion</i>	<i>Molecules of biological importance</i>	<i>Possible biological role</i>
Nickel	Adrenal glands (25 ng g ⁻¹) Bone (33 ng g ⁻¹) Kidney (10 ng g ⁻¹) Thyroid (30 ng g ⁻¹)	Urine as low molecular weight complexes	Binding of Ni ²⁺ by various ligands including amino acids (especially histidine and cysteine), proteins (especially albumin), and a macroglobulin called nickeloplasmin important in transport and excretion. Ni ²⁺ component of urease; Ni ³⁺ essential for enzymatic hydrogenation, desulfurization, and carboxylation reactions in mostly anaerobic microorganisms	Cofactor or structural component in specific metalloenzymes; role in a metabolic pathway involving vitamin B ₁₂ and folic acid. Role similar to potassium; neurophysiological function
Rubidium	Brain (4 µg g ⁻¹) Kidney (5 µg g ⁻¹) Liver (6.5 µg g ⁻¹) Testis (20 µg g ⁻¹) Aorta (16 µg g ⁻¹) Bone (18 µg g ⁻¹) Skin (4 µg g ⁻¹) Tendon (12 µg g ⁻¹)	Urine; also significant amounts excreted through intestinal tract Urine	None identified	Role similar to potassium; neurophysiological function
Silicon	Bone (18 µg g ⁻¹) Skin (4 µg g ⁻¹) Tendon (12 µg g ⁻¹)	Urine	Silicic acid (Si(OH) ₄) is the form believed to exist in plasma; magnesium orthosilicate is probably the form in urine. The bound form of silicon has never been rigorously identified	Structural role in some mucopolysaccharide or collagen; role in the initiation of calcification and in collagen formation
Tin	Bone (0.8 µg g ⁻¹) Kidney (0.2 µg g ⁻¹) Liver (0.4 µg g ⁻¹)	Urine; also significant amounts in bile	Sn ²⁺ is absorbed and excreted more readily than Sn ⁴⁺	Role in some redox reaction
Vanadium	Bone (120 ng g ⁻¹) Kidney (120 ng g ⁻¹) Liver (120 ng g ⁻¹) Spleen (120 ng g ⁻¹) Testis (200 ng g ⁻¹)	Urine; also significant amount in bile	Vanadyl (VO ²⁺), vanadate (H ₂ VO ⁴⁻ or VO ³⁻) and peroxovanadyl [V-O-O]; VO ²⁺ complexes with proteins, especially those associated with iron (e.g., transferrin, hemoglobin)	Lower forms of life have haloperoxidases that require vanadium for activity; a similar role might exist in higher forms of life

None of the suggested biological functions or roles of any of the ultratrace elements has been conclusively or unequivocally identified in higher forms of life except for those of molybdenum.

Table 3 Human body content, and deficient, typical, and rich sources of intakes of ultratrace elements

Element	Apparent deficient intake (species)	Human body content	Typical human daily dietary intake	Rich sources
Aluminum	160 $\mu\text{g kg}^{-1}$ (goat)	30–50 mg	2–10 mg	Baked goods prepared with chemical leavening agents (e.g., baking powder), processed cheese, grains, vegetables, herbs, tea, antacids, buffered analgesics
Arsenic	<25 $\mu\text{g kg}^{-1}$ (chicks) <35 $\mu\text{g kg}^{-1}$ (goat) <15 $\mu\text{g kg}^{-1}$ (hamster) <30 $\mu\text{g kg}^{-1}$ (rat) <0.3 mg kg^{-1} (chick) 0.25–0.35 mg day^{-1} (human)	1–2 mg	12–60 μg	Shellfish, fish, grain, cereal products
Boron	<0.3 mg kg^{-1} (rat)	10–20 mg	0.5–3.5 mg	Food and drink of plant origin, especially noncitrus fruits, leafy vegetables, nuts, pulses, avocados, legumes, wine, cider, beer, peanut butter
Bromine	<0.3 mg kg^{-1} (rat)			
Cadmium	0.8 mg kg^{-1} (goat) <5 $\mu\text{g kg}^{-1}$ (goat) <4 $\mu\text{g kg}^{-1}$ (rat)	200–350 mg 5–20 mg	2–8 mg 10–20 μg	Grain, nuts, fish Shellfish, grains – especially those grown on high-cadmium soils, leafy vegetables
Fluorine	<0.3 mg kg^{-1} (goat) <0.45 mg kg^{-1} (rat) 0.7 mg kg^{-1} (rat) <32 $\mu\text{g kg}^{-1}$ (pig)	3 g	Fluoridated areas, 1–3 mg Nonfluoridated areas, 0.3–0.6 mg	Fish, tea, fluoridated water
Germanium				
Lead		3 mg Children less than age 10 years, 2 mg Adults, 120 mg 350 μg	0.4–3.4 mg 15–100 μg	Wheat bran, vegetables, leguminous seeds Seafood, plant foodstuffs grown under high-lead conditions
Lithium	<45 $\mu\text{g kg}^{-1}$ (rat) <1.5 mg kg^{-1} (goat) <15 $\mu\text{g kg}^{-1}$ (rat) <25 $\mu\text{g kg}^{-1}$ (goat) <25 $\mu\text{g day}^{-1}$ (human)	10 mg	200–600 μg 50–100 μg	Eggs, meat, processed meat, fish, milk, milk products, potatoes, vegetables (content varies with geological origin) Milk and milk products, dried legumes, pulses, organ meats (liver and kidney), cereals, and baked goods
Nickel	<30 $\mu\text{g kg}^{-1}$ (rat) <100 $\mu\text{g kg}^{-1}$ (goat) <20 $\mu\text{g kg}^{-1}$ (rat)	1–2 mg	70–260 μg	Chocolate, nuts, dried beans and peas, grains
Rubidium	180 $\mu\text{g kg}^{-1}$ (goat)	360 mg	1–5 mg	Coffee, black tea, fruits and vegetables (especially asparagus), poultry, fish
Silicon	<2.0 mg kg^{-1} (chick) <4.5 mg kg^{-1} (rat) <20 $\mu\text{g kg}^{-1}$ (rat) <10 $\mu\text{g kg}^{-1}$ (goat)	2–3 g 7–14 mg 100 μg	20–50 mg 1–40 mg 10–30 μg	Unrefined grains of high fiber content, cereal products, beer, coffee Canned foods Shellfish, mushrooms, parsley, dill seed, black pepper, some prepared foods, grains, beer, wine
Tin				
Vanadium				

cultures, and stimulates osteoblasts to form bone through activating a putative G_i protein-coupled cation sensing system.

If humans have a requirement for aluminum, for which there is currently no evidence, it probably is much less than 1.0 mg day^{-1} . Aluminum toxicity apparently is not a concern for healthy individuals. Cooking foods in aluminum cookware does not lead to detrimental intakes of aluminum. High dietary ingestion of aluminum probably is not a cause of Alzheimer's disease. However, high intakes of aluminum through sources such as buffered analgesics and antacids by susceptible individuals (e.g., those with impaired kidney function including the elderly and low-birth-weight infants) may lead to pathological consequences and thus should be avoided. For most healthy individuals, an aluminum intake of 125 mg day^{-1} should not lead to toxicological consequences.

Arsenic

Arsenic deprivation has been induced in chickens, hamsters, goats, pigs, and rats. In the goat, pig, and rat, the most consistent signs of deprivation were depressed growth and abnormal reproduction characterized by impaired fertility and elevated perinatal mortality. Other notable signs of deprivation in goats were depressed serum triacylglycerol concentrations and death during lactation. Myocardial damage was also present in lactating goats. Other signs of arsenic deprivation have been reported, including changes in mineral concentrations in various organs. However, listing all signs reported to be caused by arsenic deficiency may be misleading because studies with chicks, rats, and hamsters have revealed that the nature and severity of the signs are affected by a number of dietary and other factors. For example, female rats fed a diet that is conducive to kidney calcification have more severe calcification when dietary arsenic is low; kidney iron was also elevated. Male rats fed the same diet do not show these changes.

Other factors that affect the response to arsenic deprivation include methionine, arginine, choline, taurine, and guanidoacetic acid. In other words, the signs of arsenic deprivation were changed and generally enhanced by nutritional stressors that affected sulfur amino acid or labile methyl-group metabolism; this suggests that arsenic has a biochemical function that affects these substances. Further evidence for this suggestion is the finding that arsenic deprivation slightly increases liver S-adenosylhomocysteine (SAH) and decreases liver S-adenosylmethionine (SAM) concentrations in animal models, thus resulting in a decreased SAM/SAH ratio; SAM and SAH are involved in methyl transfer. Additionally, arsenite can induce the isolated cell production of certain proteins known as heat shock proteins. The control of production of these proteins in response to arsenite apparently is at the transcriptional level, and involves changes in the methylation of core histones. It also has been shown that arsenic can increase the methylation of the *p53* promoter, or DNA, in human lung cells.

It has been suggested, based on animal data, that a possible arsenic requirement for humans eating 8.37 MJ (2000 kcal) would be $12\text{--}25 \text{ } \mu\text{g day}^{-1}$; this is near the typical daily intake shown in Table 3. Because of mechanisms for the homeostatic regulation of arsenic (including methylation, then excretion in

urine), its toxicity through oral intake is relatively low; it is actually less toxic than selenium, an ultratrace element with a well-established nutritional value. Toxic quantities of inorganic arsenic generally are reported in milligrams. For example, reported estimated fatal acute doses of arsenic for humans range from 70 to 300 mg or approximately $1.0\text{--}4.0 \text{ mg kg}^{-1}$ body weight. Some forms of organic arsenic are virtually nontoxic; a 10 g kg^{-1} body weight dose of arsenobetaine depressed spontaneous motility and respiration in male mice, but these symptoms disappeared within 1 h. Results of numerous epidemiological studies have suggested an association between chronic overexposure to arsenic and the incidence of some forms of cancer; however, the role of arsenic in carcinogenesis remains controversial. Arsenic does not seem to act as a primary carcinogen, and is either an inactive or extremely weak mitogen. In the USA, a standard known as a reference dose (RfD; lifetime exposure that is unlikely to cause adverse health effects) of $0.3 \text{ } \mu\text{g kg}^{-1}$ body weight per day, or $21 \text{ } \mu\text{g day}^{-1}$ for a 70-kg human, has been suggested for inorganic arsenic. Because of safety factors in the determination, the RfD for arsenic conflicts with the possible arsenic requirement; this conflict is similar to that for some other mineral elements including zinc. These conflicts are currently being addressed by nutritionists and toxicologists.

Boron

Listing the signs of boron deficiency for animal models is difficult because most studies have used stressors to enhance the response to changes in dietary boron. Thus, the response to boron deprivation varied as the diet changed in its content of nutrients such as calcium, phosphorus, magnesium, potassium, and vitamin D. Although the nature and severity of the changes varied with dietary composition, many of the findings indicated that boron deprivation impairs calcium metabolism, brain function, and energy metabolism. Studies also suggest that boron deprivation impairs immune function and exacerbates adjuvant-induced arthritis in rats. Feeding low boron to humans ($<0.3 \text{ mg day}^{-1}$) altered the metabolism of macrominerals, electrolytes, and nitrogen, as well as oxidative metabolism, and produced changes in erythropoiesis and hematopoiesis. Boron deprivation also altered electroencephalograms to suggest depressed behavioral activation and mental alertness, depressed psychomotor skills and cognitive processes of attention and memory, and enhanced some effects of estrogen therapy such as increases in concentrations of serum 17β -estradiol and plasma copper. Other findings suggest that boron may have an essential function. *In vitro* it competitively inhibits oxidoreductase enzymes, which require pyridine or flavin nucleotides, and enzymes such as serine proteases, which form transition state analogs with boronic acid or borate derivatives. Boron has an essential function in plants, in which it influences redox actions involved in cellular membrane transport. This latter finding supports the hypothesis that boron has a role in cell membrane function or stability such that it influences the response to hormone action, transmembrane signaling, or transmembrane movement of regulatory cations or anions. Another finding in support of this hypothesis is that boron influences the transport of

extracellular calcium into and the release of intracellular calcium in rat platelets activated by thrombin.

An analysis of both human and animal data has resulted in the suggestion by a World Health Organization (WHO) publication that an acceptable safe range of population mean intakes of boron for adults could well be 1.0–13 mg day⁻¹. In other words, 1.0 mg probably covers any requirement and 13 mg will not lead to any toxicological consequences. However, the US and Canada concluded in 2002 that there was still insufficient evidence to establish a clear biological function for boron in humans, so no recommended dietary intake was set for those countries. Boron has a low order of toxicity when administered orally. Toxicity signs in animals generally occur only after dietary boron exceeds 100 µg g⁻¹. The low order of toxicity of boron for humans is shown by the use of boron as a food preservative between 1870 and 1920 without apparent harm. It was reported in 1904 that when doses equivalent to more than 0.5 g of boric acid were consumed daily, disturbances in appetite, digestion, and health occurred. It was concluded in this report that this quantity of boron per day was too much for an average person to receive regularly. The upper limit (UL) for the US and Canada has been set at 20 mg day⁻¹ based on extrapolation from animal studies.

Bromine

It has been reported that a dietary deficiency of bromide results in depression of growth, fertility, hematocrit, hemoglobin, and life expectancy, and increases in milk fat and spontaneous abortions in goats. Other biological actions that suggest bromine could possibly act in an essential role include the findings that bromide alleviates growth retardation caused by hyperthyroidism in mice and chicks, and insomnia exhibited by many hemodialysis patients has been associated with bromide deficit.

If humans have a requirement for bromide, which has not yet been shown to be the case, based on deficient intakes for animals it is probably no more than 1.0 mg day⁻¹. Bromine ingested as the bromide ion has a low order of toxicity; thus bromine is not of toxicological concern in nutrition.

Cadmium

Deficiency of cadmium reportedly depresses growth of rats and goats. Other *in vitro* biochemical actions that suggest cadmium could possibly act as an essential element include the finding that it has transforming growth factor activity and stimulates growth of cells in soft agar.

If humans have a requirement for cadmium, which is still uncertain, based on deficient intakes for animals it is probably less than 5 µg day⁻¹. Although cadmium may be an essential element at these extremely low amounts, it is of more concern because of its toxicological properties. Cadmium has a long half-life in the body and thus high intakes can lead to accumulation, resulting in damage to some organs, especially the kidney. The toxicological aspects of cadmium have been discussed earlier.

Fluorine

Reported unequivocal or specific signs of fluoride deficiency are almost nonexistent. A study with goats indicated that a fluoride deficiency decreases life expectancy and caused pathological histology in the kidney and endocrine organs. Most of the evidence accepted as showing a need for fluoride comes from studies in which it was orally administered in pharmacological doses. Pharmacological doses of fluoride have been shown to prevent tooth caries, improve fertility, hematopoiesis and growth in iron-deficient mice and rats, prevent phosphorus-induced nephro-calcosinosis, and perhaps prevent bone loss leading to osteoporosis.

Although fluoride is not generally considered an essential element in the classical sense for humans, it still is considered a beneficial element. Because of this, in the US–Canada, the AI has been set, on the basis of reducing dental caries without adverse effects, at 0.01 mg day⁻¹ for infants 0–6 months; 0.5 mg for 6–12 months; 0.7 mg for 1–3 years; 1 mg for 4–8 years; 2 mg for 9–13 years; 3 mg for 14–18 years; 3 mg for women and 4 mg for men. These intakes provide amounts of fluoride that will give protection against dental caries and generally not result in any consequential mottling of teeth; they should not be considered intakes that are needed to prevent a nutritional deficiency of fluoride. Chronic fluoride toxicity through excessive intake mainly through water supplies and industrial exposure has been reported in many parts of the world. Chronic toxicity resulting from the ingestion of water and food providing in excess of 2.0 mg day⁻¹ is manifested by dental fluorosis or mottled enamel ranging from barely discernible with intakes not much above 2.0 mg day⁻¹ to stained and pitted enamel with much higher amounts. Crippling skeletal fluorosis apparently occurs in people who ingest 10–25 mg day⁻¹ for 7–20 years. The UL (milligram per day) is 0.7 mg for 0–6 months, 0.9 mg for 7–12 months, 1.3 mg for 1–3 years, and 2.2 mg for 4–8 years, and 10 mg for all older age groups, including pregnant and lactating women.

Germanium

A low germanium intake has been found to alter bone and liver mineral composition and decrease tibial DNA in rats. Germanium also reverses changes in rats caused by silicon deprivation, and is touted as having anticancer properties because some organic complexes of germanium can inhibit tumor formation in animal models.

If humans have a requirement for germanium, based on animal deprivation studies, it is probably less than 0.5 mg day⁻¹. The toxicity of germanium depends on its form. Some organic forms of germanium are less toxic than inorganic forms. Inorganic germanium toxicity results in kidney damage. Some individuals consuming high amounts of organic germanium supplements contaminated with inorganic germanium have died from kidney failure. Although germanium has long been believed to have a low order of toxicity because of its diffusible state and rapid elimination from the body, until more knowledge is obtained about the intakes at which germanium becomes toxic, they probably should not greatly exceed those found in a typical diet. An intake of no

more than 5.0 mg day^{-1} would meet any possible need for germanium and most likely will be below the level found to have toxicological consequences.

Lead

A large number of findings have come from one source that suggests that a low dietary intake of lead is disadvantageous to pigs and rats. Apparent deficiency signs found include: depressed growth; anemia; elevated serum cholesterol, phospholipids, and bile acids; disturbed iron metabolism; decreased liver glucose, triacylglycerols, LDL-cholesterol and phospholipids; increased liver cholesterol; and altered blood and liver enzymes. A beneficial action of lead ($2 \text{ } \mu\text{g g}^{-1}$ vs. 30 ng g^{-1} diet) is that it alleviates iron deficiency signs in young rats.

If humans have a requirement for lead, which has not yet been demonstrated to be the case, it is probably less than $30 \text{ } \mu\text{g day}^{-1}$ based on animal deprivation studies. Although lead may have beneficial effects at low intakes, lead toxicity is of more concern than lead deficiency. Lead is considered one of the major environmental pollutants because of the past use of lead-based paints and the combustion of fuels containing lead additives. The toxicological aspects of lead are discussed elsewhere.

Lithium

Lithium deficiency reportedly results in depressed fertility, birthweight, and life span, and altered activity of liver and blood enzymes in goats. In rats, lithium deficiency apparently depresses fertility, birthweight, litter size, and weaning weight. Other *in vitro* biochemical actions suggesting that lithium could possibly act as an essential element include the stimulation of growth of some cultured cells, and having insulinomimetic action. Lithium is best known for its pharmacological properties; it is used to treat manic-depressive psychosis. Its ability to affect mental function perhaps explains the report that incidence of violent crimes is lower in areas with high-lithium drinking water.

If humans have a requirement for lithium, based on animal deprivation studies it is probably less than $25 \text{ } \mu\text{g day}^{-1}$, which is much less than the usual dietary intake (see Table 3). Lithium is not a particularly toxic element, but the principal disadvantage in the use of lithium for psychiatric disorders is the narrow safety margin between therapeutic and toxic doses. Approximately 500 mg lithium per day is needed to raise serum concentrations to be effective in these disorders; this is close to the concentration where mild toxicity signs of gastrointestinal disturbances, muscular weakness, tremor, drowsiness, and a dazed feeling begin to appear. Severe toxicity results in coma, muscle tremor, convulsions, and even death.

Molybdenum

The evidence for the essentiality of molybdenum is substantial and conclusive. Molybdenum functions as a cofactor in enzymes that catalyze the hydroxylation of various substrates.

Aldehyde oxidase oxidizes and detoxifies various pyrimidines, purines, pteridines, and related compounds. Xanthine oxidase/dehydrogenase catalyzes the transformation of hypoxanthine to xanthine, and xanthine to uric acid. Sulfite oxidase catalyzes the transformation of sulfite to sulfate. Attempts to produce molybdenum deficiency signs in rats, chickens, and humans have resulted in only limited success, and no success in healthy humans. Deficiency signs in animals are best obtained when the diet is supplemented with massive amounts of tungsten, an antagonist of molybdenum metabolism. Nonetheless, reported deficiency signs for goats and pigs are depressed food consumption and growth, impaired reproduction characterized by increased mortality in both mothers and offspring, and elevated copper concentrations in liver and brain. A molybdenum-responsive syndrome found in hatching chicks is characterized by a high incidence of late embryonic mortality, mandibular distortion, anophthalmia, and defects in leg bone and feather development. The incidence of this syndrome was particularly high in commercial flocks reared on diets containing high concentrations of copper, another molybdenum metabolism antagonist.

Examples of nutritional standards that have been set for molybdenum are the current US-Canada recommendations, which are the following: Adequate Intake for infants aged 0–0.5 years, $2 \text{ } \mu\text{g}$ and aged 0.5–1 years, $3 \text{ } \mu\text{g}$; RDA for children 1–3 years, $17 \text{ } \mu\text{g}$; 4–8 years, $22 \text{ } \mu\text{g}$; 9–13 years, $34 \text{ } \mu\text{g}$; 14–18 years, $43 \text{ } \mu\text{g}$; women from 19 to >70 years, $34 \text{ } \mu\text{g}$; and men aged 19–>70 years, $45 \text{ } \mu\text{g}$. The recommended intake is $50 \text{ } \mu\text{g day}^{-1}$ in pregnancy and lactation. These values were set using balance data in adults with extrapolation to the other groups. Usual dietary intakes are substantially higher than these recommendations. Large oral doses are necessary to overcome the homeostatic control of molybdenum; thus, it is a relatively nontoxic nutrient. The UL for children 1–3 years is $300 \text{ } \mu\text{g}$, for 4–8 years, $600 \text{ } \mu\text{g}$, and 9–13 years, $1100 \text{ } \mu\text{g}$. For adolescents the UL is $1700 \text{ } \mu\text{g}$, and for adults, $2000 \text{ } \mu\text{g}$, including pregnant and lactating women, based on doses that caused reproductive damage in animals.

Nickel

Based on recent studies with rats and goats, nickel deprivation depresses growth, reproductive performance, and plasma glucose, and alters the distribution of other elements in the body, including calcium, iron, and zinc. As with other ultratrace elements, the nature and severity of signs of nickel deprivation are affected by diet composition. For example, vitamin B₁₂ status affects signs of nickel deprivation in rats, and the effects suggest that vitamin B₁₂ must be present for optimal nickel function. The nickel function also may involve folic acid because an interaction between these two affected the vitamin B₁₂ and folic acid-dependent pathway of methionine synthesis from homocysteine. Nickel might function as a cofactor or structural component in specific metalloenzymes in higher organisms because such enzymes have been identified in bacteria, fungi, plants, and invertebrates. These nickel-containing enzymes include urease, hydrogenase, methylcoenzyme M reductase, and carbon monoxide dehydrogenase. Moreover, nickel can activate numerous enzymes *in vitro*.

Based on a lack of human studies, no recommended intake levels have been set for humans. Life-threatening toxicity of nickel through oral intake is unlikely. Because of excellent homeostatic regulation, nickel salts exert their toxic action mainly by gastrointestinal irritation and not by inherent toxicity. Based on extrapolation from animal studies, the UL has been set for the US and Canada at the following doses of soluble nickel salts: 1–3 years, 0.2 mg; 4–8 years, 0.3 mg; 9–13 years, 0.6 mg; and all adolescents and adults, 1 mg.

Rubidium

Rubidium deficiency in goats reportedly results in depressed food intake and life expectancy, and increased spontaneous abortions. If rubidium is required by humans, the requirement probably would be no more than a few hundred micrograms per day, based on animal data. Rubidium is a relatively nontoxic element and thus is not of toxicological concern from the nutritional point of view.

Silicon

Most of the signs of silicon deficiency in chickens and rats indicate aberrant metabolism of connective tissue and bone. For example, chicks fed a silicon-deficient diet exhibit structural abnormalities of the skull, depressed collagen content in bone, and long-bone abnormalities characterized by small, poorly formed joints and defective endochondral bone growth. Silicon deprivation can affect the response to other dietary manipulations. For example, rats fed a diet low in calcium and high in aluminum accumulated high amounts of aluminum in the brain; silicon supplements prevented the accumulation. Also, high dietary aluminum depressed brain zinc concentrations in thyroidectomized rats fed low dietary silicon; silicon supplements prevented the depression. This effect was not seen in nonthyroidectomized rats. Other biochemical actions suggest that silicon is an essential element. Silicon is consistently found in collagen, and in bone tissue culture it has been found to be needed for maximal bone prolylhydroxylase activity. Silicon deficiency decreases ornithine aminotransferase, an enzyme in the collagen formation pathway, in rats. Finally, silicon is essential for some lower forms of life in which silica serves a structural role and possibly affects gene expression.

Much of the silicon found in most diets probably occurs as aluminosilicates and silica from which silicon is not readily available. Owing to lack of evidence for a biological role for silicon in humans, no recommended intakes have been set. Silicon is essentially nontoxic when taken orally. Magnesium trisilicate, an over-the-counter antacid, has been used by humans for more than 40 years without obvious deleterious effects. Other silicates are food additives used as anticaking or antifoaming agents.

Tin

A dietary deficiency of tin has been reported to depress growth, response to sound, and feed efficiency, alter the

mineral composition of several organs, and cause hair loss in rats. Additionally, tin has been shown to influence heme oxygenase activity and has been associated with thymus immune and homeostatic functions.

Owing to lack of data no recommended intakes have been set for tin. Inorganic tin is relatively nontoxic. However, the routine consumption of foods packed in unlacquered tin-plated cans may result in excessive exposure to tin, which could adversely affect the metabolism of other essential trace elements including zinc and copper. Because 50 mg day⁻¹ of tin was found to affect zinc and copper metabolism, routine intakes near this amount probably should be avoided.

Vanadium

Vanadium-deprived goats were found to exhibit an increased abortion rate and depressed milk production. Approximately 40% of kids from vanadium-deprived goats died between days 7 and 91 of life with some deaths preceded by convulsions; only 8% of kids from vanadium-supplemented goats died during the same time. Also, skeletal deformations were seen in the forelegs, and forefoot tarsal joints were thickened. In rats, vanadium deprivation increases thyroid weight and decreases growth. Other biochemical actions support the suggestion that vanadium could possibly act in an essential role. *In vitro* studies with cells and pharmacological studies with animals have shown that vanadium has insulin-mimetic properties; numerous stimulatory effects on cell proliferation and differentiation; effects on cell phosphorylation–dephosphorylation; effects on glucose and ion transport across the plasma membrane; and effects on oxidation–reduction processes. Some algae, lichens, fungi, and bacteria contain enzymes that require vanadium for activity. The enzymes include nitrogenase in bacteria, and bromoperoxidase, iodoperoxidase, and chloroperoxidase in algae, lichens, and fungi, respectively. The haloperoxidases catalyze the oxidation of halide ions by hydrogen peroxide, thus facilitating the formation of a carbon–halogen bond. The best known haloperoxidase in animals is thyroid peroxidase. Vanadium deprivation in rats affects the response of thyroid peroxidase to changing dietary iodine concentrations. Given that a functional role for vanadium has not been determined in humans, no recommended intakes have been set.

Vanadium can be a relatively toxic element. Green tongue, cramps, and diarrhea, and neurological effects have occurred in humans ingesting vanadium salts. Based on renal damage in animals, the UL for adults is 1.8 mg vanadium salts per day, with insufficient data to set a UL for other age groups.

Dietary Sources

The requirements for the ultratrace elements will be met if a person consumes a diet based on the dietary guidelines recommended. For some areas of the world, especially in developing countries where traditional, monotonous diets are based primarily on a cereal (particularly rice) or tuber staple, the intake of several ultratrace elements (e.g., boron and molybdenum) could possibly be low. Reported typical dietary

intakes (mostly for industrialized countries) and rich sources of the ultratrace elements are shown in **Table 3**.

See also: Aluminum. Chromium. Cofactors: Organic. Dental Disease: Etiology and Epidemiology. Food Safety: Heavy Metals. Iodine: Physiology, Dietary Sources, and Requirements. Selenium. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements

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URBAN NUTRITION

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Introduction

For most readers of this encyclopedia, the notion of urban living and lifestyle would be considered as the demographic norm or a 'ground state.' In fact, the history of human biological and cultural evolution was documented in rural areas. However, as described below, the world's population is galloping toward the supremacy of urbanization, and with this demographic revolution comes a series of implications for diet and nutrition. The context for the topic of urban nutrition includes the manner in which access to food and purchasing power is distributed among the urban populations, the agricultural efforts within and without the cities to supply food to the urban masses, trends and fashions in marketing and consumption of foods, and the ecological and environmental influences generated in the agrarian countryside and in the urbs by themselves.

Urbanization

It took from the origin of man until the middle of the nineteenth century for the population of the Earth to reach a billion inhabitants. The population has increased to seven-fold in the present. During 90% of the duration of *Homo sapiens'* evolution, human endeavor was related to obtaining food, reproducing and rearing young, and defending themselves from predators and enemies being the other components of survival. In the past, humans were part of nomadic tribal groups roving over the hunting and foraging ranges that provided them their food, the pelts or fiber for their clothing, and other necessities. Approximately 40 000 years ago, mankind witnessed the advent of the pastoralist lifestyle, with tending of grazing and milk-producing livestock across available pasture areas constituting an alternative form of livelihood.

It was only 10 000 years ago that humans first domesticated plants, with the emergence of the peasant, agrarian lifestyle. Land tenancy with the occupation of specific terrains became a human attribute. Moreover, for the first time in human history, a rural family's effort could produce enough food to feed more than just a family unit. Agriculture was the precondition for human 'civilization,' as it freed up some members of the population for nonfood-gathering activities, such as artisans, traders, clergy, politicians, and warriors, who could live in the society, supported and fed by the livestock, crops, and produce supplied by the farmers. These new classes

did not live in dispersed terrains across the countryside but rather created communities that were larger and more complex than a homestead or tribal village. The first towns represent either the seats of rulers or the temple complexes of religious elites. As trade expanded, towns and cities developed along the routes of commerce. These were often situated along waterways and at coastal harbors, or scattered along caravan routes. With the rise of warrior classes, protective walls and fortifications began to surround such communities. The Mediterranean and Asia Minor were the sites of the cities of antiquity, such as Babylon, Athens and Sparta, Alexandria, Rome, and Carthage.

The evolution of mercantile trade in Europe became a motive for constructing even larger cities, as exemplified by the city-states of Renaissance Italy. Finally, the Industrial Age gave a whole new charge and dimension to cities. The change of production from artisan guilds to factory manufacture altered the character of urban life. Crowded teeming cities with tenements, as described by Dickens, arose in Europe at the beginning of the nineteenth century. These early industrial metropolises were smoky, grimy, smelly, unsanitary places, and ripe for the occurrence of occupational mishaps and the transmission of contagious diseases. Part of the obnoxious odors and some of the recurrent diseases of the urban domain were due to the decomposition of the food supply. Until the era of preservation by refrigeration and or advancing food technology, foodstuffs were perishable and the urban food supply was precarious. Fresh fruits and vegetables were available largely to those still living in the countryside.

The United Nations defines an 'urban center' as a concentrated population of at least 20 000 inhabitants. By this definition, only 5% of the world's population was urbanized at the turn of the nineteenth century, 13% at the turn of the twentieth century, and 47% by the year 2000. In 1950, the urban population of developing countries was 17%; by the year 2000, it had reached 44%. In 1950, the world's population was estimated to be 2.4 billion and 83% lived in rural areas. The global population has risen to 7.0 billion in 2012. The degree of urbanization varies from region to region. In the USA, 75% of the population is now urban, whereas in the African continent, urbanization hovers at approximately 40%.

It is projected that most of the world's population would be living in urban areas by 2015; and a decade later, two-thirds of the inhabitants of developing countries will be urban population. The term megacities refer to metropolitan populations of more than 8 million inhabitants; 80% of them are in developing countries. Mumbai, Lagos, New Delhi, Karachi,

Manila, and Jakarta were megacities on trajectories to have doubled their metropolitan populations between 2000 and 2015. Five megacities are in Asia and one in Africa.

Contemporary Food Supplies and Diets for the Cities

Barriers to a safe food supply for the urban populace have fallen with the advent for the modern city of refrigerated transport and storage, and sewage treatment and disposal. For developing countries, westernization of the dietary patterns of cities is an interesting consequence and a reinforcing cycle of supply and demand. More perishable foods can reach urban tables, not only from the interior of countries but also from overseas. Currently, issues of food supplies are generally more an issue of accessibility at the household level than availability in the urban marketplace. Moreover, access to refrigeration has been associated with a major decrease in the use of traditional conservation methods, such as salting, pickling, and smoke curing of food for preservation, as well as improved microbiological quality. In this respect, urbanization may be a factor in reducing the incidence of carcinogenesis associated with mycotoxins, salt, and organic by-products, as well as the incidence of episodes of some food-borne diseases.

Accessibility (the ability to obtain items in the market) is more important in determining what gets onto the tables of urban households. Inequality of resources and wealth within the urban populations gives rise to food insecurity within households in the urban area. Often a pattern of geographical distribution demarcates the zones with the highest risk of insecure and inadequate household food supplies. Generally, the greater diversity of items that one is able to include in one's diet, the lower is one's risk of experiencing food insecurity.

Urbanization and Nutritional Transition

Whatever the era in the evolution of cities, from antiquity to the present, it is likely that the cuisine and dietary fare of urban populations has always differed from that in tribal, nomadic, or agrarian settings of the same countries. On the one hand, if the town were a coastal port or fishing village, consumption of fish and seafood would be higher than in the interior countryside, whereas populations of trading centers would have more access to exotic, imported items, such as Oriental spices and teas or Caribbean rums and molasses. On the other hand, as most food is produced in the countryside, the agrarian producers, themselves, have first access to the crops and foodstuffs produced, whereas the cities can only gain that which is transported and offered there for sale. Generally, then, the basic staples in the cities are those that are traditional to the farmers that serve them.

Despite a history of poorly mechanized industry in urban factories that required heavy physical labor, an even greater daily energy output was required for rural agricultural pursuits. Hence, the amount of dietary energy needed for child growth or to maintain energy balance is generally lower for urban populations. Until recently, the variety of foods and their diversity across food groups was more limited in urban

markets than on farms and plantations. The meat in urban centers was more likely to be dried and salted than fresh, and one or two staples, grains, or tubers would provide the bulk of dietary energy. One might generalize that until the advent of certain recent developments, the quality of the rural peasant diet was superior to that of the urban masses.

In recent years, electricity, food technology, domestic and international food trade, and concentration of wealth in urban areas have changed this panorama in recent years by a process called 'nutrition transition,' a term introduced by Barry Popkin in the 1990s, which has come to dominate particularly the urban populations of today's developing nations. More, rather than less, variety of food is the reality of modern cities. Sweetened and flavored processed foods have higher appeal than coarse staple roots and cereals. The twentieth century saw a meteoric rise in the demand for and production of cooking oils and fat-based spreads, while refrigeration meant that fluid milk did not have to come directly from the udder of a dairy animal to one's doorstep to be safe and available. Processed foods – bottled, canned, and frozen – entered the market with the advent of the food sciences and food technology, and sweets and desserts became a larger component of daily fare. Inexpensive vegetable oils from corn, soy, safflower, cottonseed, and more recently from the palm fruit and coconut, entered the international commerce for frying, or were hydrogenated and solidified into margarines and shortening for spreading and baking.

Most recently, a finer differentiation among commercially manufactured foods has emerged. Attributed to Carlos Monteiro and his colleagues in Brazil, a category of ultra-processed foods has been defined. Ready-to-heat or ready-to-eat items are considered as ultraprocessed foods. According to the authors, the processes include 'salting, sugaring, baking, frying, deep frying, curing, smoking, pickling, canning, frequent use of preservatives and cosmetic additives, addition of synthetic vitamins and of minerals, and sophisticated types of packaging.' This is contrasted to lightly processed foods and unprocessed (natural) foods. These dietary changes are not restricted to the affluent elite of developed countries, but are part of the change affecting the urban middle and lower classes in developing countries to an ever-increasing extent. However, at the same time, those living in abject poverty due to both urban unemployment and rural landlessness were unable to participate in any of this varied and energy-dense fare. This is the setting for 'nutrition transition' defined by Popkin as 'the rapid shift in the structure of diet in low-income countries and the coexisting problems of under- and overnutrition.' With respect to the overnutrition aspect, developing country residents are participating in the dietary patterns associated with increased risk of chronic diseases.

Urban Agriculture

The aforementioned demographic changes will produce a steep change in the ratio of food consumers and food producers. Urban agriculture, using marginal lands within the confines of the metropolitan area, has implications for this shift. Urban agriculture can save up to 20% on outlays of cash for food for poor families at the expense of 1–2 days of labor

per week. Urban gardening is seen as a means to improve public health not only through improving economic and food security, but also in providing exercise, psychological and community well-being, and environmental stewardship. The growing of plants on roofs has positive implications for air quality and water conservation in the city. The types of plants that are grown in urban settings are largely foods that contribute micronutrients, and are much less likely to be the staples that provide the bulk of energy and protein. For reasons of sanitation and proliferation of zoonotic diseases, as well as waste disposal, domestic livestock in the cities are a much more remote option, although aquaculture with treated waste waters could provide fish, crustaceans, and molluscs toward meeting the protein needs of urban populations. It has been suggested that a more holistic and ultimately more ecologically friendly system for food security can derive from a rational promotion of food production within and around the urb.

Street-Vended Ready-to-Eat Foods

Roads and highways may be a phenomenon of rural communication but the street is an urban entity. Street foods are defined by the Food and Agricultural Organization as 'ready-to-eat foods and beverages prepared and/or sold by vendors, especially in streets and other similar public places.' Opportunity, and at times the necessity, to consume one or more daily meals away from one's home has evolved with urban life. For the affluent and middle-class population, cafeterias and restaurants serve the clientele looking for a meal outside of the household. For low-income populations, street foods fill this dietary niche. One-quarter or more of daily calories are often consumed outside of poor urban homes by adult workers and high-school children.

There are two main concerns about street-vended foods: their energy density and their microbiological safety. For the schoolchild market, ready-to-eat foods tend to be sweetened and often fried. A number of pathogenic organisms have been routinely isolated from street food samples, but if the comparative standards are foods from the homes of the same consumers, street-vended foods are generally no more offensive. Municipal authorities may be skeptical of this source because it comes from the informal (unregulated and untaxed) economy.

Nutrition and Health in the Urban World

The way in which people eat is a major determinant of their nutritional stores and status, but issues of lifestyle, health, and pollution modify and influence the nutriture of individuals and populations. Each of these factors contributes to the distribution of the simultaneous under- and overnutrition states that characterize nutrition transition. The diseases conditioned by undernutrition, such as infections, and from overnutrition, such as metabolic syndrome and cancer, are also concurrently impelled by the bipolar nutritional factors, leading to the so-called double-burden of disease.

Labor market shocks impact on maternal pursuits, child work, and schooling. Rural children have traditionally worked in both household and farm chores as a part of an integrated family-production pattern. In urban areas, child labor in factories has adverse occupational risks. When urban mothers seek income-generating activities outside of the home, monetary resources may be bolstered but child rearing and child caring and meal provision can be disrupted. Breast-feeding is one form of meal provision for the infant, which can be influenced adversely by maternal work obligations. In general, less exclusive breast-feeding and shorter total lactation are seen in urban mothers as compared with their counterparts in the countryside.

Urban poverty and undernutrition are growing partly because of the influx of uneducated migrants from the countryside. Spatial distribution of the pockets of poverty can often be identified on a map of the city. The unempowered and abjectly poor tend to congregate in the least desirable and most precarious areas of the urban landscape. It has been established that such groups have scarce municipal services and low incomes. The relative social power of women in such households is vastly inferior to that of the men. True food insecurity is a legitimate concern in these zones of urban poverty, as shown in recent studies in West Africa.

Finally, environmental pollution, some of which has its negative effect on the population by contaminating the food supply, cannot be ignored. In Jakarta, where leaded gasoline is still the norm, individuals living on the streets get affected. Poor air quality (smog) can provoke respiratory distress with a cascade effect on appetite, regular eating, and nutrient retention.

Nutritional Deficiencies of Urban Populations

At a physiological level, nutritional status is a function of the intake, absorption, and retention of nutrients. Any of the micronutrient deficiencies common to human populations can be seen in residents of towns and cities. Household food insecurity or poor caring practices can explain low intakes of total macronutrients or diets with poor micronutrient density and bioavailability. Sanitation may be better in the urban setting and disease-related wastage of nutrients may contribute less to the process of general undernutrition. Rates of stunting and wasting are expected to be less common in urban areas compared with rural areas, and survey statistics bare that out. However, exclusive breast-feeding, a major protection against early malnutrition, may be more difficult to maintain in urban areas due to cultural norms and increased maternal work outside the home.

Iron deficiency is prevalent in infants, young children, fertile women, and pregnant and lactating women, independent of social class or setting. If lead exposure is present in the cities, this can aggravate iron content status, especially in the young population. In the broadest generality, micronutrient deficiencies are less frequent among urban populations, but a residual prevalence of poor status with respect to riboflavin, zinc, folic acid, and vitamin B₁₂ is common in low-income segments of urban populations. Intervention programs are logistically more accessible to urban populations,

but relief agencies often direct and target their assistance to the countryside, bypassing the problem that may exist right outside their headquarters buildings in Third World capitals. An important caveat is that related to the demographic pattern of human immunodeficiency virus (HIV)/acquired immune deficiency syndrome (AIDS) in a given nation. Transmission can be more either in urban or rural areas, depending on the specific society in question. Wherever HIV/AIDS is more prevalent, the companion risk of AIDS-related micronutrient deficiency will prevail as well, along with the household stigmatization and food insecurity concomitant with the diagnosis.

Social determinants related to poverty in urban communities and households constitute the underlying reasons for any impaired growth and poorer anthropometric indices. Discrepancies in women's status, that is, their social and economic power relative to men in the same households and societies, has a profound influence on the status of their children and the risk of the latter to suffer stunting and wasting. Greater equality between the sexes generally results in overall improved prenatal, obstetrical, and child care; better complementary feeding and treatment of illness; and higher immunization rates. Urban settings would generally tend to foster less traditional social views and provide women with more options for self-realization. Moreover, campaigns and programs for empowering women through education and entry into the work force would be logistically easier to maintain in towns and cities. Hence, the better general nutritional status of children in cities may be a consequence of trends toward a greater equality of women and improved decision-making power in favor of their children's nutritional evolution.

Survival through and beyond infancy and preschool years with generally more favorable nutritional status than one's rural counterparts is likely to be reflected in continued better nutriture into childhood and adolescence. A phenomenon of family disintegration, more commonly seen in urban communities, has led to the proliferation of street children who live in the street. Several aspects of the stereotypical portrait of their lifestyle, including abuse of glue and illicit drugs and participation in child prostitution, would point to increased risks of organic damage and sexually transmitted disease, including HIV/AIDS. However, studies indicate that street children have more disposable income and a more diverse diet than other urban children from equally humble origins but dependent on meager household means.

The urban elderly are another generational group of concern in terms of their risk to suffer nutritional deficiencies. Their age, *per se*, makes them more susceptible to undernutrition and social isolation, with the weakening of extended family traditions. It afflicts more urban than rural elders. Their situation in the urban context can be more nutritionally precarious.

Nutritional Excess in Urban Populations

The most important nutritional excesses to consider are those of overweight. This is officially defined as a body mass index (BMI) higher than 25 kg m^{-2} . For children, overweight begins at the 85th percentile of normative curves and obesity at the

95th percentile, as defined on the 2000 BMI charts of the US Center for Disease Control and Prevention. Worldwide, overweight and obesity are reaching epidemic proportions, and for several reasons, the urban milieu predisposes to their development.

Changes in the pattern of meals and diet, including skipping breakfast, changing to away-from-home snacking for formal family sit-down meals, and increasingly energy-dense fare, are also considered contributors to urban excess weight on the input side of the equation. In terms of energy expenditure, physical activity patterns are altered in the traditional rural focus role of food production to that of urban areas. Much of the increase in the number of overweight individuals in cities is ascribed to sedentariness, with motorization of transport and mechanization of labor and household chores. Secure areas for outdoor activities are diminishing in low-income urban areas. For both adults and children, sports and active recreational pursuits are being replaced by television watching and computer entertainment, including Internet, in urban settings. A new and troubling association of stunted child-overweight mother pairs has been identified, but it is not restricted to urban families. However, low stature seems to be a risk factor for overnutrition as confirmed in China, Singapore, Brazil, and Mexico, especially in their urban areas.

Diet, Nutrition, and Quality of Life

It is probably safe to conclude that suffering nutritional deficiency and dying a premature death from infectious illness is more likely to occur among the rural agrarian peasantry, nomadic pastoralist, or tribal groups than among the urban masses. But for rural individuals who survive accidental and infectious deaths, their plant-based diets and rigorous lifelong physical activity patterns makes the goal of 'dying healthy in old age' a stronger possibility. With extended life expectancy among urban populations, ensuring the quality of life in later life is important as both a humanistic and economic consideration. Cardiovascular and malignant diseases produce lingering debility and dependency, robbing individuals of well-being and placing a burden of health care on relatives and governmental resources. Global and sustainable interventions to reduce sedentarism and to improve dietary practices in the growing segment of the world's population living in cities is an imperative for social and economic stability going forward.

Conclusions

As the world's population grows through the billions, and shifts to an even more expansive urban population, urban nutrition emerges as an ever more important concern of nutritional epidemiology and public health nutrition. The essential complexity of providing for human needs in densely populated settings has repercussions for the resulting nutriture of the urban populations and for the nature of their environments, with the more global consequence of a soaring food-consumer to food-provider ratio. Although there are

Table 1 Opportunities in urban nutrition research

Basic description of diet and nutrition in selected townships and metropoli
Rural–urban comparisons
Role of family and household relationships
Influence of rural-to-urban migration on nutritional status and dietary status
Universality and generalizability of dietary factors for degenerative diseases
Contributions of urban agriculture to the urban food supply
Influence of urban pollution on the food supply and nutritional status
Nutritional and health status of street children

agricultural and logistical avenues to make urban diets ever more varied and diverse, there are economic pressures to make them more processed and energy dense. The latter consequences are conducive to greater risk of noncommunicable, chronic diseases. However, in really poor societies in which the urban slums dominate the metropolitan landscape, energy and nutrient deficiencies can still be prominent epidemiological features. Rather than using the 'lessons' from the rural experience in developing countries, it is important to direct one's reading, one's research, or both to the study of the urban milieu in order to gain insights for addressing the challenges of urban nutrition. **Table 1** outlines a framework of opportunities for urban nutrition research that is derived from the considerations in this review.

See also: Breast Feeding. Iron: Physiology, Dietary Sources, and Requirements. Obesity: Definition, Etiology, and Assessment

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VEGETARIAN DIETS

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Introduction

Vegetarian eating patterns are the norms in many parts of the world, whereas in Western countries they are an exception. Currently, approximately 2.5% of adults in the US and 4% of adults in Canada follow some sort of self-described vegetarian diet. Approximately a fifth of American adults report that they do without animal protein at more than four meals a week. This article examines the healthfulness of vegetarian eating patterns and practices, and their benefits and risks in Western countries. Vegetarian patterns in other countries, particularly developing countries, are quite different and not reviewed here.

Table 1 provides a list of commonly used definitions. These terms are important since 'vegetarian' covers a multitude of disparate characteristics that are interpreted in various ways. More precise definitions make it easier to assess the nutritional and health implications of various dietary patterns.

History

Vegetarian diets go back many millennia. Until 50 years ago, in Western countries the common vegetarian eating patterns involved avoidance of animal flesh (meat and poultry) at a minimum, and most adherents' motivations were religious or moral. Categorization of vegetarian patterns was relatively straightforward and consisted simply of differentiating between those who ate no animal foods at all (vegan vegetarians), those who also consumed milk and milk products (lacto vegetarians), and those who ate eggs as well (lacto-ovo vegetarians). This simple categorization scheme broke down in the 1960s and 1970s when new patterns of vegetarian eating emerged as a result of Westerners' greater exposure to the cuisines of other cultures, the influence of new variants of Eastern religions and philosophical systems with a vegetarian tradition, the popularity of economic, environmental, and sustainability arguments for eating fewer animal foods, and evidence that vegetarian eating was associated with decreased risks of certain chronic diseases. Those who follow vegetarian patterns today have rationales that involve food preferences, environmental, economic,

sustainability, and health concerns rather than solely religious or ethical reasons for doing so. Partial or semi-vegetarians often initially adopt a vegetarian diet for health reasons and in some instances their commitments then broaden or are augmented by other reasons for sustaining or further restricting their diets.

Many authoritative bodies now suggest that 'plant-based' diets confer a health advantage. There is increasing evidence from large observational studies that plant-based eating patterns rich in vegetables, beans, nuts, whole grains, fish, and plant oils have cardiovascular benefits. The mass media provides much favorable publicity about phytochemicals in plant foods that supposedly have beneficial health effects. At the same time, fears and concerns about the healthfulness of animal foods have been triggered by publicity on the bovine spongiform encephalopathy (BSE) epidemic in the UK and Europe, epidemics of hoof and mouth disease in cattle, recent SARS, and swine influenza epidemics that originated in animals and spread to people, and massive outbreaks of animal food borne illness, such as Salmonellosis caused by contamination of shell eggs. Worries are also rife among aging populations that 'Western' diets rich in red meat and other animal foods also contain saturated fat and cholesterol that increase risks of heart disease, and that other components of animal foods cause cancers, diabetes, and other diseases. Such health concerns have probably contributed to the increased prevalence of vegetarian eating.

As a result of these influences, not only plant-based, but also meatless and vegetarian eating patterns have grown in popularity among Westerners who are much better informed and concerned about nutrition than they were years ago. Although 'plant-based' diets do not conform strictly to the definition of a vegetarian diet, they are clearly different from usual intake patterns in that the type and amount of animal foods is substantially decreased, and many who eat them now consider themselves to be 'vegetarians'.

Vegetarian Patterns and Practices

'Vegetarian diet' does not fully describe the variety in nutrient intakes and health status of followers of such eating patterns in

Table 1 Common types of vegetarian dietary patterns categorized by animal food use

Pattern	Animal Food use and other Characteristics
Omnivorous	All culturally acceptable and economically available animal foods are consumed with no specific prohibitions on any food group.
Plant-based diet, DASH diet, 'flexitarian' diet	Variously defined. The Dietary Approaches to Stop Hypertension (DASH) diet recommended in the Dietary Guidelines for Americans 2010 is high in low-fat-milk (2–3 servings per day), low in lean meat, poultry, fish (6 oz total per day), eggs, and very limited in red meat. It is high in fruits, vegetables, whole grains, nuts, and legumes, and limited in sugar-sweetened foods and beverages, sodium, added fats, and saturated/trans fats. Flexitarian diet refers to a plant-based diet that includes meat, dairy, eggs, poultry, and fish on occasion or only in small quantities. Other food groups are rarely avoided, but specific foods (e.g., those high in 'added sugars', sodium, trans fat, saturated fat, 'processed', nonorganic, etc. may be avoided).
Meat avoiders, meatless, or semi-vegetarian	Limit or avoid red meat and other flesh foods; may also restrict poultry, fish, and seafood. Diets are similar in most respects to nonvegetarian diets.
Lacto-ovo vegetarian	Avoid all meat, poultry, and often fish, but milk products (especially low fat products) and eggs are consumed. Iron may be limiting; it can be obtained from iron-fortified cereals or iron supplements.
Lacto vegetarian	Avoid all meat, fish, poultry, and eggs. Nutrient considerations same as lacto-ovo vegetarians.
Macrobiotic	Numerous restrictions including avoidance of all meat, poultry, milk, and eggs; fish is consumed in small amounts. Sugar, other refined sweeteners, members of the nightshade family (peppers, egg plant, tomatoes, and potatoes), and tropical fruits are avoided. Current versions of the diet are less restrictive than those of 40 years ago, but deficiencies of energy, iron, calcium, vitamin B ₁₂ , vitamin D, and other nutrients may still arise in weanlings, pregnant women, young children, and adolescents during the growth spurt if diets are nutritionally unplanned and dietary supplements and fortified foods are avoided.
Vegan	Avoidances include all animal products including meat, fish, poultry, eggs, and dairy products. Some may also refuse to use animal products (leather, fur, lipstick) in daily life. Without careful planning, energy, vitamins B ₁₂ and D, choline, essential fatty acids, and bioavailable sources of iron may be low. Concentrated sources of energy-dense foods, such as sugars and fats are helpful for increasing energy intakes. Vitamins B ₁₂ , D and calcium are available in fortified soymilk, fortified cereals, and dietary supplements of these nutrients. Usually protein is adequate if a variety of protein sources is consumed.
Vegetarian	Range of dietary patterns, all of which include avoidance of some or all animal foods to varying degrees, with or without additional restrictions, additions, or prohibitions.
Other patterns	Raw food eaters and 'living food' eaters avoid animal foods and eat raw plant foods (fruits, vegetables, nuts, legumes, and cereals) as well as special health foods, such as wheatgrass or carrot juice. <i>Fruitarians</i> consume diets mostly of fruits, nuts, honey, and olive oil. <i>Rastafarians</i> eat a near-vegan diet and avoid alcohol, salt-preserved foods, and additives. <i>Yogic groups</i> (Transcendental Meditation, Hare Krishna, Bab Ram Das, etc.) vary in their eating patterns but are often lacto vegetarian.

Western countries. Today, myriad vegetarian eating patterns exist that cannot be easily described by focusing on a single dimension, such as animal food intake (see Table 1). The impact of these patterns on nutritional status and health requires more complete characterization of diet and other aspects of lifestyle than a simple description of, which foods are left over after others have been omitted from the diet. It is also important to characterize what is eaten in greater detail to obtain an accurate nutrient profile of the adequacy of the eater's diet. Traditional vegetarian diets were poor sources of energy, protein of high biological value, vitamins D and B₁₂, zinc, bioavailable iron, omega-3 fatty acids, choline, and sometimes iodine. However, today a wide variety of plant foods are now available year round in most Western countries, along with fortified foods and dietary supplements that can fill these gaps.

Differences Between Vegetarian Eating and Vegetarianism

Vegetarian eating is often confused with vegetarianism. Most vegetarians in the world today have eating styles that are dictated chiefly by culture, habit, health, economic and environmental concerns, food preferences, cost, and taste rather

than moral philosophy, religion, and ethics. A small minority of vegetarians also subscribe to vegetarianism, a larger belief system that is reflected in their eating pattern but that also encompasses many other aspects of lifestyle and thought. Vegetarianism involves philosophy, values, beliefs, or religious convictions rather than simply food choices. Its adherents often hold strong, deeply held convictions about the moral, metaphysical, ethical, religious, or political appropriateness of vegetarian eating. Often those, such as vegans with the most restrictive avoidances of animal and other foods have the most deeply held views. Advocates of vegetarianism have become more militant in recent years in Western countries. Some, such as Seventh Day Adventists and some yogic groups, simply hope to enlist additional followers to what they believe is the optimal diet from the ethical, health, and environmental standpoint. Others are more vocal, strident, and occasionally violent advocates who proselytize broader agendas, including animal rights, reform of agricultural and animal husbandry practices, and nonuse of experimental animals in addition to health and environmental concerns. In the US these latter groups include People for the Ethical Treatment of Animals (PETA), the PETA foundation, the Animal Liberation Front, and the Physicians' Committee for Responsible Medicine (PCRM). They finance advertising, journal articles, and

educational efforts to encourage adoption of their views. Extensive efforts are directed toward youth. Similar groups are also active in other countries.

Nutritional Features of Well-Planned Vegetarian Diets

The nutritional benefits of vegetarian diets on health are several. Plant foods are generally nutrient dense for the calories they provide. There are good sources of vitamins B, E, C, potassium, magnesium, copper, manganese, dietary fiber, omega 6 polyunsaturated and monounsaturated fat, and complex carbohydrates. They are also rich in plant sterols and a number of other phytochemicals, such as the flavonoids, lignans, glucosinolates, and isothiocyanates, and possibly also in compounds, such as curcumin in the spices of some vegetarians' use that may have beneficial health effects. Some are high in protein, such as legumes. Although some of the proteins in single plant foods, such as cassava are 'incomplete' in their amino acid profiles in that they alone do not meet human amino acid needs, different plant protein sources or small amounts of animal protein can complement each other supplying missing amino acids and permitting these needs to be met. Since most vegetarian diets in Western countries contain a number of different plant protein sources, adequacy or quality of protein, even on vegan diets are rarely a problem. At the same time, vegetarian diets are generally low in saturated and trans-fats and in cholesterol, which are also positive attributes from the health standpoint. Potentially they may fall short in vitamins D, B₁₂, zinc, bioavailable iron, omega 3 fatty acids, choline, and iodine. However, today it is easy for vegans and vegetarians to obtain nutrients that might otherwise be low or lacking in their diets. A wide variety of plant foods is at their disposal. There is also widespread availability of a variety of soy and other meat alternatives and analogs for animal products, and of fortified plant foods, including soy milks (with added vitamins B₁₂ and D, as well as highly bioavailable calcium), calcium-fortified orange juice, and highly fortified breakfast cereals. Dietary supplements offer other easily available alternatives to remedying nutrient shortfalls.

Most of the differences between the health of vegetarians and nonvegetarians are due to nutritional characteristics of what they eat and avoid. Other rationales attributing healthful aspects to vegetarian diets go beyond the nutrients, that the diets contain or lack but there is less evidence to support them. For example, vegetarian diets are often claimed to be evolutionarily more in line with dietary patterns in Paleolithic times than are present 'Western' diets and therefore better suited to our genes. However, very little morphologic, bone, or artifactual evidence is available on the actual diets of early humans. Most early hominids died young of infectious or other diseases rather than living into middle and old age when the chronic degenerative diseases so common today occur. What is clear is that the advent of farming and agriculture led to rapid population growth, and at least initially to increase in morbidity and mortality because of crowding, changes in patterns of disease transmission, and many other factors including diet.

Another rationale for vegetarian eating is that animal foods, particularly meat, increase risks of many infectious and chronic diseases. This will be discussed further in the next

section. A third contention is that it is the synergy of foods that are plentiful in vegetarian diets and particularly fruits and vegetables working together as a 'package' (e.g., abundant fruits, vegetables, legumes, nuts, and other plant foods) that confers a special and added health advantage. Generally, Western vegetarian diets eaten today tend to be low in saturated fat and cholesterol and high in polyunsaturated fats, complex carbohydrates, dietary fiber, a variety of plant proteins, which complement each other well and provide good biological value, some animal protein as well as magnesium, potassium, folic acid, and antioxidant nutrients, such as vitamin E and selenium. They also tend to be relatively low in food energy. Vegetarian diets are also relatively high in non-nutrient phytochemical bioactives (phytosterols, flavonoids, and isothiocyanates) that may provide health benefits. There are a few clinical studies that do show that in comparison to a low-fat fast food nonvegetarian diet matched on type of fat, macronutrients and cholesterol, low-density lipoprotein (LDL) cholesterol levels were lower in the vegetarian diet, suggesting that other components in the vegetarian diet might also have positive effects on this intermediary marker of coronary heart disease. However, the hypothesis that 'food synergy' (e.g., the orchestrated effects of plant foods and constituents working together in the vegetarian pattern) provides very large additional benefits over the health effects of each constituent alone or with other foods requires verification.

Morbidity and Mortality of Vegetarian Versus Omnivorous Diets

Well-planned vegetarian diets have nutritional profiles that are in line with recent expert recommendations, which, if sustained, may reduce risks of some chronic degenerative diseases and obesity. Thus, the diet-related risks of a number of chronic degenerative diseases (coronary artery disease, hypertension, type 2 diabetes) associated with caloric excess and intakes of certain of these nutrients may be decreased on vegetarian diets. The differences between vegetarians and omnivores in other lifestyle characteristics that affect disease risk also play a role in patterns of illness and disease between them. For example, many vegetarians are health conscious, eschew tobacco, alcohol, and recreational drugs, avoid overexposure to the sun, and lead physically active lives, and so risk factors for diseases that are influenced by those factors are decreased. Other differences that may be residual confounders are lesser exposure to second-hand smoke, less consumption of sugars, whole grains, nuts, and legumes that are protective, and great amounts of food energy and diets high in refined sugars. If any of these factors influence disease it could be them, rather than the use or nonuse of animal foods, which is responsible for the positive health effects.

In the past few decades, many observational epidemiological studies have been done to determine if vegetarians are more healthy or live longer than nonvegetarians.

With respect to morbidity, food-borne illness easily occurs with mishandling of animal foods, and historically many types of food-borne illness (Salmonellosis, Campylobacter, Brucellosis, parasites) were a major problem in animal foods

and could be avoided by not eating them. Now that animal food handling and preparation practices are more advanced, the dangers of these diseases have decreased, although continued vigilance is necessary.

Some conditions and disease risks are clearly lower; for example, constipation is less of a problem in vegetarians (especially vegans) than in omnivores, perhaps due to their higher intakes of dietary fiber and more active lifestyles. Vegetarians generally tend to have lower body mass indices (BMIs) than do nonvegetarians, and consequently have lower risks of chronic diseases for which obesity is a risk factor, such as type 2 diabetes. There are now several studies showing effects of various animal foods, and particularly meat, on type 2 diabetes, but although some studies implicate all meats, others only do so for processed meats. Whether meat is still associated, when the comparison group is healthy and weight conscious nonvegetarians remains to be determined.

Animal foods and especially red and other meats (saturated fats and cholesterol), poultry (unless lean and skinned), eggs, crustaceans like shrimp and lobster (rich in cholesterol), and whole fat dairy products (high in saturated and total fat) have been indicted as increasing risks of coronary artery disease. Their ill effects appear to stem primarily from their content of saturated fat, cholesterol, and high amounts of food energy that they contribute to the diets of eaters. Also meat eaters tend to have low intakes of nuts, fruits and vegetables, plant sterols, dietary fiber, and other dietary components that may lessen coronary artery disease risk. Total serum cholesterol and LDL cholesterol tend to be lower. Ischemic heart and coronary heart disease are often decreased among vegetarians, particularly among vegans compared to carnivores. Blood pressure and hypertension are also generally lower among vegetarians, perhaps because of their lower weights, lesser sodium, and higher potassium intakes.

Excessive food energy, alcohol, and fat pose greater cancer risks than do synthetic compounds in Western diets, and so if vegetarian diets are low in them there might be effects on some cancers. Cancer morbidity is lower in some vegetarians, such as Seventh-Day Adventists but it is primarily in the smoking and alcohol-related cancers, as might be expected because many vegetarians also do not use tobacco or drink alcohol. Modest (<10%) protection against some cancers seems to be associated with decreased weight. Westerners eat a great deal of meat compared to the rest of the world's population. Red meat has recently been singled out for attention in cancer causation because of its composition (often high fat, high saturated fat, high salt, and high in heme iron), preservation methods (forming N nitroso compounds or interacting with heme to form carcinogens), and styles of preparation (such as benzo(a)pyrene, heterocyclic amines and polycyclic aromatic compounds, adducts, and other possible carcinogens produced in meats broiled and fried at high temperatures) may expose eaters to carcinogens and cancer promoters. Some of these substances have been shown to be carcinogenic in experimental animals. Colon cancer is of particular interest, since the World Cancer Research Foundation in 2007 concluded that to lessen cancer risks intakes of red meat should be limited and processed meat should be avoided based on its summary of the epidemiological evidence. That analysis suggested that meat, especially preserved and processed meat, had

a modest effect on some cancers, particularly colorectal cancers, but this analysis has been criticized and more recent studies are mixed. One very large study of older Americans suggests a modest association between meat intake and mortality in a prospective study in the US. The results on breast cancer are mixed and do not favor an effect. The notion that dairy products and particularly milk contain sufficient estrogens or some other substances that are cancer promoters continues to be debated, but the human and experimental animal evidences are weak. More and better studies are needed to definitively rule meat, or specific types of meat, in or out as the culprit in these cancers.

There is some mixed evidence that osteoporosis and fracture risks are greater in vegans, perhaps due to their low calcium and vitamin D intakes but their more active lifestyles often compensate for this. The associations between animal food intakes and other illnesses, including diverticular disease of the colon, gallbladder disease, and appendicitis are more speculative.

Fewer studies have been done on specific and all-cause mortality and longevity among vegetarians. Smoking-related mortality is lower among groups of vegetarians who do not use tobacco, but most of these effects do not appear to be due to diet. Obesity-related mortality is also reduced. Once these factors have been accounted for, other differences between vegetarians and nonvegetarians or between vegetarians who differ in the degree to which they restrict animal foods in mortality or longevity are not dramatic.

At present the evidence is insufficient to say that well-planned meatless or vegetarian diets have distinct health advantages over well-planned plant-based dietary patterns. Causal inference is difficult in making comparisons between vegetarians and nonvegetarians: residual confounding in diet and the many differences between vegetarians and health-conscious omnivores, including education, lifestyles, and many other health related behaviors, all of which cannot be controlled for in observational studies. However, it is abundantly clear that most Western vegetarians who subsist on well-planned regimens appear to be at least as healthy as their omnivorous counterparts, and that many omnivores eat diets that do not conform to expert recommendations. Whether vegetarians have an advantage over health conscious non-vegetarians is still uncertain.

Nutritional Adequacy

In English-speaking North America, dietary reference intake (DRI) standards that embody what is known about nutrient requirements have been issued by the Food and Nutrition Board, Institute of Medicine, National Academy of Sciences and Health, Canada. Similar but not identical reference standards are available in other countries to assess and plan nutritious diets. Nutritional adequacy is defined as meeting nutrient needs, such as the recommended dietary allowances (RDAs) or the adequate intakes (AI), while avoiding excess and staying below the safe upper levels of intakes (UL) and keeping within the acceptable macronutrient distribution ranges (AMDR) specified by expert groups (in the US, fat 20–35%, protein 10–35%, and carbohydrate 45–55% of

calories). Most vegetarians have little difficulty in meeting these standards with a little planning.

From the nutritional standpoint the animal food groups (e.g., meat, fish/seafood, poultry/fowl, eggs, milk, and milk products) are nutrient-dense foods high in micronutrients that are low or lacking in plant foods. In traditional diets, depending on the particular animal food group under consideration, these nutrients that might fall short included protein of high biological value, highly bioavailable iron, zinc, calcium, vitamins A, D, B₁₂, and B₆, riboflavin, choline, omega 3 fatty acids, and iodine. If animal food groups are entirely eliminated, intakes of these nutrients often become deficient. Today, the widespread availability of foods fortified with these nutrients and appropriate micronutrient-containing dietary supplements provide options that can help to make up these lacks if they are used. Although dietary diversity in the number of food groups they consume is less among vegetarians, diversity within food groups is often considerable and may be sufficient to provide adequate amounts of nutrients. Variety within the remaining food groups (such as fruits, vegetables, nuts, and legumes) may even be increased on vegetarian diets compared to omnivore choices. However, when vegetarians use these options they must make sure that the nutrients they obtain are those that are low in their diets, rather than employing a 'shotgun' approach of a little more of many nutrients, and that the nutrient dose in the product is sufficient to meet nutrient needs. For example, for an older woman, a single glass of calcium-fortified orange juice and a serving of spinach, or a multivitamin-mineral supplement does not contain enough calcium to meet her nutrient needs, and a specific calcium supplement might be more appropriate. Many vegetarians prefer food-based solutions and are reluctant to use dietary supplements. However, in some instances, such as among infants who are breastfed for many months with no other source of vitamin D, a dietary supplement of water-miscible vitamin D may be acceptable and it protects the infant from vitamin D deficiency.

Eating patterns appear to be more closely associated with health outcomes than are intakes of nutrients alone, perhaps because of the synergistic effects of nutrients working with each other or because other bioactives, such as phytochemicals, also have health effects. Vegetarians' intakes of bioactives rich in fruits and vegetables, such as the flavonoids, antioxidants, and dietary fiber are often much higher than those of omnivores. If these substances prove to have beneficial effects on health, such increased intakes may be important. To date only a few effects have been demonstrated.

From the nutritional standpoint there are beneficial trade-offs from vegetarians' limited intakes of animal foods. In Western diets, animal foods are the major sources of dietary constituents that are excessive in Western diets, such as calories, fat, saturated and trans-fat, cholesterol, and sodium, and they are low in polyunsaturated fats and dietary fiber. Consumption of fewer animal foods, especially if it leads to lesser intakes of these constituents and greater intakes of other nutrients and bioactives, may have positive effects on overall nutritional status. The beneficial effects of vegetarian eating patterns on nutritional status vary depending on the food group(s) avoided, the degree of limitation, substitutions of other rich food sources, use of fortified foods and dietary supplements containing

the lacking nutrients, and other changes in dietary intake or lifestyles that occur at the same time. The nutritional goal in planning a vegetarian diet is to maximize the benefits and minimize the health risks by a judicious choice of the type and amount of animal foods or other sources of needed nutrients that are acceptable to the eater.

Adequate Vegetarian Dietary Patterns

Dietary practices among vegetarians are highly variable. Most vegetarians are at little or no risk of dietary inadequacy from their eating patterns and do not merit special assessment although they may benefit from general dietary advice adopted to their eating preferences. For example, neither a man who regards himself as a semi-vegetarian (also referred to as a meat avoider) because he avoids red meat most of the time nor a lacto-ovo vegetarian woman with no other major dietary avoidances are unlikely to need further dietary assessment.

Some characteristics of sound and adequate vegetarian diet patterns include the following:

- Limited animal food avoidances, only sporadic avoidances, or abstinence rather than complete animal food avoidance.
- Consumption of a wide variety of animal food groups and a wide variety of foods within each group.
- Use of a nutritionally sound food guide for diet planning; sound vegan, and vegetarian food guides are available that ensure that nutrient needs are adequate, balanced, and moderate
- Regular use of foods fortified with nutrients likely to fall short in the diet and vitamin or mineral supplements in RDA or Daily Reference Value (DRV) amounts to meet nutrient needs plus use of a nutritionally sound food guide if the individual follows a vegan pattern, or has multiple food avoidances.
- Membership in a group (such as Seventh Day Adventists) or family with a long tradition of adherence to healthy vegetarian eating styles and attitudes toward health.

Possibly Inadequate Vegetarian Dietary Patterns

For vegetarians who are likely to be at high risk of dietary inadequacy, further and more complete dietary assessment and planning may be needed. The entire pattern of intake (including avoidances, substitutions and additions of foods, and use of dietary supplements) must be examined to obtain a full profile of nutrient adequacy or inadequacy. The presence or absence of other lifestyle practices with potentially beneficial health impacts (nonsmoking, abstinence from alcohol, high levels of physical activity) can also have impacts on health. Individual assessment is recommended for those at special risk in nutritionally vulnerable physiological groups (due to age, life stage (pregnancy, lactation), or illness) or because they adhere to very limited patterns (such as avoiding many animal food groups (vegans) and other food groups, or have multiple other food avoidances (e.g., all 'processed' nonorganic, cooked, or canned foods, 'nonwhite' foods, and 'non-natural' foods) so that only 'whole', 'nonprocessed',

'organic' and 'natural' foods are acceptable, further limiting options for planning an acceptable diet.

Characteristics that may indicate inadequate or unbalanced vegetarian diets and the need for further assessment include:

- Entire or very extensive avoidance of animal food groups.
- Refusal to eat fortified plant foods or to use nutrient-containing dietary supplements, or processed foods.
- Refusal to eat 'processed' or 'nonorganic' foods.
- Frequent fasting, vomiting, purging, or drastically altered diet during illness.
- Lack or avoidance of conventional health and medical care (e.g., prescribed medications, vaccinations, mental, or dental health visits), use of only alternative medical care.
- Nutritional vulnerability due to age or physiological condition (infancy, weaning and toddlerhood, rapid growth, puberty, pregnancy, lactation, chronic illness, or recovery from illness, old age, frailty).
- Low weight for height, body mass index (BMI) $< 18.5 \text{ kg m}^{-2}$, $> 4\text{--}7 \text{ kg}$ (10–15 lb) unintentional weight loss, or rapid unintentional weight loss of $> 5\%$ in a month.
- Deeply held beliefs in alternative philosophical or religious systems that rigidly restrict food choice and prescribe vegetarian diets that fail to meet the DRIs.

Key Nutritional Concerns for Vegetarians

Of particular concern with respect to risk of inadequacy for vegetarians and especially vegans and others with extreme restrictions are energy, vitamins B₁₂ and D, riboflavin, omega 3 fatty acids, calcium, iron, zinc, choline, and iodine. Vegetarians of all types can easily meet current recommendations for these nutrients if they are willing to use nutrient sources in fortified foods (such as, e.g., highly fortified cereals, calcium fortified soy milk, and juices, B₁₂ fortified yeast, vitamin A fortified margarine) or specific micronutrient supplements containing enough of the micronutrient that is falling short. However, some vegetarians are unwilling to use these options, increasing risks of deficiency and making dietary planning more difficult. With respect to macronutrients, vegetarian and particularly vegan diets tend to be low in energy, total fat, saturated fat, cholesterol, and sodium. If processed foods are avoided added sugars are also low. Current recommendations for acceptable macronutrient distribution ranges (AMDR) in the US are for fat 20–55% of calories and for protein 10–35% of calories, with added sugars no more than 25% of calories, and the remainder from other carbohydrates.

Key Nutrients for Vegetarians Over the Life Cycle

Well-planned vegan and vegetarian diets can meet nutritional needs at all stages of the life cycle including pregnancy, lactation, infancy, childhood, and adolescence. There are very few longitudinal studies of individuals on the more restrictive vegetarian diets; an exception is a Dutch cohort of macrobiotic vegetarians who were followed from birth to adolescence, and who showed very poor growth and continue to have some

health problems. More studies are needed so that long-lasting effects of diet early in life can be better ascertained.

Some vegetarian parents inadvertently feed their children with diets that are inadequate. The problem is not that nutritionally adequate diets cannot be planned, but that the eater's or cook's ideologies and concerns may get in the way. Under such circumstances health problems have arisen and continue to do so, especially among infants and children on vegan diets that are limited in other foods as well.

Vegan diets present more problems of micronutrient adequacy than do other vegetarian diets across the life cycle and particularly in infants and children because more food groups are eliminated, sources of vitamins B₁₂, D and bioavailable iron, calcium, and zinc may be lacking, the caloric density of the diet is lower, but bulk is higher and weaning is often very late. Vegan diets may also be low in calcium, iron, and zinc, and the forms of these minerals may not be highly bioavailable. Sources of riboflavin and choline also need to be identified.

Vegetarian infants are usually breastfed. They generally thrive until 4–6 months of age, and continue to do so if they remain at the breast and receive complementary feedings of nutritionally complete heat-treated cow's milk-based, fortified soy formulas, or other developmentally appropriate feedings that are high in nutrients, sufficient in energy, and low in bulk. Soy milk is not appropriate under 1 year of age. In countries where home prepared or commercial infant formulas and weaning foods do not provide adequate amounts of micronutrients, certain dietary supplements such as iron or vitamins B₁₂ and D may be needed. Today, more fortified vegan foods are available for infants and other groups than in the past. Good food or supplemental sources of vitamin B₁₂ and D (especially above approximately 40° N latitude where exposure to sunlight is not likely to be sufficient), linolenic acid (an omega 3 fatty acid to ensure that docosahexaenoic acid (DHA) intakes are satisfactory), riboflavin, calcium, and bioavailable forms of iron, and zinc must be included in the diets of weanlings and young children. Protein intakes are usually adequate if many different plant proteins are fed and energy intakes are sufficient. Growth monitoring is helpful. When soy milk is used later in childhood, especially if the child is a vegan, it should be fortified with vitamins D, B₁₂, and calcium. For children during the pubertal growth spurt, energy, calcium, iron, vitamins D and B₁₂, as well as iron are of particular concern with respect to dietary adequacy. Pregnant and lactating vegetarian women have increased nutrient needs for these and other nutrients that can usually be dealt with by dietary planning. Elderly people who are vegetarians may have problems meeting micronutrient needs because they have low energy requirements owing to physical inactivity, whereas need for calcium, vitamin D, and vitamin B₁₂ increases.

Conclusions

Vegetarian diets should be planned in accordance with the DRI or other authoritative nutritional recommendations so that they are healthful and nutritionally adequate. When vegetarian diets are unplanned, the nutrients that are likely

to fall short usually differ somewhat from those of unplanned omnivorous diets. In some cases these deficits can be easily remedied by dietary counseling. In others differences between ideologies about life, diet, and nutrient needs are such that acceptable dietary strategies are difficult. Nutrition scientists and practitioners can help vegetarians who seek their advice by screening the nutritional status of high-risk individuals, by identifying acceptable food sources of specific nutrients for the eater and when asked, by suggesting dietary modifications that may be necessary to meet individual needs when intakes fall short, and by monitoring the vegetarians' progress.

See also: Adolescents: Nutritional Problems of Adolescents. Cancer: Epidemiology and Associations Between Diet and Cancer. Meat, Poultry, and Meat Products: Nutritional Value. Phytochemicals: Classification and Occurrence. Religious Customs, Influence on Diet. Supplementation: Dietary Supplements. Vitamin B₁₂: Physiology, Dietary Sources, and Requirements

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VITAMIN A

Contents

Deficiency and Interventions

Physiology, Dietary Sources, and Requirements

Deficiency and Interventions

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Glossary

Dark adaptation The process by which the eye, through vitamin A-enabled photoreceptor activation, adapts vision under low lit conditions, measured by electroretinography and various measures of pupillary responsiveness to light.

Visual cycle The metabolic pathway in the rod photoreceptor cells in the retina of eye that converts vitamin A into its active vitamers involved in light perception and transmitting visual images to the brain.

Vitamin A deficiency disorders (VADD) All health consequences attributable entirely or partially to vitamin A deficiency.

Vitamin A status The body's vitamin A nutriture expressed as a clinical, biochemical or functional indicator, with conventional cutoffs to convey presence and severity of deficiency. Low serum vitamin A (hyporetinolemia) is the most widely used indicator of vitamin A status (typically classified as a concentration below 0.70 $\mu\text{mol/l}$).

Xerophthalmia From xeros-dryness, -ophthalmia-pertaining to the eye. Ocular manifestations of vitamin A deficiency, including night blindness, Bitot's spots, corneal ulceration and necrosis (keratomalacia).

Introduction

Vitamin A (VA) is an essential nutrient in the human diet. As a result, deficiency can develop when its intake is chronically low, as has occurred in undernourished societies throughout human history. Its existence as a public health problem is readily manifested as eye disease. Night blindness, the mildest ocular condition of VA deficiency, has been recognized since antiquity, as depicted in bas-relief on the wall of the Egyptian pyramid in Saqqara, dating to the Middle Kingdom, and in writings of Hippocrates in the 4th century BC who recognized and treated the condition with animal liver (a major dietary source of VA). Corneal destruction and its consequent blindness, as well as milder stages of conjunctival xerophthalmia, were linked to dietary insufficiency in the eighteenth and nineteenth centuries, as cod liver oil emerged as a treatment for night blindness, 'Bitot's spots' and corneal necrosis (keratomalacia) more than a century ago. Discovery in 1913 of 'fat soluble factor A,' an ether-soluble compound in butter and egg yolk required for growth, general health, and vision in animals, ushered in the past century of vitamin discovery, advanced treatment and prevention of xerophthalmia, synthesis of VA and its analogs, understanding the vitamin's metabolic functions in the visual cycle, epithelial, immune,

hematopoietic, and osteoid cell function and its avertable public health importance.

VA Deficiency Disorders (VADD)

VA is essential to maintain normal retinal function, and in regulating cellular proliferation, differentiation, and energy utilization. Effects of deficiency are most readily observed in rapidly dividing, bipotential cells such as epithelial linings of the body. These regulatory roles give rise to specific effects of hypovitaminosis A that include disturbed photoreceptor function that can lead to night blindness, metaplasia, and keratinization of mucosal surfaces that lead to conjunctival and corneal xerosis as well as epidermoid metaplasia and mucociliary defects throughout the respiratory, genitourinary, and gastrointestinal tracts as well as glandular ducts. Embryo-fetal deficiency may impair multiple organs, including the lungs and cardiovascular, that is only beginning to emerge as a public health concern. Postnatal VA deficiency may impair development and functioning of innate and adaptive arms of the immune system that can weaken or render less-controlled complex host defenses against infection, thereby increasing severity, clinical morbidity, and risk of death. Collectively, all pathophysiological consequences attributed in varying degrees

to VA depletion are termed 'Vitamin A Deficiency Disorders' or VADD (Figure 1).

Biochemical Depletion

Tissue depletion of VA, while not a disorder per se, likely precedes functional consequences of deficiency. In uncomplicated hypovitaminosis A, plasma retinol tends to be homeostatically controlled until body (primarily liver) stores become lower after which the plasma retinol concentration declines. Plasma retinol may also decrease in response to chronic inflammation and clinically significant infection, in parallel with raised concentrations of positive acute phase proteins, increased tissue VA delivery, reduced hepatic mobilization via retinol-binding protein, and increased urinary loss. Plasma retinol gradually normalizes during recovery from

infection in the presence of adequate hepatic stores. If VA is deficient, imposed infection can leave the host more tissue-depleted and at-risk. Despite nondietary influences, plasma or serum retinol measurement remains the most common biochemical index of VA status. VA deficiency is generally diagnosed at a serum retinol concentration below $0.70 \mu\text{mol l}^{-1}$ (or $20 \mu\text{g dl}^{-1}$), below which often 20% to >50% of concentrations lie in a VA-deficient population, compared to <3% in well-nourished societies. A serum retinol level $<0.35 \mu\text{mol l}^{-1}$ ($10 \mu\text{g dl}^{-1}$) indicates severe deficiency. Decrements in serum retinol below these cutoffs can be expected to increase the severity of xerophthalmia and infectious illness. Other indices of tissue retinol depletion include the relative dose response, a before–after test-dose difference in serum retinol that indirectly reflects hepatic retinol adequacy, breast milk retinol concentration for assessing both maternal status and intake adequacy of breastfed infants, and stable isotopic dilution to assess the total body VA pool.

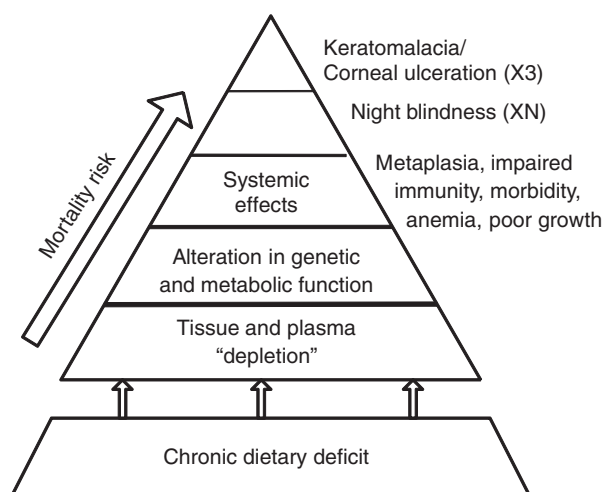


Figure 1 Concept of VADD, due primarily to underlying chronic dietary deficit in preformed VA and proVA carotenoids. Reproduced from West Jr KP (2002) Extent of vitamin A deficiency among preschool children and women of reproductive age. *Journal of Nutrition* 132: 2857S–2866S, with permission from The American Society for Nutrition.

Xerophthalmia

Conjunctival and corneal epithelia deprived of VA undergo keratinizing metaplasia. Columnar epithelial cells on the ocular surface become squamous and mucus-producing goblet cells disappear, providing the histopathologic mechanisms for deficiency-induced xerotic (drying) changes to the ocular surfaces. VA deficiency is also required for rod vision in dim light. VA deficiency-induced night blindness often occurs with histopathologic changes on the ocular surface. Thus, night blindness and clinical eye signs both are listed under one xerophthalmia classification scheme (Table 1).

XN and Dark Maladaptation

VA, as retinaldehyde, is an essential photosensitive pigment in rod cells of the retina that respond to light (become 'bleached') by releasing the VA ligand from the protein rhodopsin, thereby initiating neural impulses to the brain that permits vision under conditions of low illumination. The utilization and recycling of VA in this process is known as the visual (or retinoid) cycle. Hypovitaminosis A restricts rhodopsin production that, in turn, raises the scotopic (low light)

Table 1 WHO^a Classification and minimum prevalence criteria for xerophthalmia and VA deficiency as a public health problem

Definition (code)	Minimum prevalence (%)	Highest-risk period
Children 1 to 5 years of age		
Night blindness (XN)	1.0	2–6 yr
Conjunctival xerosis (X1A)	—	—
Bitot's spots (X1B)	0.5	2–6 yr
Cornea xerosis (X2)/Corneal ulceration (X3A)/keratomalacia (X3B)	0.01	1–3 yr
Xerophthalmic corneal scar (XS)	0.05	>1 yr
Deficient serum retinol ($<0.70 \mu\text{mol l}^{-1}$)	15.0	<5 yr
Pregnant/lactating women		
XN during most recent pregnancy	5.0	Third trimester
Low serum retinol ($<1.05 \mu\text{mol l}^{-1}$)	20.0	Third trimester

^aWorld Health Organization

Source: Adapted from Sommer A and Davidson FR (2002) Assessment and control of vitamin A deficiency: The Anecny Accords. *Journal of Nutrition* 132: 2845S–2850S, and West Jr KP (2002) Extent of vitamin A deficiency among preschool children and women of reproductive age. *Journal Nutrition* 132: 2857S–2866S. Alpha-numeric characters in () denote the WHO classification scheme for xerophthalmia.

visual threshold. Dark adaptometry is becoming possible with new field instrumentation that can detect a preclinical, lagging pupillary response to graded light intensity that can occur in a VA deficient state. Gradually, a perceptive threshold is reached that leads to recognition of XN, the earliest symptom of xerophthalmia. It is marked by an inability to move about in the dark. Young children between 1 and 5 years of age and pregnant women appear to be at greatest risk of XN. Where VA deficiency is endemic, there is often a local term for XN that translates into 'evening' or 'twilight' blindness or 'chicken eyes' (which lack rod cells and also cannot see at night), making the condition readily detectable by history. Typically, gestational XN resolves spontaneously with child birth and expulsion of the placenta, likely related to the lessening of maternal metabolic demands for VA.

X1A and X1B

Early xerosis of the conjunctiva can be detected by filter paper impression cytology, showing distorted, enlarged, and non-contiguous sheaths of epithelial cells, and disappearance of goblet cells. In advanced VA deficiency, confluent xerosis appears clinically as a dry, unwettable surface of the bulbar conjunctiva (X1A). The affected areas are usually overlaid with superficial white, cheesy, or foamy patches of triangular or oval shape that consist of desquamated keratin and bacteria (often the *xerosis bacillus*). These are known as X1B. They are nearly always bilateral, found temporal (and, in more advanced cases, also nasal) to the corneal limbus and are more reliably diagnosed than X1A. X1B are not blinding but are reflective of chronic moderate-to-severe systemic depletion of VA.

Corneal Xerophthalmia

Corneal xerophthalmia is manifested in severe VA deficiency. The earliest corneal lesions appear as superficial punctate defects, evident with a slit lamp, that with advanced deficiency become more numerous and concentrated. The cornea is considered xerotic (X2) when the keratopathy covers large areas of its surface rendering a hazy, nonwettable, lusterless, and irregular appearance on handlight examination. Stromal edema may be present. In more severe cases, thick, elevated X2 plaques may form. Usually both eyes are affected. X3A can be sharply demarcated, round, or oval defects that are usually shallow but may also perforate the cornea. Healed ulcers form a leukoma (scar) or adherent leukoma if the iris has plugged the perforated ulcer. Most ulcers occur peripheral to the visual axis and, thus, may not threaten central vision if treated in time. X3B refers to a full-thickness softening and necrosis of the corneal stroma that can cause protruding, opaque, yellow-to-gray lesions to form (Figure 2). These may reduce or slough off leaving a descemetocoele following VA treatment. X3B usually impairs vision in the involved eye although the degree of visual loss depends on the location, thickness, and extent of corneal necrosis and the resultant scar. Owing to the generally malnourished and ill state of children with corneal xerophthalmia, fatality of hospitalized cases ranges from 4% to 25%.

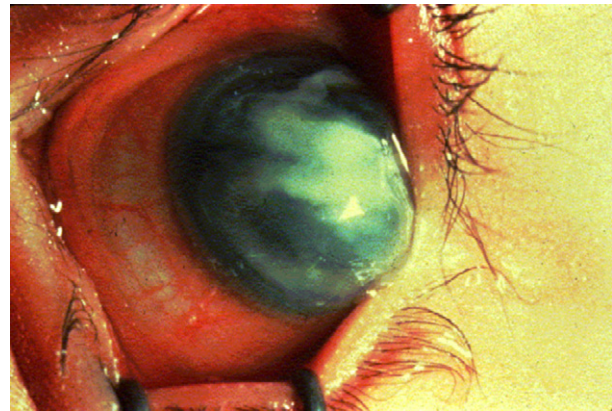


Figure 2 Keratomalacia. Reproduced from Sommer A (1995) *Vitamin A Deficiency and its Consequences: A Field Guide to Detection and Control*, 3rd edn. Geneva: World Health Organization.

Other VADD: Infection, Anemia, and Poor Growth

Infection

A synergism exists between hypovitaminosis A and infection, each exacerbating the other, representing a classic 'vicious cycle.' In this context, infection may be considered both a cause of VA deficiency and, in terms of severity and sequelae, a consequence, or 'disorder.' Xerophthalmia or severe hyporetinolemia have been consistently associated in cross-sectional assessment with higher risks of diarrhea, fever, and other infections though an acute phase reaction to infection can lower the circulating retinol level and directionality can be difficult to establish.

VA deficiency presumably raises risk of infection by compromising 'barrier' epithelial defenses and impairing regulatory innate, cell-mediated and antibody-mediated immune mechanisms. Especially, there is a growing literature implicating roles for retinoic acid in suppressing pathological and autoimmune responses to inflammation, which could underlie its role in controlling severity of infection. Epidemiological corollary evidence exists. VA-deficient South Asian preschoolers (i.e., with mild xerophthalmia) were twice as likely to develop acute respiratory infection and (in Indonesia) three times more likely to develop diarrhea over subsequent 3–6-month periods. Deficient children are also more likely to die. This was so among Indonesian preschool children whose risk of mortality rose with increased severity of mild eye signs (Figure 3). In Nepal, siblings of cases were more likely to develop xerophthalmia and twofold more likely to die than children living in unaffected households, reflecting a clustering of child mortality risk within VA-deficient households. Measles is an illness that can deteriorate VA status which, in malnourished children, can lead to corneal xerophthalmia, more severe complications, and increased fatality. Affecting lifelong ability is the emergence of VA deficiency in exacerbating severity of middle ear infection that may consequently increase the risk of hearing loss.

Anemia and Poor Growth

Children with xerophthalmia tend to be anemic relative to peers without eye disease. VA supplementation can often

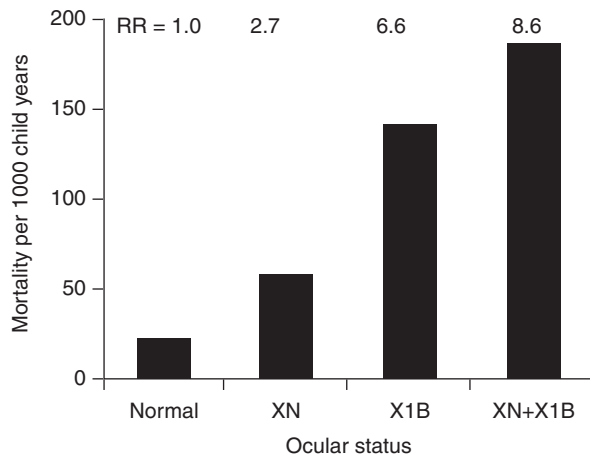


Figure 3 Risk of mortality among ~3500 Indonesian preschool children by ocular status at the outset of each 3-month interval. RR, relative risk of mortality. Adapted from Sommer A, Tarwotjo I, Hussaini G, and Susanto D (1983) Increased mortality in children with mild vitamin A deficiency. *Lancet* 2: 585–588.

improve hemotological indicators of iron status and reduce anemia. Mechanisms involved in this interaction are not clear but could involve enhanced iron absorption, storage, and transport as well as direct effects on hematopoiesis in the presence of adequate iron stores.

VA deficiency decelerates growth in animals and has been observed to be associated with both stunting and wasting malnutrition in children, possibly reflecting plausible roles for the vitamin in osteogenesis and energy metabolism. Trials, however, have shown inconsistent effects of VA supplementation on child growth, possibly due to variations in the extent of infection, seasonality in dietary protein and energy adequacy, exclusion criteria, and entry levels of VA status among study children. It appears that VA supplementation can influence ponderal and linear growth, as well as body composition, in children for whom VA deficiency is a 'growth limiting' nutritional deficit.

Epidemiology

The epidemiology of VA deficiency is mostly understood in relation to hyporetinolemia and xerophthalmia (Table 1), especially the noncorneal stages involving XN and X1B due to X1A. The latter eye signs may be common in the absence of effective prophylaxis, specific and likely to reflect more widespread hypovitaminosis A. Although both conditions are stages of 'mild' nonblinding xerophthalmia, they typically result from moderate-to-severe systemic VA deficiency. Preschool children and women of reproductive age are at highest risk of being VA deficient, best understood in terms of how the condition distributes by person, place, and time.

Person (High-Risk Groups)

Despite uncertainty about an appropriate cutoff for hyporetinolemia in the first months of life, infants in most poor

populations are born with low VA status and, without adequate VA from breast milk and complementary foods remain deficient throughout early childhood. Current estimates suggest that VA deficiency (serum retinol $<0.70 \mu\text{mol l}^{-1}$) afflicts 33%, or 190 million preschool-aged children in the developing world, of whom 5 million (~1% of all) exhibit XN. The number of extant or annually occurring cases of X1B is less well known due to few ocular surveys having been conducted since the 1990s. In one major country where recent data exist, India, an overall rate of ~1% was observed. Milder stages of xerophthalmia typically affect children beyond the first year of age, as breast milk is replaced by less nutritious food from household diet. Boys tend to be at higher risk, thereby possibly reflecting gender differences in dietary practices.

Risk corneal xerophthalmia (Figure 2) tends to peak in the second through fourth years of life, typically following the epidemics of acute infection such as severe measles. Informally, it appears that numbers of cases globally have dropped, likely attributable to high coverage of at-risk child populations with semiannual VA supplementation, which reduces risk of corneal disease by ~90%. A second factor has also likely been a steadily increased and sustained coverage of vaccination against measles, a major precipitant of corneal xerophthalmia.

VA deficiency has been noted to affect women living in undernourished settings, especially in relation to pregnancy, appearing to be driven by heightened nutrient demands of gestation. Globally, some 19 million pregnant women are estimated to be VA deficient (serum retinol concentration $<0.70 \mu\text{mol l}^{-1}$), of whom nearly 10 million are night blind. This latter symptom not only identifies women at high risk of hyporetinemia but also anemia, wasting malnutrition, offspring mortality, and maternal morbidity and mortality (Figure 4). Widespread maternal VA deficiency has also been observed to coexist with HIV infection and AIDS throughout sub-Saharan Africa. However, modest, absent, or inconsistent effects of maternal VA supplementation on infant and maternal health, survival, or transmission suggest the deficiency may be more a consequence than determinant of disease severity.

Place (Geographic Clustering)

Regions of the world with the greatest burden of VA deficiency remain Southern Asia and sub-Saharan Africa which, together, account for two-thirds of all cases of mild xerophthalmia (XN) and over 75% of biochemically VA-deficient children.

The geographic distribution of VA deficiency in preschool-aged children, based on joint distributions of preschool xerophthalmia and hyporetinolemia, reflects a persistent, peri-equatorial distribution, is presented in Figure 5. Within a region or other locality, VA deficiency, best characterized for xerophthalmia, clusters in association with indices of underdevelopment, low availability of food sources of VA and disease patterns. In Indonesia, villages where cases of xerophthalmia were detected tended to be poorer than xerophthalmia-free communities and, within a village, cases arose more frequently from households of low socioeconomic standing than children with normal eyes (controls) (Table 2).

In multiple surveys in Africa and Asia preschool children incur an approximate twofold higher risk of having or developing xerophthalmia in villages where at least one other child has been diagnosed compared to villages where xerophthalmia has not been previously seen (Table 3). More striking is a

7–13-fold higher risk of xerophthalmia in siblings of cases compared to children at home with no previous history of xerophthalmia. Maternal XN and childhood xerophthalmia often coexist in households and communities. Spatial clustering seems to arise mostly from shared dietary practices at

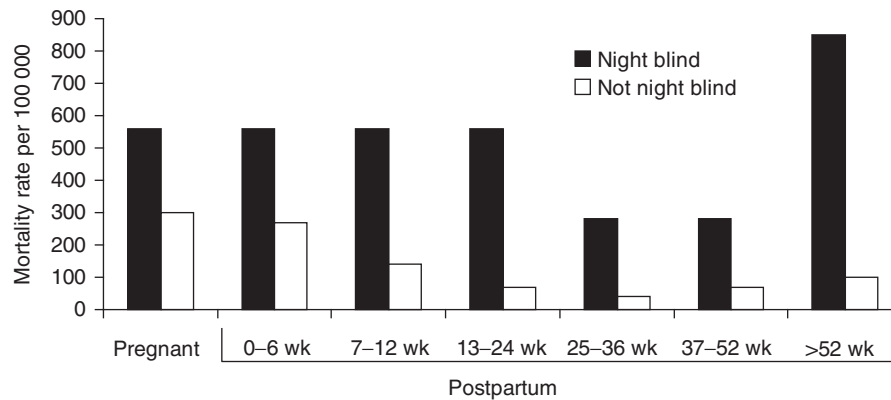


Figure 4 Mortality rates of rural Nepalese women (per 100 000 pregnancies) during and for up to 2 years following pregnancy according to whether mothers experienced night blindness ($n=361$) or not ($n=3052$) during pregnancy. Reproduced from Christian P, *et al.* (2000) Night blindness during pregnancy and subsequent mortality among women in Nepal: Effects of vitamin A and B-carotene supplementation. *American Journal of Epidemiology* 152: 542–547, with permission from OUP.

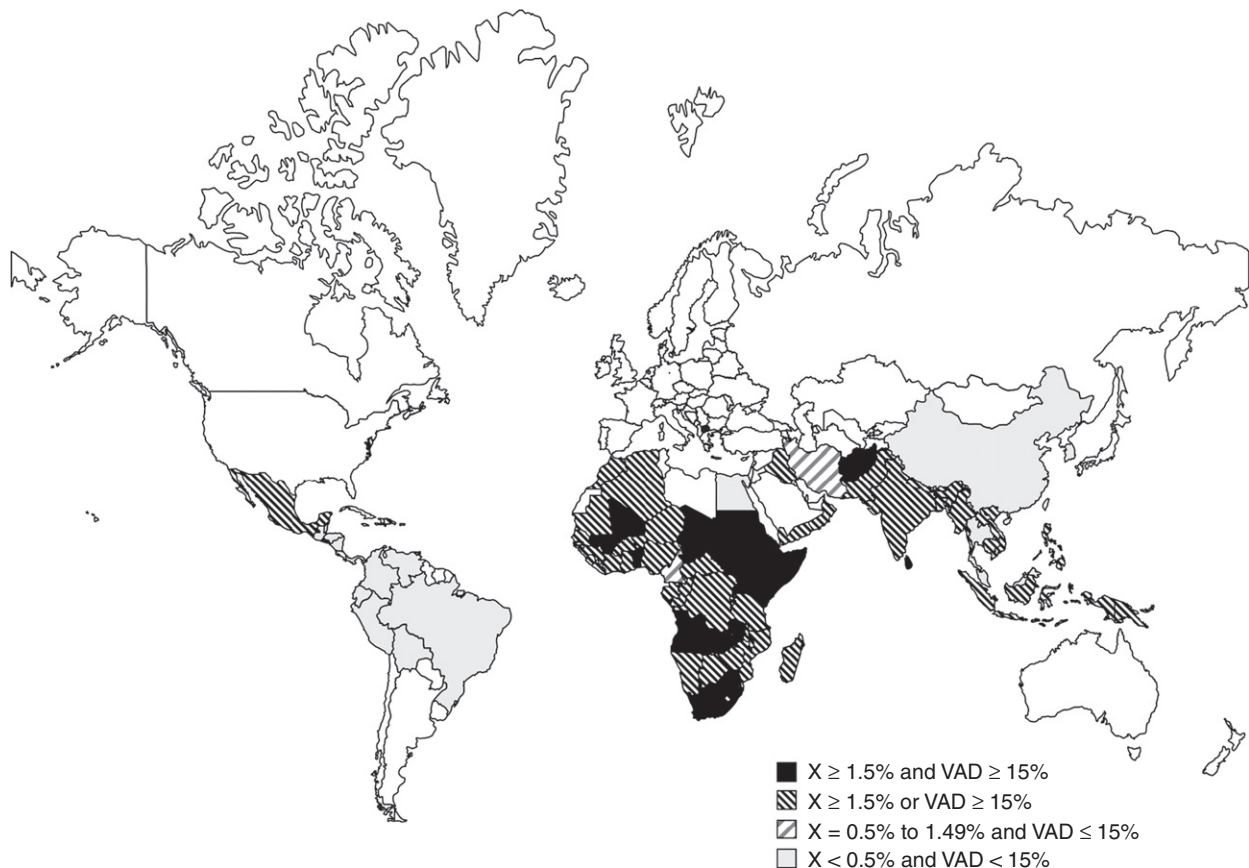


Figure 5 Global geographic distribution of xerophthalmia (X, all clinical stages) and VA deficiency (VAD, serum retinol concentrations $< 0.70 \mu\text{mol l}^{-1}$) in preschool-aged children. Reproduced from West Jr KP (2002) Extent of vitamin A deficiency among preschool children and women of reproductive age. *Journal of Nutrition* 132: 2857S–2866S, with permission from The American Society for Nutrition.

Table 2 Household characteristics of xerophthalmia cases, controls and the remaining Aceh study population

Household characteristic	Cases (%) (N= 466)	Village-matched controls (%) (N= 466)	Aceh study households (%) (N= 15 915)
Unprotected water source	47.5	43.8	41.1 ^a
No private latrine	86.7	83.6	71.3 ^a
Bamboo house walls	47.1	33.5	31.6 ^a
Household head farms	57.3	55.5	53.4
Mother has <6 yr of education	94.3	86.6	80.3 ^a
History of child death	12.1	9.7	7.5 ^a

^aSignificant linear trend in proportions, $p < 0.001$.

Source: Adapted from Mele L, West Jr KP, Kusdiono, Pandji A, *et al.* and the Aceh Study Group (1991) Nutritional and household risk factors for xerophthalmia: A case-control study. *American Journal of Clinical Nutrition* 53: 1460–1465.

Table 3 Age-adjusted village and household odds ratios for risk of xerophthalmia among preschool children^a

	Malawi		Zambia		Indonesia		Nepal	
	n	OR ^b	n	OR	n	OR	n	OR
Village	50	1.2 (1.0–1.5) ^c	110	1.7 (0.9–3.2)	460	1.8 (1.4–2.2)	40	2.3 (1.6–3.4)
Household	2899	7.3 (3.2–16.7)	2449	7.9 (3.5–17.8)	16337	10.5 (7.0–15.7)	2909	13.2 (6.0–29.0)

^aNumbers of children <6 years of age in each country: Malawi (n=5441); Zambia (n=4316); Indonesia (n=28 586); and Nepal (n=4764).

^bPairwise odds ratio based on alternating logistic regression.

^c95% confidence intervals in parentheses.

Source: Adapted from Katz J, Zeger SL, West Jr KP, Tielsch JM, and Sommer A (1993) Clustering of xerophthalmia within households and villages. *International Journal of Epidemiology* 22: 709–715.

homes and villages rather than other exposures that lead to common infections. The exception to this observation is likely to be in households and communities afflicted by HIV/AIDS.

Time (Periodicity)

Occurrence of xerophthalmia can follow predictable, though not parallel, seasonal patterns in different parts of the world. Typically, a seasonal peak in VA deficiency emerges from a convergence of causal risk factors. In South Asia, for example, a distinct peak in the incidence of mild xerophthalmia occurs during the late dry and early monsoon seasons (April–July). This peak follows a postharvest growth spurt in the cool dry season. It also coincides with a general scarcity of proVA-rich vegetables and fruits and a seasonal rise in the incidence of diarrhea, respiratory infection and measles. In this area of the world, the seasonal peak is often curbed abruptly midway through the ‘mango season,’ reflecting a likely impact of widespread consumption of this beta-carotene rich fruit. Periodicity, where it exists, can help identify causes and target prevention to specific times of the year.

Causal Agents (Diet and Infection)

VA deficiency results from consuming a diet that is chronically inadequate in VA in relation to need, often exacerbated by infection. A low dietary fat intake (e.g., <5% of calories) may restrict absorption of proVA carotenoids from vegetables and fruits and thus also predispose certain poor populations to deficiency.

Breastfeeding and Diet

Breast milk is an infant’s most important initial dietary source of VA. Commonly, breast milk from marginally nourished mothers contains ~500 µg of retinol activity equivalents (RAE) per liter, thereby delivering 325 µg RAE per day to infants typically consuming ~650 ml day⁻¹. An ‘adequate intake’ of 400–500 µg RAE has been set as a dietary guide for infants, making this usual level of VA intake from breast milk marginal during the first year and, beyond infancy, marginally above an estimated deficient intake threshold of 210 µg RAE, but usually sufficient to prevent clinical signs. Studies in Asia and Africa show breastfed infants and toddlers to be 65–90% less likely to develop xerophthalmia than nonbreast-fed peers of the same age. Children who had begun weaning ~1 month earlier and completed breastfeeding ~6 months earlier than nonxerophthalmic children. Even among those breastfeeding, a higher daily frequency may confer further protection against xerophthalmia in undernourished settings.

The choice of foods offered to complement breast feeding may also affect risk of VA deficiency. Indonesian preschoolers were at a two- to sixfold higher risk of xerophthalmia if food sources of VA such as dark green leaves, mango or papaya, egg, meat or fish with liver, and milk, and other dairy products were not routinely given during the first year they were provided complementary foods, initiating a pattern that likely places children at risk throughout their early years of life. Across undernourished regions of the world, less frequent intakes of preformed VA and proVA carotenoid rich foods are observed by children with, versus absent, a history of xerophthalmia (Figure 6). Similar dietary differences are evident among women with versus without a history of maternal XN.

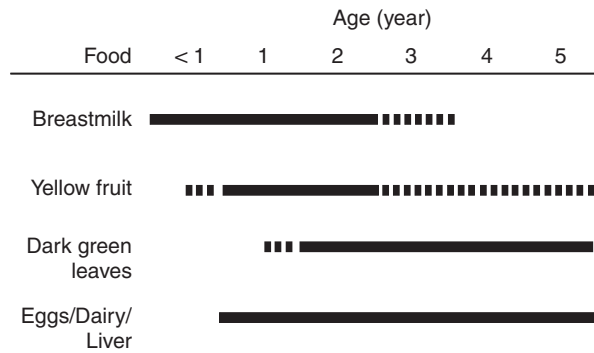


Figure 6 Foods that protect against hypovitaminosis A (xerophthalmia), based on numerous studies. Dark line, strong evidence; dashed line, suggestive evidence. Reproduced from Sommer A and West Jr KP (1996) *Vitamin A deficiency: Health, Survival and Vision*. New York: Oxford University Press.

Infection

Because a vicious cycle exists between VA deficiency and infection, infection can be viewed as a cause of deficiency. Prospective studies show that severe infections such as measles, chicken pox, diarrhea, and acute respiratory illness decrease serum as well as apparent hepatic levels of retinol and increase risk of xerophthalmia. In some settings, measles has been observed to increase by as much as >13-fold the risk of children developing corneal xerophthalmia. In Indonesia, young children with diarrhea and acute respiratory infections were twice as likely to develop mild xerophthalmia (XN or X1B) than disease-free children. Similar patterns temporal patterns between infection and XN have been observed in undernourished pregnant women. Explanations for a role of infection as a cause of VA deficiency include decreased absorption of VA, increased metabolic requirements, impaired retinol transport, greatly increased renal excretion during the acute-phase response, and slow normalization of these mechanisms coupled with a chronically decreased VA intake during extended recovery or repeated illness.

Impact of Interventions

VA deficiency can be prevented through direct supplementation, fortification of commonly eaten food items in factories or at home, or other food-based approaches that include home gardening, small animal husbandry, nutrition education, and broad agronomic approaches such as biofortifying staple crops with proVA beta-carotene. Most evaluations to date have assessed impact of supplementation and, occasionally fortification, on VA status, xerophthalmia, mortality, and morbidity.

VA Status

The impact of VA prophylaxis on status may vary by indicator, dosage, and mode of delivery of the supplement, as well as the population context, including level of initial deficiency and other risk factors. A single, high-potency supplement (210 μ mol, 60 mg RAE, or 200 000 IU) has typically been shown to elevate serum retinol to near adequacy in

deficiency-prone child populations for periods of up to a few months. Continuous intake of a half to full recommended allowance of VA through fortified foods gradually improves and sustains adequate serum and breast milk retinol concentrations or, when assessed by indirect means, hepatic retinol adequacy. Regular consumption of proVA food sources (dark green leaves, yellow vegetables, and fruits) has variable, though generally positive, effects on VA status. Dietary proVA carotenoid intake appears most efficacious in raising serum retinol from deficient concentrations to minimally adequate levels but often fails to optimize VA status. Variations in food matrix, methods of storage and preparation, amounts of pre-formed VA and fat in the diet, gut integrity and function, protein energy and VA status of the host, and genetic predisposition are among factors that may affect bioconversion to VA. Single nucleotide polymorphisms for beta-carotene monooxygenase, the enzyme responsible for cleaving beta-carotene to retinaldehyde in the VA bioconversion pathway, are beginning to be reported in populations that may help explain high interindividual variability in the bioconversion of dietary carotenoids, generally estimated to occur at ratio of 12:1 when consuming a mixed vegetarian diet.

Xerophthalmia

High-potency VA delivered to preschool children every 4–6 months, as widely practiced in low income countries, is ~90% efficacious in preventing both corneal and noncorneal xerophthalmia. Prophylactic failure (~10%) may reflect inadequacy of dosage for some children who are severely VA-deficient or become ill. Xerophthalmia, on the other hand, virtually disappears in societies consuming adequate amounts of VA-fortified foods. Supervised treatment with proVA-rich vegetables and fruit has been reported to cure or improve noncorneal xerophthalmia.

Mortality

There has been extensive investigation into the effects of VA on mortality over the past two decades in preschool-aged children, and more recently in young infants and women of reproductive age. The impact of VA supplementation on preschool child mortality has been firmly established through the conduct of eight controlled community trials in the 1980s and early 1990s specifically designed to address this outcome, involving ~165 000 children 6–72 months of age on three subcontinents (Table 4). In six trials, children 6 months to 6 years of age were supplemented every 4–6 months with an oral dose of VA containing 60 mg retinol equivalents (RE) (or 200 000 IU). Half this dosage was provided to children <12 months of age. One study, in India, provided a small weekly dose to children and the other, in Indonesia, supplied half of a recommended allowance of VA to children in treatment villages through a routinely marketed fortified monosodium glutamate product (a meal flavor enhancer). Rates of mortality in supplemented groups were compared to rates among children in concurrent control groups. Six of the eight trials showed reductions of 19% to 54% in preschool child mortality beyond either 6 or 12 months of age. Meta-analyses of data from these trials, alone or with additional, often smaller studies meeting inclusion criteria, have estimated the reduction in mortality to range from 23% to 34%. Cumulative

Table 4 VA mortality prevention trials

Target group/location	VA dosage ^a	Number	% Change ^b
<i>Young neonates</i>			
Bandung, Indonesia ^c	15 mg RAE at birth	2067	↓64% ^v
Madurai, India ^d	14.4 mg RAE at birth	11 619 ^t	↓22% ^v
Gaibandha, Bangladesh ^e	15 mg RAE at birth	15 937	↓15% ^v
Guinea-Bissau ^f	15 mg RAE at birth	4345	↑6%
<i>Infants 1–5 mo</i>			
Sarlahi, Nepal ^g	30 mg RAE	4617	↑4%
Jumla, Nepal ^h	15 mg RAE	1058	↑1%
<i>Infants < 6 mo</i>			
Sarlahi, Nepal ^g	7 mg RAE/wk to mothers	15 987	↑4%
<i>Children 6–72 mo</i>			
Aceh, Indonesia ⁱ	60 mg RAE/6 mo	29 236	↓34% ^v
West Java, Indonesia ^j	0.81 mg RAE/day	11 220	↓46% ^v
Tamil Nadu, India ^k	2.5 mg RAE/week	15 419	↓54% ^v
Hyderabad, India ^l	60 mg RAE/6 mo	15 775	↓6%
Sarlahi, Nepal ^m	60 mg RAE/4 mo	28 640	↓30% ^v
Jumla, Nepal ^h	60 mg RAE/5 mo	7197	↓29% ^v
Khartoum, Sudan ⁿ	60 mg RE/6 mo	29 615	↑6%
Northern Ghana ^o	60 mg RE/4 mo	21 906	↓19% ^v
<i>Pregnant/lactating women^p</i>			
Sarlahi, Nepal ^g	7 mg RAE/week	22 189 ^u	↓40% ^v
Brong Ahafo, Ghana ^r	7.5 mg RAE/week	78 835 ^u	↓8%
Gaibandha, Bangladesh ^s	7 mg RAE/week	59 666 ^u	↑15%

^aRAE, Retinol activity equivalents; trials providing 60 mg RE gave a half dose to infants <12 months.

^bPercent change in mortality rate among VA recipients compared to controls of similar age or life-stage for all trials.

^cHumphrey JH, Agoestina T, Wu L, *et al.* (1996) Impact of neonatal vitamin A supplementation on infant morbidity and mortality. *Journal of Pediatrics* 128: 489–496.

^dRahmathullah L, Tielsch JM, Thulasiraj RD, *et al.* (2003) Impact of supplementing newborn infants with vitamin A on early infant mortality: community based randomised trial in southern India. *British Medical Journal* 327: 254–260.

^eKlemm RDW, Labrique AB, Christian P, *et al.* (2008) Newborn vitamin A supplementation reduced infant mortality in rural Bangladesh. *Pediatrics* 122: e242–e250.

^fBenn CS, Diness BR, Roth A, *et al.* (2008) Effect of 50 000 IU vitamin A given with BCG vaccine on mortality in infants in Guinea-Bissau: randomised placebo controlled trial. *British Medical Journal* 336: 1416–1420.

^gWest Jr KP, Katz J, Shrestha SR, *et al.* (1995) Mortality of infants <6 mo of age supplemented with vitamin A: a randomized, double-masked trial in Nepal. *American Journal of Clinical Nutrition* 62: 143–148.

^hDaulaire NMP, Starbuck ES, Houston RM, *et al.* (1992) Childhood mortality after a high dose of vitamin A in a high risk population. *British Medical Journal* 304: 207–210.

ⁱSommer A, Tarwotjo I, Djunaedi E, *et al.* (1986) Impact of vitamin A supplementation on childhood mortality: A randomized controlled community trial. *Lancet* 1: 1169–1173.

^jMuhilal, *et al.* (1988) Vitamin A-fortified monosodium glutamate and health, growth and survival of children: a controlled field trial. *American Journal of Clinical Nutrition* 48: 1271–1276.

^kRahmathullah L, Underwood BA, Thulasiraj RD, *et al.* (1990) Reduced mortality among children in Southern India receiving a small weekly dose of vitamin A. *New England Journal of Medicine* 323: 929–935.

^lVijayaraghavan R, *et al.* (1992) Vitamin A supplementation and childhood mortality (Letter). *Lancet* 340: 1358–1359.

^mWest Jr KP, Pokhrel RP, Katz J, *et al.* (1991) Efficacy of vitamin A in reducing preschool child mortality in Nepal. *Lancet* 338: 67–71.

ⁿHerrera MG, Nestel P, El Amin A, *et al.* (1992) Vitamin A supplementation and child survival. *Lancet* 340: 267–271.

^oGhana Vast Study Team (1993) Vitamin A supplementation in northern Ghana: effects on clinic attendances, hospital admissions and child mortality. *Lancet* 342: 7–12.

^pPercent changes are due to VA only, in all cause mortality West Jr KP, Katz J, Khatry SK, *et al.* (1999) Double blind, cluster randomised trial of low dose supplementation with vitamin A or carotene on mortality related to pregnancy in Nepal. *British Medical Journal* 318: 570–575

^qKirkwood BR, Hurt L, Amenga-Etego S, *et al.* (2010) Effect of vitamin A supplementation in women of reproductive age on maternal survival in Ghana (ObaapaVitA): a cluster-randomised, placebo-controlled trial. *Lancet* 375: 1640–49.

^rWest Jr KP, Christian P, Labrique AB, *et al.* (2011) Effects of vitamin A or beta-carotene supplementation on pregnancy-related mortality and infant mortality in rural Bangladesh: A cluster-randomized trial. *Journal of American Medical Association* 305: 1986–95.

^sChild-years of observation.

^tNumber of pregnancies.

^uIndicates statistically significant differences ($p < 0.05$).

mortality curves from trials with positive results show a characteristic departure in the mortality rate of VA supplemented from control groups (Figure 7). Causes of death known to respond to VA include measles, diarrhea, possibly malaria, and

other febrile illnesses. In particular, VA has consistently been shown to reduce fatality from severe measles, by ~50%. A lack of effect on fatality from acute lower respiratory infection has been a perplexing but consistent finding across studies.

A large dose of VA (50 000 IU), given shortly after birth, appears to reduce risk of mortality in the first half of infancy. Trials carried out to date in Indonesia, India, and Bangladesh have reported significant 64%, 22%, and 15% reductions, respectively, in infant mortality following newborn VA versus placebo receipt (Table 4), with findings from South India revealing attenuation of deaths attributed to diarrhea and fever. In contrast, two African trials, in Guinea-Bissau (Table 4) and Zimbabwe, have not reduced infant mortality, suggesting a potential mediating role of local context, including prominent infections such as human immunodeficiency virus (HIV) as was present in the latter study. Additional trials are underway in South Asia and sub-Saharan Africa. Plausible explanations for the observed impact may include maturational effects of VA on an immature immune system, gut, and airway that could enhance resistance to infection months later.

Finally, improving intake of VA, either preformed or as proVA beta-carotene in amounts approximating a recommended dietary intake, may in some settings also reduce mortality related to pregnancy where risks of deficiency and mortality are exceedingly high (e.g., ~700 or more deaths per 100 000 pregnancies). In rural Nepal, weekly VA supplementation lowered mortality related to pregnancy by 40%, largely

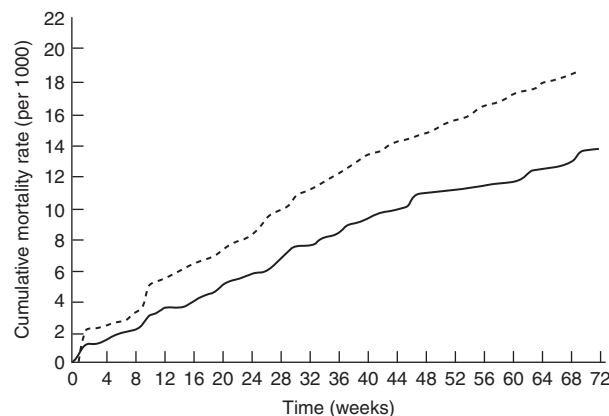


Figure 7 Cumulative mortality of children randomized to 4-monthly placebo control (dashed line) versus 200 000 IU of vitamin A (solid line) during a large community trial in Sarlahi District, rural Nepal. Reproduced from Sommer A and West Jr KP (1996) *Vitamin A Deficiency: Health, Survival and Vision*. New York: Oxford University Press.

explained by lowering severity of infections among mothers prone to XN. In contrast, large trials of similar design conducted in Ghana and Bangladesh have failed to reduce maternal mortality (Table 4), linked to lower risks of mortality and VA deficiency, reflected by improved diets in the latter two countries.

Morbidity

Direct effects of VA on 'morbidity' have been difficult to establish, possibly due to variation in disease sensitivity to VA and inherent problems in measuring incidence, duration, and severity of morbidity in community studies. Available data suggest VA has little impact on prevalence of common childhood morbidities, but rather holds the potential to attenuate severity of infections such as measles, diarrhea, dysentery, and *falciparum* malaria. In Ghana, VA supplementation decreased childhood clinic visits for illness (relative risk (RR)=0.88), hospitalization rates for severe disease (RR=0.62) and the severity of illness among children admitted with diarrhea, compared to placebo recipients. In Brazil, prior VA receipt had no effect on children's diarrheal episodes of 1–2 days' duration (RR=0.97) but was significantly protective against episodes lasting ≥ 3 days with ≥ 4 stools per day (RR=0.91) and episodes of ≥ 3 day with ≥ 5 stools per day (RR=0.80). In an early VA trial in Sarlahi, Nepal, long term follow-up has revealed a 42% lower risk in hearing loss from middle ear infections. However, as with acute lower respiratory infection-associated mortality, multiple trials have found little effect of VA on recovery from pneumonia, a surprising finding given decades of animal experiments linking VA deficiency to keratinization and, presumably, greater susceptibility to pathogen invasion and infection, of the respiratory tract.

Management

Treatment

Children with xerophthalmia and measles should be treated immediately with oral, high-potency VA (200 000 IU) according to WHO and International Vitamin A Consultative Group (IVACG) guidelines Table 5 and provided other supportive nutritional and medical therapy, as indicated. Corneal lesions should be topically treated with a suitable antibiotic (e.g., tetracycline or chloramphenicol) to prevent bacterial infection. Corneal xerophthalmia typically improves with VA treatment within a week, and resolving within 4 weeks,

Table 5 VA treatment and prevention schedules

Age	Treatment at diagnosis ^a	Prevention Dosage	Frequency
<6 mo	50 000 IU	50 000 IU	Once within 3 day after birth ^c
6–11 mo	100 000 IU	100 000 IU	Every 4–6 months
12–59 mo	200 000 IU	200 000 IU	Every 4–6 months
Women	By severity of eye signs ^b	200 000 IU	2 doses 24 h apart # 6 weeks after delivery

^aTreat all cases of xerophthalmia and measles on days 1 and 2; give an additional dose for xerophthalmia on day 14. For severe malnutrition give 1 dose on day 1.

^bFor women of reproductive age, give 200 000 IU only for corneal xerophthalmia on days 1, 2, and 14; for night blindness or X1B give 10 000 IU per day or 25 000 IU per week for >3 months.

^cBased on limited number of trials in South Asia, to date, showing a ~20% reduction in infant mortality with an oral supplement given shortly after birth.

Source: Reproduced from Ross DA (2002) Recommendations for vitamin A supplementation. *Journal of Nutrition* 131: 2902S–2906S.

depending on size, thickness and location of the lesion, and nutritional and health status of the patient. XN typically disappears within 24 h of treatment. Most X1B respond within 2–5 days and disappear within 2 weeks after VA treatment, though may persist in older children. Severely wasted children should be given a single large oral dose (200 000 IU). It is also judicious to give children with severe diarrhea, dysentery, respiratory infection, and measles a single, large oral dose of VA. Large-dose VA is indicated for women of reproductive age with corneal disease. For XN, smaller daily (10 000 IU) or weekly (25 000 IU) doses are recommended for at least 3 months.

Prevention

Health consequences, or disorders, of VA deficiency can be prevented via direct supplementation of target groups, food fortification at central production facilities or by adding nutrient-dense powders to meals at home, biofortification of staple crops, and other agricultural or dietary approaches, including garden and education programs that encourage exclusive and extended breast-feeding and improved dietary quality at home.

Administration of large-dose, oral VA (200 000 IU), adjusted to age (Table 5), on a ~6 monthly basis is a common preventive in most undernourished societies. A half-dose is dispensed to infants 6–11 months of age and a quarter dose given to younger infants to minimize the risk of toxicity in high risk areas, although a more effective alternative to reducing infant mortality appears to lie in dosing infants shortly after birth. Periodically providing a large dose of VA helps to increase nutrient liver stores from where it is mobilized into circulation, as needed. Supplements can be provided during routine health care (e.g., for growth monitoring, immunization, and other extension services) or more extensively and systematically on a regular (e.g., semiannual) basis, especially through national campaigns often called Child Health Days, which routinely achieve 80% or greater coverage. Nearly four decades of distributing billions of large-dose VA supplements for prevention, coupled with marked declines in mortality attributed to the intervention, attests to the acceptance, potential effectiveness, and safety of this approach. Gastrointestinal side effects may occur in ~5% of recipient children.

Increasingly, commercial fortification of dietary ingredients is becoming possible in low-income, deficiency-prone countries, and able to offer a quarter to a full day's recommended allowance of VA. Potential food vehicles should be technically fortifiable at planned levels, and consumed within a range that could be both effective in deficient groups while remaining safe for nutritionally adequate or overconsuming segments of a population. VA fortification has been effectively scaled-up using a few products in few countries so far, but will likely rise with opportunity. Successful products have included sugar,

nonfat milk powder, wheat flour nonrefrigerated margarine, and vegetable oils. A slow but steadily rising trend in food fortification will likely occur in years ahead as processed foods become more widely consumed and as the food industry becomes engaged in solving VA and other micronutrient deficiency problems.

Biofortification of major staple grains and tubers, such as rice, maize, and sweet potatoes, with beta-carotene have been shown to be potentially effective in improving VA status in poor settings. Dietary diversification is widely held to be the most culturally appropriate and potentially sustainable approach to preventing VA deficiency. Although pilot trials show efficacy of a variety of dietary approaches in improving VA intake and status, data on effectiveness and cost of population food-based interventions remain lacking. Dietary intakes can be improved through home and school gardening initiatives, nutrition education, and social marketing of locally available food sources of VA. However, effective dietary change requires a thorough understanding of local cultural, food system, and behavioral factors that increase the risk of VA deficiency.

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Physiology, Dietary Sources, and Requirements

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Glossary

Retinoic acid The active metabolite of vitamin A in most tissues of the body.

Retinol activity equivalent (RAE) The unit of dietary vitamin A quantification.

Retinol-binding protein The transport protein for vitamin A (retinol) in plasma.

Visual cycle The reactions that recycle retinol and 11-*cis*-retinal in the rods and cones of the retina.

Vitamin A deficiency Clinical manifestations that include low plasma retinol, loss of visual function, alterations to epithelia, and abnormal gene expression.

Vitamin A: Physiology, Dietary Sources, and Requirements

Vitamin A (retinol) is a fat-soluble micronutrient that is required by all vertebrates for vision and the normal functioning of epithelial tissues, the immune system, and reproduction. Vitamin A was discovered in 1913 as an essential growth and survival factor for young rats. The active compound was shown to be a minor lipid present in eggs, butter, whole milk, and fish liver oils. Soon thereafter, vitamin A was characterized chemically as all-*trans*-retinol, a 20-carbon lipid alcohol that is present only in foods of animal origin, often referred to as 'preformed vitamin A.' A second form of vitamin A was isolated from deep-yellow and green leafy vegetables and characterized as β -carotene, a 40-carbon hydrocarbon, referred to as provitamin A. The nutritional requirement for vitamin A can be fully met by a diet containing only preformed retinol (as for carnivores), only provitamin A carotenoids (as for herbivores and humans who consume a strictly vegetarian diet), or a mixture of both as is typical for omnivores. These carotenoids include mainly β -carotene and less amounts of α -carotene and β -cryptoxanthin.

Although retinol or its esters and β -carotene are the nutritional forms of vitamin A provided by diet, both must undergo metabolic reactions to generate the two principal bioactive molecules: 11-*cis*-retinal, required for vision, and retinoic acid, a metabolite that functions as a regulator of gene expression in nearly all tissues. Retinoic acid has important effects on embryogenesis, immune function, reproduction, and the maintenance of healthy cells in essentially all tissues. Adequate dietary vitamin A is therefore thought to be important for the prevention of cancer and degenerative diseases.

Besides the natural forms of vitamin A, a large number of structurally related analogs have been synthesized as potential therapeutic agents. The term 'retinoid' is now used to describe both the natural forms of vitamin A and synthetic analogs, even though the latter typically do not possess all of the biological properties of vitamin A.

Major Molecules in Vitamin A Biochemistry and Physiology

Vitamin A and its metabolites comprise numerous molecules that differ in isomeric form and oxidation state. The major

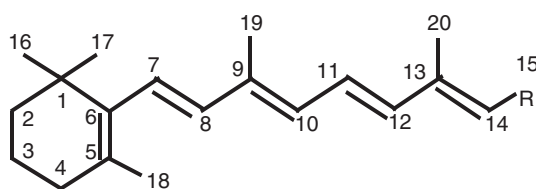
forms are the retinol, its esters, retinal, and retinoic acid. Additional polar metabolites of retinol and retinoic acid are often formed before excretion. The all-*trans* isomer is the most abundant and most stable isomer. In the retina, 11-*cis*-retinal is the functional form, acting as an essential component of rhodopsin (see the Section on Vision).

Retinol is considered the parent molecule of the vitamin A family (Figure 1). Its double bonds are typically in the all-*trans* conformation although tissues and plasma may also contain low levels of 9-*cis* and 13-*cis* retinol. In foods and most storage tissues of the body, retinol is esterified with a long-chain fatty acid and stored as retinyl ester. Fatty acid esters of retinol, especially retinyl palmitate, oleate, and stearate, predominate in most tissues.

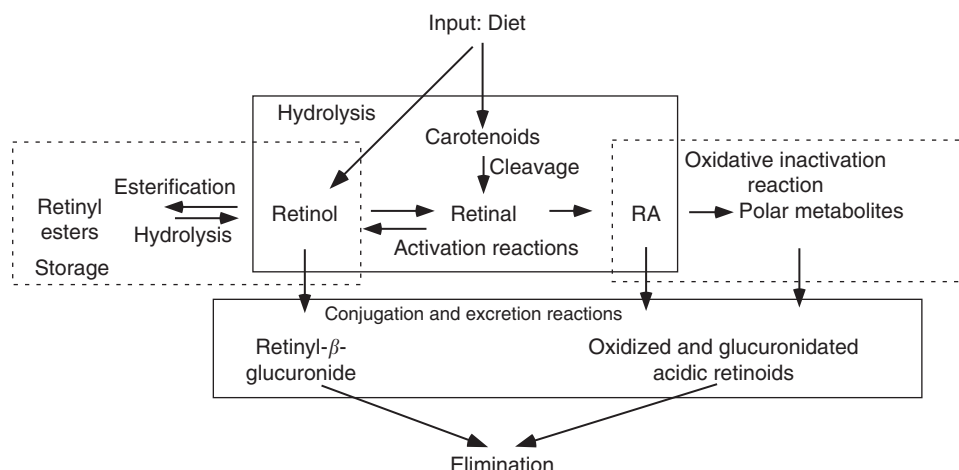
Additionally, natural variant forms of retinol are present in some foods and human tissues, including vitamin A₂ (3,4-didehydroretinol), which is found in tissues of freshwater fish and produced metabolically in human skin, and α -retinol, an isomer of retinol. These forms have lower vitamin A activity than retinol itself.

Oxidative metabolism is essential for the physiological functions of vitamin A. Figure 2 illustrates key processes in retinol metabolism. Retinol is oxidized within cells to generate retinal, which is generally a transient metabolite that can either be reduced back to form retinol, allowing for cycling between these two forms, or further oxidized in an irreversible reaction to form retinoic acid. All-*trans*-retinoic acid is the major acidic form of vitamin A with widespread effects on gene expression. 9-*cis*-Retinoic acid has also been proposed to function in gene regulation but its *in vivo* activity remains uncertain. 13-*cis*-Retinoic acid is found naturally, probably as a metabolite, as well as being clinically useful as a prodrug that can be slowly converted to all-*trans*-retinoic acid.

Polar metabolites of retinol or retinoic acid are formed by the addition of hydroxyl or keto groups to the cyclohexenyl ring structure, generally at positions 4 or 18, or by formation of epoxides such as 5,6 epoxy-retinoic acid. Retinol, retinoic acid, and more polar metabolites may be conjugated with glucuronic acid, forming retinyl and retinoyl- β -glucuronide. Conjugation renders these metabolites water soluble and they are found mostly in bile and to some extent in urine, suggesting they are formed as terminal excretion products. Generally, their biological activity is low and their biologic half-life is short.

Retinol (all-*trans*) and related forms

$R = \text{CH}_2\text{OH}$, retinol
 $R^2 = \text{CH}_2\text{O-fatty acid}$, retinyl ester
 $R^3 = \text{CHO}$, retinal
 $R^4 = \text{COOH}$, retinoic acid
 $R^5 = \text{COO-glucuronide}$

Figure 1 Structure of all-*trans*-retinol and several related forms.**Figure 2** Schematic of principal reactions of vitamin A metabolism.

Transport

The solubility of most retinoids in plasma and cells depends on their association with specific proteins.

Retinol-Binding Protein (RBP4)

Plasma retinol is transported by a specific 21-kDa transport protein, RBP (gene name *RBP4*). Most RBP is synthesized in the liver (see the Section on Hepatic Vitamin A Uptake, Storage, and Release), but some extrahepatic organs also produce it. Each RBP molecule binds a single molecule of all-*trans*-retinol in a noncovalent manner; this complex is referred to as holo-RBP. Holo-RBP associates in plasma with a cotransport protein, transthyretin (TTR), to form a molecular complex of approximately 75 kDa.

Cellular RBPs

Several cellular RBPs are present in the cytoplasm of many types of cells. These proteins, designated CRBP-I, -II, -III, and -IV, and cellular retinoic acid-binding proteins, CRABP-I and -II, are similar in structure and size (~14.6 kDa). Each contains a single binding site that preferentially binds a particular form of retinoid (retinol, retinal, or retinoic acid), often preferring a specific isomer. Each protein is expressed in different tissue-specific patterns. These proteins function as chaperones that confer aqueous solubility on their

lipophilic retinoid and target them to specific enzymes for metabolism.

Nuclear Retinoid Receptors

Two families of nuclear retinoid receptor proteins, retinoic acid receptor (RAR) and retinoid X receptor (RXR), reside mainly in the nucleus where they form dimers capable of binding to specific DNA sequences in retinoid-responsive target genes. The RAR (RAR α , β , and γ) and RXR (RXR α , β , and γ) are transcription factors that become activated on binding of ligand, e.g., all-*trans*-retinoic acid for RARs and potentially 9-*cis*-retinoic acid for RXRs. Ligand binding facilitates the recruitment of proteins that modify chromatin and either positively or negatively regulate gene transcription. The RXR also form heterodimers with several other nuclear hormone receptors, namely those for vitamin D, thyroid hormone, certain fatty acids and lipophilic hormones, and xenobiotic agents. Numerous posttranslational protein modifications of the RAR and RXR have been described which further modulate their activity.

Absorption and Metabolism

Intestinal Metabolism

Dietary retinyl esters must be hydrolyzed in the lumen of the small intestine, emulsified, and incorporated into lipid micelles before retinol can be absorbed into the mucosa

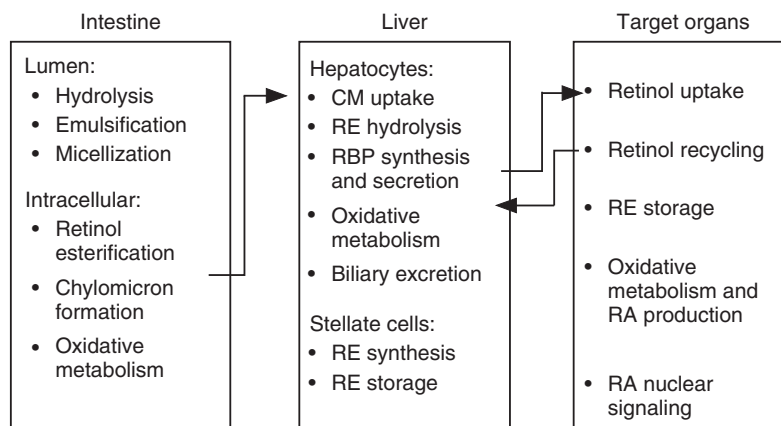


Figure 3 Major physiological processes occurring in the intestine, liver, and target organs. Target organs include eyes, immune system, reproductive organs, and other epithelial tissues including the intestine and liver. CM, chylomicron; RA, retinoic acid; RBP, retinol-binding protein; RE, retinyl ester.

(Figure 3). Several retinyl ester hydrolase (REH) enzymes are present in pancreatic juice or situated on the brush border of duodenal and jejunal enterocytes. For greatest efficiency, these processes require an adequate amount of bile salts and a small quantity of dietary fat, approximately 5%, consumed concomitantly. The retinol molecules then diffuse into the enterocyte where they are bound to CRBP-II and then esterified by the enzyme lecithin:retinol acyltransferase (LRAT). Animals lacking LRAT have been shown to absorb vitamin A poorly, consistent with observations that retinyl ester formation is important for vitamin A absorption. The overall efficiency of intestinal retinol absorption is quite high, approximately 70–90%, and not significantly downregulated when vitamin A consumption increases. Thus, it is possible to absorb too much vitamin A when it is consumed in excess.

β -Carotene is absorbed less efficiently, on the order of 9–22%, than retinol. Provitamin A carotenoids are cleaved in the enterocytes by carotene monooxygenases (BCOs), generating retinal that can be rapidly metabolized to retinol, then esterified. However, humans absorb approximately one-third of ingested β -carotene intact, without cleavage, and this β -carotene is directly incorporated into chylomicrons.

Although most retinol and β -carotene is transformed to retinyl ester in the intestine, a small fraction is oxidized to retinoic acid and then absorbed into the portal vein and systemic circulation. The metabolism of small amounts of dietary 9-*cis*- β -carotene may be a source of 9-*cis*-retinoids in tissues.

Hepatic Vitamin A Uptake, Storage, and Release

After chylomicrons enter lymph and plasma, their triglyceride component is rapidly hydrolyzed, producing smaller chylomicron remnants which still contain newly absorbed vitamin A. Small amounts of the newly absorbed vitamin A, or β -carotene, from chylomicrons and remnants may enter adipose and other tissues including the mammary glands during lactation; however, most chylomicron remnants are cleared rapidly into liver parenchymal cells (hepatocytes) by receptor-mediated endocytosis (Figure 3). The majority of the retinyl ester molecules delivered in this manner undergo rapid

hydrolysis, after which retinol is transferred by a process still not well defined into hepatic stellate cells (HSCs) where the retinol is bound to CRBP-I and esterified by LRAT. The HSCs contain predominantly retinyl palmitate and retinyl stearate which, along with other lipids, form numerous small lipid droplets that are characteristic of HSCs. When vitamin A nutrition is adequate, >90% of the body's total vitamin A resides in HSCs. Extrahepatic tissues also contain small numbers of similar-appearing stellate cells, suggesting the presence of a network of vitamin A-storing cells throughout the body.

Retinyl esters in HSCs can be hydrolyzed, apparently in response to signals that vitamin A is required in other tissues, and the liberated retinol is then transferred back to hepatocytes where it combines with newly synthesized RBP, and is secreted through the Golgi apparatus. Before or shortly after secretion into plasma, holo-RBP binds noncovalently with a tetramer of TTR to form the holo-RBP–TTR transport complex described earlier.

Plasma Concentrations

The concentration of retinol is kept within a nearly constant range, staying close to ~ 2 and $1.7 \mu\text{mol}$ retinol per liter of plasma in adults, and somewhat lower in children. RBP concentrations are generally somewhat higher and thus RBP is normally 80–90% saturated with retinol. Apo-RBP (i.e., RBP without retinol) displays a reduced affinity for TTR and therefore it is readily filtered in the kidneys, and then catabolized or lost in urine.

In the postprandial period after ingestion of vitamin A, a significant proportion of total plasma retinol is present as retinyl ester. As most blood specimens are collected in the fasting state, these postprandial retinyl esters are usually not measured.

Relationship of Plasma Retinol to Liver Retinol Concentration

The relationship of plasma vitamin A to liver vitamin A concentration has been studied in humans and animals. The steady levels of plasma retinol are generally maintained at a

nearly constant concentration even though liver vitamin A concentrations may vary widely, reflecting close homeostatic regulation. If liver vitamin A reserves fall below a concentration of approximately 20–30 μg retinol per gram liver, then the secretion of holo-RBP from the hepatocytes into plasma is compromised. Under these conditions plasma retinol (holo-RBP) levels fall, until either additional vitamin A is consumed or until nearly all of the vitamin A in liver is used. Clinical signs of vitamin A deficiency begin to manifest themselves when plasma retinol becomes inadequate to support the secretion of holo-RBP and thus to maintain normal functions in peripheral tissues.

As liver and plasma retinol levels fall, RBP synthesis in the liver still continues. If retinol is then administered, it can immediately combine with preexisting apo-RBP and be rapidly secreted into plasma as holo-RBP. A useful test for adequate versus inadequate liver vitamin A stores has been developed based on this finding of rapid secretion of holo-RBP when the liver's vitamin A is insufficient and apo-RBP has built up.

Changes due to Metabolic Disturbances

When the rate of protein synthesis falls, as in states of protein or energy malnutrition, the concentrations of plasma retinol, RBP, and TTR also fall. Plasma RBP and TTR levels are sometimes used as clinical indicators of visceral protein synthesis. During infection or inflammation, the biosynthesis of RBP and TTR is also compromised, even if liver vitamin A reserves may be adequate. Other nutritional and metabolic disturbances can lead to similar decreases in plasma retinol, RBP, and TTR. Thus, laboratory values for plasma retinol must be interpreted cautiously, because not all low levels signal a deficiency of vitamin A.

The consumption of vitamin A over time in amounts that greatly exceed needs, or acute intake of very large doses results in elevated liver vitamin A levels, and potentially in hypervitaminosis A, discussed in the Section on Hypervitaminosis A and Vitamin A Toxicity. This condition is associated with persistent liver levels $>300 \mu\text{g}$ retinol per gram liver. The levels of plasma holo-RBP still remain almost normal, but total vitamin A in plasma increases due to the presence of retinyl esters carried by plasma lipoproteins. Clinically, the presence of retinyl esters in fasting plasma state is a signal of possible hypervitaminosis A.

Vitamin A Kinetics

RBP and TTR each have a relatively short half-life in the circulation, ~ 0.5 and 2–3 days, respectively. Thus the maintenance of relatively stable plasma concentrations necessitates that they be nearly continuously synthesized.

Studies of plasma retinol kinetics have shown that each molecule of retinol circulates through the plasma compartment several times before it is irreversibly degraded (see the Section on Tissue Retinoid Metabolism). In one study of a healthy young man who consumed 105 μmol of retinyl palmitate in a test meal, 50 μmol of retinol passed through his plasma per day, whereas only 4 $\mu\text{mol d}^{-1}$ was degraded. Unlike retinol, the RBP protein does not appear to be recycled, implying that new

molecules of RBP must be synthesized for the continued recycling of retinol from the periphery back to the liver. Some extrahepatic tissues, such as kidney and adipose, contain RBP mRNA at levels ~ 5 –10% that in liver. The kidney evidently plays a very significant role in the recycling and conservation of retinol after the glomerular filtration of holo-RBP. Holo-RBP can bind to renal epithelial cells and cross the epithelium by transcytosis, which appears to be a mechanism for the recovery of retinol after filtration. Adipose tissue may be another source of plasma RBP. Additionally, adipose-derived RBP has been reported to have adipokine-like activity.

Overall, the body is very efficient at conserving retinol, but relatively inefficient in degrading and eliminating retinoids when vitamin A is consumed in amounts that substantially exceed requirements. Intakes in excess of physiological needs thus result in a build up of retinyl esters in tissues, especially in, but not limited to, the liver.

Tissue Retinoid Metabolism

Recently, a novel protein has been discovered which serves as a receptor for holo-RBP in certain tissues. The protein, known as Stra6, is located on the plasma membrane of certain cells or organs, including the retinal pigment epithelium (RPE), lungs, and other tissues. However, the liver does not appear to express Stra6, yet studies have shown that retinol recycles from plasma to liver, suggesting the existence of other uptake mechanisms as well.

Although the majority of the body's vitamin A is stored in the liver, many organs contain small depots of retinyl esters, which are thought to be critical for the generation of bioactive retinoids formed by oxidative metabolism (see [Figure 2](#)). Several enzymes of the alcohol dehydrogenase, the short-chain dehydrogenase/reductase, and aldehyde dehydrogenase gene families may participate in these oxidative reactions. However, most of these enzymes are also capable of oxidizing other substrates and therefore elucidating the specific oxidative metabolism of retinoids has been difficult.

Retinoic acid is generally present in tissues at nanomolar concentrations, far lower than that of retinol. The half-life of retinoic acid is very short, a few hours or less, implying that it must be generated almost continuously to maintain appropriate cellular concentrations. Some tissues, such as brain, derive most of their retinoic acid through metabolism whereas other tissues such as liver obtain retinoic acid mostly by uptake from plasma.

Both retinol and retinoic acid can be oxidized on their ring structure, usually at carbon 4, to yield hydroxy and oxo metabolites. The oxidative metabolism of retinoic acid is in part autoregulated because elevated concentrations of retinoic acid induce the expression of cytochrome P450 enzymes, especially CYP26A1, a retinoic acid 4-hydroxylase that is capable of converting retinoic acid to polar metabolites. Ring-oxidized metabolites of retinol and retinoic acid are normally present at low concentrations in plasma, probably because they are readily removed by the liver and conjugated with glucuronic acid and readily excreted. Although tissue retinoid levels are normally maintained within a narrow range by a balance of production and oxidation, it is possible for homeostatic mechanisms to be overwhelmed when vitamin A or retinoids

are taken in excess (see the Section on Hypervitaminosis A and Retinoid Toxicity).

Physiological Actions

Vision

The RPE constitutes a layer of epithelial cells underlying the photoreceptors that take up retinol bound to RBP from choroid capillaries and convert it into retinyl esters for storage and ultimately to be used for the generation of 11-*cis*-retinal. As per requirement, 11-*cis*-retinal is transported by interstitial RBP (IRBP) to the adjacent layer of rod and cone photoreceptor cells where 11-*cis*-retinal combines covalently with the protein opsin in rods to generate the visual pigment rhodopsin, and, similarly, with proteins known as iodopsins in cones to form red-, green-, and blue-sensitive pigments. 11-*cis*-Retinal is very efficient in absorbing light. Although retinal in organic solvent absorbs maximally in the ultraviolet range at ~ 365 nm, the absorption maximum is shifted into the visible range by the combination of 11-*cis*-retinal with opsin. The rod cell outer segment is densely packed with membrane disks that, overall, contain some 10^8 molecules of rhodopsin per cell. Nevertheless, the small quantity of vitamin A present in the retina would be inadequate to maintain vision if it were not for the presence of the visual cycle, which constitutes a recycling process in which 11-*cis*-retinal is regenerated after it has been photobleached by light. Some of these recycling reactions take place in the rods and cones and others in the RPE. The regeneration of 11-*cis*-retinal, referred to as dark adaptation, is relatively slow (on the order of minutes) as compared with the very rapid process of photoisomerization. However, vision normally continues without a period of blindness as long as retinol can be drawn from the retinyl ester storage pool in the RPE, rapidly isomerized to 11-*cis*-retinol, reoxidized to 11-*cis*-retinal, and passed by IRBP to the rod cells where rhodopsin can be regenerated. When the RPE's supply of retinyl esters is low, as occurs during the development of vitamin A deficiency, the visual cycle slows significantly. The clinical outcome is night blindness, a loss of the ability to quickly adapt to darkness again after exposure to bright light. Night blindness is an early indicator of incipient vitamin A deficiency.

The cornea is an avascular tissue, however holo-RBP is present in the lacrimal glands and tears, and this vitamin A is likely to provide the substrate for the local formation of retinoic acid, which then maintains corneal cell integrity. Retinoid deficiency results in a loss of goblet cells (mucin-secreting cells). The presence of corneal xerosis and Bitôt's spots (foamy deposits of cells and bacteria, usually at the outer quadrants of the eye) is an indicator of severe vitamin A deficiency. Persons with these symptoms need vitamin A immediately to prevent corneal ulceration, which results in lifelong blindness.

Functions in Cell Differentiation

Epithelial tissues (the skin, respiratory tract, immune system, reproductive organs, etc.) are especially sensitive to a lack of vitamin A, and often to an excess of retinoids as well. The

systemic effects of vitamin A deficiency include dryness of the skin (follicular hyperkeratosis), loss of goblet cells in the trachea and respiratory tract, and a generalized flattening of epithelia (squamous metaplasia, sometimes with keratinization). The hematopoietic system is also affected, as are reproductive organs. In the testes, spermatogenesis is inhibited by vitamin A deficiency.

Although either a lack or an excess of retinoids is known to affect many organ systems, the developing embryo and the immune system have been studied most intensively. During development, retinoids are required from the early, post-gastrulation stage of development. Retinoic acid has been proposed to be an essential morphogen whose concentration is a key determinant of the expression of several developmentally important genes, particularly *Hox* genes that are critical for the formation of the body pattern. Some *Hox* genes contain a retinoic acid-response element (RARE) and thus can respond directly to retinoic acid. Both deficiency and excess of vitamin A, as well as excess of retinoid analogs, can cause severe developmental defects.

For the immune system, studies in vitamin A-deficient animal models have demonstrated many abnormal immune responses, including reduced T-cell counts and an altered pattern of T-cell subsets. The functions of cytotoxic cells, such as cytotoxic T cells, natural killer cells, and macrophages, are often low. Similarly, it is often the case that the production of cytokines that regulate T-cell immunity and antibody production by B cells is also low, or dysregulated, in vitamin A deficiency. The migration of T and B cells to sites of immune stimulation may also be impaired by vitamin A deficiency.

In children at risk of vitamin A deficiency, vitamin A either given prophylactically or as therapy during illness has resulted in a significant reduction in mortality and in the severity of diseases such as measles.

Dietary Sources and Nutritional Equivalency

Preformed vitamin A is present at highest concentration in liver and fish oils; it is present at lower concentrations in nonorgan meats and eggs (Table 1). Vitamin A, usually as retinyl palmitate for improved stability, is also used in food fortification and supplement preparation. In the USA, approximately two-thirds of vitamin A is consumed preformed and the rest is consumed as carotenoids, but the proportions vary widely depending on dietary patterns.

Units of Nutritional Activity

Because vitamin A exists in multiple forms and the efficiency of utilization of some of its forms, including carotenoids, is lower than that of preformed vitamin A, it is necessary to express 'the total bioactivity of vitamin A' in terms of equivalents. The currently accepted nutritional equivalents, established in 2001 by the Institute of Medicine (IOM), are expressed in retinol activity equivalents (RAE), where 1 μ g RAE corresponds to the activity of 1 μ g of all-*trans*-retinol or 2 μ g of β -carotene in oily solution. It is now considered that, on

average, carotenoids must be ingested in the following amounts to provide the equivalent nutritional value of 1 µg of all-*trans*-retinol (1 RAE):

1. Two micrograms of supplemental β -carotene (in an oily, easily absorbed solution).
2. Twelve micrograms of β -carotene, or 24 µg of α -carotene or β -cryptoxanthin in fruits and vegetables (due to association of carotenoids with the food matrices, and therefore reduced digestibility).

Food labels still use the older international unit (IU), which is outdated by the above-mentioned considerations. One IU equals 0.3 µg of all-*trans*-retinol. Another indicator of

nutritional value used in food labeling is the percentage daily value (%DV). This is a less quantitative but convenient means to compare foods for their nutritional value, and is used in **Table 1** to compare differences among vitamin A-containing foods.

Recommended Dietary Allowances (RDAs) and Tolerable Upper Intake Levels for Vitamin A

RDAs for the USA and Canada were updated by the IOM in 2001. Guidelines were also established for a tolerable upper intake level (UL). The 2001 RDA and UL for vitamin A for various life stages are listed in **Table 2**. The UL is defined as the highest intake of a nutrient that is likely to pose no risk of adverse health effects in nearly all healthy individuals. For vitamin A, the UL applies specifically to 'preformed vitamin A' (e.g., retinol, but not carotenoids) from foods, fortified foods, and supplements combined. For several life stage groups, the UL is less than three times the RDA (**Table 2**). The UL was based on epidemiological studies showing teratogenic effects of excessive vitamin A taken during pregnancy, and on minimizing the risk of liver damage due to hypervitaminosis A. For children, the RDA and UL values were scaled down based on body weight. The recommendations of the Food and Agricultural Organization/World Health Organization (FAO/WHO) tend to be slightly lower than the RDAs defined by the IOM.

Hypervitaminosis A and Vitamin A Toxicity

Hypervitaminosis A is a rare but serious and sometimes fatal condition. It can arise acutely after consumption of very large amounts of preformed vitamin A, or slowly after the persistent intake of lesser, but still excessive, amounts of preformed vitamin A. Most cases have resulted from excessive use of vitamin A-containing supplements, whereas similar symptoms have been reported in some patients taking prescription retinoids for therapy. The clinical signs of vitamin A toxicity include nausea and vomiting, headache, dizziness, blurred vision, lack of muscular

Table 1 Food sources of vitamin A

Food	%DV
<i>Animal sources of preformed vitamin A</i>	
Liver, beef, cooked, 3 oz	545
Liver, chicken, cooked, 3 oz	245
Fat-free milk, fortified with vitamin A, 1 cup	10
Cheddar cheese, 1 oz	6
Milk, whole, 3.25% fat, 1 cup	5
<i>Plant sources of β-carotene and other provitamin A carotenoids</i>	
Carrots, boiled, 1/2 cup slices	270
Carrot, 1 raw (7½-in long)	175
Cantaloupe, raw, 1 cup	110
Spinach, raw, 1 cup	55
Apricot nectar, canned, 1/2 cup	35
Papaya, raw, 1 cup cubes	30
Tomato juice, canned, 6 oz	15
Peach, raw, 1 medium	6

%DV = Daily Value. DVs are reference numbers based on the recommended dietary allowance (RDA). They were developed to help consumers determine if a food contains a lot or a little of a specific nutrient. The DV for vitamin A is 5000 IU (1500 µg retinol). Most food labels do not list a food's vitamin A content. The percent DV (%DV) listed on the table above indicates the percentage of the DV provided in one serving. Percent DVs are based on a 2000-calorie diet.

Source: <http://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/#en18> (last accessed May 20 2011).

Table 2 Recommended dietary allowances (RDAs) for vitamin A in micrograms (µg), retinol activity equivalents (RAE), and international units (IUs), and tolerable upper intake levels (UL, µg retinol per day) for children and adults

Age (years)	Children	Men	Women	Pregnancy	Lactation
<i>RDA (µg RAE per day)</i>					
1–3	300 µg or 1000 IU				
4–8	400 µg or 1333 IU				
9–13	600 µg or 2000 IU				
14–18	900 µg or 3000 IU	700 µg or 2330 IU	750 µg or 2500 IU	1200 µg or 4000 IU	
≥ 19	900 µg or 3000 IU	700 µg or 2330 IU	770 µg or 2565 IU	1300 µg or 4335 IU	
<i>UL (µg retinol per day)</i>					
1–3	600 µg				
4–8	900 µg				
9–13	1700 µg				
14–18		3000 µg	2800 µg	2800 µg	2800 µg
≥ 19		3000 µg	3000 µg	3000 µg	3000 µg

Source: <http://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional/> (last accessed 24 December 2010).

coordination, abnormal liver function, and pain in weight-bearing bones and joints.

There is little that can be done to treat vitamin A toxicity besides eliminating the intake of vitamin A or use of retinoids, and there is no antidote. For prevention, care should be exercised to avoid overconsumption of preformed vitamin A including in supplements (see ULs, [Table 2](#)).

Individuals who consume large amounts of carotenoid-rich foods or juices may develop yellowing of the skin (carotenoderma), especially in fatty tissues and the palms of the hands. This condition is considered benign and the yellow color will gradually subside after intake of carotene is reduced to a normal level.

See also: Bioavailability. Carotenoids: Chemistry, Sources and Physiology; Health Effects. Vitamin A: Deficiency and Interventions

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VITAMIN B₆

Physiology

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Glossary

Acrodermatitis In rats, a specific dermatitis associated with vitamin B₆ deficiency, leading to scaly paws and ears. Formerly used as the basis for a biological assay of vitamin B₆.

Ataxia Inability to coordinate voluntary muscle movement as a result of neurological damage.

Decarboxylation The removal of carbon dioxide from an amino acid to yield an amine.

Glycogen phosphorylase The enzyme that catalyses the stepwise removal of glucose units (as glucose 1-phosphate) from the storage carbohydrate glycogen in liver and muscle.

Hyperhomocysteinemia An elevated blood concentration of homocysteine, a risk factor for cardiovascular disease.

Myelin The lipid sheath around nerve fibers.

Neuropathy Damage to nerves.

Racemization The reaction in which the D- and L-isomers of an amino acid are interconverted.

Schiff base The product of condensation between an amino group and an aldehyde or ketone.

Transamination The reaction in which the amino group of an amino acid is transferred onto an oxo-acid (keto-acid), yielding the corresponding amino acid, and forming the oxo-acid carbon skeleton of the donor amino acid

Dietary Forms, Biological Availability, and Metabolism

The main form of vitamin B₆ in foods is pyridoxal phosphate, bound to enzymes. There is also a small amount of pyridoxamine phosphate. In plant foods, a significant amount of the vitamin is present as pyridoxine.

A number of plants contain relatively large amounts of pyridoxine glycosides, which are only approximately 50% biologically available. Approximately 15% of the total vitamin B₆ in typical diets is present as glycosides, some of which is hydrolyzed by intestinal mucosal glycosidases. Some can be absorbed intact, and may be a substrate for tissue glycosidases, or may be excreted into the urine unchanged. The overall dietary vitamin B₆ is approximately 75% biologically available.

A proportion of the vitamin B₆ in foods may be biologically unavailable, especially after heating, as a result of the formation of (phospho)pyridoxyllysine by reduction of the aldimine (Schiff base) by which pyridoxal phosphate is bound to the ε-amino groups of lysine residues in proteins. Although some of this pyridoxyllysine may be useable, because it is a substrate for pyridoxine phosphate oxidase, it is also a vitamin B₆ antimetabolite, and, even at relatively low concentrations, can accelerate the development of deficiency in experimental animals maintained on deficient diets. In the 1950s, there was an outbreak of vitamin B₆ deficiency among infants fed on formula that had been overheated in manufacture, resulting in the formation of relatively large amounts of pyridoxyllysine.

Digestion and Absorption

Pyridoxal phosphate bound as a Schiff base to lysine in dietary proteins is released on digestion of the protein. The phosphorylated vitamers are dephosphorylated by membrane-bound alkaline phosphatase in the intestinal mucosa; pyridoxal, pyridoxamine, and pyridoxine are all absorbed rapidly by passive diffusion and even very high doses are well absorbed. Intestinal mucosal cells have pyridoxine kinase and pyridoxine phosphate oxidase so that there is net accumulation by metabolic trapping. Much of the ingested pyridoxine is released into the portal circulation as pyridoxal, after dephosphorylation at the serosal surface.

Metabolism and Transport

Much of the absorbed vitamin is taken up by the liver, although other tissues can also take up the unphosphorylated vitamers from the circulation. Uptake is by carrier-mediated diffusion, followed by metabolic trapping as phosphate esters. Pyridoxine and pyridoxamine phosphates are oxidized to pyridoxal phosphate. All tissues have pyridoxine kinase activity, but pyridoxine phosphate oxidase is found only in the liver, kidney, and brain (Figure 1).

Pyridoxine phosphate oxidase is a flavoprotein, and its activity declines markedly in riboflavin deficiency. Despite this central role of riboflavin in vitamin B₆ metabolism, blood and tissue concentrations of pyridoxal phosphate are not affected by riboflavin deficiency, and riboflavin nutrition appears to have little effect on vitamin B₆ nutritional status.

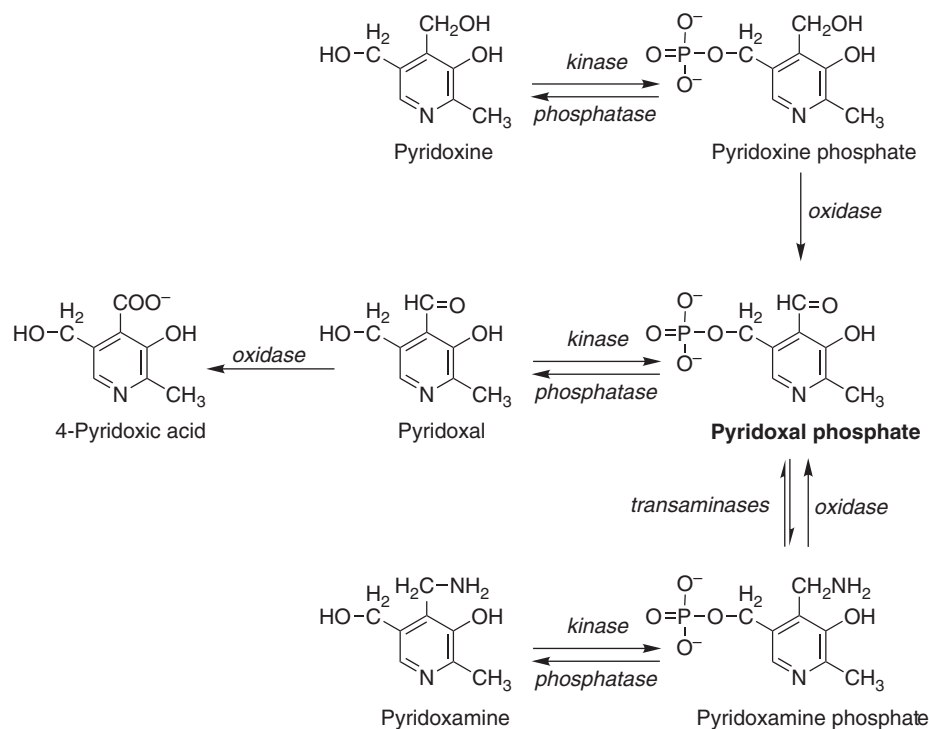


Figure 1 Metabolism of vitamin B₆.

Pyridoxine phosphate oxidase is inhibited by its product, pyridoxal phosphate. This is not simple product inhibition, but involves binding at a specific inhibitor site on the enzyme. The normal intracellular concentration of free pyridoxal phosphate causes significant inhibition, which indicates that this is a physiologically important mechanism in the control of tissue pyridoxal phosphate.

Pyridoxine is rapidly phosphorylated in the liver and other tissues. Pyridoxal phosphate does not cross cell membranes, and uptake and efflux of the vitamin in most tissues is as pyridoxal. Pyridoxal phosphate is exported from the liver bound to albumin. Much of the free pyridoxal phosphate in the liver is hydrolyzed to pyridoxal, which is also exported, and circulates bound both to albumin and to hemoglobin in erythrocytes. Free pyridoxal remaining in the liver is rapidly oxidized to 4-pyridoxic acid, which is the main excretory product of the vitamin.

Extrahepatic tissues take up pyridoxal from the plasma. Pyridoxal phosphate is hydrolyzed to pyridoxal, which can cross cell membranes, by extracellular alkaline phosphatase, and then trapped intracellularly by phosphorylation.

Tissue concentrations of pyridoxal phosphate are controlled by the balance between phosphorylation and dephosphorylation. The activity of phosphatases acting on pyridoxal phosphate is greater than that of the kinase in most tissues. This means that pyridoxal phosphate that is not bound to enzymes will be dephosphorylated and hence will leave the cell by diffusion. Thus, there is little accumulation of pyridoxal phosphate in tissues, other than that which is bound to enzymes and other proteins (e.g., hormone receptors).

Free pyridoxal either leaves the cell or is oxidized to 4-pyridoxic acid by aldehyde dehydrogenase, which is present in all tissues, and also by hepatic and renal aldehyde oxidase. 4-Pyridoxic acid is the main excretory product of vitamin B₆, and its excretion reflects recent intake more than the state of underlying tissue reserves of the vitamin. Small amounts of pyridoxal and pyridoxamine are also excreted in the urine, although much of the active vitamin B₆ that is filtered at the glomerulus is reabsorbed in the kidney tubules. High doses of pyridoxine are largely excreted unchanged.

Storage and Body Reserves

There is no specific storage of vitamin B₆ in the body; as discussed in the Section on Metabolism and Transport, pyridoxal phosphate that is not bound to enzymes is rapidly dephosphorylated, oxidized to 4-pyridoxic acid, and excreted.

The total body pool of vitamin B₆ is of the order of 1000 μmol (167 mg), 15 $\mu\text{mol kg}^{-1}$ bodyweight. Approximately 80% of this is in muscle, associated with glycogen phosphorylase. This does not seem to function as a true reserve of the vitamin and is not released from muscle in times of deficiency, nor does it turn over as rapidly as pools in other tissues.

Muscle pyridoxal phosphate is released into the circulation (as pyridoxal) in starvation, as muscle glycogen reserves are exhausted, and there is less requirement for glycogen phosphorylase activity. Under these conditions, it is available for redistribution to other tissues, especially the liver and kidney,

to meet the increased requirement for transamination of amino acids for gluconeogenesis.

Metabolic Functions of Vitamin B₆

The metabolically active vitamer is pyridoxal phosphate, which is involved in many reactions of amino acid metabolism, where the carbonyl group is the reactive moiety, in glycogen phosphorylase, where it is the phosphate group that is important in catalysis, and in the release of hormone receptors from tight nuclear binding, where again it is the carbonyl group that is important.

The Role of Pyridoxal Phosphate in Amino Acid Metabolism

The various reactions of pyridoxal phosphate in amino acid metabolism (Figure 2) all depend on the same chemical principle: the ability to stabilize amino acid carbanions and hence to weaken bonds around the α -carbon of the substrate. This is achieved by the reaction of the α -amino group with the carbonyl group of the coenzyme to form a Schiff base (aldimine).

Pyridoxal phosphate is bound to enzymes, in the absence of the substrate, by the formation of an internal Schiff base to the ϵ -amino group of a lysine residue at the active site. Thus,

the first reaction between the substrate and the coenzyme is transfer of the aldimine linkage from this ϵ -amino group to the α -amino group of the substrate.

The ring nitrogen of pyridoxal phosphate exerts a strong electron-withdrawing effect on the aldimine, and this leads to weakening of all three bonds around the α -carbon of the substrate. In nonenzymic model systems, all the possible pyridoxal-catalyzed reactions are observed: α -decarboxylation, aminotransfer, racemization, and relevant side-chain elimination and replacement reactions. By contrast, enzymes generally show specificity for the reaction pathway followed; which bond is cleaved will depend on the orientation of the Schiff base relative to reactive groups of the catalytic site. However, a number of decarboxylases and enzymes that catalyze side-chain elimination reactions of amino acids undergo gradual inactivation as a result of catalyzing the half-reaction of transamination, leaving (catalytically inactive) pyridoxamine phosphate at the catalytic site.

α -Decarboxylation

If the electron-withdrawing effect of the ring nitrogen is primarily centered on the α -carbon-carboxyl bond, the result is decarboxylation of the amino acid with the release of carbon dioxide. The resultant carbanion is then protonated, and the primary amine corresponding to the amino acid is displaced

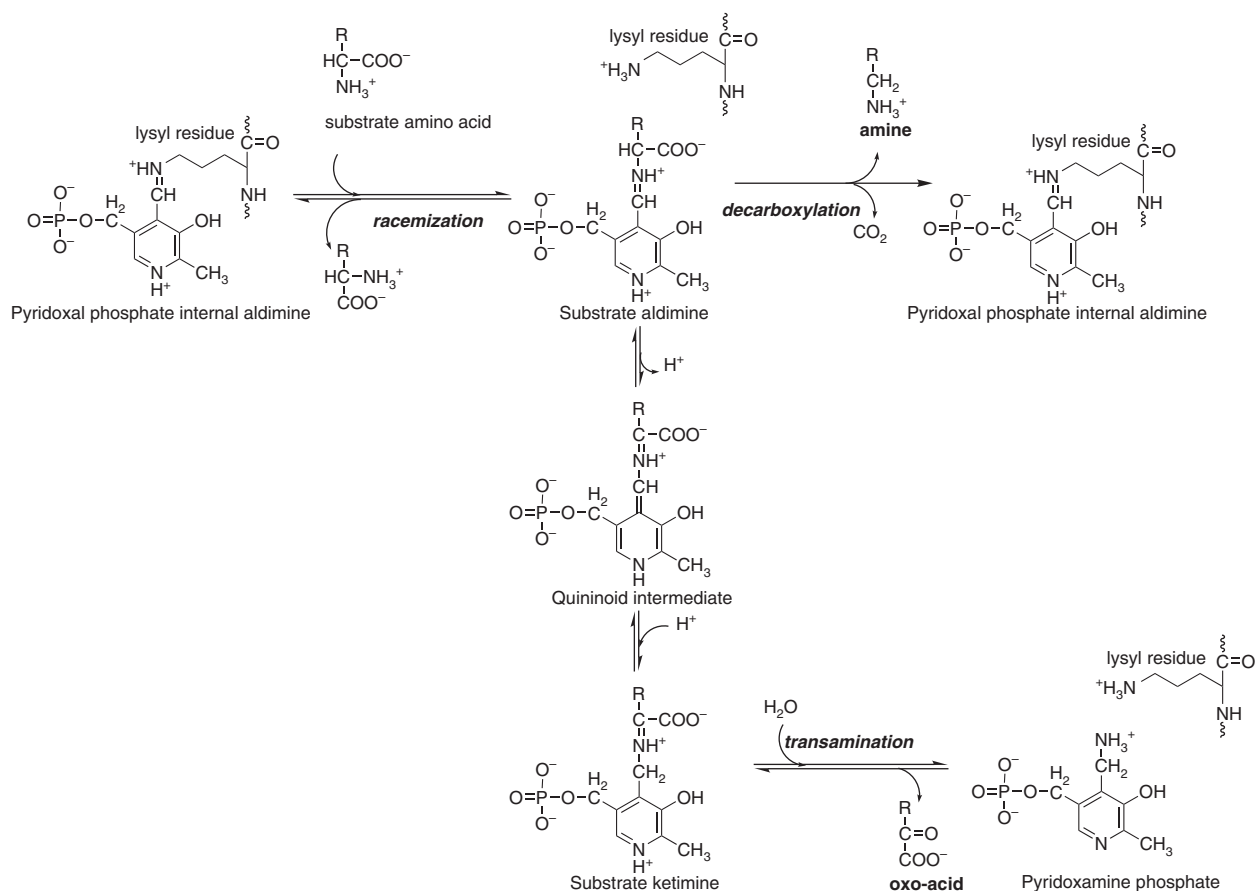


Figure 2 Roles of vitamin B₆ in amino acid metabolism.

by the lysine residue at the active site, with reformation of the internal Schiff base.

A number of the products of the decarboxylation of amino acids are important as neurotransmitters and hormones (5-hydroxytryptamine, the catecholamines dopamine, nor-adrenaline and adrenaline; histamine, and γ -aminobutyrate (GABA)) and as the diamines and polyamines involved in the regulation of DNA metabolism.

Racemization of Amino Acids

Deprotonation of the α -carbon of the amino acid leads to tautomerization of the Schiff base to yield a quinonoid ketimine. The simplest reaction that the ketimine can undergo is reprotonation at the now symmetrical α -carbon. Displacement of the substrate by the reactive lysine residue results in the racemic mixture of D- and L-amino acid.

Amino acid racemases have long been known to be important in bacterial metabolism, because several D-amino acids are required for the synthesis of cell wall mucopolysaccharides. D-Aspartate and D-serine are mammalian neurotransmitters, D-aspartate plays a role in signaling during brain development, and there are racemases in the hypothalamus and other brain regions.

Transamination

Hydrolysis of the α -carbon-amino bond of the ketimine formed by deprotonation of the α -carbon of the amino acid results in the release of the 2-oxo-acid corresponding to the amino acid substrate, and leaves pyridoxamine phosphate at the catalytic site of the enzyme. In this case, the internal Schiff base to the reactive lysine residue is not reformed. This is the half-reaction of transamination. The process is completed by the reaction of pyridoxamine phosphate with a second oxo-acid substrate, forming an intermediate ketimine, followed by the reverse of the reaction sequence shown in Figure 2, releasing the amino acid corresponding to this second substrate after displacement from the aldimine by the reactive lysine residue to reform the internal Schiff base.

Transamination (Figure 3) is of central importance in amino acid metabolism, providing pathways for the catabolism of all of the amino acids, except lysine, which does not

undergo transamination. Many of these reactions are linked to the amination of 2-oxoglutarate to glutamate or glyoxylate to glycine, which are substrates for oxidative deamination, reforming the oxo-acids. Transamination reactions also provide a pathway for the synthesis of those amino acids, for which there is an alternative source of the oxo-acid (the nonessential amino acids). Indeed, the nonessential amino acids can be defined as those whose oxo-acids can be formed other than from the amino acid itself.

Side-Chain Elimination and Replacement Reactions

The third bond in the Schiff base aldimine that can be labilized by the electron-withdrawing effect of the ring nitrogen of pyridoxal phosphate is that between the α -carbon and the side chain of the amino acids, resulting in a variety of $\alpha - \beta$ elimination and $\beta - \gamma$ replacement reactions.

The Role of Pyridoxal Phosphate in Glycogen Phosphorylase

Glycogen phosphorylase catalyzes the sequential phosphorylolytic cleavage of glycogen to release glucose-1-phosphate; it is thus the key enzyme in the utilization of muscle and liver reserves of glycogen.

Unlike other pyridoxal phosphate-dependent enzymes, in which the carbonyl group is essential for catalysis, the internal Schiff base between pyridoxal phosphate and lysine in glycogen phosphorylase is not broken during the reaction. The catalytic region of the coenzyme is the 5'-phosphate group. The initial stage in the phosphorylolytic cleavage of glycogen is protonation of the glycosidic oxygen of the polysaccharide by inorganic phosphate. The resultant oxycarbonium ion is stabilized by the inorganic phosphate. The role played by pyridoxal phosphate is that of a proton shuttle or buffer to stabilize the oxycarbonium-phosphate ion pair, allowing covalent binding of the phosphate to the oxycarbonium ion, to form glucose-1-phosphate.

The Role of Pyridoxal Phosphate in Steroid Hormone Action

Pyridoxal phosphate plays a role in modulating the action of those hormones that act by binding to a nuclear receptor

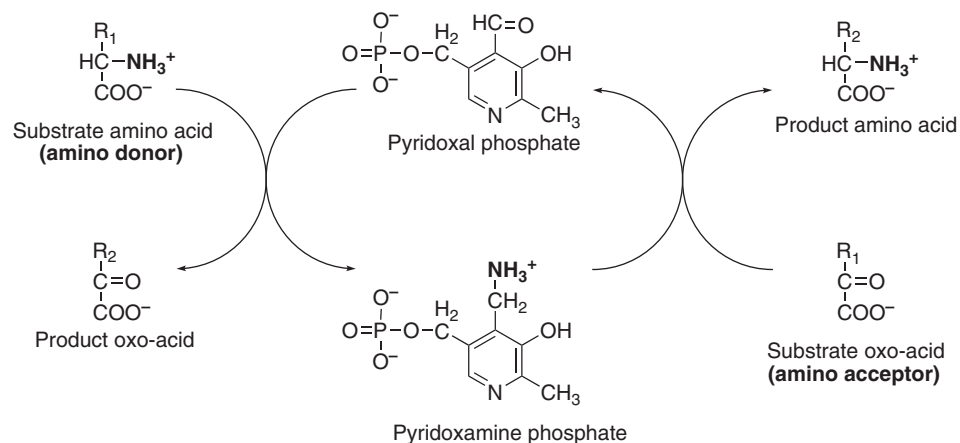


Figure 3 The role of vitamin B₆ in transamination reactions.

protein, inducing transcription of DNA, and hence regulating gene expression and protein synthesis. Such hormones include androgens, estrogens, progesterone, glucocorticoids, calcitriol (the active metabolite of vitamin D), retinoic acid and other retinoids, and thyroid hormone. Target-tissue specificity of hormone action is ensured by the presence of receptor proteins with a zinc finger motif, which are responsible for both nuclear uptake of the hormone and the interaction with DNA and nucleoproteins to initiate gene expression.

Pyridoxal phosphate reacts with a lysine residue in the receptor protein, and displaces the hormone–receptor complex from tight nuclear binding. *In vitro*, reaction with pyridoxal phosphate also inhibits the binding of receptor protein to isolated DNA and chromatin. The effect is specific for the phosphorylated vitamer, suggesting that there is a specific pyridoxal-phosphate-binding site on the receptor protein, and it occurs at low concentrations of pyridoxal phosphate, of the same order of magnitude as in tissues under normal conditions.

This suggests that pyridoxal phosphate acts as a cofactor in the release of hormone–receptor complexes from tight nuclear binding, resulting in the release of the receptor from the nucleus, termination of hormone action, and recycling receptor protein for further uptake of hormone.

In experimental animals, vitamin B₆ deficiency results in increased and prolonged nuclear uptake and retention of steroid hormones in target tissues, and there is enhanced sensitivity to hormone action. Deficient animals show greater induction of uterine peroxidase, and considerably greater suppression of the hypothalamic secretion of luteinizing hormone, by estrogens than do vitamin B₆-supplemented controls. In vitamin B₆-deficient male animals, there is an increased mitotic response of the prostate to low doses of testosterone. Deficient male animals have a higher activity of ornithine decarboxylase (an androgen-induced enzyme) in the liver and deficient females have higher renal ornithine transaminase (an estrogen-induced enzyme). The induction of hepatic tyrosine transaminase and tryptophan dioxygenase by glucocorticoids is also enhanced in vitamin B₆-deficient animals.

In a variety of cells in culture that have been transfected with a glucocorticoid, estrogen, or progesterone response element linked to a reporter gene, acute vitamin B₆ depletion (by incubation with 4-deoxypyridoxine) leads to a two-fold increase in the expression of the reporter gene in response to hormone action. Conversely, incubation of these cells with high concentrations of pyridoxal, leading to a high intracellular concentration of pyridoxal phosphate, results in a halving of the expression of the reporter gene in response to hormone stimulation.

Criteria of Adequacy and Assessment of Nutritional Status

Plasma Concentrations of the Vitamin

The fasting plasma concentration of either total vitamin B₆ or, more specifically, pyridoxal phosphate, is widely used as an

Table 1 Indices of vitamin B₆ nutritional status

	<i>Adequate status</i>
Plasma total vitamin B ₆	> 40 nmol (10 µg) l ⁻¹
Plasma pyridoxal phosphate	> 30 nmol (7.5 µg) l ⁻¹
Erythrocyte alanine aminotransferase activation coefficient	< 1.25
Erythrocyte aspartate aminotransferase activation coefficient	< 1.80
Erythrocyte aspartate aminotransferase	> 0.13 units (8.4 µkat) l ⁻¹
Urine 4-pyridoxic acid	> 3.0 µmol per 24 h > 1.3 mmol per mol creatinine
Urine total vitamin B ₆	> 0.5 µmol per 24 h > 0.2 mmol per mol creatinine
Urine xanthurenic acid after a 2-g tryptophan load	< 65 µmol per 24 h increase
Urine cystathionine after a 3-g methionine load	< 350 µmol per 24 h increase

index of vitamin B₆ nutritional status. The generally accepted criteria of adequacy are shown in **Table 1**.

Conditions that involve increased plasma activity of alkaline phosphatase may result in reduced plasma pyridoxal phosphate, without affecting the tissue concentrations of pyridoxal phosphate or vitamin B₆ nutritional status as assessed by other criteria. There is a compensatory increase in the circulating concentration of pyridoxal, which is the main form of tissue uptake. Despite the decline in plasma pyridoxal phosphate in pregnancy, which has been widely interpreted as indicating vitamin B₆ depletion, the plasma concentration of (pyridoxal phosphate plus pyridoxal) is unchanged. This suggests that determination of plasma pyridoxal phosphate alone may not be a reliable index of vitamin B₆ nutritional status.

Urinary Excretion of 4-Pyridoxic Acid

Approximately half of the normal dietary intake of vitamin B₆ is excreted as 4-pyridoxic acid. Urinary excretion of 4-pyridoxic acid will largely reflect recent intake of the vitamin rather than underlying nutritional status; the criteria for the assessment of 4-pyridoxic acid excretion are shown in **Table 1**.

Coenzyme Saturation of Transaminases

Various pyridoxal phosphate-dependent enzymes compete with each other for the available pool of coenzyme. Thus, the extent to which an enzyme is saturated with its coenzyme provides a means of assessing the adequacy of the body pool of coenzyme. This can be determined by measuring the activity of the enzyme before and after the activation of any apoenzyme present in the sample by incubation with pyridoxal phosphate added *in vitro*. Both erythrocyte aspartate and alanine transaminases are commonly used; the results are expressed as either the percentage stimulation of activity by added pyridoxal phosphate or the activation coefficient – the

ratio of activity with added coenzyme to that without added coenzyme.

It seems to be normal for a proportion of pyridoxal phosphate-dependent enzymes to be present as an inactive apoenzyme, without a coenzyme. This may be a mechanism for metabolic regulation. It is possible that increasing the intake of vitamin B₆, so as to ensure complete saturation of pyridoxal phosphate-dependent enzymes, may not be desirable.

Metabolic Loading Tests

A direct test of the adequacy of an individual's intake to meet his or her idiosyncratic metabolic requirement is the ability to metabolize a test dose of a substrate whose metabolism is dependent on the vitamin. For vitamin B₆, two metabolic loading tests can be used, although neither can be considered to be reliable for population studies of vitamin B₆ status.

The Tryptophan Load Test

The oxidative pathway of tryptophan metabolism is shown in **Figure 4**. Kynureninase is a pyridoxal phosphate-dependent enzyme, and in vitamin B₆ deficiency, its activity is lower than that of tryptophan dioxygenase. This means that there is a

considerable accumulation of both hydroxykynurenine and kynurenine, sufficient to allow greater than usual metabolic flux through kynurenine transaminase, resulting in increased formation of kynurenic and xanthurenic acids. Although kynurenine transaminase is also pyridoxal phosphate dependent, it is relatively unaffected in vitamin B₆ deficiency. Kynureninase is very sensitive to vitamin B₆ deficiency because it undergoes a slow inactivation as a result of catalyzing the half-reaction of transamination in addition to its normal reaction. The resultant enzyme with pyridoxamine phosphate at the catalytic site is catalytically inactive and can only be re-activated if there is an adequate concentration of pyridoxal phosphate to displace the pyridoxamine phosphate.

Xanthurenic and kynurenic acids are easy to measure in urine, so that the ability to metabolize a test dose of 2 g or 5 g of tryptophan has been widely adopted as a convenient and sensitive index of vitamin B₆ nutritional status. However, induction of tryptophan dioxygenase by glucocorticoid hormones will result in a greater rate of formation of kynurenine and hydroxykynurenine than the capacity of kynureninase, and will thus lead to increased formation of kynurenic and xanthurenic acids – an effect similar to that seen in vitamin B₆ deficiency. Such results may be erroneously interpreted as indicating vitamin B₆ deficiency in a variety of subjects whose

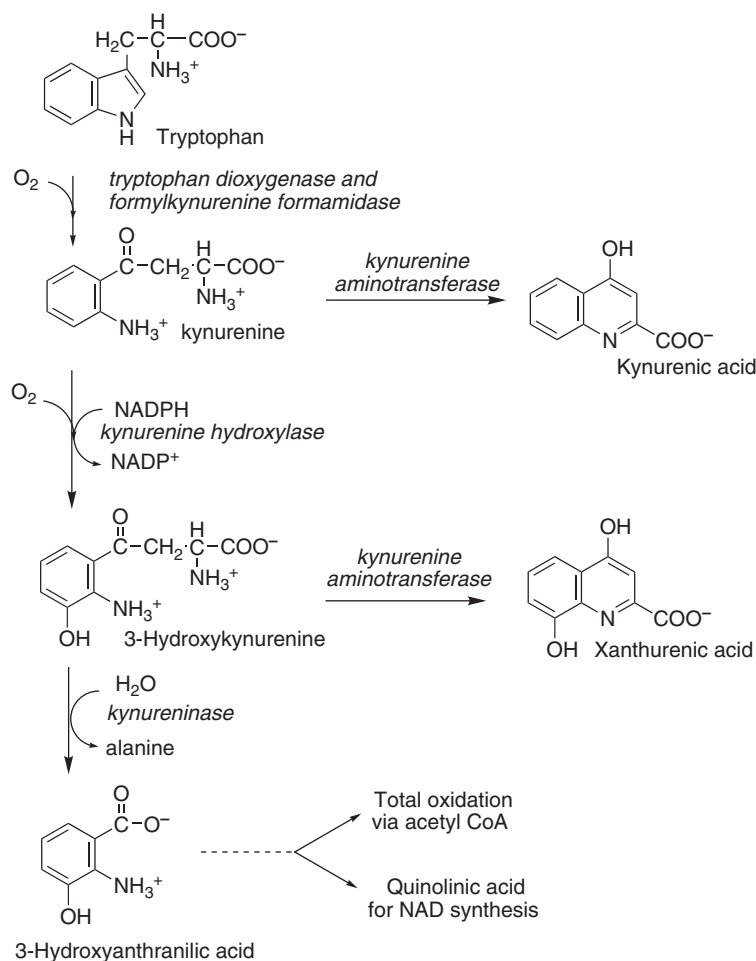


Figure 4 The oxidative pathway of tryptophan metabolism – the basis of the tryptophan load test for vitamin B₆ status.

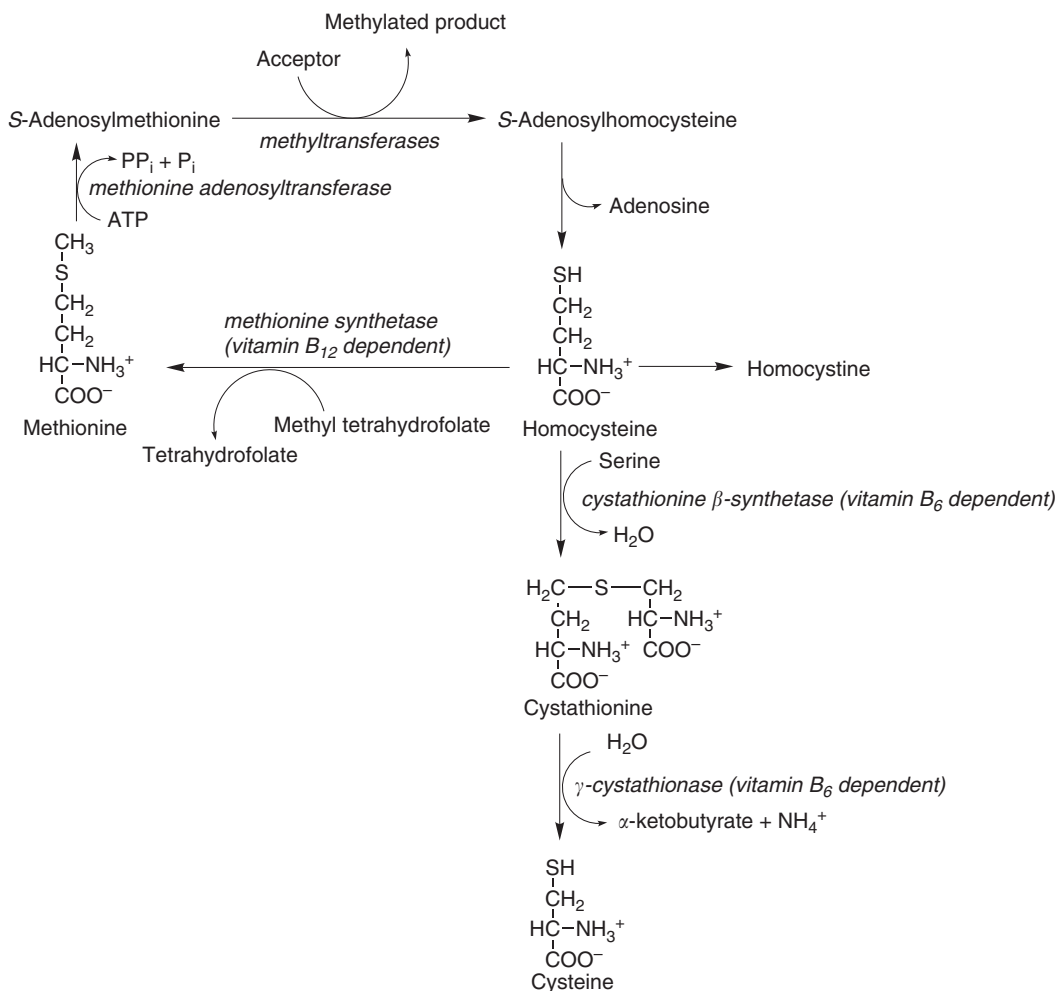


Figure 5 The pathway of methionine metabolism – the basis of the methionine load test for vitamin B₆ status.

problem is increased glucocorticoid secretion as a result of stress or illness, not vitamin B₆ deficiency.

Inhibition of kynureninase, for example, by estrogen metabolites, also results in the accumulation of kynurenine and hydroxykynurenine and hence increased formation of kynurenic and xanthurenic acids, again yielding results that falsely suggest vitamin B₆ deficiency. This has been widely, but incorrectly, interpreted as estrogen-induced vitamin B₆ deficiency – it is in fact simple competitive inhibition by estrogen metabolites of the enzyme that is the basis of the tryptophan load test.

There is normally a considerable excess of either apokynureninase or kynureninase that has undergone transamination and has pyridoxamine phosphate at the catalytic site, in the liver. This can be activated by (relatively high concentrations of) pyridoxal phosphate. The abnormalities of tryptophan metabolism associated with increased activity of tryptophan dioxygenase, or inhibition of kynureninase by estrogen metabolites, are thus corrected by the administration of high doses of vitamin B₆, although they are not in fact due to deficiency.

This means that although the tryptophan load test may be an appropriate index of status in controlled depletion/repletion studies to determine vitamin B₆ requirements, it is not an appropriate index of status in population studies.

The Methionine Loading Test

The metabolism of methionine, shown in **Figure 5**, includes two pyridoxal phosphate-dependent steps, catalyzed by cystathionine synthetase and cystathionase. In vitamin B₆ deficiency, there is an increase in the plasma concentration of homocysteine, and increased urinary excretion of cystathionine and homocysteine, both after a loading dose of methionine and under basal conditions. The ability to metabolize a test dose of methionine therefore provides an index of vitamin B₆ nutritional status. Because measurement of homocysteine and cystathionine is technically less easy than measurement of xanthurenic and kynurenic acids, the methionine load test has been less widely used than the tryptophan load test.

Approximately 10–25% of the population has a genetic predisposition to hyperhomocysteinemia, which is a risk

factor for atherosclerosis and coronary heart disease, as a result of polymorphism in the gene for methylenetetrahydrofolate reductase. As discussed below, there is little evidence that supplements of vitamin B₆ reduce fasting plasma homocysteine in these subjects, and like the tryptophan load test, the methionine load test may be an appropriate index of status in controlled depletion/repletion studies to determine vitamin B₆ requirements, but not in population studies.

Requirements and Recommendations

The total body pool of vitamin B₆ is of the order of 15 μmol (3.7 mg) kg^{-1} of bodyweight. Isotope tracer studies suggest that there is a turnover of approximately 0.13% per day, and hence a minimum requirement for replacement of 0.02 μmol (5 μg) kg^{-1} of bodyweight, approximately 350 $\mu\text{g day}^{-1}$ for a 70-kg adult.

Most studies of vitamin B₆ requirements have followed the development of abnormalities of tryptophan (and sometimes also methionine) metabolism during depletion and normalization during repletion with graded intakes of the vitamin. Although the tryptophan and methionine loading tests are unreliable as indices of vitamin B₆ nutritional status in field studies, under the controlled conditions of depletion/repletion studies, they do indeed provide a useful indication of the state of vitamin B₆ nutrition.

Although approximately 80% of the total body pool of vitamin B₆ is associated with muscle glycogen phosphorylase, this pool turns over relatively slowly. The major metabolic role of the remaining 20% of total body vitamin B₆, which turns over considerably more rapidly, is in amino acid metabolism. Therefore, *a priori*, it seems likely that protein intake will affect vitamin B₆ requirements.

Individuals maintained on (experimental) vitamin B₆-deficient diets develop abnormalities of tryptophan and methionine metabolism faster, and their blood vitamin B₆ declines more rapidly, when their protein intake is high. Similarly, during repletion of deficient subjects, tryptophan and methionine metabolism and blood vitamin B₆ are normalized faster at low than at high levels of protein intake.

Depletion/repletion studies suggest a mean requirement of 13 μg of vitamin B₆ per gram of dietary protein; the UK Reference Nutrient Intake (RNI) and the EU Population Reference Intake (PRI) are based on 14–16 $\mu\text{g g}^{-1}$ of protein. At average intakes of approximately 100 g of protein per day, this yields an RNI or a PRI of 1.4–1.6 mg of vitamin B₆.

The relevance of the studies involving low and high protein intakes to normal nutrition is unclear – they were conducted using extremes of protein intake: 40 g day^{-1} for the low protein intake, which is barely adequate to maintain nitrogen balance, and 150 g day^{-1} for the high intake, which is considerably higher than the average intakes of protein. The correlation of plasma pyridoxal phosphate with intake of pyridoxine in depletion/repletion studies is the same as that with pyridoxine per gram dietary protein, suggesting that protein intake within the normal range is not relevant to determining vitamin B₆ requirements. On this basis, the US Estimated Average Requirement (EAR) is 1.1 mg day^{-1} and the Recommended Daily Amount (RDA) is 1.3 mg.

Possible Benefits of Higher Levels of Intake

The identification of hyperhomocysteinemia as an independent risk factor in atherosclerosis and coronary heart disease has led to suggestions that higher intakes of vitamin B₆ than are currently considered adequate to meet requirements may be desirable. As shown in Figure 5, homocysteine is intermediate in methionine metabolism, and may have one of two metabolic fates: remethylation to methionine (a reaction that is dependent on vitamin B₁₂ and folic acid) or onward metabolism leading to the synthesis of cysteine (the vitamin B₆-dependent trans-sulfuration pathway).

Among elderly survivors of the Framingham study cohort (aged 67–96), hyperhomocysteinemia was most significantly correlated with low folate status, but there was also a significant association with low vitamin B₆ status. However, a number of studies have shown that while folate supplements lower fasting homocysteine in moderately hyperhomocysteinemic subjects, 10 mg day^{-1} vitamin B₆ has little additional effect.

Vitamin B₆ supplements do, however, reduce the peak plasma concentration of homocysteine following a test dose of methionine. This can probably be explained by the kinetics of the enzymes involved. The K_m of cystathionine synthetase is 10-fold higher than that of methionine synthetase. Under basal conditions, little homocysteine is metabolized by way of the trans-sulfuration pathway. It is only after a loading dose of methionine, when homocysteine increases to relatively high levels, that the activity of cystathionine synthetase, rather than the availability of its substrate, is limiting for trans-sulfuration.

It is thus unlikely that intakes of vitamin B₆ above amounts that are adequate to prevent metabolic signs of deficiency will be beneficial in lowering plasma concentrations of homocysteine, and in any case, most intervention studies of vitamin supplementation to lower plasma homocysteine have shown no effect on death from myocardial infarction, although some have shown a reduction in the incidence of stroke.

Vitamin B₆ Requirements of Infants

Estimation of the RDA for vitamin B₆ of infants presents a problem, and there is a clear need for further research to achieve a realistic estimate of infants' requirements. Human milk, which must be assumed to be adequate for infant nutrition, provides only approximately 40–100 $\mu\text{g l}^{-1}$ or 3–8 μg of vitamin B₆ per gram of protein – very much lower than the apparent requirement for adults. There is no reason why infants should have a lower requirement than adults, and indeed because they must increase their total body pool of the vitamin as they grow, they might be expected to have a proportionally higher requirement than adults.

A first approximation of the vitamin B₆ needs of infants emerged from studies of those who convulsed as a result of gross deficiency caused by overheated infant milk formula in the 1950s. At intakes of 60 $\mu\text{g day}^{-1}$, there was an incidence of convulsions of 0.3%. Provision of 260 $\mu\text{g day}^{-1}$ prevented or cured convulsions, but 300 $\mu\text{g day}^{-1}$ was required to normalize tryptophan metabolism. This is almost certainly a considerable overestimate of requirements, because pyridoxyllysine,

formed by heating the vitamin with proteins, has antivitamin activity, and would therefore result in a higher apparent requirement.

Based on the body content of 15 μmol (3.7 mg) of vitamin B₆ kg^{-1} of bodyweight, and the rate of weight gain, the minimum requirement for infants over the first 6 months of life would appear to be 100 μg (417 nmol) day^{-1} to establish tissue reserves.

Pharmacological Uses and Toxicity of Vitamin B₆ Supplements

Supplements of vitamin B₆ ranging from 25 to 500 mg day^{-1} , and sometimes higher, have been recommended for the treatment of a variety of conditions in which there is an underlying physiological or biochemical mechanism to justify the use of supplements, although in most cases, there is little evidence of efficacy. Such conditions include postnatal depression, depression, and other side effects associated with oral contraceptives, hyperemesis of pregnancy, and the premenstrual syndrome.

Supplements have also been used empirically, with little or no rational basis, and little or no evidence of efficacy, in the treatment of a variety of conditions, including acute alcohol intoxication, atopic dermatitis, autism, dental caries, diabetic neuropathy, Down's syndrome, Huntington's chorea, schizophrenia, and steroid-dependent asthma. There is some evidence that vitamin B₆ supplements in pregnancy reduce the incidence of dental caries.

Doses of 100 mg day^{-1} have been reported to be beneficial in the treatment of the carpal tunnel syndrome or what has been called tenosynovitis. Although some studies suggest that there is some beneficial effect, two systematic reviews show that vitamin B₆ is no more effective than placebo.

Vitamin B₆ has been reported to be effective in suppression of lactation, although other reports have shown no difference from placebo. Because the vitamin suppresses the increase in prolactin induced by treatment with the dopamine receptor antagonist pimozide, and because lactation is also suppressed by the dopamine agonist bromocriptine, it has been suggested that it acts to stimulate dopaminergic activity in the hypothalamus. However, it is more likely that its action is by reduction in target-tissue responsiveness to the steroid hormones that stimulate prolactin secretion.

Doses of 50–200 mg day^{-1} have an antiemetic effect, and the vitamin is widely used, alone or in conjunction with other antiemetics, to minimize the nausea associated with radiotherapy and to treat pregnancy sickness. There is no evidence that vitamin B₆ has any beneficial effect in pregnancy sickness, nor those women who suffer from morning sickness have lower vitamin B₆ nutritional status than other pregnant women. There have been reports of a teratogenic effect of vitamin B₆ used to treat morning sickness, but all of these involved the use of the vitamin together with the sedative Debendox, and there is no evidence that vitamin B₆ itself is teratogenic. However, because it downregulates responsiveness to steroid hormones and retinoids, it is possible that high levels of vitamin B₆ intake may affect embryonic or fetal development.

Vitamin B₆ and the Side Effects of Oral Contraceptives

Although estrogens do not cause vitamin B₆ deficiency, the administration of vitamin B₆ supplements has beneficial effects on some of the side effects of both administered and endogenous estrogens. The supplements act in two main areas: in normalizing glucose tolerance and as an antidepressant.

Impairment of glucose tolerance is common in pregnancy and might indeed be severe enough to be classified as gestational diabetes, which generally resolves at parturition, although in some subjects it may persist, pregnancy having been the trigger for the development of maturity-onset diabetes. High-estrogen oral contraceptives may also cause impaired glucose tolerance. This seems to be the result of increased tissue and blood concentrations of xanthurenic acid, because of the inhibition of kynureninase by estrogen metabolites. Xanthurenic acid forms a complex with insulin, which has little or no hormonal activity. Vitamin B₆ supplements may have a beneficial effect on glucose tolerance by activating apokynureninase or kynureninase that has been inactivated by undergoing transamination.

One of the relatively common side effects of high-estrogen oral contraceptives is depression, affecting approximately 6% of women in some studies. This frequently responds well to the administration of relatively large amounts of vitamin B₆ (generally more than 40 mg day^{-1}). Postnatal depression also responds to similar supplements in some studies.

Again, this does not seem to be due to correction of vitamin B₆ deficiency, but rather to a direct effect of pyridoxal phosphate on the metabolism of tryptophan. High concentrations of pyridoxal phosphate attenuate the response to glucocorticoid hormones; tryptophan dioxygenase is a glucocorticoid-induced enzyme, and thus its synthesis and activity will be reduced by high intakes of vitamin B₆. This reduces the oxidative metabolism of tryptophan and increases the amount available for the synthesis of 5-hydroxytryptamine in the brain. Increased brain 5-hydroxytryptamine synthesis has a mood-elevating effect.

Vitamin B₆ in the Premenstrual Syndrome

The studies showing a beneficial action of vitamin B₆ in overcoming depression associated with oral contraceptives have led to the use of the vitamin in depression and other pathology associated with endogenous estrogens, in the premenstrual syndrome. There is no evidence of poorer vitamin B₆ nutritional status in women who suffer from premenstrual syndrome.

There are few well-controlled studies of the effects of vitamin B₆ in premenstrual syndrome. In general, those who have been properly controlled report little benefit from doses between 50 and 200 mg day^{-1} compared with placebo, although some studies do claim a beneficial effect. Interestingly, meta-analysis of controlled cross-over trials shows that whichever treatment is used second, active vitamin or placebo, is (marginally) more effective. There is no obvious explanation for this observation.

Despite the lack of evidence of efficacy, vitamin B₆ is widely prescribed (and self-prescribed) for the treatment of premenstrual syndrome.

Toxicity of Vitamin B₆

Animal studies have demonstrated the development of signs of peripheral neuropathy, with ataxia, muscle weakness, and loss of balance in dogs given 200 mg pyridoxine HCl per kg of bodyweight for 4075 days, and the development of a swaying gait and ataxia within 9 days at a dose of 300 mg kg⁻¹ of bodyweight. At a dose of 50 mg kg⁻¹ of bodyweight, there are no clinical signs of toxicity; but histologically, there is a loss of myelin in the dorsal nerve roots. At higher doses, there is more widespread neuronal damage, with loss of myelin and degeneration of sensory fibers in peripheral nerves, the dorsal columns of the spinal cord, and the descending spinal tract of the trigeminal nerve. The clinical signs of vitamin B₆ toxicity in animals regress after withdrawal of these massive doses, but sensory nerve conduction velocity, which decreases during the development of the neuropathy, does not recover fully. The mechanism of the neurotoxic action of vitamin B₆ is unknown.

The development of sensory neuropathy has been reported in patients taking 2–7 g of pyridoxine HCl per day. Although there was residual damage in some patients, withdrawal of these extremely high doses resulted in a considerable recovery of sensory nerve function. Other reports have suggested that intakes as low as 50 mg day⁻¹ are associated with neurological damage, although these have been based on patients reporting symptoms rather than on detailed neurological examination.

Based on studies including detailed neurological examination that show no adverse effects of intakes up to 200 mg day⁻¹, the US Institute of Medicine has set a tolerable upper level of intake of pyridoxine from supplements of 100 mg day⁻¹. The UK Food Standards Agency adopted a more cautious approach and, by extrapolation from animal studies, set a safe upper level for daily consumption over a lifetime of 10 mg day⁻¹, while noting that there is no evidence of adverse effects at doses up to 200 mg day⁻¹.

Vitamin B₆ Deficiency

Gross clinical deficiency of vitamin B₆ is more or less unknown. The vitamin is widely distributed in foods, and intestinal flora synthesize relatively large amounts, at least some of which is believed to be absorbed and hence available.

In vitamin B₆-deficient experimental animals, there are more or less specific skin lesions (e.g., acrodynia in the rat) and fissures or ulceration at the corners of the mouth and over the tongue, as well as a number of endocrine abnormalities, defects in the metabolism of tryptophan, methionine and other amino acids, hypochromic microcytic anemia (the first step of heme biosynthesis is a pyridoxal phosphate-dependent reaction), changes in leukocyte count and activity, a tendency of epileptiform convulsions, and peripheral nervous system damage resulting in ataxia and sensory neuropathy. Classical clinical signs of human vitamin B₆ deficiency (which was almost certainly multivitamin deficiency) include seborrheic dermatitis, depression, and confusion as well as hypochromic microcytic anemia. In controlled studies of vitamin B₆ depletion, electroencephalogram abnormalities develop after a few days and resolve on repletion with the vitamin.

There was an outbreak of vitamin B₆ deficiency in the early 1950s among infants who had been fed a milk formula that had undergone severe heating in manufacture. The probable result of this was the formation of pyridoxyllysine by reaction between pyridoxal phosphate and the ε-amino groups of lysine in proteins. In addition to a number of metabolic abnormalities, many of the affected infants convulsed. They responded to the administration of vitamin B₆ supplements.

Investigation of the neurochemical basis of the convulsions in vitamin B₆ deficiency helped to elucidate the role of γ-aminobutyrate (GABA) as a neurotransmitter; GABA is synthesized by the decarboxylation of glutamate. More recent studies have suggested that the accumulation of hydroxykynurenine in the brain may be the critical factor precipitating convulsions in deficiency; GABA is depleted in the brains of deficient adult and neonate animals, whereas hydroxykynurenine accumulation is considerably more marked in neonates than in adults – only neonates convulse when having vitamin B₆ deficiency. GABA depletion may be a necessary but not a sufficient condition for convulsions when having vitamin B₆ deficiency.

Vitamin B₆ Dependency Syndromes

A small number of cases have been reported of patients with genetic defects that result in an abnormally high requirement for vitamin B₆ in order to maintain the activity of the affected enzyme (Table 2). Such vitamin B₆ dependency syndromes have been reported in cases of xanthurenic aciduria, homocystinuria, hypochromic sideroblastic anemia, ornithinemia, and infantile convulsions. The molecular basis of the defects appears to be a severely impaired affinity of the affected enzyme for its cofactor, and patients respond well to doses of 500–1000 mg of vitamin B₆ per day. Apart from the affected enzyme, other biochemical indices of vitamin B₆ nutritional status are normal in these patients. Interestingly, there are very few reports of peripheral neuropathy among such patients treated with high doses of vitamin B₆ for many years.

Groups at Risk of Deficiency

A number of studies have shown that between 10% and 20% of the apparently healthy population have low plasma concentrations of pyridoxal phosphate or an abnormal

Table 2 Vitamin B₆-responsive inborn errors of metabolism

	<i>Enzyme affected</i>
Convulsions of the newborn	Unknown
Cystathioninuria	Cystathionase (see Figure 5)
Gyrate atrophy with ornithinuria	Ornithine-δ-aminotransferase
Homocystinuria	Cystathionine synthase (see Figure 5)
Primary hyperoxaluria, type I	Peroxisomal alanine-glyoxylate transaminase
Sideroblastic anemia	δ-Aminolevulinate synthase (↓ heme synthesis)
Xanthurenic aciduria	Kynureninase (see Figure 4)

erythrocyte transaminase activation coefficient, suggesting vitamin B₆ inadequacy or deficiency. In most studies, only one of these indices of vitamin B₆ nutritional status has been assessed. Where both indices have been assessed, although each shows approximately 10% of the population apparently inadequately provided with vitamin B₆, few of the subjects show inadequacy by both criteria. Approximately 10–25% of the US population has an intake of vitamin B₆ that is below the EAR, rising to almost 50% of pregnant women.

There is a decrease in the plasma concentration of vitamin B₆ with increasing age, and some studies have shown a high prevalence of abnormal transaminase activation coefficients in elderly subjects, suggesting that the elderly may be at risk of vitamin B₆ deficiency. It is not known whether this reflects an inadequate intake, a greater requirement, or changes in the tissue distribution and metabolism of the vitamin with increasing age, but the (US) EAR and RDA are higher for individuals over the age of 50.

Drug-Induced Vitamin B₆ Deficiency

A number of drugs that react with carbonyl compounds are capable of causing vitamin B₆ deficiency on prolonged use. These include the antituberculosis drug isoniazid (iso-nicotinic acid hydrazide), penicillamine, and the anti-parkinsonian drugs, benserazide and carbidopa. In general, the main effect is impairment of tryptophan metabolism by inhibition of kynureninase, and hence the development of the niacin deficiency disease, pellagra. The condition therefore responds to the administration of either vitamin B₆ or niacin. Isoniazid also causes peripheral neuropathy, which responds to vitamin B₆ supplements, but not to niacin.

Estrogens and Vitamin B₆ Nutritional Status

There have been many reports of abnormal tryptophan metabolism in women taking estrogens as oral contraceptives and menopausal hormone replacement therapy. These have been widely interpreted as evidence of estrogen-induced vitamin B₆ deficiency. However, this is the result of inhibition of kynureninase by estrogen metabolites, not estrogen-induced deficiency of the vitamin. Where other indices of vitamin B₆ status have been reported, they have been generally unaffected by contraceptive use, again suggesting an effect on tryptophan metabolism *per se*, rather than on vitamin B₆ nutritional status.

In many cases, the metabolism of tryptophan has been normalized by the administration of vitamin B₆ supplements of the order of 20–50 mg day⁻¹, compared with an RDA of 1.4–1.6 mg day⁻¹. It was noted earlier that there is an apparent excess of apokynureninase in the liver and therefore the administration of vitamin B₆ supplements will increase

kynurenine metabolism even when there is no preexisting deficiency.

See also: Amino Acids: Metabolism. Cofactors: Organic. Dietary Guidelines, International Perspectives. Drug–Nutrient Interactions. Homocysteine. Niacin and Pellagra. Nutrient–Gene Interactions: Molecular Aspects. Nutritional Requirements of Infants. Riboflavin

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VITAMIN B₁₂

Physiology, Dietary Sources, and Requirements

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Glossary

Cobalamins Compounds that are collectively known as vitamin B₁₂ and contain cobalt in a corrin ring. The cobalt can be attached to one of several sidegroups, including cyanide. Cyanocobalamin is the stable form of the vitamin manufactured for use as supplements and a food fortificant.

Macrocytosis When red blood cells (erythrocytes) are larger than normal.

Transcobalamins Carrier proteins which bind and transport vitamin B₁₂.

Introduction

The cobalamins are a group of closely related and interconvertible compounds with a complex structure that are collectively known by the common name of vitamin B₁₂. Recommended biochemical nomenclature restricts the term 'vitamin B₁₂' for the particular form of cobalamin known as cyanocobalamin. All cobalamins belong to the broader family of corrinoids, which share the characteristic of consisting of a planar four-membered pyrrole ring (corrin ring) containing a

central cobalt atom. Cobalamins are distinguished from other corrinoids by possessing both alpha (lower) and beta (upper) axial ligands that are attached to the central cobalt atom (**Figure 1**). The lower ligand consists of a base (5,6-dimethylbenzimidazole) attached to a sugar (ribose), which in turn is attached to a phosphate and an amino-propyl group that ultimately is tethered back to the corrin ring. In the naturally occurring cobalamins the upper ligand is variably a cyano-, hydroxo-, aquo-, methyl-, or adenosylgroup, giving rise to the correspondingly named chemical forms of the vitamin.

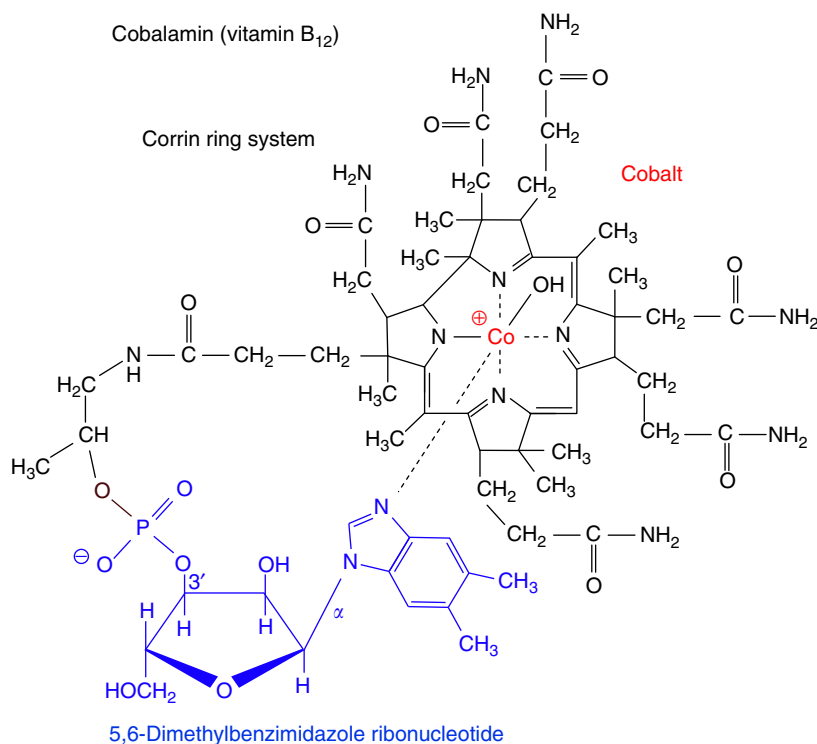


Figure 1 Chemical structure of Cobalamin.

Of these, methylcobalamin and deoxyadenosylcobalamin are the forms that function as coenzymes for metabolic reactions. These are highly sensitive to destruction by light. Cyanocobalamin is a stable form and is therefore used in therapeutic preparations. Hydroxo or aquocobalamin are intermediates during the synthesis of the coenzyme forms. Other forms including sulphito-, nitrito-, and glutathionyl derivatives of cobalamin have also been described, but their precise role in metabolism is not known.

Biochemistry and Metabolic Functions

Only two reactions in humans and other animals are known to require cobalamin (Figure 2). One, isomerization of methylmalonyl CoA, which requires deoxyadenosylcobalamin, catalyzed by the enzyme methylmalonyl CoA mutase and is mitochondrial. The other reaction is the transmethylation of homocysteine by 5-methyl-tetrahydrofolate to methionine, catalyzed by the enzyme methionine synthase (*N*⁵-methyl homocysteine methyl transferase), which requires methylcobalamin as coenzyme and is located in the cytosol. It is through their essential roles in this important metabolic reaction that cobalamin and folate interact and are linked with respect to their importance in nutrition. In addition, there are major similarities in the effects of their deficiencies in humans. These will be discussed below. Considering this 'metabolic crossroad' for the two vitamins, it may be pointed out that without adequate supplies of both nutrients, the synthesis of methionine and its derivative *S*-adenosylmethionine (SAM) is disrupted, with consequent profound effects on normal cellular function. Methionine is a key and essential amino acid and normal supply depends critically on recycling through the remethylation pathway (Figure 3). Moreover, SAM is the universal methyl donor, essential for more than 100 transmethylation reactions involving amino acid, nucleotide,

neurotransmitter, and phospholipid metabolism as well as detoxification reactions.

Apart from methionine the other product of the methionine synthase reaction, which is almost completely irreversible, is tetrahydrofolate (THF). This constitutes the first step by which folate enters bone marrow and other cells from plasma, for its conversion into the various intracellular forms of reduced folate containing a series of one-carbon substituents (Figure 3). The active forms of these folate congeners are all polyglutamated by an enzyme, folate polyglutamate synthetase, which cannot use methyl-THF as substrate. THF is the obligate substrate for polyglutamate addition. Consequently, when the methionine synthase reaction is blocked as a result of cobalamin deficiency, there is 'THF starvation'. Methyl-THF accumulates in the plasma, although intracellular folate concentrations fall due to failure of formation of the critical intracellular folate polyglutamates because of 'methyl-folate trapping'. This theory explains the abnormalities of folate metabolism, which occur in cobalamin deficiency (high concentrations of serum folate, low red blood cell folate), and also why the anemia that occurs in cobalamin deficiency will temporarily or partially respond to folic acid in large doses. The explanation of why the serum cobalamin falls in folate deficiency may also be related to impairment of the methionine synthase reaction resulting from reduced formation of methylcobalamin, the predominant circulating form of cobalamin in plasma.

Physiology

The recommended dietary allowance (RDA) for cobalamin in adults, proposed by the Food and Nutrition Board of the Institute of Medicine in 1997, is 2–4 µg. Cobalamins do not occur in plants and are synthesized by certain bacteria, fungi, and algae, which constitute the ultimate source of all

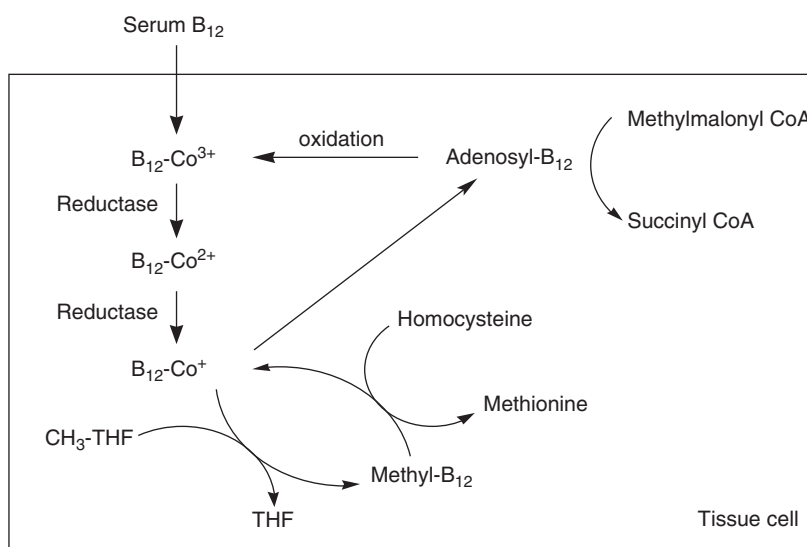


Figure 2 Mammalian intracellular reactions requiring cobalamin. Conversion of methylmalonyl CoA to succinyl CoA is mitochondrial and requires cobalamin in the form of adenosyl-B₁₂. Conversion of homocysteine to methionine is cytosolic and requires cobalamin in the form of methyl-B₁₂.

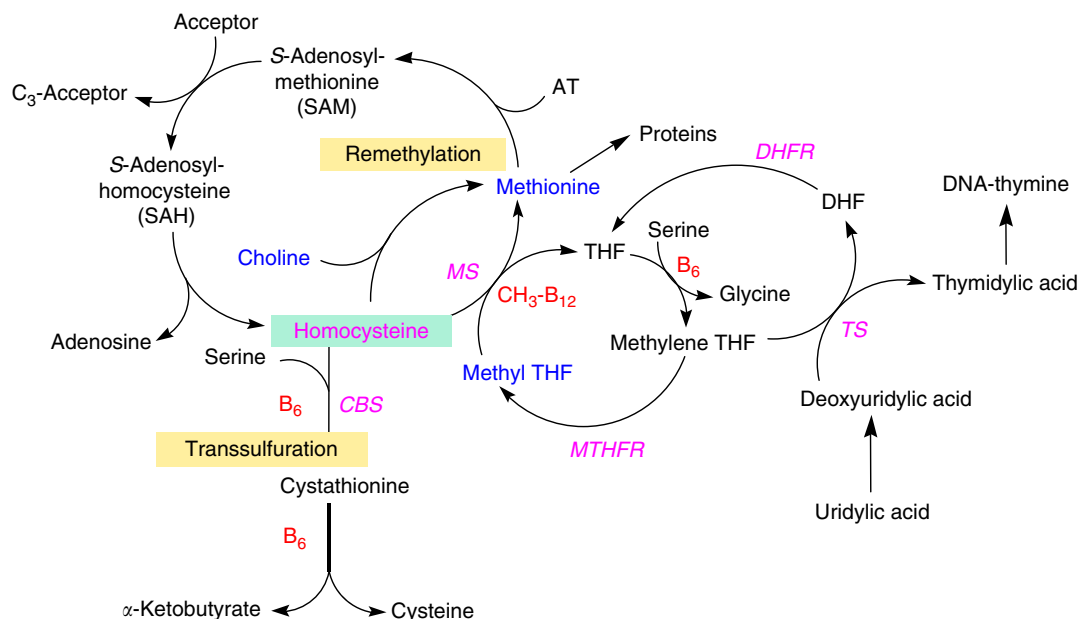


Figure 3 The remethylation and transsulfuration pathways of homocysteine metabolism showing the role of cobalamin in the form of methyl-B₁₂ (CH₃-B₁₂) in the production of methionine. Other B vitamins (B₆, folate) and required enzymes for related pathways are shown. MS, methionine synthase; MTHFR, methylene tetrahydrofolate reductase; TS, thymidylate synthase; DHFR, dihydrofolate reductase; CBS, cystathionine beta synthase; THF, tetrahydrofolate.

cobalamins found in nature. Cobalamins enter the food chain through herbivorous animals that harbor cobalamin-producing microorganisms in their upper gastrointestinal tract (e.g., the 'first stomach' of ruminants). Consumption of the meat or products of these animals supplies cobalamin in the diet for other animals. Dietary sources of cobalamin in humans are restricted to meat, poultry, fish, shellfish, eggs, and dairy products. Cobalamin is resistant to destruction by cooking, unlike the heat labile folates. On account of the exceedingly small daily requirement for cobalamin, in the order of 2–3 µg, and the relatively large body store of the vitamin (3000–5000 µg in individuals in developed countries), complete absence of intake or absorption of cobalamin is preceded by a long lag of up to 3–5 years before depletion of body cobalamin stores reaches a critical point that begins to result in the manifestations of cobalamin deficiency. This is not the case in developing countries where the onset of depletion may be much more rapid because of initially lower stores. Still, complete lack of dietary intake of cobalamin is somewhat rare and occurs only in strict vegans who shun all animal foods, including dairy products and eggs.

Cobalamin absorption is a complex mechanism that consists of several steps and involves several protein chaperones and receptors, defects of which can result in reduced or absent uptake of dietary cobalamin. The related and continuous processes of ingestion, digestion, and absorption of cobalamin comprising the assimilation of the vitamin are arbitrarily divided into six steps. During the first step of mastication and swallowing of food, dietary cobalamin becomes mixed with a binding protein derived from saliva belonging to the family of cobalamin-binding proteins known as haptocorrins. Cobalamin in foods is generally complexed to proteins that must first be digested to release the bioavailable vitamin.

In the second step release of cobalamin takes place largely in the stomach, under the influence of gastric hydrochloric acid and proteolytic digestion by pepsin. It is during this process and in the acid environment of the stomach that salivary haptocorrin preferentially binds and protects food cobalamin. Another specific cobalamin-binding protein, known as intrinsic factor, is secreted by the parietal cells of the stomach, but is unable to bind the cobalamin still tightly complexed to haptocorrin. During the third step, which occurs in the duodenum, cobalamin is released from its complex with haptocorrin through the combined effects of pancreatic bicarbonate, which neutralizes the gastric acid, and the proteolytic action of the enzymes trypsin and chymotrypsin that digest haptocorrin and thus enable the binding of the free cobalamin by gastric intrinsic factor. In the fourth step, the intrinsic factor-cobalamin complex, having traversed the full length of the small intestine, arrives at the luminal surface of the terminal ileum. There, it comes in contact with specialized receptors. In the presence of calcium, the complex attaches to the receptor consisting of two distinct proteins coded by the genes cubulin and amnionless that is necessary to complete the assimilation process. Both proteins are essential for the internalization of the intrinsic factor-B₁₂ complex through the process of receptor-mediated endocytosis. Through this process, cobalamin together with intrinsic factor is escorted by the receptor and taken into lysosomes. Here the intrinsic factor-cobalamin complex is released and intrinsic factor is degraded through the action of acid hydrolysis by lysosomal peptidases. The final fifth step is poorly understood but first involves the release from lysosomes and then the metabolism of cobalamin to its methyl and deoxyadenosyl derivatives. It is currently believed that cobalamin enters the plasma in the form of methylcobalamin. The assimilation of food containing

vitamin B₁₂ is a lengthy process, as evidenced by the 6–8 h taken for orally administered cobalamin to first appear in the plasma and several additional hours for the process to be completed. Recent evidence indicates that cobalamin leaves the cell through an exit portal that is part of the ABC drug transport system, ABCC1 (also known as the Multidrug Resistance Protein, MRP1), present in the basolateral membrane of the intestinal epithelium as well as in other cells.

After cobalamin enters the plasma via the MRP1 it becomes bound to the cobalamin binding protein, transcobalamin (previously known as transcobalamin II to distinguish it from transcobalamins I and III which, together with the salivary cobalamin binding protein and other cobalamin binders present in secretions are now referred to collectively as the haptocorrins). The properties of transcobalamin and the haptocorrins are summarized in **Table 1**. The fraction of cobalamin bound to transcobalamin accounts for only 10–30% of the total plasma cobalamin. The major residual fraction of the plasma cobalamin is attached to haptocorrin. The function of haptocorrins is not known, but rapidly proliferating cells including bone marrow precursors can obtain cobalamin only from transcobalamin. Consequently, the critical fraction of the serum cobalamin is the transcobalamin-bound portion, known as holotranscobalamin. Conditions that alter the amount or distribution of cobalamin on these binding proteins can critically affect delivery and transport. Therefore, conditions that lead to an increase in haptocorrins,

such as chronic granulocytic leukemia, characterized by markedly increased numbers of granulocytes (haptocorrins are produced in granulocytes), can give rise to an apparently normal serum cobalamin level even in patients who have severe underlying cobalamin deficiency, through redistribution of reduced body cobalamin stores. Conversely, insufficient holotranscobalamin can result in cobalamin deficiency even if the total serum cobalamin level is apparently normal. This occurs in infants and children affected by congenital transcobalamin deficiency, which is associated with severe megaloblastic anemia. Levels of transcobalamin may be affected by a number of factors. Lowering of holotranscobalamin can result in tissue cobalamin deficiency with a normal total serum cobalamin level. Now that sufficiently sensitive and robust methods are available to measure holotranscobalamin levels in serum, several studies have reported that there is a good inverse correlation between serum holotranscobalamin concentration and serum levels of the metabolites methylmalonic acid and homocysteine, which are described below.

Causes, Mechanisms, and Effects of Cobalamin Deficiency

There are several causes of cobalamin deficiency that range in severity and frequency of occurrence. These are summarized in **Table 2**. In general, causes of cobalamin deficiency can be

Table 1 Properties of human plasma cobalamin binding proteins

	<i>Haptocorrins (TC I + III)</i>	<i>Transcobalamin (TC II)</i>
Source	Granulocytes	Endothelial cells
Transport functions	Storage, excretion of B ₁₂ analogs, antimicrobial	Cellular B ₁₂ uptake
Binding specificity	Low specificity, binds B ₁₂ analogs	Binds B ₁₂ with higher specificity
Membrane receptors	Nonspecific asialoglycoprotein receptors on hepatocytes	Specific receptors on most cells
Saturation	High (mainly 'holo')	Low (mainly 'apo')
Fraction of total B ₁₂	70–90%	10–30%
Plasma clearance	Slow ($t_{1/2}$ ~ 10 days)	Rapid ($t_{1/2}$ ~ 6 min)
Molecular Weight	60 000	38 000–45 000

Table 2 Causes of Vitamin B₁₂ deficiency

1. Dietary^a
 - a. Veganism or very low intake of animal source foods
2. Gastric
 - a. Atrophic gastritis and food B₁₂ malabsorption
 - b. Autoimmune gastritis/gastric atrophy (classical pernicious anemia)
 - c. Extensive gastric disease or resection
3. Ileal
 - a. Extensive ileal disease (Crohn disease, inflammatory bowel disease, tuberculous enteritis), or resection for these diseases
 - b. Luminal disturbances (chronic pancreatic disease and gastrinoma) and parasites (giardiasis, bacterial overgrowth, and fish tapeworm).
4. Chemical/Drug
 - a. Nitrous oxide
 - b. PAS, metformin, colchicine
5. Congenital/Inherited
 - a. Intrinsic factor deficiency/defect ('Juvenile' pernicious anemia).
 - b. Intrinsic factor receptor deficiency/defect (Immerslund-Gräsbeck disease).
 - c. Transcobalamin (TC) II deficiency or polymorphisms^a
 - d. Cobalamin mutants (C-G).

^aSuboptimal cobalamin status caused by lowered intake or TC polymorphisms can predispose to more rapid onset of deficiency when other pathological causes of cobalamin deficiency occur.

divided into those caused by absent or markedly reduced dietary intake and those caused by malabsorption, either gastric or ileal. The most frequent cause of severe and clinically important cobalamin deficiency is pernicious anemia, caused by an autoimmune destruction of the gastric mucosa with consequent failure of intrinsic factor production. Less common causes include chemical inactivation and inherited defects in cobalamin absorption or metabolism.

In all situations resulting from impairment of cobalamin absorption, the time to onset of deficiency depends on several factors, including the size of the body store, the extent of impairment of absorption (partial or complete), and, in diseases like pernicious anemia and others affecting the small intestine, the rate of progression of the disease. In general, however, cobalamin deficiency resulting from malabsorption develops sooner than is the case in the dietary deficiency encountered among vegans. This difference may be explained by the existence of a considerable enterohepatic recirculation of cobalamin. Biliary cobalamin is efficiently reabsorbed in vegans compared with patients with pernicious anemia or other forms of malabsorption, because the intrinsic factor-dependent mechanism is intact.

Deficiency of cobalamin, when severe, affects all rapidly growing (DNA-synthesizing) tissues. After the marrow, the next most affected tissues are the epithelial cell surfaces of the gastrointestinal tract (mouth, stomach, and the small intestine). Affected cells are large, with increased numbers of multinucleated and apoptotic dying cells. The gonads are also affected and infertility occurs in patients with cobalamin deficiency. Cobalamin deficiency may also be associated with skin hyperpigmentation and has also been described in association with osteoporosis with reduced plasma bone-derived alkaline phosphatase and osteocalcin levels in the plasma.

Cobalamin deficiency may cause bilateral peripheral neuropathy or degeneration (demyelination) of the posterior and pyramidal tracts of the spinal cord and, less frequently, atrophy of the optic nerve or cerebral symptoms. Cobalamin-deficient patients typically display sensory disturbances (paraesthesiae), muscle weakness, difficulty in walking and sometimes dementia, psychotic disturbances or visual impairment. Long-term nutritional cobalamin deficiency in infancy leads to poor brain development and impaired intellectual development. The effects of cobalamin deficiency on the blood and on the nervous system may occur separately or in combination and their severity is often inversely rather than directly correlated. The biochemical basis for cobalamin neuropathy, however, remains obscure. Its occurrence in the absence of methylmalonic aciduria in TCII deficiency, and in monkeys given the anesthetic agent nitrous oxide, suggests that the neuropathy is related to a defect in homocysteine-methionine conversion. Accumulation of S-adenosylhomocysteine in the brain, resulting in inhibition of transmethylation reactions has been suggested as the mechanism.

Psychiatric disturbance is common in cobalamin deficiencies. Like the neuropathy, this has been attributed to a failure of the synthesis of SAM, due to reduced conversion of homocysteine to methionine. SAM is needed for methylation of biogenic amines (e.g., dopamine), as well as of proteins, phospholipids, and neurotransmitters in the brain.

Diagnosis of Cobalamin Deficiency

Cobalamin deficiency is suspected in individuals who display the typical manifestations of deficiency of the vitamin as described in the Section above, Causes, Mechanisms and Effects of Cobalamin Deficiency. In addition to the symptoms that may be experienced related to anemia (easy fatigue, shortness of breath, palpitations) and neuropathy (sensory and motor disturbances and memory loss) there are features that may be detected by a physician, including skin pallor (from anemia), abnormalities in neurological examination (sensory loss, abnormal balance and reflexes, and mental changes), and epithelial changes (skin pigmentation and smooth tongue). On the basis of any combination of such changes, cobalamin deficiency may be suspected but confirmation is necessary using laboratory tests because other conditions may give rise to effects that closely resemble cobalamin deficiency. The need to confirm suspected cobalamin deficiency applies also to individuals who have abnormalities in their blood count measurements.

The standard screening test for cobalamin deficiency consists of direct measurement of circulating levels of cobalamin. Serum levels less than 150p mol l⁻¹ are considered deficient and levels 150–250p mol l⁻¹ are considered borderline. Serum or plasma cobalamin concentration can be measured in several ways and this has evolved from early microbial growth assays through competitive binding assays that were first radioisotopic and are now enzyme linked or based on chemiluminescence detection. The sensitivity and specificity of these assays are imperfect, such that measurement of serum cobalamin levels does not always detect the presence of deficiency, nor does the finding of a low serum cobalamin always connote true deficiency. There are several reasons for this including the distribution of cobalamin between the binding proteins in circulation (Table 1), imperfections in the assays for its measurement, and various poorly understood factors relating to exchange of cobalamin between cellular and circulatory compartments. Regarding the distribution of cobalamin between plasma-binding proteins, because transcobalamin is responsible for cobalamin delivery to cells, the fraction of the total cobalamin that is associated with transcobalamin (holoTC), even though small in comparison with the haptocorrin-associated fraction, is more likely to be indicative of cobalamin status than is the total serum cobalamin. Some studies bear this out, although the clinical utility of measuring holoTC levels has not yet been convincingly demonstrated.

The other approach to identification of cobalamin deficiency is indirect, based on the detection of raised levels of compounds in the blood or urine that require adequate tissue levels of cobalamin for their metabolic disposal. The compounds most commonly measured for identification of possible cobalamin deficiency are methylmalonic acid and homocysteine. These are the substrates in two cobalamin-dependent reactions shown in Figure 2. Because of the identification of these metabolic roles for cobalamin, it has been apparent that deficiency of cobalamin or disturbances in its metabolism would result in accumulation of these substances and clinical assays for these metabolites is now available. Of the two compounds, elevation of the levels of methylmalonic acid is more specific for identification of cobalamin deficiency;

Table 3 Laboratory identification of cobalamin deficiency

Test	Finding	Major limitations
Serum/plasma cobalamin concentration	Low ($<150 \text{ pmol l}^{-1}$)	Normal levels in some deficient subjects; slight to moderately low levels may not connote deficiency
Serum/plasma holotranscobalamin (holo TC II)	Low ($<35 \text{ pmol l}^{-1}$)	Test not yet widely available; insufficient validation of usefulness
Serum/plasma or urine methylmalonic acid	Raised ($>350 \text{ nmol l}^{-1}$)	Levels raised in renal insufficiency
Plasma homocysteine	Raised ($>12 \mu\text{mol l}^{-1}$)	Levels raised in folate and in vitamin B ₆ deficiencies, renal insufficiency, hypothyroidism

Table 4 Inherited disorders affecting cobalamin metabolism and their effects

Cobalamin protein	Effects of deletion or mutation
Intrinsic factor	Cobalamin malabsorption (juvenile pernicious anemia)
Cubulin/amnionless complex	Selective malabsorption of cobalamin, autosomal recessive megaloblastic anemia (MGA1, Imerslund-Gräsbeck disease)
Transcobalamin	Severe cobalamin deficiency
Haptocorrin	No apparent abnormality
Cobalamin reducing and activating enzymes (mut ⁺ and mut ⁻ , cobalamin mutants C-G)	Varying degrees of disruption in one or both cobalamin-dependent pathways

however, renal insufficiency can cause raised levels of methylmalonic acid in the blood. In addition to cobalamin deficiency, several other conditions also can cause raised homocysteine levels in the blood, including deficiencies of folate and vitamin B₆, lowered levels of thyroid hormone, and renal insufficiency.

Table 3 shows the idealized usefulness of the various tests commonly employed for the detection of cobalamin deficiency.

Inborn Errors of Cobalamin Metabolism

There are several known but rare inherited molecular defects resulting in absence or structural defects of proteins required for normal absorption, transport, or metabolism of cobalamin. These include the intestinal binding proteins, gastric

intrinsic factor and its ileal receptor complex cubulin and amnionless, the plasma binders transcobalamin and haptocorrin, the enzymes that are required for conversion of cobalamin to its coenzymatically active methyl and deoxyadenosyl forms, and enzyme complexes involved in the catalysis of the two cobalamin-dependent reactions responsible for conversion of homocysteine to methionine and methylmalonate to succinate, respectively. Individuals who inherit a defective gene from each parent for any one of the proteins that are critical for cobalamin metabolism suffer from varying degrees of impairment of normal cobalamin-related status (see **Table 4**), closely mimicking the various manifestations of cobalamin deficiency described above. These disorders usually become manifest at an early age.

See also: Folic Acid. Homocysteine

Further Reading

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VITAMIN C

Contents

Deficiency States

Physiology, Dietary Sources, and Requirements

Deficiency States

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Glossary

Clinical deficiency Often results from severe prolonged tissue (nutrient) deficiency, which may in turn result from inadequate (nutrient) intake, but may also arise from impaired absorption, increased turnover and/or excretion; increased tissue demand, etc. Deficiency signs are directly observable; symptoms can be elicited by testing, and the response of these to intervention (e.g., nutrient supplementation) can help to confirm a diagnosis of deficiency.

Deficiency (clinical or biochemical) Arises when the amounts of an essential nutrient in (tissues of) the body falls below a critical minimum level. Biochemical (tissue)

depletion then occurs, and may be followed by clinical (i.e., pathological) signs and symptoms of deficiency. Deficiency of an essential nutrient in the diet may lead to tissue- and clinical-deficiency, but impaired absorption, increased losses, increased tissue demands, etc., may also lead to a functional (tissue) deficiency, even when the diet-content is adequate.

Nutrient (e.g., vitamin) status Commonly assessed by measuring the concentration of the nutrient in an accessible body fluid such as serum or urine, or else the functionality of an enzyme or biochemical pathway (functional status). Published 'normal ranges' enable the results to be classified as (e.g.,) deficient, low, normal, or high.

Scurvy: The History and Discovery of Vitamin C

Scurvy is traditionally associated with long sea voyages, which often lasted for several years; the seamen's diets were confined to whatever could be stored at room temperature for long periods. In the absence of refrigeration, their diets typically consisted of dried biscuits and other dry cereal foods (wheat flour and oatmeal), salted meat, dried peas, cheese, butter, and ale, i.e., whatever could be dried and preserved in adverse tropical climates. The signs and symptoms described in classical accounts of scurvy, written long before its cause was understood, included lassitude, swollen joints, putrid and bleeding gums, failure of wound healing and the opening of old wounds and sores, intradermal bleeding due to capillary fragility, heart failure, and sudden death (**Table 1**). Although nowadays we distinguish the symptoms of true scurvy (now known to be produced specifically by vitamin C deficiency) from conditions such as beriberi (thiamin deficiency, associated with edema of the lower limbs), vitamin A deficiency (associated with night-blindness and corneal lesions), and rickets (vitamin D deficiency caused mainly by a lack of exposure to sunlight in children), in the older literature these conditions were often not recognized as distinct. Signs and

symptoms of scurvy occurred on land in times of siege or during prolonged military campaigns where accesses to fresh foods were severely restricted. Although some medical practitioners and political leaders became convinced that scurvy had a dietary cause, and that its cure or prevention were possible by including fresh plant materials such as scurvy grass, decoctions of evergreen needles, etc., in the diet (**Table 2**), many believed, right up to the beginning of the twentieth century, that factors such as 'foul vapours or infections were to be blamed. Indeed, before the recognition and discovery of essential micronutrients in the early years of the twentieth century, which confirmed that small amounts of certain complex organic molecules are needed in the diet to maintain health; it was thought that food is needed to supply only energy, protein, certain minerals, and water.

Although treatments for scurvy stretch back over many centuries, a definitive dietary cure is attributable to James Lind, whose controlled intervention trial on board HMS Salisbury, followed by 'A Treatise of the Scurvy' in 1753, provided decisive evidence that persuaded the British Admiralty to insist on the inclusion of citrus fruit regularly in the British naval diet (**Table 2**). Lind showed that a 'rob' or decoction of

Table 1 Signs, symptoms, and biochemical indicators of vitamin C deficiency*Signs of clinical scurvy:*

Bleeding gums and looseness of teeth.

Skin petechiae (intradermal bleeding due to capillary fragility); perifollicular hemorrhages; larger sheet hemorrhages, especially of the skin of the limbs and trunk; hematomas; purpura; bruising.

Ecchymoses (failure of eruption of hair from hair follicles); hyperkeratosis of hair follicles.

Breakdown and bleeding from previously healed wounds.

Swollen painful joints, with effusions and arthralgia; hemarthrosis; myalgia

Edema (especially pedal); dyspnea; dry eyes and mouth.

Abnormal long bone development in children; swelling ('beading') of rib cage; swelling of long-bone joints.

Intracranial hemorrhages in children; 'bulging eyes'; gums often affected if teeth already erupted.

Clinical symptoms of scurvy:

Fatigue, lassitude, joint pain, 'pithed frog' appearance in babies, for example, after prolonged feeding of condensed milk.

Abnormal X-ray appearance of long bones in children, especially at the epiphyses; subperiosteal bleeding; osteolysis; osteonecrosis.

Capillary fragility, as measured by the Hess test (pressure cuff on limb); nonspecific anemia in many cases.

Heart failure in severe cases, potentially leading to death.

Biochemical indicators of vitamin C deficiency:

Plasma vitamin C (ascorbate) concentrations $< 11 \mu\text{mol/l}$

Buffy coat vitamin C concentrations $< 15 \mu\text{g}/10^8$ cells

Suggested, but not yet verified, functional tests, for example, abnormalities of hydroxylation and cross-links of nascent collagen; of carnitine formation, of vitamin E recycling; of purine oxidation, etc.

oranges could rapidly cure the disease, and his discovery rapidly brought about dramatic reductions in the incidence and mortality due to scurvy.

In the first decade of the twentieth century, two Norwegian scientists, Holst and Frohlich, found that guinea pigs, like humans, reproducibly become scorbutic if fed a diet that lacks the 'antiscorbutic principle' which was, by now, known to be abundantly present in fresh fruit and vegetables. Few other mammals share this unusual dietary requirement, which, we now know, is solely attributable to the lack of a key enzyme, L-gulonolactone oxidase, which is needed in the multistep biosynthetic pathway for the synthesis of ascorbic acid from common sugars such as glucose and galactose. This animal model of dietary-induced scurvy provided a very important tool for the study of the disease.

Paradoxically, crystalline ascorbic acid (vitamin C) was first isolated, not from a well-established antiscorbutic plant source such as fruit or green leaves, but instead from an animal source, namely adrenal glands, where high concentrations of the vitamin are also found. Indeed, the original motivation for the isolation of the crystalline material (by Albert Szent-Gyorgyi in Gowland Hopkins' laboratory in Cambridge) was a serendipitous accident, arising out of his attempts to isolate a new adrenal hormone. Charles Glen King in Pittsburgh then showed that this easily-oxidized sugar derivative (initially named 'hexuronic acid') was indeed the long-sought antiscorbutic principle, i.e., vitamin C or L-ascorbic acid.

Table 2 Historical timeline of investigations into scurvy and discovery of vitamin C (ascorbic acid)

Tudor England: Leaders on certain long naval voyages successfully made use of plant foods (e.g., scurvy grass; young pine needles) as an *ad hoc* remedy for the prevalent scourge of scurvy.

Mid-eighteenth century: James Lind (a Scottish physician) carried out the first controlled trial of scurvy remedies at sea; he demonstrated efficacy of citrus fruit (oranges, lemons) and published 'Treatise of the Scurvy', 1753. Sir Gilbert Blane (end of eighteenth century) caused British naval diets to be supplemented with citrus (lemons, limes), thereby reducing prevalence of scurvy and greatly improving sailors' health – although limes eventually proved less effective than lemons, and scurvy reappeared as a result of failure to realise this.

Nineteenth century: Emergence of 'Barlow's disease' – scurvy in babies mainly fed with condensed milk.

Early twentieth century: Holst and Frohlich developed a guinea-pig model of scurvy, permitting investigation of the antiscorbutic potency of various foods.

1929: Albert Szent-Gyorgyi in Cambridge isolated 'hexuronic acid' from adrenal extracts, which proved identical to 'ascorbic acid', or 'vitamin C', the elusive antiscorbutic substance present in citrus fruit and plants, then being studied by Charles Glen King in Pittsburgh. 'Vitamin C' was thus isolated and named.

1930s: Norman Haworth and Tadeus Reichstein achieved chemical synthesis of vitamin C (ascorbic acid) from common sugars (glucose, galactose), thereby proving its chemical structure and elucidating its unique chemical and physiological (nutritional) properties.

1940s: Wartime studies showed that 10 mg vitamin C per day is sufficient to prevent and cure clinical scurvy in adult males.

Mid-twentieth century: Biochemical studies in several laboratories elucidated multiple roles of vitamin C: (1) as a cofactor for certain key enzymes, involved in specific oxidation and hydroxylation pathways of intermediary metabolism; (2) as a redox-modulator and provider of protection against certain types of (especially oxygen-radical-mediated) oxidative damage.

The vitamin was isolated in 1928, and its chemical structure was proven by de novo synthesis from common sugars a few years later (Table 2).

Degradation, Turnover, and Factors that Induce Increased Requirements for Vitamin C

The instability of vitamin C in air, and especially in neutral or alkaline aqueous solution, is attributable to the fact that in the presence of oxygen or other oxidizing agents it readily undergoes two successive one-electron oxidation steps to produce (reversibly, see below) another unstable product, dehydroascorbate, which readily undergoes an irreversible lactone ring-hydrolysis to yield 2,3-diketogulonic acid. Thus the vitamin is readily destroyed, both in foods during storage and (at a lower rate because of efficient recycling mechanisms) in the body. Diketogulonic acid is one of several degradation products of vitamin C that cannot be reconverted to the vitamin and are further degraded to stable excretory products, such as oxalic acid, by oxidative catabolism. Of all the micronutrients that are essential for human health and

survival, vitamin C is the most easily destroyed during drying and other traditional methods of preserving food. Citrus fruits contain other organic acids that inhibit this process of oxidation by lowering the pH of the fruit juice. This enables them, and extracts of them, to preserve at least some of their vitamin content for several weeks and even months of storage and thereby helps them to prevent and cure scurvy.

It remains largely a mystery why some people succumb to scurvy after a modest period of very low intake, whereas others survive for much longer. It has been speculated that some people may be able to produce all of the enzymes of the vitamin C synthetic pathway, including gulonolactone oxidase. However, this now seems unlikely and it is more probable that the retention and recycling mechanisms for the vitamin are more efficient in some people than in others. For example, smokers have a higher turnover of endogenous vitamin C than nonsmokers, mainly because of the free-radical oxidant species in cigarette smoke. People with infections also have increased vitamin C turnover, which is associated with the liberation of pro-oxidant substances (such as hypochlorous acid) that are used by the body to kill bacteria. Some people have genetic variants of the vitamin C transporters (see the following section); others have isoforms of certain blood proteins such as haptoglobins, both of which may be associated with relatively low levels of vitamin C in the blood. Very occasionally, there arise nonlethal mutations of vitamin C-dependent pathways whose abnormalities can be treated with high vitamin C intakes. A well-characterized example is Ehlers–Danlos syndrome, type VI, which is associated with impaired collagen lysyl hydroxylation and presents a variety of clinical and biochemical connective-tissue (collagen-related) defects. However, much more research is needed to determine, which of many possible genetic and environmental factors modulate the turnover of vitamin C in the body and to determine individual requirements and hence relative resistance to scurvy. Although 100–200 mg of the vitamins per day is needed to approach saturation of the tissues of humans, the amount needed to prevent or cure scurvy is less than 10 mg day⁻¹, as was shown by experiments involving prolonged periods of feeding with depleted diets in the middle of the twentieth century (Table 2). Today, overt clinical scurvy is rare. It is occasionally seen in refugee camps or in elderly people with poor diets that are devoid of the usual sources of the vitamin. The latter high-risk group contains many individuals who are unable to chew fresh fruit and vegetables because of poorly-fitting dentures or poor gastric tolerance of acidic or fibrous foods (see final section).

An essential dietary requirement for vitamin C is shared by only a small number of vertebrates, including most primates, guinea pigs and agoutis, and some birds and fishes. Most mammals synthesize the vitamin in their livers from hexose sugars; birds synthesize it in their kidneys. The final enzyme in the pathway, L-gulonolactone oxidase, has been lost in several unrelated species, suggesting a vulnerable and easily mutated locus on the genome. Presumably this mutation was neutral or advantageous during the natural selection of man's ancestors, when human and related-primate diets were rich in plant sources of the vitamin.

Well-Established Metabolic Functions of Vitamin C that are Impaired by Deficiency

Studies of guinea pigs (and other species that require a dietary source of vitamin C) have revealed that, when deprived of the vitamin, characteristic lesions of growing bones, failure of wound-healing of skin and bones, capillary defects, and other lesions arise, all of which point to a failure of the new synthesis of or repair processes for, connective tissues and especially the protein collagen, which is the major extracellular protein and comprises a third of all the protein in the body (Table 2). As the biochemical pathway of collagen biosynthesis became better understood, during the middle years of the twentieth century, it became clear that hydroxylated amino-acids, comprising two different hydroxylated forms of proline and one of lysine, occurred uniquely in collagen. These were not coded by the genome or inserted by the amino-acid-assembly machinery of the cell but instead were created by 'post-translational' amino-acid hydroxylation processes that took place after the nascent procollagen polypeptide chain had been synthesized on the polysomal messenger RNA. Some of the prolyl residues of the procollagen molecule were then hydroxylated to hydroxyprolyl residues, and some of the lysyl residues were hydroxylated to hydroxylysyl residues. The hydroxylated prolyl residues are essential for subsequent collagen triple-helix formation and hence for the secretion of nascent collagen; the hydroxylated lysyl residues form part of the essential pyridinoline-type crosslinks that stabilize the collagen fibers, especially those in bone. In the absence of sufficient vitamin C, these hydroxylation reactions rapidly fail, because the ferrous iron at the active center of the 'mixed function oxidase' enzymes that catalyze them is rapidly inactivated by oxidation. Vitamin C, specifically, is needed to keep the essential ferrous residues at the hydroxylase-enzyme active centers in the reduced, active, form. In the absence of the vitamin, these enzymes are inactivated only after a few cycles of hydroxylation. Hydroxyproline formation in the C1q component of complement, an important component of the immune system, is also vitamin C-dependent.

The essential function of vitamin C in collagen maturation can go a long way toward explaining many of the clinical lesions of scurvy (Table 1). However, the vitamin may also act directly on the transcription and translation of collagen mRNA and on the synthesis of other parts of the cell machinery that are needed for the formation of normal connective tissues.

Vitamin C plays a cofactor-like role in the reactions of several other enzymes that split molecular oxygen, notably members of the group of enzymes that are classified as 'mixed-function oxidases.' Two enzymes containing ferrous iron that are involved in carnitine biosynthesis (trimethyl lysine hydroxylase and γ -butyrobetaine hydroxylase) fall into this category. Aspartate β -hydroxylase, which is needed for the postsynthetic modification of protein kinase C, also requires vitamin C. Another enzyme that requires vitamin C is the copper-containing dopamine β -hydroxylase, and in the reaction that it catalyzes, ascorbic acid reduces cupric to cuprous copper at the active site. Peptidyl glycine hydroxylase (peptidyl α -amidase) is also a copper-containing enzyme requiring vitamin C as cosubstrate. Vitamin C can increase the activities of several other enzymes, usually by a nonspecific reducing or protective

action that is also shared by some other cellular reductants. Newly identified roles for the vitamin include cell-signaling; nucleic acid and histone dealkylation, and proteoglycan deglycanation (e.g., via turnover of glypican-1, which is involved in cell growth and differentiation). One such newly identified ascorbate-dependent reaction is a dioxygenase-dependent hydroxylation of prolyl and asparaginyl residues in the α -subunit of hypoxia-inducible transcription factor 1 (HIF-1). The asparagine-hydroxylation modulates interaction with other activators, whereas the proline-hydroxylation targets HIF-1 for destruction in proteasomes; the unhydroxylated HIF-1 increases expression of most glycolytic enzymes and two glucose transporters (which also transport dehydroascorbate).

During its function, ascorbic acid is oxidized in two successive reversible one-electron steps, and most, if not all, of its essential biological actions are centered around this redox cycle. The first oxidation product is the free-radical form of the vitamin, which is known variously as 'monodehydroascorbate,' 'semidehydroascorbate,' or 'ascorbate free radical' (AFR). Although this intermediate shares with most other free radicals, the properties of having a relatively short half life and a high degree of chemical reactivity, it is more stable than many other free radicals, contrasting with the highly reactive and damaging radicals such as hydroxyl or superoxide radical that arise from molecular oxygen. By reacting with, and thus quenching, these damaging oxygen free radicals, ascorbate can act as a free-radical chain terminator and can thereby protect vulnerable macromolecules such as DNA, lipids, and proteins from oxidative damage by free-radical chain reactions. Such reactions would otherwise cause extensive damage, including genetic damage (to DNA), the formation of potentially atherogenic oxidized lipids, and oxidative inactivation of enzymes. For this reason, ascorbic acid is thought to possess important 'protective' antioxidant properties that are not directly connected with its other cofactor-like or cosubstrate-like roles in enzyme reactions. Ascorbate probably also protects host tissues against damage by oxidants such as hypochlorous acid that are produced in the normal course of bacterial-killing action of white cells.

The second one-electron oxidation step in ascorbate oxidation produces dehydroascorbate from the free-radical intermediate AFR. Both of these oxidized forms can be recycled to ascorbate either by nonenzymatic reactions with glutathione as the reductant (electron acceptor) or by pyridine nucleotide-dependent enzymatically catalyzed reactions. Thus, the two sequential one-electron oxidation steps from ascorbate to dehydroascorbate are fully reversible *in vivo*. However, the subsequent spontaneous nonenzymatic reaction comprising of hydrolysis of the 1,4-lactone ring is not reversible, so that the product of this reaction, diketogulonic acid, has no provitamin activity. Normally, approximately 3% of the vitamin C in the body is degraded every day and this loss must be replaced from the diet. Nevertheless, many weeks at or near zero intake are usually needed to reach scorbutic levels.

An important aspect of functional adequacy of nutrients is their efficient transport across cell membranes and hence between tissue pools. Reduced ascorbic acid is transported by two distinct sodium-vitamin C transporters, SVCT1, which is found mainly in epithelial tissue such as intestine, liver, and

kidney and SVCT2, which is widely distributed among tissues. Naturally-occurring genetic variants affecting SVCT1 have recently been shown to affect circulating concentrations of the vitamin in human populations, and thus, presumably, vitamin C status and requirements. The oxidized form, dehydroascorbate, is transported by the glucose transporter systems, GLUT1 and GLUT2, and glucose and other sugars that share these transporters compete with it, a fact that is relevant for some diseases, such as diabetes, in which glucose transport and concentrations are abnormal.

Measurement of Vitamin C Status; Biochemical Tests for Adequacy and Deficiency

In species (such as humans) that cannot synthesize vitamin C in their bodies, the vitamin concentration in tissues and blood compartments (plasma, erythrocytes, and white blood cells) varies characteristically with the dietary intake of the vitamin. Because the blood-compartment concentrations mirror the concentrations in most other cells and tissue compartments, tissue vitamin C status can be monitored by measuring the concentration in plasma or blood, even though the blood intracellular concentrations are generally lower than those in most tissues. The concentration ratios between extracellular and various intracellular compartments are determined by active transport systems (SVCT 1 and 2, see previous section), that concentrate the vitamin inside many cell types. At high intakes of the vitamin, the intestinal absorption process is overwhelmed, so that some of the ingested vitamins remain unabsorbed and is destroyed in the lower intestine by intestinal bacteria. The maximum steady-state level in plasma can be temporarily exceeded following a high bolus intake, but the excess vitamin is rapidly excreted in the urine once the renal threshold for filtration and reabsorption is exceeded. These safety mechanisms limit the maximum concentration of the vitamin to which the tissues are normally exposed.

For many years, the best biochemical measure of vitamin C status was considered to be the buffy coat, or total white-cell concentration of the vitamin (Table 1), expressed as micrograms or micromoles per 10^8 white cells, the cell count in the assay sample being estimated by an electronic cell counter. This status index varied predictably with total body vitamin C stores during controlled (e.g., animal) depletion studies. However, in practice it has proved to be a difficult test to use in human studies and especially in surveys, as it requires complex laboratory operations to be performed immediately after collecting the blood. It is also difficult to harmonize this test between laboratories, and, because it measures the average vitamin C content across several different white-cell types, whose individual proportions and relative vitamin C contents may vary considerably, its interpretation was not always straightforward. In addition, infection affects the values obtained. For all of these reasons, this assay has fallen out of favor and is now rarely used. The concentration of the vitamin in erythrocytes or whole blood is not an ideal alternative, partly because hemoglobin can catalyze the oxidative destruction of the vitamin *in vitro* and partly because erythrocyte concentrations do not mirror other body compartments in a simple manner.

Serum or plasma vitamin C has therefore become the most commonly used status assay. To avoid short-term fluctuations caused by recent bolus intakes from food or supplements, it is preferable to collect an overnight-fasting blood sample. Because the vitamin is extremely easily oxidized, the sample must be carefully preserved unless the assay can be performed immediately. The usual approach is to add freshly prepared metaphosphoric acid, usually at between 2 and 5% w/v, which precipitates plasma proteins, chelates transition-metal ions, and provides a protective acidic environment of a suitable pH. If stored, the samples must be kept at a low temperature, for example, at -25°C for not more than a week or two or at -80°C for up to 1–2 years. There are many alternative physicochemical and chemical assay methods for measuring vitamin C in extracts of plasma or serum. These include (1) the measurement of its chemical reducing action on reducible dyes such as dichlorophenol indophenol or (2) the formation of either a colored osazone, or a fluorescent derivative with orthophenylene diamine, after conversion to dehydroascorbate. Quantitation by absorbance or by electrochemical detection after separation by high-performance liquid chromatography is favored by many workers. This procedure has the advantage of being relatively specific (i.e., free from most forms of interference) and highly sensitive, but it is more time-consuming than the simpler nonchromatographic methods. Different methods may differ with respect to their specificity and their sensitivity to problems of interference as well as in the precautions that are needed to avoid oxidative destruction of the vitamin during the assay. Careful validation and robust quality-control procedures are essential.

Plasma or serum levels below $11\ \mu\text{mol l}^{-1}$ ($<0.2\ \text{mg per } 100\ \text{ml}$) are considered to be evidences of biochemical deficiency, and if this is severe and prolonged, the risk of clinical deficiency, i.e., scorbutic signs and symptoms, gradually increases. Intakes below $20\ \text{mg day}^{-1}$ are likely to result in plasma levels in this range. Studies of human volunteers in the middle of the twentieth century showed clearly that an intake of $10\ \text{mg}$ vitamin C per day in a healthy adult is sufficient to

prevent clinical scurvy, and this small amount is also sufficient to cure scorbutic signs and symptoms (Table 2).

Assay methods based on urinary excretion of vitamin C have been used to study status, but they are too cumbersome and difficult to interpret to be useful in population studies. There are no well-established functional assays available to define vitamin C status and requirements at present. An older method known as the 'Hess test,' which measures relative capillary fragility under pressure or suction (Table 1), is useful only if subclinical scurvy is present and is rarely attempted today. Studies of collagen crosslinks or oxidative damage to macromolecules such as DNA or lipids may yield evidences about functional status in the future, but this remains a research challenge and is not yet an available option for routine studies or surveys.

Occurrence of Low Intakes and Poor Biochemical Status in Present-Day Societies

Although scurvy is rare, biochemical evidence of poor vitamin C status is not uncommon in certain high-risk groups in different human populations. Studies in The Gambia in West Africa, for instance, have shown that there is a regular seasonal cycle of availability of foods rich in vitamin C, with a good availability in the dry season alternating with a severe shortage during the rainy season. Plasma, buffy-coat, and breast-milk concentrations are all, on average, adequate in the dry season but are severely reduced during the rains. Functional and health-related parameters also deteriorate during the rains, but it has so far proved to be difficult to reverse this deterioration by vitamin C supplements alone. Therefore robust evidence of health consequences of this seasonal availability cycle has not yet been obtained.

From recent surveys in the UK, Table 3 shows the prevalence of low intakes of vitamin C (estimated from the proportion of participants receiving less than the lower reference nutrient intake (LRNI), which is the amount deemed to be sufficient for only a few people in a population group, namely

Table 3 Prevalence of low vitamin C intakes and low plasma vitamin C concentrations in Britain at the end of the twentieth century

Age group	LRNI(mg day^{-1})	Intake less than LRNI	Less than $11\ \mu\text{mol l}^{-1}$ plasma vitamin
<i>Preschool 1.5–4.5 years</i>	8	8/723 = 1.1%	24/723 = 3.3%
<i>Young people 4–18 years</i>			
4–10 years	8	1/423 = 0.2%	6/422 = 1.4%
11–14 years	9	0/307 = 0%	4/307 = 1.3%
15–18 years	10	1/271 = 0.4%	8/271 = 3.0%
<i>Adults 19–64 years</i>			
19–24 years	10	1/212 = 0.5%	11/151 = 7.3%
25–34 years	10	0/429 = 0%	8/307 = 2.6%
35–49 years	10	0/571 = 0%	8/414 = 1.9%
50–64 years	10	0/512 = 0%	8/366 = 2.2%
<i>Adults 65 years and above</i>			
Free-living 65–79 years	10	8/606 = 1.3%	88/606 = 14.5%
Free-living 80+ years	10	7/274 = 2.5%	45/274 = 16.4%
Institution-living	10	2/248 = 0.8%	98/248 = 39.5%

The LRNI is the Lower Reference Nutrient Intake, deemed to be sufficient for only that 2.5% of the population who have the smallest requirements, and a plasma concentration of $11\ \mu\text{mol l}^{-1}$ is the cut-off for biochemical deficiency.

the 2.5% with the lowest requirements). Also shown in **Table 3** is the prevalence of plasma concentrations below the lower cut-off in normality, set at 0.2 mg dl^{-1} or $11 \text{ } \mu\text{mol l}^{-1}$. This is shown for several subgroups of the British population of different ages, from data collected in three nationally representative population surveys during the decade 1990s. It is clear from these results that a very few people were getting less than the LRNI for vitamin C over a 4 days or 7 days period of weighed-intake estimates of their diets. Low plasma levels were likewise relatively uncommon in the younger age groups; however, they were more common in older people and were especially prevalent, at almost 40%, in older people living in institutions such as nursing homes. Many of these relatively low plasma levels seen in frail older people are likely to be caused by factors other than very low intakes of the vitamin, such as reduced efficiency of the vitamin C transporters, especially SVCT1 or increased vitamin turnover. In the UK, unlike The Gambia, there was relatively little evidence of a major seasonal variation in vitamin C intake or status at the end of the twentieth century.

Vitamin C absorption does not appear to be abnormally low in healthy older people. However, the multiple pathologies associated with old age (and with debility at any age) are associated with increased turnover of the vitamin. Older people with very low levels of vitamin C are at higher risk of dying sooner than those with high levels, although short-term vitamin supplements generally fail to reverse this increased risk. It thus appears that vitamin C status can act as a barometer of health as well as being a marker of adequacy of vitamin C intake. Further research is needed to determine the key mechanisms that affect the rate of vitamin C turnover and its control in different age groups and different metabolic states. Because frail older people are at high risk of developing pressure sores and of needing surgery for a variety of ailments, there seems to be a potential public-health advantage in protecting vitamin C stores, especially in this vulnerable older age group.

See also: Biochemical Indices. Vitamin C: Physiology, Dietary Sources, and Requirements

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Physiology, Dietary Sources, and Requirements

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Glossary

Carnitine β -Hydroxy- γ -trimethylammonium butyrate, biosynthesized by methylation of lysine, important for the transport of fatty acids into the mitochondrion for oxidation.

Flavoprotein Any enzyme (or other protein) with a prosthetic group formed from riboflavin (vitamin B₂) or one of its derivatives.

Glutathione A tripeptide, γ -glutamyl-cysteinyl-glycine that acts as a major redox cofactor, being oxidized to yield the hexapeptide lined by a disulfide bridge.

Granulocytes A class of white blood cells characterized by conspicuous cytoplasmic granules.

Hydroxymethylglutaryl CoA reductase The first and rate-limiting enzyme of cholesterol biosynthesis. The target of statin drugs for the treatment of hypercholesterolemia.

Hypercholesterolemia Elevated blood concentration of cholesterol.

Leukocytes White blood cells of various kinds.

Teleost fish Bony, as opposed to cartilaginous, fishes.

Introduction

Ascorbic acid is a vitamin (vitamin C) for only a limited number of species: human beings and other primates, bats, the guinea pig, a number of birds, and teleost fishes. In other species ascorbic acid is not a vitamin but is an intermediate in glucuronic acid catabolism, and its rate of synthesis bears no relation to physiological requirements for ascorbate. Species for which ascorbate is a vitamin lack the enzyme gulonolactone oxidase (EC 1.1.3.8) and have an alternative pathway for glucuronic acid metabolism, via reduction and the pentose phosphate pathway.

Ascorbic acid functions as a relatively nonspecific, radical-trapping antioxidant, and also reduces the tocopheroxyl radical formed by oxidation of vitamin E. It has a specific metabolic function as the coenzyme for dopamine β -hydroxylase and peptidyl glycine hydroxylase, and it is required to maintain the iron of 2-oxoglutarate-dependent hydroxylases in the reduced state. It also enhances the absorption of inorganic iron from foods.

Absorption, Transport, and Storage

In species for which ascorbate is not a vitamin, intestinal absorption is by sodium-independent facilitated diffusion, whereas in human beings and guinea pigs there is sodium-dependent active transport of the vitamin at the brush border membrane, with a sodium-independent mechanism at the basolateral membrane. Dehydroascorbate is absorbed passively in the intestinal mucosa, and is reduced to ascorbate before transport across the basolateral membrane.

At intakes up to about 100 mg day⁻¹, 80–95% of dietary ascorbate is absorbed, falling from 50% of a 1-g to 25% of a 6-g and 16% of a 12-g dose. Unabsorbed ascorbate is a substrate for intestinal bacterial metabolism.

Ascorbate and dehydroascorbate circulate in the bloodstream both in free solution and bound to albumin. Approximately 5% of plasma vitamin C is normally in the form of dehydroascorbate. Ascorbate enters cells by sodium-dependent active transport; dehydroascorbate is transported

by insulin-dependent glucose transporters and is accumulated intracellularly by reduction to ascorbate. In poorly controlled diabetes mellitus, tissue uptake of dehydroascorbate is impaired because of competition by glucose, as well as insulin resistance or lack of insulin, which is required for activity of the transporters, and there may be functional deficiency of vitamin C despite an apparently adequate intake.

Approximately 70% of blood-borne ascorbate is in plasma and erythrocytes (which do not concentrate the vitamin from plasma). The remainder is in white cells, which have a marked ability to concentrate ascorbate; mononuclear leukocytes achieve 80-fold concentration, platelets 40-fold, and granulocytes 25-fold, compared with the plasma concentration.

There is no specific storage organ for ascorbate; apart from leukocytes (which account for 10% of total blood ascorbate), the only tissues showing a significant concentration of the vitamin are the adrenal and pituitary glands. Although the concentration of ascorbate in muscle is relatively low, skeletal muscle contains much of the body pool of 5–8.5 mmol (900–1500 mg) of ascorbate.

Metabolism and Excretion

As shown in **Figure 1**, oxidation of ascorbic acid proceeds by a one-electron process, forming monodehydroascorbate, which disproportionates to ascorbate and dehydroascorbate. Most tissues also contain monodehydroascorbate reductase (EC 1.6.5.4), a flavoprotein that reduces the radical back to ascorbate. In the cytosol dehydroascorbate is reduced by either reduced nicotinamide adenine dinucleotide (NADH) or reduced nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzymes; in mitochondria it is reduced by complex 3 of the electron transport chain. It is also reduced nonenzymically by reaction with glutathione. At neutral pH dehydroascorbate is unstable and is rapidly hydrated to diketogulonate, but little is lost in this way.

Both ascorbate and dehydroascorbate are filtered at the glomerulus, then reabsorbed by the same transporters as are involved in intestinal absorption; the sodium-dependent

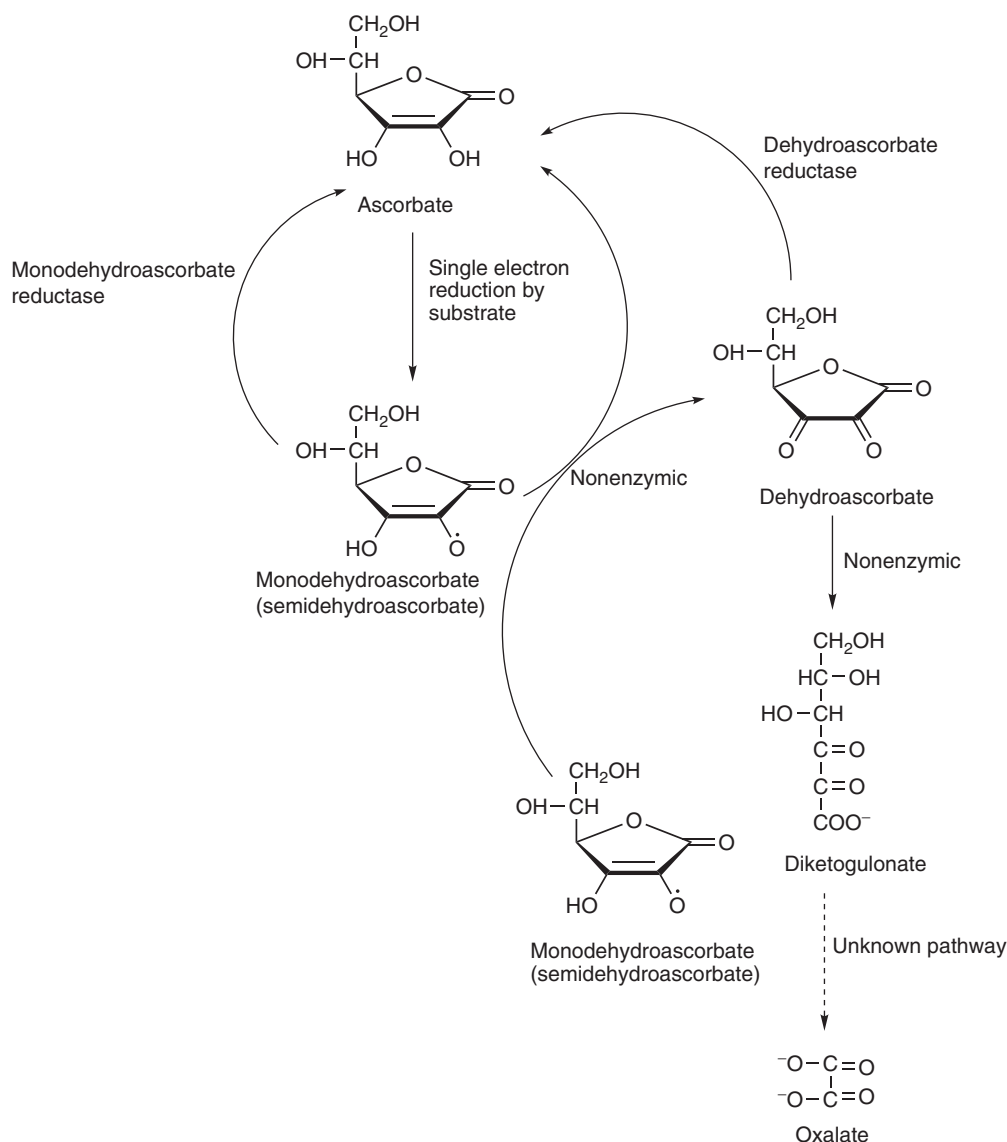


Figure 1 The metabolism of ascorbate.

vitamin C transporter for ascorbate and the glucose transporter for dehydroascorbate. When glomerular filtration exceeds the capacity of the transport systems, at a plasma concentration of ascorbate above about $85 \mu\text{mol l}^{-1}$, the vitamin is excreted in the urine in amounts proportional to intake.

Approximately 1.5% of dietary ascorbate is excreted as oxalate; this may be a factor in development of oxalate renal stones. The pathway of oxalate formation from ascorbate is not known, and it is likely that there is nonenzymic cleavage of diketogulonate to oxalate. Ascorbate also increases the intestinal absorption of dietary oxalate.

Metabolic Functions of Ascorbic Acid

Ascorbic acid has specific and well-defined roles in two classes of enzymes: copper-containing hydroxylases and the

2-oxoglutarate-linked, iron-containing hydroxylases. It also increases the activity of a number of other enzymes *in vitro* – a nonspecific reducing action rather than reflecting a metabolic function of the vitamin. In addition, ascorbic acid has a number of less specific effects due to its action as a reducing agent and oxygen-radical quencher. There is also evidence that ascorbate has a role in regulating the expression of connective tissue protein (and some other) genes; its mechanism of action is unknown.

Copper-Containing Hydroxylases

Dopamine β -hydroxylase (EC 1.14.17.1) is a copper-containing enzyme involved in the synthesis of the catecholamines noradrenaline and adrenaline from tyrosine in the adrenal medulla and central nervous system. The active enzyme contains Cu^+ ,

which is oxidized to Cu^{2+} during the hydroxylation of the substrate; reduction back to Cu^+ specifically requires ascorbate, which is oxidized to monodehydroascorbate. In the chromaffin granules, monodehydroascorbate is reduced by transmembrane electron transport via cytochrome b_{561} , with electrons provided by ascorbate in the cytosol. The resultant monodehydroascorbate in the cytosol is reduced back to ascorbate by a mitochondrial outer membrane reductase.

More than half of the peptide hormones undergo post-synthetic modification to form a carboxyl terminal amide, which is essential for biological activity. One function of this amidation is to render the peptides more hydrophobic, and so enhance receptor binding. The amide group is derived from a glycine residue that is to the carboxyl side of the amino acid that will become the amidated terminal of the mature peptide. The reaction is catalyzed by peptidyl glycine hydroxylase (peptidyl α -amidase, EC 1.14.17.3).

The first step in the reaction is hydroxylation of the glycine residue to yield hydroxyglycine. The hydroxylase is a copper-containing oxygenase that uses ascorbate to reduce the two copper ions to Cu^+ ; these then activate oxygen, with incorporation of one atom into hydroxyglycine and reduction of the other to water. This is followed by the cleavage of the peptide bond, with amidation of the amino acid to the amino side of the hydroxyglycine residue, and release of glyoxylate. These two activities occur in a single bifunctional protein.

2-Oxoglutarate-Linked, Iron-Containing Hydroxylases

A number of iron-containing hydroxylases (listed in Table 1) share a common reaction mechanism, in which hydroxylation of the substrate is linked to decarboxylation of 2-oxoglutarate. Ascorbate is required for the activity of all of these enzymes, but it does not function as either a stoichiometric substrate or a conventional coenzyme (which would not be consumed in the reaction).

Proline and lysine hydroxylases are required for the post-synthetic modification of collagen, and proline hydroxylase also for the postsynthetic modification of osteocalcin in bone and the C_1 component of complement, as well as a number of other proteins that have a collagen-like domain.

Aspartyl β -hydroxylase catalyzes hydroxylation of aspartyl and asparaginyl residues in epidermal growth factor (EGF) and a number of other proteins that have an EGF-like domain,

including several of the vitamin K-dependent blood clotting factors.

Trimethyllysine and γ -butyrobetaine hydroxylases are required for the synthesis of carnitine, and many of the early signs of scurvy may be due to carnitine deficiency.

The best studied of this class of enzymes is procollagen proline hydroxylase; it is assumed that the others follow essentially the same mechanism. As shown in Figure 2, the first step is the binding of oxygen to the enzyme-bound iron, followed by attack on the 2-oxoglutarate substrate, resulting in decarboxylation to succinate, leaving a ferryl radical at the active site of the enzyme. This catalyzes the hydroxylation of proline, restoring the free iron to undergo further reaction with oxygen.

It has long been known that ascorbate is oxidized during the reaction, but not stoichiometrically with hydroxylation of proline and decarboxylation of 2-oxoglutarate. The purified enzyme is active in the absence of ascorbate, but after some 5–10 s (approximately 15–30 cycles of enzyme action) the rate of reaction falls. The loss of activity is due to a side-reaction of the highly reactive ferryl radical in which the iron is oxidized to Fe^{3+} , which is catalytically inactive – so-called uncoupled decarboxylation of 2-oxoglutarate. Activity is only restored by ascorbate, which reduces the iron back to Fe^{2+} .

The Role of Ascorbate in Iron Absorption

Inorganic dietary iron is absorbed as Fe^{2+} , and not as Fe^{3+} ; ascorbic acid in the intestinal lumen not only maintains iron in the reduced state but also chelates it, increasing absorption considerably. A dose of 25 mg of vitamin C taken together with a meal increases the absorption of iron approximately 65%, whereas a 1-g dose gives a ninefold increase. This is an effect of ascorbic acid present together with the test meal; neither intravenous administration of vitamin C nor supplements several hours before the test meal affects iron absorption, although the ascorbate secreted in gastric juice should be effective. This is not a specific effect of ascorbate; a variety of other reducing agents including alcohol and fructose also enhance the absorption of inorganic iron. In addition, ascorbate is the electron donor for the intracellular ferric reductase in intestinal mucosal cells.

Inhibition of Nitrosamine Formation

Oral bacteria can reduce nitrate to nitrite which, under the acidic conditions of the stomach, can react with amines in foods to form carcinogenic *N*-nitrosamines. In addition to dietary sources, a significant amount of nitrate is formed endogenously by the metabolism of nitric oxide – 1 mg per kg bodyweight per day (about the same as the average dietary intake), increasing 20-fold in response to inflammation and immune stimulation, and nitrate is secreted in saliva.

Ascorbate reacts with nitrite forming NO , NO_2 , and N_2 , so preventing the formation of nitrosamines. In addition to ascorbate in foods, there is considerable secretion of ascorbate in the gastric juice, and inhibition of gastric secretion for treatment of gastric ulcers, as well as reducing vitamin B_{12}

Table 1 Vitamin C dependent, 2-oxoglutarate-linked hydroxylases

Aspartyl β -hydroxylase	EC 1.14.11.16
Histone demethylase	EC 1.14.11.27
<i>p</i> -Hydroxyphenylpyruvate hydroxylase	EC 1.14.11.27
Procollagen lysine hydroxylase	EC 1.14.11.4
Procollagen proline 3-hydroxylase	EC 1.14.11.7
Procollagen proline 4-hydroxylase	EC 1.14.11.2
Pyrimidine deoxynucleotide dioxygenase	EC 1.14.11.3
Thymidine dioxygenase	EC 1.14.11.10
Thymine dioxygenase	EC 1.14.11.6
Trimethyllysine hydroxylase	EC 1.14.11.8
γ -Butyrobetaine hydroxylase	EC 1.14.11.1

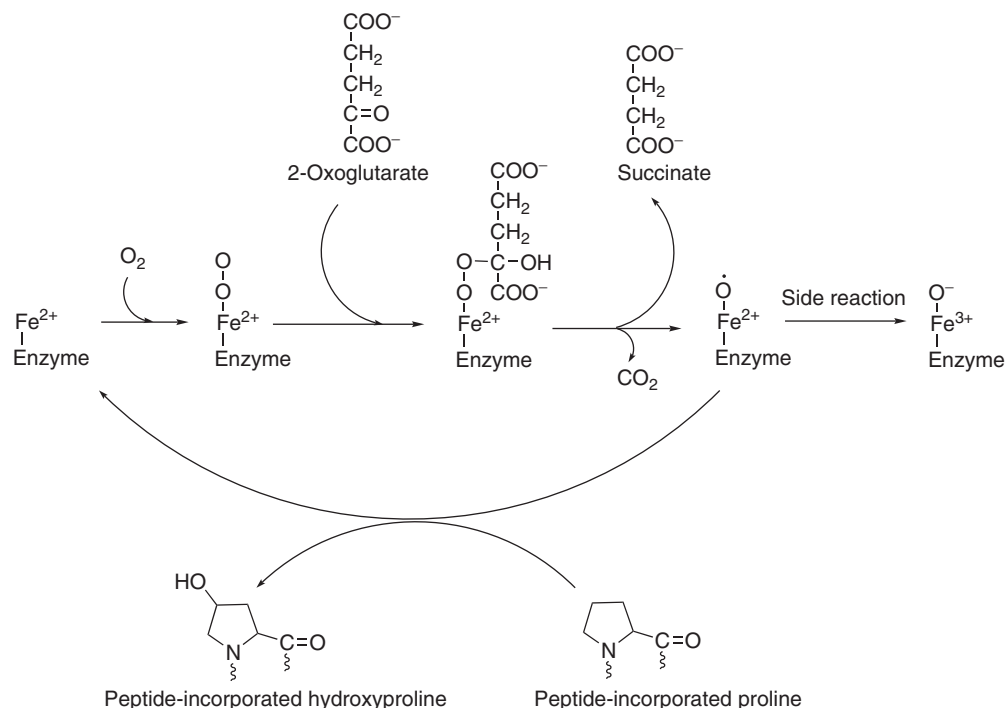


Figure 2 The reaction of procollagen proline hydroxylase.

absorption, also inhibits this presumably protective gastric secretion of ascorbate.

However, although ascorbate can deplete nitrosating compounds under anaerobic conditions, the situation may be reversed in the presence of oxygen. Nitric oxide reacts with oxygen to form N_2O_3 and N_2O_4 , both of which are nitrosating reagents, and can also react with ascorbate to form NO and monodehydroascorbate. It is thus possible for ascorbate to be depleted, with little or no significant effect on the total concentration of nitrosating species.

Antioxidant and Prooxidant Actions of Ascorbate

Chemically, ascorbate is a potent reducing agent, both reducing hydrogen peroxide and also acting as a radical-trapping antioxidant, reacting with superoxide and a proton to yield hydrogen peroxide, or with the hydroxyl radical to yield water. In each case the product is monodehydroascorbate, which, as shown in **Figure 1**, undergoes dismutation to ascorbate and dehydroascorbate. In studies of ascorbate depletion in men there is a significant increase in abnormalities of sperm deoxyribonucleic acid (DNA), suggesting that vitamin C may have a general, nonspecific radical-trapping antioxidant function.

Ascorbate also acts to reduce the tocopheroxyl radical formed by the oxidation of vitamin E in cell membranes and plasma lipoproteins. It thus has a vitamin E sparing antioxidant action, coupling lipophilic and hydrophilic antioxidant reactions.

The antioxidant efficiency of ascorbate is variable. From the chemistry involved, it would be expected that overall 2 mol of tocopheroxyl radical would be reduced per mole of ascorbate, because of the reaction of 2 mol of monodehydroascorbate to

yield ascorbate and dehydroascorbate. However, as the concentration of ascorbate increases, the molar ratio decreases, and only at very low concentrations of ascorbate it tends toward the theoretical ratio. This is because, as well as its antioxidant role, ascorbate can be a source of hydroxyl and superoxide radicals.

At high concentrations, ascorbate can reduce molecular oxygen to superoxide, being oxidized to monodehydroascorbate. Both Fe^{3+} and Cu^{2+} ions are reduced by ascorbate, again yielding monodehydroascorbate; the resultant Fe^{2+} and Cu^+ are reoxidized by reaction with hydrogen peroxide to yield hydroxide ions and hydroxyl radicals. Thus, as well as its antioxidant role, ascorbate has prooxidant action; the net result will depend on the relative rates of formation of superoxide and hydroxyl radicals by autooxidation and metal-catalyzed reactions of ascorbate, and the trapping of these radicals by ascorbate.

It seems likely that the prooxidant actions of ascorbate are of relatively little importance *in vivo*. Except in cases of iron overload there are almost no transition metal ions in free solution, they are all bound to proteins, and because the renal transport system is readily saturated, plasma and tissue concentrations of ascorbate are unlikely to rise to a sufficient extent to lead to significant radical formation.

Assessment of Vitamin C Status

The early method of assessing vitamin C nutritional status was by testing the extent of saturation of the body's reserves, by giving a test dose of 500 mg (2.8 mmol), and measuring the amount excreted in the urine. In a subject with high status, more or less all of the test dose is recovered over a period of 5–6 h.

Table 2 Plasma and leukocyte ascorbate concentrations as criteria of vitamin C nutritional status

		<i>Deficient</i>	<i>Marginal</i>	<i>Adequate</i>
Whole blood	mmol l ⁻¹	<17	17–28	>28
	mg l ⁻¹	<3.0	3.0–5.0	>5.0
Plasma	mmol l ⁻¹	<11	11–17	>17
	mg l ⁻¹	<2.0	2.0–3.0	>3.0
Leukocytes	pmol per 10 ⁶ cells	<1.1	1.1–2.8	>2.8
	μg per 10 ⁶ cells	<0.2	0.2–0.5	>0.5

More sensitive assessment of status is achieved by measuring the concentration of the vitamin in whole blood, plasma, or leukocytes. Criteria of adequacy are shown in **Table 2**. The determination of ascorbate in whole blood is complicated by nonenzymic oxidation of the vitamin by hemoglobin, and most studies rely on plasma or leukocyte concentrations of ascorbate.

A problem arises in the interpretation of leukocyte ascorbate concentrations because of the different capacity of different classes of leukocytes to accumulate the vitamin. Granulocytes are saturated at a concentration of approximately 530 pmol per 10⁶ cells, whereas mononuclear leukocytes can accumulate 2.5-times more ascorbate. A considerable mythology has developed to the effect that vitamin C requirements are increased in response to infection, inflammation, and trauma, based on reduced leukocyte concentrations of ascorbate in these conditions. However, the fall in leukocyte ascorbate can be accounted for by an increase in the proportion of granulocytes in response to trauma and infection (and hence a fall in the proportion of mononuclear leukocytes). Total leukocyte ascorbate is not a useful index of vitamin C status without a differential white cell count.

There is increased formation of 8-hydroxyguanine (a marker of oxidative radical damage) in DNA during (short term) vitamin C depletion, and the rate of removal of 8-hydroxyguanine from DNA by excision repair, and hence its urinary excretion, is affected by vitamin C status. This suggests that measurement of urinary excretion of 8-hydroxyguanine may provide a biomarker of optimum status, as a basis for estimating requirements.

Requirements

Although the minimum requirement for ascorbate is firmly established, there are considerable differences between the reference intakes published by different national and international authorities. Depending on the chosen criteria of adequacy, and assumptions made in interpreting experimental results, it is possible to produce arguments in support of reference intakes ranging from 30 to 100 mg day⁻¹. Studies of intakes associated with reduced risks of cancer and cardiovascular disease suggest an average requirement of 90–100 mg day⁻¹, and a reference intake of 120 mg day⁻¹.

Minimum Requirement

The minimum requirement for vitamin C was established in the 1940s in a depletion/repletion study, which showed that

an intake of less than 10 mg day⁻¹ was adequate to prevent the development of scurvy, or to cure the clinical signs. At this level of intake, wound healing is impaired, and optimum wound healing requires a mean intake of 20 mg day⁻¹. Allowing for individual variation, this gives reference intake of 30 mg day⁻¹, which was the UK figure until 1991 and the World Health Organization/Food and Agriculture Organization figure until 2001.

Requirements Estimated from the Plasma and Leukocyte Concentrations of Ascorbate

The plasma concentration of ascorbate shows a sigmoidal relationship with intake. Below approximately 30 mg day⁻¹ it is extremely low and does not reflect increasing intake any significant extent. As the intake rises above 30 mg day⁻¹, the plasma concentration begins to increase sharply, reaching a plateau of 70–85 μmol l⁻¹, at intakes between 70 and 100 mg day⁻¹, when the renal threshold is reached and the vitamin is excreted quantitatively with increasing intake.

The point at which the plasma concentration increases more or less linearly with increasing intake represents a state where reserves are adequate and ascorbate is available for transfer between tissues. This corresponds to an intake of 40 mg day⁻¹ and is the basis of the UK, EU, and FAO figures. At this level of intake the total body pool is approximately 5.1 mmol (900 mg). It has been argued that setting requirements and reference intakes on the basis of the steep part of a sigmoidal curve is undesirable, and a more appropriate point would be the intake at which the plasma concentration reaches a plateau, at an intake of approximately 100–200 mg day⁻¹.

The US/Canadian reference intakes of 75 mg for women and 90 mg for men are based on studies of leukocyte saturation during depletion/repletion studies.

Requirements Estimated from Maintenance of the Body Pool of Ascorbate

An alternative approach to estimating requirements is to determine the fractional rate of catabolism of total body ascorbate; an appropriate intake would then be that required to replace losses and maintain the body pool.

Clinical signs of scurvy are seen when the total body pool of ascorbate is below 1.7 mmol (300 mg). The pool increases with intake, reaching a maximum of approximately 8.5 mmol (1500 mg) in adults – 114 μmol (20 mg) per kg bodyweight. The basis for the 1989 United States Recommended Daily Amount (US RDA) of 60 mg was the observed mean fractional turnover rate of 3.2% of a body pool of 20 mg per kg bodyweight per day, with allowances for incomplete absorption of dietary ascorbate and individual variation.

It has been argued that a total body pool of 5.1 mmol (900 mg) is adequate; it is threefold higher than the minimum required to prevent scurvy, and there is no evidence that there are any health benefits from a body pool greater than 600 mg. The observed body pool of 8.5 mmol in depletion/repletion studies was found in subjects previously consuming a self-selected diet, with a relatively high intake of vitamin C, and

therefore might not represent any index of requirement. Assuming a total body pool of 5.1 mmol and catabolism of 2.7% per day, allowing for efficiency of absorption and individual variation gives a reference intake of 40 mg day⁻¹.

Because the fractional turnover rate was determined during a depletion study, and the rate of ascorbate catabolism varies with intake, it has been suggested that this implies a rate of 3.6% per day before depletion. On this basis, and allowing for incomplete absorption and individual variation, various national authorities arrive at a reference intake of 80 mg.

The rate of ascorbate catabolism is affected by intake, and the requirement to maintain the body pool cannot be estimated as an absolute value. A habitual low intake, with a consequent low rate of catabolism, will maintain the same body pool as a habitual higher intake with a higher rate of catabolism.

Dietary Sources and High Intakes

It is apparent from the list of rich sources of vitamin C in Table 3 that the major determinant of vitamin C intake is the consumption of fruits and vegetables; deficiency is likely in people whose habitual intake of fruit and vegetables is very low. However, clinical signs of deficiency are rarely seen in developed countries. The range of intakes by healthy adults in Britain reflects fruit and vegetable consumption: the 2.5 percentile intake is 19 mg day⁻¹ (men) and 14 mg day⁻¹ (women), whereas the 97.5 percentile intake from foods (excluding supplements) is 170 mg day⁻¹ (men) and 160 mg day⁻¹ (women). Smokers may be at increased risk of deficiency; there is some evidence that the rate of ascorbate catabolism is twofold higher in smokers than in nonsmokers.

There is a school of thought that human requirements for vitamin C are considerably higher than those discussed above. The evidence is largely based on observation of the vitamin C intake of gorillas in captivity, assuming that this is the same as their intake in the wild (where they eat considerably less fruit than under zoo conditions) and then assuming that because they have this intake, it is their requirement – an unjustified assumption. Scaling this to human beings suggests a requirement of 1–2 g day⁻¹.

Intakes in excess of approximately 80–100 mg day⁻¹ lead to a quantitative increase in urinary excretion of unmetabolized ascorbate, suggesting saturation of tissue reserves. It is difficult to justify a requirement in excess of tissue storage capacity.

A number of studies have reported low ascorbate status in patients with advanced cancer – perhaps an unsurprising finding in seriously ill patients. One study has suggested, on the basis of an uncontrolled open trial, that 10-g daily doses of vitamin C resulted in increased survival. Controlled studies have not demonstrated any beneficial effects of high dose ascorbic acid in the treatment of advanced cancer.

High doses of ascorbate are popularly recommended for the prevention and treatment of the common cold. The evidence from controlled trials is unconvincing, and meta-analysis shows no evidence of a protective effect against the incidence of colds. There is, however, consistent evidence of a beneficial effect in reducing the severity and duration of symptoms. This may be

Table 3 Rich sources of vitamin C

	Portion (g)	mg per portion	mg 100 g ⁻¹
Blackcurrants	80	160	200
Oranges	250	125	50
Orange juice	200	100	34
Strawberries	100	60	60
Grapefruit	140	56	40
Melon	200	50	25
Green peppers	45	45	100
Sweet potato	150	38	25
Loganberries	85	34	40
Spinach	130	33	25
Red currants	80	32	40
White currants	80	32	40
Pineapple	125	31	25
Brussels sprouts	75	30	40
Mangoes	100	30	30
Satsumas	100	30	30
Tangerines	100	30	30
Turnips	120	30	25
Gooseberries	70	28	40
Potato chips	265	27	10
Broccoli	75	26	35
Swedes	120	24	20
Spring greens	75	23	31
Artichokes, globe	220	22	10
Potatoes	140	21	15
Avocados	130	20	15
Leeks	125	20	16
Lemons	25	20	80
Okra	80	20	25
Peas	75	20	27
Raspberries	80	20	25
Tomato juice	100	20	20
Plantain, green	85	17	20
Bilberries	80	16	20
Blackberries	80	16	20
Kidney	150	15	10
Tomatoes	75	15	20
Bananas	135	14	10
Cauliflower	65	13	20
Beans, broad	75	11	15
Cabbage	75	11	15
Nectarines	110	11	10
Parsnips	110	11	10
Rhubarb	100	10	10

Source: Reproduced from Food Standards Agency (2002) *McCance and Widdowson's the Composition of Foods*, 6th summary edn. Cambridge: Royal Society of Chemistry.

due to the antioxidant actions of ascorbate against the oxidizing agents produced by, and released from, activated phagocytes, and hence a decreased inflammatory response.

Scorbutic guinea pigs develop hypercholesterolemia. Although there is no evidence that high intakes of vitamin C result in increased cholesterol catabolism, there is evidence that monodehydroascorbate inhibits hydroxymethylglutaryl CoA reductase, resulting in reduced synthesis of cholesterol, and high intakes of ascorbate may have some hypocholesterolemic action. There is limited evidence of benefits of high intakes of vitamin C in reducing the incidence of stroke, but inconsistent evidence with respect to coronary heart disease.

Regardless of whether or not high intakes of ascorbate have any beneficial effects, large numbers of people habitually take between 1 and 5 g day⁻¹ of vitamin C supplements. There is little evidence of any significant toxicity from these high intakes. Once the plasma concentration of ascorbate reaches the renal threshold, it is excreted more or less quantitatively with increasing intake. However, ascorbate can react non-enzymically with amino groups in proteins, leading to glycation, as occurs in poorly controlled diabetes, and there is some evidence that diabetics who take vitamin C supplements are at increased risk of cardiovascular disease.

Because the rate of ascorbate catabolism increases with increasing intake, it has been suggested that abrupt cessation of high intakes of ascorbate may result in rebound scurvy, because of 'metabolic conditioning' and a greatly increased rate of catabolism. Although there have been a number of anecdotal reports, there is no evidence that this occurs.

See also: Antioxidants. Cancer: Carcinogenic Substances in Food. Diabetes Mellitus: Dietary Management. Iron: Physiology, Dietary Sources, and Requirements. Nutritional Aspects of Bone. Vitamin C: Deficiency States. Vitamin E: Physiology and Health Effects

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VITAMIN D

Physiology, Dietary Sources, and Requirements

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Introduction

Vitamin D is a fat-soluble vitamin that is recognized for its importance for bone health. Vitamin D is neither a vitamin nor a nutrient because exposure to sunlight can provide the body's requirement for vitamin D. We take vitamin D for granted because it is casual exposure to sunlight that provides most humans with their vitamin D requirement. This recognition and the fortification of milk and other foods including some margarines, cereals, and orange juice with vitamin D have eradicated vitamin D deficiency rickets as a significant health problem for children in the US and countries that practise this fortification process. However, it is now recognized that both children and adults are at risk for developing vitamin D deficiency. The daily requirement for vitamin D is 400 IU day⁻¹ for children 0–1 year, 600 IU day⁻¹ for children over 1 year and all adults <70 years old, and 800 IU day⁻¹ for those >70 years. However, without adequate sun exposure, at least 1000 IU day⁻¹ may be needed. Once vitamin D is formed in the skin or ingested in the diet, it enters the bloodstream and travels to the liver and kidney where it is hydroxylated on carbons 25 and 1 to form 25-hydroxyvitamin D (25(OH)D) and 1,25-dihydroxyvitamin D (1,25(OH)₂D), respectively. 25-Hydroxyvitamin D is the major circulating form of the vitamin that is measured to determine the vitamin D status of patients. 1,25-Dihydroxyvitamin D is the biologically active form of vitamin D that is responsible for maintaining calcium homeostasis and bone health. It is now recognized that vitamin D deficiency may increase the risk of many chronic diseases, including cancers of the breast, prostate, and colon, type I diabetes mellitus, multiple sclerosis, rheumatoid arthritis, and heart disease.

Origin and Structure of Vitamin D

As the industrial revolution began to take hold in Northern Europe in the fifteenth century, it was quickly associated with a new disease that caused severe growth retardation and bony deformities in young children (Figure 1). This disease was commonly known as rickets or 'English disease' and plagued the children of the industrialized cities in Europe and North America for more than 250 years. Although Sniadecki in 1822 and Palm in 1890 both recognized that it was lack of exposure to sunlight that was the likely cause of rickets in children, Huldschinsky, in 1919, was the first to prove that exposure of the skin to ultraviolet radiation could cure rickets. Within 2

years, Hess and Unger reported that exposure of several rachitic children to sunlight was adequate for curing this bone-deforming disease.

Steenbock and Black and Hess independently recognized that exposure of animals and their food to ultraviolet radiation imparted antirachitic activity. This led to the recommendation for the ultraviolet irradiation of foods as a means of fortifying them with vitamin D. This resulted in the addition of provitamin D to milk followed by ultraviolet irradiation. As soon as it was possible to commercially synthesize vitamin D in large quantities, it was added directly to milk and other foods.

The first vitamin D was isolated from the irradiation of the yeast sterol ergosterol (Figure 2). This vitamin D was thought to be identical to that produced in the skin of animals and humans. However, studies revealed that when vitamin D produced from yeast was fed to chickens, they were unable to utilize it and developed rickets. When chickens were fed natural vitamin D from fish liver oil, rickets was prevented. This led to the conclusion that vitamin D originating from yeast was different from that in fish liver oil and animal and human skin. In 1937, this mystery was solved when the structure of provitamin D from pig skin was determined. A structural analysis revealed that provitamin D derived from ergosterol differed from that derived from pig skin. The provitamin D (ergosterol; provitamin D₂) that came from yeast had a double bond between carbons 22 and 23 and a methyl group on carbon 24. The provitamin D in animal skin had a side chain that was identical to cholesterol, that is, it did not contain either a double bond or methyl group on carbons 22–23 and 24, respectively, and was identified as 7-dehydrocholesterol (provitamin D₃) (Figure 2). The vitamin Ds generated from ergosterol and 7-dehydrocholesterol were called ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃), respectively.

Production of Vitamin D in the Skin

During exposure to sunlight, the ultraviolet B photons with energies between 290 and 315 nm are absorbed by provitamin D₃ (7-dehydrocholesterol) in the skin. This absorption results in a photolysis of the B-ring of provitamin D₃ resulting in the formation of previtamin D₃ (Figure 3). However, because previtamin D₃ is thermodynamically unstable, it quickly undergoes an isomerization (rearrangement) of its triple bond system to form vitamin D₃. This isomerization process is enhanced in skin cells because the previtamin D₃ is synthesized



Figure 1 This is a typical presentation of a child with rickets. The child is suffering from severe muscle weakness, has bony deformities including bowed legs, and knob-like projects in the middle of his ribcage called the rachitic rosary. Reproduced from Fraser D and Scriver CR (1979) Disorders associated with hereditary or acquired abnormalities of vitamin D function: Hereditary disorders associated with vitamin D resistance or defective phosphate metabolism. In: De Groot LJ, *et al.* (eds.) *Endocrinology*, pp. 797–808. New York: Grune and Stratton.

in the cell membrane, which restricts its movement thereby accelerating the transformation of previtamin D_3 to vitamin D_3 . Once vitamin D_3 is formed in the skin cell membrane, it is no longer restricted in its movement and freely translocates into the extracellular space to find its way into the dermal capillary bloodstream where it is bound to a specific vitamin D-binding protein (Figure 3).

An increase in skin pigmentation and zenith angle of the sun (change in latitude, season, and time of day) and the topical application of a sunscreen can markedly diminish or even prevent the production of vitamin D_3 in the skin. Over the age of ~ 65 years, there is a three- to fourfold decline in the synthetic capacity of the skin to produce vitamin D_3 . Excessive exposure to sunlight cannot cause vitamin D_3 intoxication because once previtamin D_3 and vitamin D_3 are made

in the skin, excessive quantities are rapidly destroyed by sunlight (Figure 3).

Absorption, Metabolism, and Excretion of Vitamin D

Vitamin D (vitamin D without a subscript represents either vitamin D_2 or D_3) is fat soluble and, therefore, once ingested vitamin D_2 and vitamin D_3 are incorporated into the chylomicron fraction and absorbed in the small intestine into the lymphatic system. Both dietary vitamin D_2 and vitamin D_3 , and cutaneous vitamin D_3 enter the circulation and are bound to a specific α_1 -globulin known as the vitamin D-binding protein (DBP). It is believed that this protein acts as a buffering system whereby it helps maintain circulating concentrations of $25(OH)D$ so that the free unbound form of $25(OH)D$ can enter into the renal tubular cells to be metabolized. 25 -Hydroxyvitamin D bound to DBP is transported by megalin into the renal tubular cell where $25(OH)D$ is then dissociated from DBP and enters the mitochondria to be metabolized.

Neither vitamin D_2 nor vitamin D_3 possess any intrinsic biologic activity on calcium metabolism. They both require a hydroxylation on carbon 25 to form $25(OH)D$ (Figure 4). When given as a 1000-IU supplement, vitamin D_2 is as effective as vitamin D_3 in raising serum $25(OH)D$ levels. $25(OH)D$ is the major circulating form of vitamin D, and at physiologic concentrations, it too has little biologic activity on calcium metabolism. It must undergo a hydroxylation on carbon 1 in the kidney to form $1,25(OH)_2D$, the biologically active form of vitamin D (Figure 4). The metabolism of $25(OH)D$ to $1,25(OH)_2D$ is tightly regulated by parathyroid hormone (PTH), fibroblast growth factor 23 (FGF23), and serum phosphorus levels (Figure 5). PTH and low serum phosphorus levels increase the production of $1,25(OH)_2D$, whereas FGF23 suppresses its production.

$25(OH)D$ and $1,25(OH)_2D$ act as substrate for a 24-hydroxylase (an enzyme that attaches an hydroxyl on carbon-24), which is found in the kidney and other target tissues for $1,25(OH)_2D$. Once $1,25(OH)_2D$ is hydroxylated on carbon 24, this is the first step in its degradation to a water-soluble acid, calcitric acid (Figure 4). Vitamin D and calcitric acid are excreted in the bile.

Biologic Functions of Vitamin D on Calcium Metabolism

$1,25(OH)_2D$ interacts with a specific nuclear receptor that is commonly known as the vitamin D receptor (VDR) and is one of the many members of the super family of steroid hormone receptors that includes retinoic acid, thyroid hormone, glucocorticoids, and sex steroids. Once $1,25(OH)_2D$ interacts with the VDR, the complex forms a heterodimer with retinoic acid X receptor (RXR) (Figure 6). This new complex sits on specific segments of vitamin D responsive genes known as vitamin D responsive elements (VDREs) to either increase or decrease transcriptional activity of the vitamin D-sensitive genes such as osteocalcin, calcium binding protein (calbindin), PTH, and osteonectin (Figure 6).

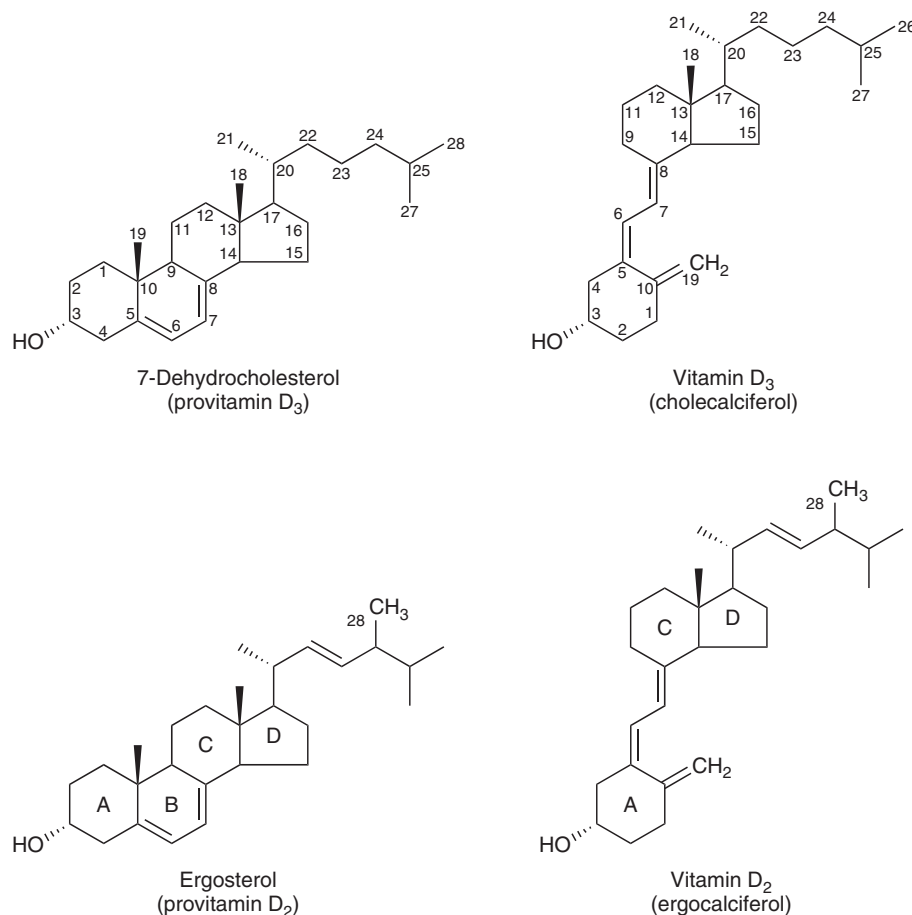


Figure 2 Structures for 7-dehydrocholesterol (provitamin D₃), ergosterol (provitamin D₂), vitamin D₃ (cholecalciferol), and vitamin D₂ (ergocalciferol). The carbons are numbered and the ring systems are labeled. Reproduced with permission from Holick MF (2007) Vitamin D deficiency. *New England Journal of Medicine* 357: 266–281.

In the intestine, $1,25(\text{OH})_2\text{D}$ enhances the absorption of dietary calcium (from ~15% to 30–40% in adults) and phosphorus (from ~60–80%) across the microvilli of the small intestinal absorptive cells (Figure 5). $1,25(\text{OH})_2\text{D}$ also interacts with osteoblasts to stimulate the expression for receptor activator NF κ B ligand (RANKL) in the bone to initiate the transformation of monocytes into mature osteoclasts (Figure 5). Thus, $1,25(\text{OH})_2\text{D}_3$ regulates serum calcium levels by enhancing the efficiency of intestinal calcium absorption and stimulating resorption of calcium from the bone. It remains controversial as to whether $1,25(\text{OH})_2\text{D}$ has any direct action on the renal handling of either calcium or phosphorus.

There are a variety of other tissues including the brain, gonads, pancreas, stomach, activated T and B lymphocytes, monocytes, and skin that have nuclear VDR. Although the exact physiologic function of $1,25(\text{OH})_2\text{D}$'s interaction with these VDRs is not well understood, it is known that *in vivo* and *in vitro* $1,25(\text{OH})_2\text{D}_3$ can inhibit proliferation and induce terminal differentiation of various normal and tumor cells including normal human keratinocytes. This is the reason why activated vitamin D compounds are now routinely used for the treatment of the hyperproliferative skin disorder psoriasis. It has been estimated that at least 200 and as many as 2000 genes are influenced directly or indirectly by $1,25(\text{OH})_2\text{D}_3$.

Evaluation for and Consequences of Vitamin D Deficiency

Vitamin D deficiency in young children causes rickets. As a child becomes vitamin D deficient, this results in a decrease in the efficiency of intestinal calcium absorption. There is a decline in blood-ionized calcium, which causes the parathyroid glands to produce and secrete more PTH. PTH tries to conserve calcium by enhancing tubular reabsorption of calcium in the kidney. However, in the face of developing hypocalcemia, which could disturb neuromuscular function and a wide variety of metabolic and cellular processes, the body calls upon $1,25(\text{OH})_2\text{D}$ and PTH to stimulate the expression of RANKL in osteoblasts to mobilize monocytic stem cells to become functional osteoclasts, which, in turn, mobilize calcium from the skeleton (Figure 5). In addition, PTH causes a loss of phosphorus into the urine causing hypophosphatemia. Thus, in early vitamin D deficiency, the serum calcium is normal; it is the low serum phosphorus that causes the extracellular CaXPO_4 to be too low for normal mineralization of bone matrix. This causes a disruption in the orderly sequence of events in the differentiation of hypertrophied chondrocytes in the epiphyseal plates resulting in their disorganization causing a widening of the epiphyseal plates (end of long bones),

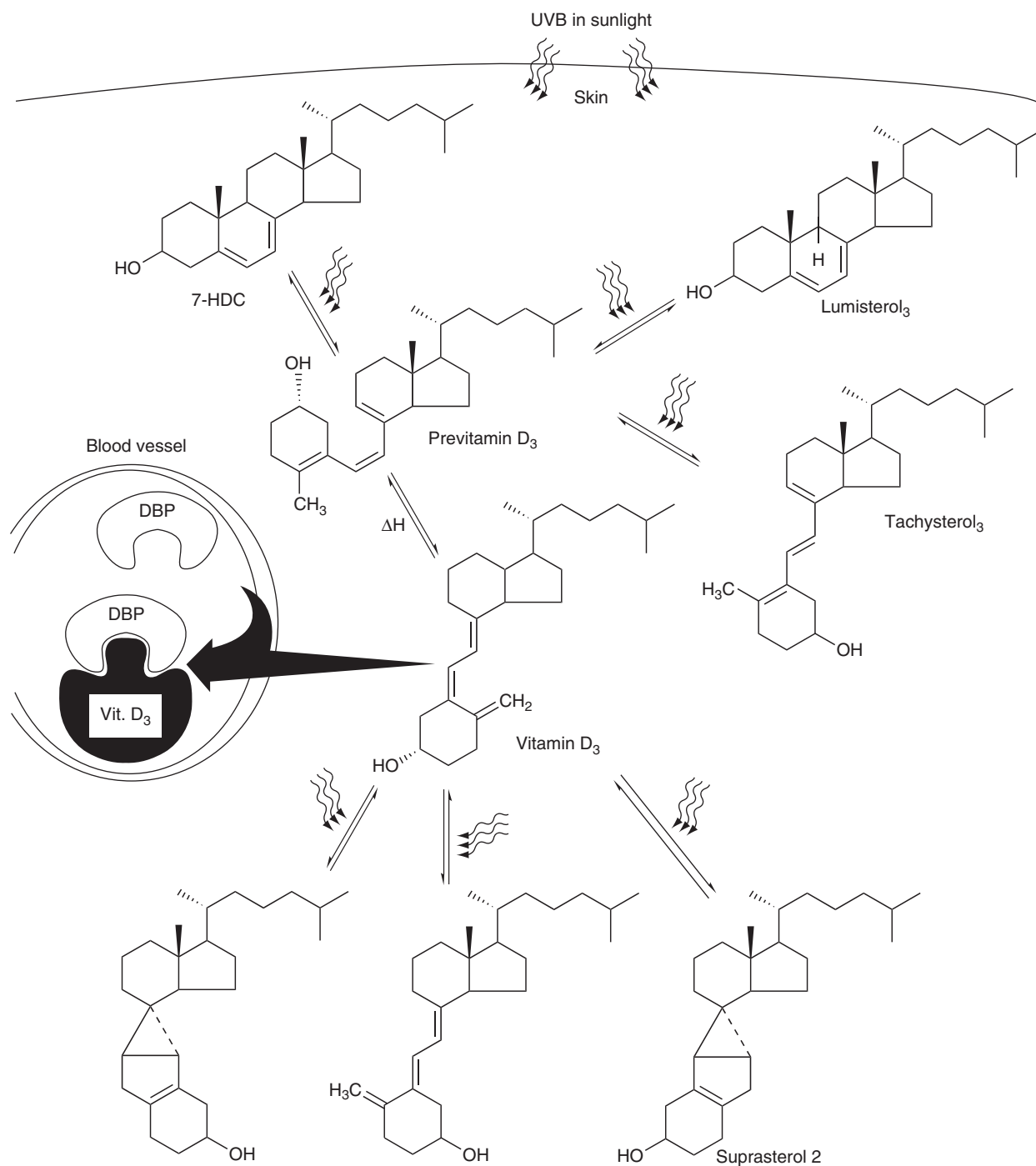


Figure 3 A schematic representation of the photochemical and thermal events that result in the synthesis of vitamin D₃ in the skin, and the photodegradation of previtamin D₃ and vitamin D₃ to biologically inert photoproducts. 7-Dehydrocholesterol (7-DHC) in the skin is converted to previtamin D₃ by the action of solar ultraviolet B radiation. Once formed, previtamin D₃ is transformed into vitamin D₃ by a heat-dependent (ΔH) process. Vitamin D₃ exits the skin into the dermal capillary blood system and is bound to a specific vitamin D-binding protein (DBP). When previtamin D₃ and vitamin D₃ are exposed to solar ultraviolet B radiation, they are converted to a variety of photoproducts that have little or no activity on calcium metabolism. Reproduced from Holick MF (1995) Vitamin D: Photobiology, metabolism, and clinical applications. In: DeGroot LJ, *et al.* (eds.) *Endocrinology*, 3rd edn, pp. 990–1013. Philadelphia: W.B. Saunders.

demineralization of the skeleton, and bony deformities (Figure 1).

Once the epiphyseal plates are closed later in adolescence, vitamin D deficiency can no longer cause bone deformities.

Instead, there is an inability to mineralize newly deposited bone matrix leading to wide osteoid seams within the trabecular and cortical bone causing the bone disease commonly known as osteomalacia. In addition, the secondary

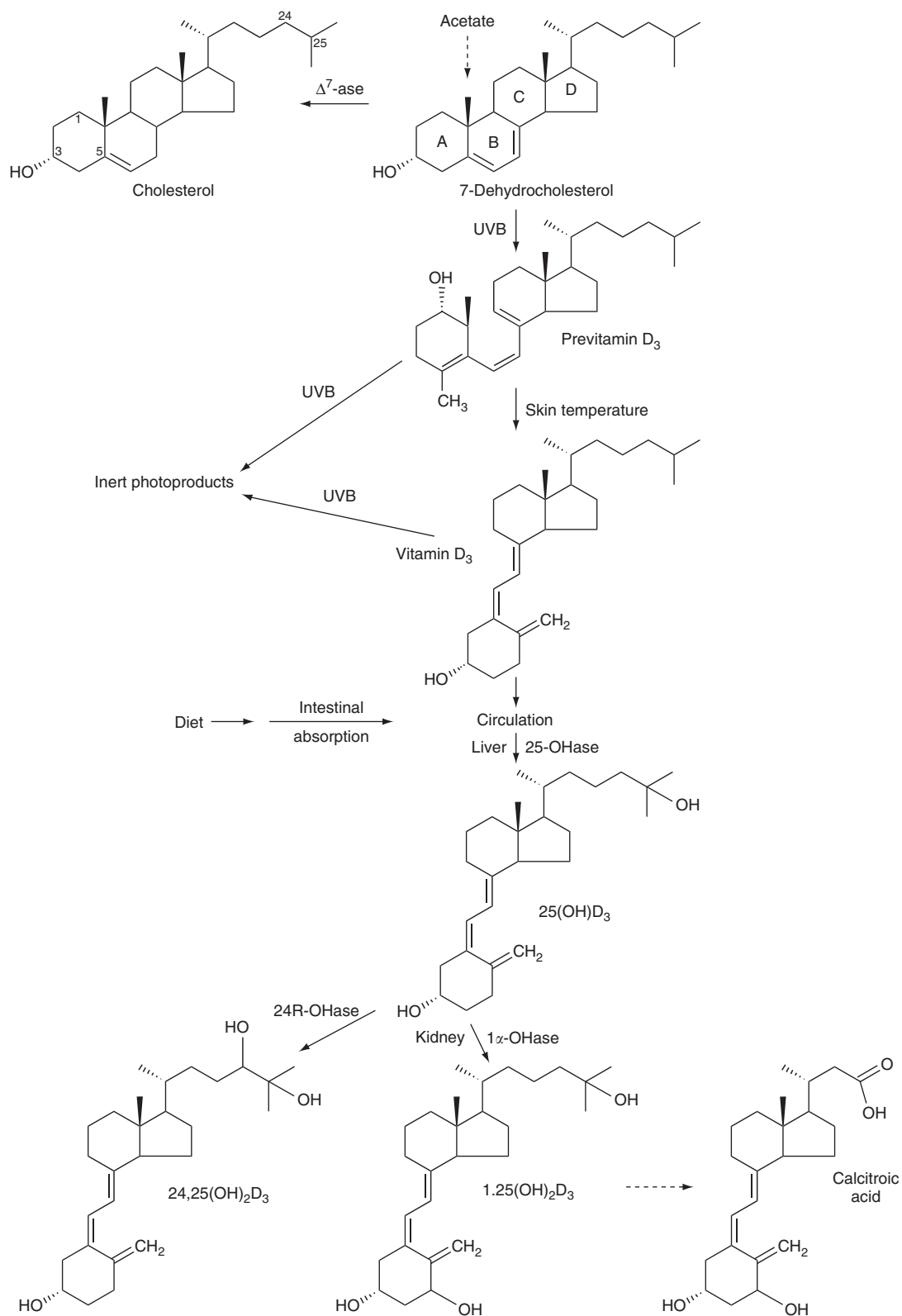


Figure 4 A schematic representation of the origin of vitamin D₃ and its metabolism in the liver by the hepatic vitamin D-25-hydroxylase. Once formed, the 25-hydroxyvitamin D₃ (25(OH)D₃) is metabolized by either a 25(OH)D-1 α -hydroxylase or a 25(OH)D-24-hydroxylase. 1,25-Dihydroxyvitamin D₃ (1,25(OH)₂D₃) can either go to its target tissues to carry out its biologic function(s), or it can be metabolized in its side-chain and degraded to calcitroic acid. Reproduced from Holick MF (1995) Vitamin D: Photobiology, metabolism, and clinical applications. In: DeGroot LJ, *et al.* (eds.) *Endocrinology*, 3rd edn, pp. 990–1013. Philadelphia: W.B. Saunders.

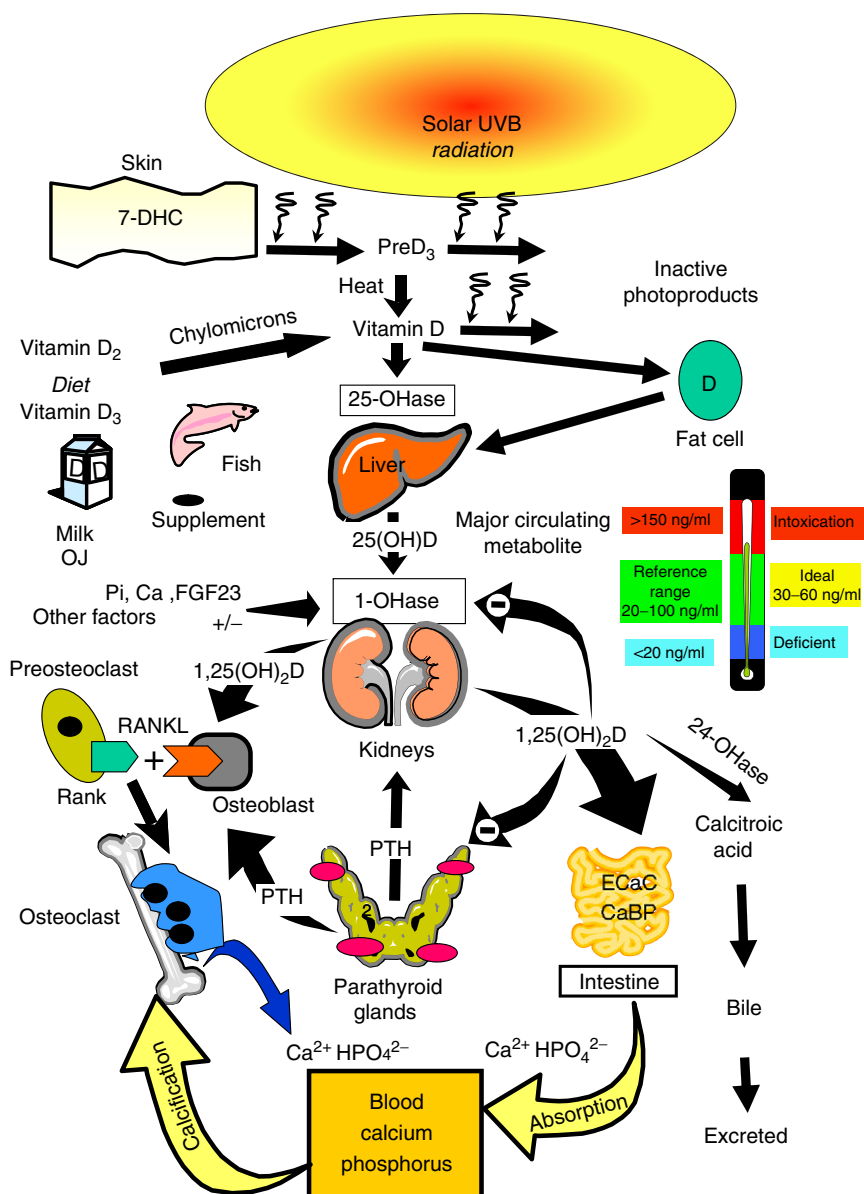


Figure 5 Schematic representation of the synthesis and metabolism of vitamin D for regulating calcium, phosphorus, and bone metabolism. During exposure to sunlight, 7-dehydrocholesterol in the skin is converted to previtamin D₃. PreD₃ immediately converts by a heat-dependent process to vitamin D₃. Excessive exposure to sunlight degrades previtamin D₃ and vitamin D₃ into inactive photoproducts. Vitamin D₂ and vitamin D₃ from dietary sources are incorporated into chylomicrons, transported by the lymphatic system into the venous circulation. Vitamin D (D represents D₂ or D₃) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D binding protein which transports it to the liver where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D (25(OH)D). This is the major circulating form of vitamin D that is used by clinicians to measure vitamin D status (although most reference laboratories report the normal range to be 20–100 ng ml⁻¹, the preferred healthful range is 30–60 ng ml⁻¹). It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1 α -hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D [1,25(OH)₂D]. Serum phosphorus, calcium, fibroblast growth factors (FGF-23), and other factors can either increase (+) or decrease (–) the renal production of 1,25(OH)₂D. 1,25(OH)₂D feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands. 1,25(OH)₂D increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize 1,25(OH)₂D to the water-soluble biologically inactive calcitric acid which is excreted in the bile. 1,25(OH)₂D enhances intestinal calcium absorption in the small intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9 K (calcium binding protein; CaBP). 1,25(OH)₂D is recognized by its receptor in osteoblasts causing an increase in the expression of receptor activator of NF κ B ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralization of the skeleton. Reproduced with permission from Holick MF (2007) Vitamin D deficiency. *New England Journal of Medicine* 357: 266–281.

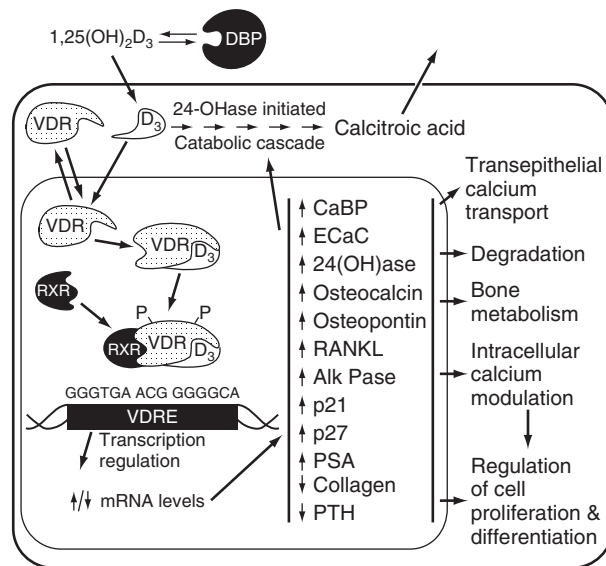


Figure 6 A schematic representation of the mechanism of action of 1,25(OH)₂D₃ in various target cells resulting in a variety of biological responses. The free form of 1,25(OH)₂D₃ enters the target cell and interacts with its nuclear vitamin D receptor (VDR), which is phosphorylated (Pi). The 1,25(OH)₂D₃-VDR complex combines with the retinoic acid X receptor (RXR) to form a heterodimer, which, in turn, interacts with the vitamin D responsive element (VDRE), causing an enhancement or inhibition of transcription of vitamin D-responsive genes including calcium-binding protein (CaBP), ECaC, 24-OHase, RANKL, alkaline phosphatase (alk Pase), prostate-specific antigen (PSA), and PTH. Reproduced with permission from Holick MF (2007) Vitamin D deficiency. *New England Journal of Medicine* 357: 266–281.

hyperparathyroidism that results from vitamin D deficiency results in the mobilization of precious calcium stores from the bone thereby exacerbating bone loss and causing osteoporosis. This can increase a person's risk for fracture.

The hallmark for determining the vitamin D status is the measurement of the circulating concentration of 25(OH)D. The 25(OH)D is low or undetectable in vitamin D deficiency and markedly elevated in vitamin D intoxication. Measurement of 1,25(OH)₂D is of little value for determining the vitamin D nutritional status because its synthesis is tightly regulated. Indeed, as a person becomes vitamin D deficient, there is an increase in the secretion of PTH which, in turn, increases the production of 1,25(OH)₂D. Thus, early in vitamin D deficiency one can see a normal fasting serum calcium, low-normal to low phosphorus, low 25(OH)D, and elevated PTH, 1,25(OH)₂D and alkaline phosphatase. In chronic vitamin D deficiency, all the above are seen with the exception that serum calcium and 1,25(OH)₂D are low-normal or low.

Nonskeletal Consequences of Vitamin D Deficiency

As early as 1941, it was appreciated that if you lived at higher latitudes in the US you were at higher risk of dying of cancer. A multitude of epidemiologic studies clearly show that if you live at higher latitudes and are more prone to vitamin D deficiency, then you are at higher risk of dying of colon, prostate,

breast, ovarian, and a variety of other cancers. It is also known that living at higher latitudes increases risk of having high blood pressure and heart disease as well as autoimmune diseases including multiple sclerosis and type I diabetes.

Essentially every cell and organ in the body requires vitamin D, that is, they all have a VDR. It is also known that most tissues in the body can activate vitamin D. Thus, maintaining adequate levels of 25(OH)D in the circulation of at least 20 ng ml⁻¹ as recently recommended by the IOM for bone health and preferably 30 ng ml⁻¹ may be necessary for various organs including colon, breast, and prostate to convert it to 1,25(OH)₂D, which in turn can help regulate various genes responsible for cell growth and differentiation (Figure 7). This could be the explanation for how vitamin D sufficiency is protective against many common cancers. The immune cells also recognize 1,25(OH)₂D₃. This may explain why children who had received 2000 IU of vitamin D a day during their first year decreased their risk of developing type I diabetes by 88%. A study in Japanese children who received 1200 IU vitamin D₃/d from December through March had a 42% reduced risk for developing influenza A infection compared to children who received a placebo pill. Increasing intake of vitamin D and sun exposure has now been associated with decreased risk of developing multiple sclerosis, rheumatoid arthritis, and even Crohn's disease.

The relationship of vitamin D to cardiovascular disease is finally being understood. 1,25(OH)₂D inhibits the production of the blood pressure hormone renin (Figure 7). It also alters cardiomyocyte growth and modulates the inflammatory response of atherosclerosis. Vitamin D deficiency has been associated with a 50% increased risk for having a heart attack and stroke, and 80% increased risk for developing peripheral vascular disease. When African-American teenagers were given 2000 IU vitamin D₃/d for 4 months, they had a significant reduction in arterial wall stiffness, a prelude to hypertension and atherosclerosis when compared to teenagers receiving 400 IU vitamin D₃/d for four months.

Recommended Dietary Intake of Vitamin D

Vitamin D is very rare in foods naturally, with the exception of fatty fish, some fish liver oils, and mushrooms exposed to UVR. Milk and some dairy products in the United States are fortified with vitamin D. Some orange juice and other juice products are fortified with calcium and 100 IU of vitamin D₃/8 oz. Multivitamin preparations that contain vitamin D are a good source of vitamin D as are pharmaceutical preparations (Table 1).

In 2010, the Institute of Medicine and the National Academy of Sciences reviewed the recommended dietary intake for calcium and vitamin D. The recommended dietary allowance (RDA) was defined as the daily intake level that is sufficient to meet nutrient requirements for nearly all (97–98%) individuals in life-stage and gender group. The RDA was meant to apply to individuals and not groups. When sufficient scientific evidence was not available to calculate an estimated average requirement (EAR), that is, a nutrient value that was estimated to meet the requirement defined by a specified indicator of adequacy in 50% of individuals in a life-stage and gender

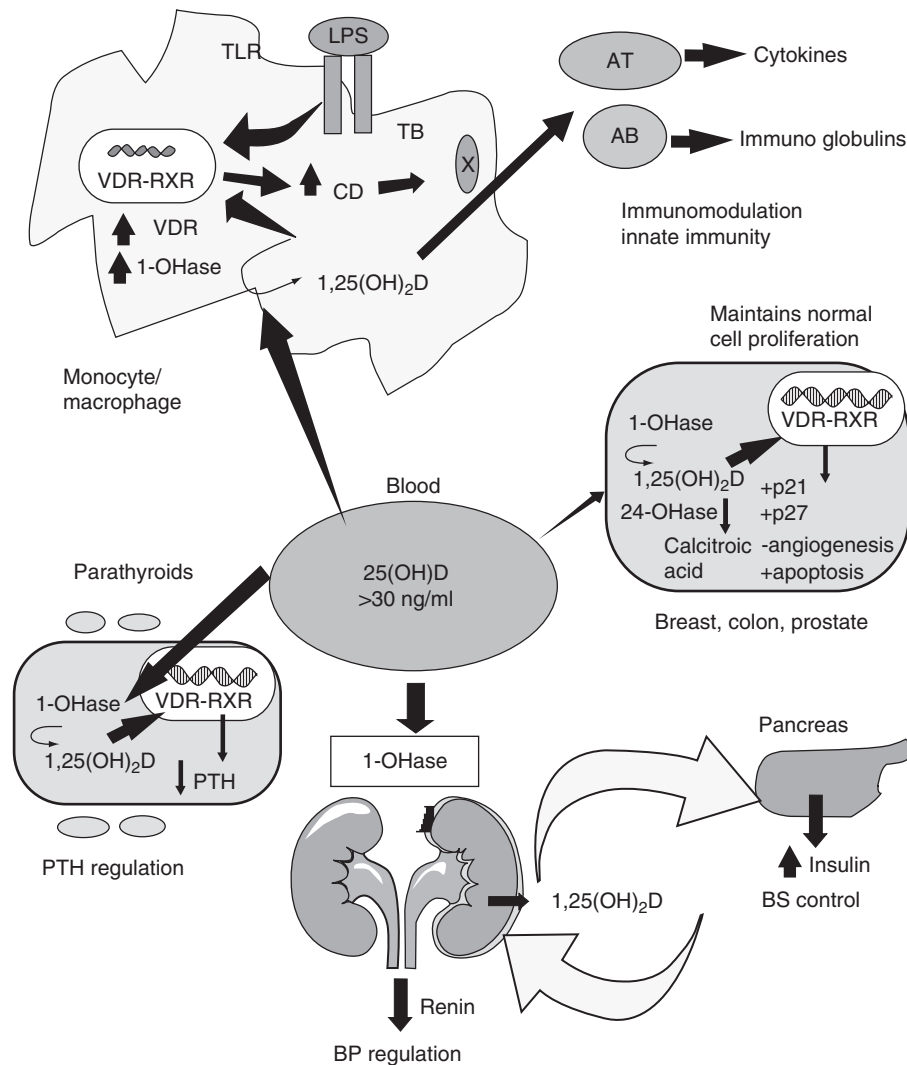


Figure 7 Metabolism of 25-hydroxyvitamin D (25(OH)D) to 1,25 dihydroxyvitamin D, 1,25(OH)₂D for nonskeletal functions. When a monocyte/macrophage is stimulated through its toll-like receptor 2/1 (TLR2/1) by an infective agent such as mycobacterium tuberculosis (TB) or its lipopolysaccharide (LPS), the signal upregulates the expression of vitamin D receptor (VDR) and the 25-hydroxyvitamin D-1-hydroxylase (1-OHase). 25(OH)D levels > 30 ng ml⁻¹ provide adequate substrate for the 1-OHase to convert it to 1,25(OH)₂D. 1,25(OH)₂D returns to the nucleus where it increases the expression of cathelicidin (CD) which is a peptide capable of promoting innate immunity and inducing the destruction of infective agents such as TB. It is also likely that the 1,25(OH)₂D produced in the monocytes/macrophage is released to act locally on activated T (AT) and activated B (AB) lymphocytes, which regulate cytokine and immunoglobulin synthesis, respectively. It is believed that the local production of 1,25(OH)₂D in the breast, colon, prostate, and other cells regulates a variety of genes that control proliferation including p21 and p27 as well as genes that inhibit angiogenesis and induced apoptosis. Once 1,25(OH)₂D completes the task of maintaining normal cellular proliferation and differentiation, it induces the 25-hydroxyvitamin D-24-hydroxylase (24-OHase). The 24-OHase enhances the metabolism of 1,25(OH)₂D to calcitroic acid which is biologically inert. Thus, the local production of 1,25(OH)₂D does not enter the circulation and has no influence on calcium metabolism. The parathyroid glands have 1-OHase activity, and the local production of 1,25(OH)₂D inhibits the expression and synthesis of PTH. The production of 1,25(OH)₂D in the kidney enters the circulation and is able to downregulate renin production in the kidney and to stimulate insulin secretion in the β -islet cells of the pancreas. Reproduced with permission from Holick MF (2007) Vitamin D deficiency. *New England Journal of Medicine* 357: 266–281.

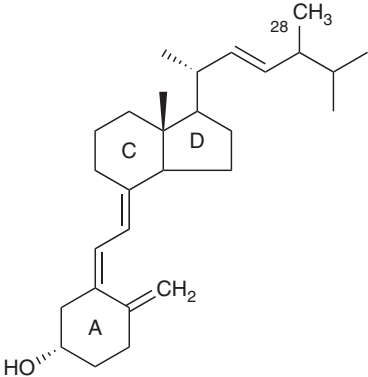
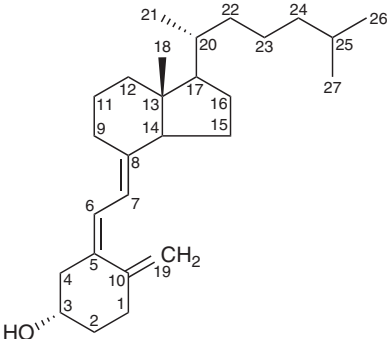
group, the Committee recommended using an adequate intake (AI). The AI is based on the observation of experimentally determined approximations of average nutrient intake by a defined population or subgroup that appears to sustain a defined nutritional state such as normal circulation nutrient values or growth. Because sunlight played such an important role in providing humans with their vitamin D requirement and, therefore, was a variable that was difficult to quantify in

studies in infants that were reviewed by the Committee, it was concluded that an AI rather than an RDA should be used for vitamin D (Table 2).

Adequate Intake for Ages 0–6 Months

It is well documented that human and cows' milk has very little vitamin D naturally. Human milk contains on average

Table 1 Sources of vitamin D₂ and vitamin D₃

Source	Vitamin D content (IU = 25 ng)
<i>Natural sources</i>	
	 
	<p>Vitamin D₂ (Ergocalciferol)</p> <p>Vitamin D₃ (Cholecalciferol)</p>
Cod liver oil	~ 400–1000 IU/tsp vitamin D ₃
Salmon, fresh wild caught	~ 600–1000 IU/3.5 oz vitamin D ₃
Salmon, fresh farmed	~ 100–250 IU/3.5 oz vitamin D ₃ , vitamin D ₂
Salmon, canned	~ 300–600 IU/3.5 oz vitamin D ₃
Sardines, canned	~ 300 IU/3.5 oz vitamin D ₃
Mackerel, canned	~ 250 IU/3.5 oz vitamin D ₃
Tuna, canned	~ 236 IU/3.5 oz vitamin D ₃
Shiitake mushrooms, fresh	~ 100 IU/3.5 oz vitamin D ₂
Shiitake mushrooms, sun dried	~ 1600 IU/3.5 oz vitamin D ₂
Egg yolk	~ 20 IU/yolk vitamin D ₃ or D ₂
Sunlight/UVB radiation	~ 20 000 IU equivalent to exposure to 1 minimal erythral dose (MED) in a bathing suit. Thus, exposure of arms and legs to 0.5 MED is equivalent to ingesting ~ 3000 IU vitamin D ₃
<i>Fortified foods</i>	
Fortified milk	100 IU/8 oz usually vitamin D ₃
Fortified orange juice	100 IU/8 oz vitamin D ₃
Infant formulas	100 IU/8 oz vitamin D ₃
Fortified yogurts	100 IU/8 oz usually vitamin D ₃
Fortified butter	56 IU/3.5 oz usually vitamin D ₃
Fortified margarine	429/3.5 oz usually vitamin D ₃
Fortified cheeses	100 IU/3 oz usually vitamin D ₃
Fortified breakfast cereals	100 IU/serving usually vitamin D ₃
<i>Pharmaceutical sources in the US</i>	
Vitamin D ₂ (ergocalciferol)	50 000 IU/capsule
Drisdol (vitamin D ₂) liquid	8000 IU/cc
<i>Supplemental sources</i>	
Multivitamin	400, 500, 1000 IU vitamin D ₃ or vitamin D ₂
Vitamin D ₃	400, 800, 1000, 2000, 5000, 10 000, and 50 000 IU

^aDesignated calciferol which usually means vitamin D₂.

Source: Reproduced from Holick MF (2007) Vitamin D deficiency. *New England Journal of Medicine* 357: 266–281.

between 10 and 50 IU l⁻¹ (0.25–1.25 µg). This is dependent on the mother's exposure to sunlight and her vitamin D intake. Several studies have suggested that infant intakes of vitamin D of between 8.5 (340 IU) and 15 µg (600 IU) day⁻¹ would provide the maximum effect on their linear growth. A study in infants from Northern China (40–47° N) found that vitamin D supplements of 2.5 (100 IU), 5 (200 IU), or 10 µg (400 IU) day⁻¹ resulted in 36, 29, and 2% of the infants being vitamin D deficient with 25(OH)D levels of less than 25 nmol l⁻¹ (10 ng ml⁻¹). None of the infants, however, had

manifestations of rickets. Chinese infants from two southern cities (22° N and 30° N) maintained normal vitamin D status on as little as 2.5 µg (100 IU) day⁻¹ of vitamin D.

There was a seasonal variation of vitamin D status of infants when they were fed human milk only and did not receive vitamin D supplements; their 25(OH)D levels decreased in the winter due to less exposure to sunlight. However, this decrease did not occur in infants receiving a vitamin D supplement of 10 µg (400 IU) day⁻¹ beginning at 3 weeks of age.

Table 2 Adequate intake (AI) and tolerable upper limit (UL) for Vitamin D

IOM Recommendations		Dr Holick's recommendations for patients at risk for vitamin D deficiency			
Life Stage Group	AI	EAR	RDA	UL	Daily Allowance (IU day ⁻¹)
Infants (months)					
0–6	~400 IU	–	–	1000 IU (25 µg)	400–1000
6–12	~400 IU	–	–	1500 IU (38 µg)	400–1000
Children (years)					
1–3	–	C	600 IU (15 µg)	2500 IU (63 µg)	600–1000
4–8	–	400 IU (10 µg)	600 IU (15 µg)	3000 IU (75 µg)	600–1000
Males (years)					
9–13	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
14–18	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
19–30	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
31–50	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
51–70	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
> 70	–	400 IU (10 µg)	800 IU (20 µg)	4000 IU (100 µg)	1500–2000
Females (years)					
9–13	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
14–18	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
19–30	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
31–50	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
51–70	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
> 70	–	400 IU (10 µg)	800 IU (20 µg)	4000 IU (100 µg)	1500–2000
Pregnancy (years)					
14–18	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
19–30	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
31–50	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
Lactation* (years)					
14–18	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
19–30	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000
31–50	–	400 IU (10 µg)	600 IU (15 µg)	4000 IU (100 µg)	1500–2000

Recommended adequate intakes (AI), estimated average requirement (EAR), recommended dietary allowance (RDA) and tolerable upper limit (UL) by the Institute of Medicine (IOM) and Dr. Holick's recommendation for Daily Allowance and safe Upper Limit (UL) for vitamin D for children and adults who are not obtaining adequate vitamin D from sun exposure and who are at risk for vitamin D deficiency.

Source: Reproduced from Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. (2011) Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 96(7): 1911–1930.

Therefore, based on the available literature, it was concluded that a minimum intake of $2.5 \mu\text{g}$ (100 IU) day^{-1} of vitamin D was adequate to prevent rickets. However, at this intake and in the absence of sunlight, infants are at risk for developing hypovitaminosis D; therefore, it was recommended that an adequate intake of $10 \mu\text{g}$ day^{-1} (400 IU) was prudent for infants during their first 6 months of life.

Adequate Intake for Ages 6–12 Months

Infants between 6 and 12 months of age who were fed human milk and exposed to an average of 35 min day^{-1} of sunshine had similar $25(\text{OH})\text{D}$ concentrations at 1 year of age whether the infants received 400 IU of vitamin D or no vitamin D supplementation. However, in Norway, in the winter, older infants who received an average of $5 \mu\text{g}$ (200 IU) day^{-1} of vitamin D had $25(\text{OH})\text{D}$ levels that were intermediate between those of infants studied at the end of the summer and formula-fed infants.

Therefore, in the absence of any sunlight exposure, an AI of $10 \mu\text{g}$ (400 IU) day^{-1} was recommended.

RDA for Ages 1–18 Years

There are no studies in the scientific literature that systematically evaluated the influence of different amounts of vitamin D on either serum $25(\text{OH})\text{D}$ or bone mineral content in this age group. Sunlight exposure is very important for this age group to obtain its required vitamin D. In South Africa, children aged 1–8 years of mixed race showed no evidence of vitamin D deficiency. A longitudinal study in Norway, where sun exposure was presumed to vary widely over a year, an intake of vitamin D of approximately $2.5 \mu\text{g}$ (100 IU) day^{-1} from fortified margarine in children aged 8–18 years was adequate to prevent vitamin D deficiency.

During puberty, there is a need to increase the efficiency of dietary calcium absorption in order to satisfy the rapid growth of the skeleton. As a result, there is an increase in the metabolism of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$. Because the blood levels of $1,25(\text{OH})_2\text{D}$ are approximately 1000 times less than $25(\text{OH})\text{D}$, this increase in metabolism does not appear to increase the requirement of vitamin D for either boys or girls between the ages of 8 and 18 years. An average daily intake of $2.5 \mu\text{g}$ (100 IU) day^{-1} prevented any evidence of vitamin D deficiency in Scandinavian children in this age group. However, intakes less than $2.5 \mu\text{g day}^{-1}$ in Turkish children aged 12–17 years resulted in a decrease in $25(\text{OH})\text{D}$ levels consistent with vitamin D deficiency. Girls of 9–17 years in Lebanon who took 2000 IU day^{-1} for 1 year had better bone density and muscle strength compared to girls who received 400 IU vitamin D_3 per day. Therefore, based on the available literature, it appears that children between 1 and 18 years obtain some of their vitamin D from exposure to sunlight. The IOM recommended they ingest $15 \mu\text{g}$ (600 IU) day^{-1} .

RDA for Ages 19–70 Years

There is only sparse literature regarding the roles that sunlight and diet play in maintaining an adequate vitamin D status for

men and women in this age group. This age group depends on sunlight for some of its vitamin D requirement. Regardless of exposure to sunlight, it was estimated by the IOM that $15 \mu\text{g}$ (600 IU) day^{-1} meets this age group's needs for bone health.

RDA for Ages 70+ Years

The IOM recommended increasing the dietary intake of vitamin D for this age group to $20 \mu\text{g}$ (800 IU) day^{-1} . This was based on several studies that demonstrated the importance of increasing dietary intakes of vitamin D to maximize bone health. There was strong evidence-based literature that demonstrated a decrease in the circulating concentration of $25(\text{OH})\text{D}$, and an increase in the PTH level correlated with an increased risk of skeletal fractures in both the hip and spine in this age group. Studies in both men and women supplemented with 10 – $25 \mu\text{g day}^{-1}$ of vitamin D demonstrated reduced bone resorption, increased bone mineral content, and a decrease in vertebral and nonvertebral fractures. An evaluation of 333 ambulatory Caucasian women (mean age 58 ± 6 years) found that serum PTH concentrations were elevated in the winter (between March and May) in women consuming less than $5.5 \mu\text{g}$ (220 IU) day^{-1} of vitamin D. There was no seasonal variation in serum PTH concentrations when vitamin D intakes were greater than $5.5 \mu\text{g}$ (220 IU) day^{-1} . When bone loss was evaluated between seasons in women (62 ± 0.5 years) who had a usual vitamin D intake of $2.5 \mu\text{g day}^{-1}$, a dietary supplement of $10 \mu\text{g day}^{-1}$ decreased spinal and hip-bone density loss. An analysis of the skeletons of German adults at autopsy revealed no evidence of vitamin D deficiency osteomalacia for those who had a $25(\text{OH})\text{D} > 30 \text{ ng ml}^{-1}$.

Thus, because this age group does not obtain as much of its vitamin D from exposure to sunlight, it is at more risk for developing vitamin D deficiency. Therefore, in the absence of exposure to sunlight, there appears to be an increased requirement for vitamin D in this age group a RDA of $20 \mu\text{g}$ (800 IU) day^{-1} was recommended.

RDA for Pregnancy and Lactation

Although there is an increase in the metabolism of $25(\text{OH})\text{D}$ to $1,25(\text{OH})_2\text{D}$, during the last trimester of pregnancy and during lactation, the IOM concluded there is nothing in the evidence-based literature to suggest that there is an increased vitamin D requirement for pregnant and lactating women. Therefore, it was recommended that the RDA of vitamin D for pregnancy and lactation follows that recommended for their age group, that is, $15 \mu\text{g}$ (600 IU) day^{-1} . However, a study in Boston reported that 81% newborns and 76% of their mothers had a $25(\text{OH})\text{D} < 20 \text{ ng ml}^{-1}$ even though they took a prenatal vitamin supplement containing 400 IU and drank two glasses of milk per day thereby ingesting 600 IU day^{-1} .

Healthy Vitamin D Intakes

There have been a multitude of studies during the past decade that suggest that the RDAs recommended for vitamin D may still be inadequate if there is no exposure to sunlight. Based on a multitude of studies, it is reasonable for children and adults

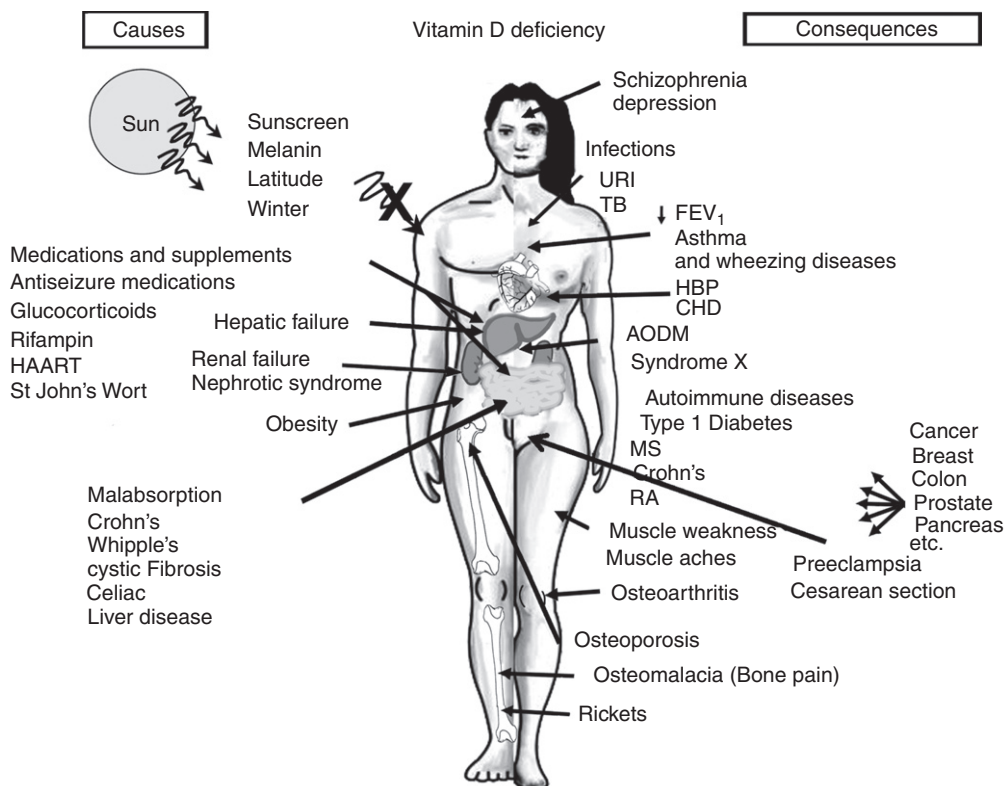


Figure 8 A schematic representation of the major causes for vitamin D deficiency and potential health consequences. Reproduced with permission from Holick MF (2010) *The Vitamin D Solution*. New York: Hudson Street Press.

to increase their vitamin D intake as noted in [Table 2](#). In order to maintain a blood level of 25(OH)D > 30 ng ml⁻¹ and not to exceed 100 ng ml⁻¹ for all the potential health benefits of vitamin D ([Figure 8](#)).

Tolerable Upper Intake Levels and Vitamin D Intoxication

An excessive intake of vitamin D can lead to vitamin D intoxication. This is characterized by a marked increase in serum 25(OH)D that is usually greater than 375 nmol l⁻¹ (150 ng ml⁻¹) and is associated with hypercalciuria and hypercalcemia. This can lead to soft tissue calcification and increased risk of kidney stones. The safe upper limits for vitamin D, as recommended by the IOM, are found in [Table 2](#).

Vitamin D intoxication usually occurs when a person ingests more than 10 000 IU of vitamin D daily for several months. A person does not need to be concerned about becoming vitamin D intoxicated if they take a multivitamin that contains 400 IU of vitamin D, drink a quart of milk that contains 400 IU of vitamin D, and is exposed to sunlight. The IOM recognized that vitamin D is not as toxic as once believed and raised the upper limit (UL) for older children and adults up to 4000 IU day⁻¹. Based on the evidence-based literature, these recommendations are conservative and could probably be raised up to 10 000 IU day⁻¹ ([Table 2](#)).

See also: Calcium. Lactation: Dietary Requirements. Pregnancy: Nutrient Requirements

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VITAMIN E

Contents

Metabolism and Requirements

Physiology and Health Effects

Metabolism and Requirements

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Glossary

α -Tocopherol transfer protein (α -TTP) The hepatic α -TTP preferentially facilitates secretion of α -tocopherol, specifically 2R- α -tocopherols, not other tocopherols or tocotrienols, from the liver into the plasma.

Ataxia with vitamin E deficiency Humans with a defect in the α -TTP gene, who are not supplemented with vitamin E, display a syndrome called ataxia with vitamin E deficiency (AVED).

Biologic activity A term that has been used historically to indicate a disconnect between vitamin E antioxidant activities and *in vivo* activities.

Fat malabsorption syndromes Fat malabsorption syndromes are various disorders that cause an inability to absorb dietary fat. These can include cystic fibrosis, short bowel syndrome, or cholestatic liver disease, as examples.

Vitamin E metabolites Tocopherols, as well as tocotrienols, are metabolized to carboxyethyl hydroxychromans (CEHCs), which can be excreted in urine or bile.

Introduction

Vitamin E is the most potent, fat-soluble antioxidant in human plasma. Although vitamin E was first discovered in 1922, its metabolic function remains an enigma. There are eight different molecular forms with vitamin E antioxidant activity, yet the body preferentially retains the α -form of tocopherol. This preference for α -tocopherol has led the Food and Nutrition Board in its 2000 dietary reference intakes (DRIs) for vitamin E to recommend that only α -tocopherol, not the other forms, meets human requirements for vitamin E. Moreover, only α -tocopherol is recognized by the hepatic α -tocopherol transfer protein (α -TTP). This protein regulates plasma α -tocopherol concentrations. Abnormalities in the α -TTP gene lead to vitamin E deficiency in humans. Vitamin E metabolism is important for increasing excretion of excess α -tocopherol, as well as non- α -tocopherol forms of vitamin E.

chromanol ring. α -Tocopherol or α -tocotrienol has 3 methyl groups, β - or γ - has 2, and δ - has 1.

The naturally occurring form of α -tocopherol is called *RRR*- α -tocopherol; or on supplement labels, *D*- α -tocopherol; or

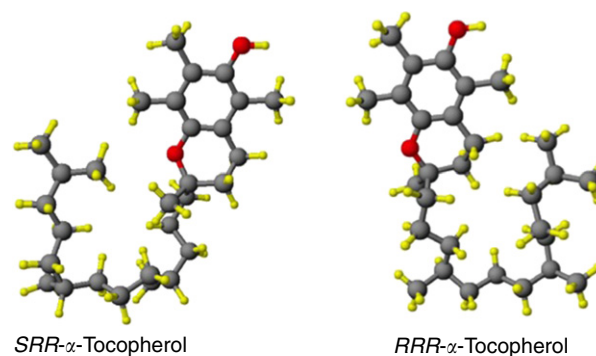


Figure 1 The naturally occurring form of α -tocopherol is called *RRR*- α -tocopherol; or more formally, 2,5,7,8-tetramethyl-2*R*-(4'*R*,8'*R*,12 trimethyltridecyl)-6-chromanol. Of the chiral centers at positions 2, 4', and 8' of α -tocopherol, position 2 is the most important for biologic activity. The *R*-conformation occurs in α -tocopherol synthesized by plants, but when α -tocopherol is chemically synthesized, the three chiral centers can each be in either the *R*- or the *S*-conformation. The α -tocopherol transfer protein prefers the 2*R*-form, not the 2*S*-form.

General Description and Scientific Name

Dietary components with vitamin E antioxidant activity include α -, β -, γ -, and δ -tocopherols and α -, β -, γ -, and δ -tocotrienols. These compounds all have a chromanol ring with a saturate, phytyl tail (tocopherols) or an unsaturated tail (tocotrienols) and vary in the number of methyl groups on the

Table 1 Estimated average requirements (EARs), recommended dietary allowances (RDAs), and average intakes (AIs) (mg day⁻¹) for α -tocopherol – adults and children

Lifestage	EAR	RDA	AI
0–6 months			4
7–12 months			6
1–3 years	5	6	
4–8 years	6	7	
9–13 years	9	11	
14–18 years	12	15	
Adult (male or female)	12	15	
Pregnant	12	15	
Lactation	16	19	

Table 2 Factors to convert IU of vitamin E to mg 2*R*- α -tocopherol

	mg IU ^{-1a}
<i>all rac</i> - α -Tocopherol and esters ^b	
<i>dl</i> - α -Tocopheryl acetate	0.45
<i>dl</i> - α -Tocopheryl succinate	0.45
<i>dl</i> - α -Tocopherol	0.45
<i>RRR</i> - α -Tocopherol and esters ^b	
D- α -Tocopheryl acetate	0.67
D- α -Tocopheryl succinate	0.67
D- α -Tocopherol	0.67

^aMultiply the IU in foods or supplements times the indicated factor to obtain the mg active vitamin E.

^bNote when the esters are synthesized the weight per IU is adjusted to include the molecular weight of the ester form. Thus, to calculate what is the mg of free tocopherol, this adjustment is not included in the calculation.

more formally, 2,5,7,8-tetramethyl-2*R*-(4'*R*,8'*R*,12 trimethyltridecyl)-6-chromanol (**Figure 1**). At positions 2, 4', and 8' of α -tocopherol are chiral carbon-centers that are in the *R*-conformation in naturally occurring α -tocopherol, but theoretically can take on either the *R*- or the *S*-conformation. Position 2 is the most important for biologic activity. Therefore, the DRIs for vitamin E are given in units of mg 2*R*- α -tocopherol (**Table 1**, see below for discussion).

The chemical synthesis of α -tocopherol results in an equal mixture of eight different stereoisomers (*RRR*, *RSR*, *RSS*, *RSS*, *SRR*, *SSR*, *SRS*, *SSS*), or more formally 2,5,7,8-tetramethyl-2*RS*-(4'*RS*,8'*RS*,12 trimethyltridecyl)-6-chromanol. To indicate that synthetic α -tocopherol is a racemic mixture, it is called *all rac*- α -tocopherol, or on supplement labels, *dl*- α -tocopherol. The first letter of the three-letter combination is the 2 position; therefore, only half of the synthetic α -tocopherol is in the active 2*R*- α -tocopherol conformation. **Table 2** lists the factors to convert IU to mg. For example, if a vitamin E supplement is labeled 400 IU and it is *dl*- α -tocopheryl acetate, then 400 times 0.45 equals 180 mg 2*R*- α -tocopherol, but if it is labeled D- α -tocopheryl acetate, then 400 times 0.67 equals 268 mg 2*R*- α -tocopherol.

Vitamin E Supplements

Most vitamin E supplements and food fortificants contain *all rac*- α -tocopherol, but can contain mixtures of tocopherols or

tocotrienols. Supplements often are sold as esters, which protect α -tocopherol from oxidation. These can be acetates, succinates, or nicotinate of α -tocopherol. Either the natural stereoisomer (*RRR*- α -tocopherol) or the synthetic (*all rac*- α -tocopherol) can be sold as an ester, for example, D- or *dl*- α -tocopheryl acetate, respectively.

Dietary Vitamin E

Vitamin E can be readily obtained from food. Generally, the richest sources are vegetable oils. Wheat germ oil, safflower oil, and sunflower oil contain predominantly α -tocopherol, whereas soy and corn oils contain predominantly γ -tocopherol. All of these oils are polyunsaturated. Good sources of mono-unsaturated oils, such as olive or canola oils, also contain predominantly α -tocopherol. Whole grains and nuts are also good sources of vitamin E. Fruits and vegetables, although rich in water-soluble antioxidants, are not good sources of vitamin E.

α -Tocopherol Equivalents

It is often assumed for the purpose of calculating vitamin E intakes from food in α -tocopherol equivalents (α -TEs) that γ -tocopherol can substitute for α -tocopherol with an efficiency of 10%. However, functionally γ -tocopherol is not equivalent to α -tocopherol, is not converted to α -tocopherol, and is rapidly depleted from the body. Therefore, some caution should be used in applying α -TEs to estimates of α -tocopherol intakes when corn or soybean oils (hydrogenated vegetable oils) represent the major oils present in foods. These oils have high γ -tocopherol contents and therefore the α -TEs are not reflective of only α -tocopherol, but are inflated because of the expectation that γ -tocopherol equals 10% α -tocopherol. Therefore, if food tables reporting α -TEs are used to estimate dietary α -tocopherol, α -tocopherol intakes are over-estimated. The 2000 DRIs no longer recommend use of α -TEs; only mg α -tocopherol and only 2*R*- α -tocopherol (half of the *dl* mg value), should be included in estimates of vitamin E intakes.

Vitamin E Actions and Metabolism

Antioxidant Activity

Vitamin E is the most potent, lipid-soluble antioxidant in human plasma and tissues. Thus, vitamin E protects polyunsaturated fatty acids within membrane phospholipids and plasma lipoproteins. When a peroxy radical forms in a membrane, it is 1000-times more likely to attack a vitamin E molecule than a polyunsaturated fatty acid (**Figure 2**). The hydroxyl group on the chromanol ring of vitamin E reacts with the peroxy radical to form the corresponding lipid hydroperoxide and tocopheroxyl radical. Thus, vitamin E acts as a chain-breaking antioxidant, preventing further autoxidation of lipids, but it forms a radical itself, which could re-initiate lipid peroxidation.

The tocopheroxyl radical has a number of possible fates. The tocopheroxyl radical can react with another radical to form nonreactive products. Alternatively, it can be further oxidized to the tocopheryl quinone, a two-electron oxidation

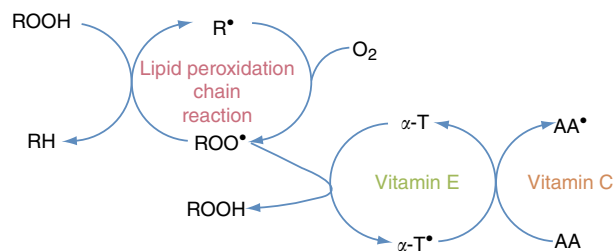


Figure 2 When a carbon-centered radical (R^\bullet) reacts with oxygen (O_2), a peroxy radical (ROO^\bullet) is formed. The peroxy radical is 1000-times more likely to attack a vitamin E (αT) molecule than a polyunsaturated fatty acid (RH). The hydroxyl group (OH) on the chromanol ring of vitamin E reacts with the peroxy radical to form the corresponding lipid hydroperoxide ($ROOH$) and tocopheroxyl radical (αT^\bullet). Thus, vitamin E acts as a chain-breaking antioxidant, preventing further autooxidation of lipids, but it forms a radical itself, which could re-initiate lipid peroxidation. The tocopheroxyl radical (αT^\bullet) can react with another radical to form non-reactive products. Alternatively, it can be further oxidized to the tocopheryl quinone, a two-electron oxidation product. Another possibility is “vitamin E recycling”, where the tocopheroxyl radical is restored to its unoxidized form by other antioxidants such as vitamin C or ubiquinol, or with thiols, such as glutathione. The latter possibility is the most likely one.

product. Another possibility is vitamin E recycling, where the tocopheroxyl radical is restored to its unoxidized form by other antioxidants such as vitamin C or ubiquinol, or with thiols, such as glutathione. This process will deplete these other antioxidants. For this reason, it is important to maintain a good intake of other dietary antioxidants.

Biologic Activity

Biologic activity is a term that has been used historically to indicate a disconnect between vitamin E antioxidant activities and *in vivo* activities. Observations in rodent experiments carried out in the 1930s, formed the basis for determining the biologic activity of vitamin E. Although the various vitamin E forms have somewhat similar structures and antioxidant activities, they differed in their abilities to prevent or reverse specific vitamin E deficiency symptoms (e.g., fetal resorption, muscular dystrophy, and encephalomalacia). α -Tocopherol with three methyl groups and a free hydroxyl group on the chromanol ring with the phytyl tail meeting the ring in the *R*-orientation (**Figure 1**) had the highest biological activity. This specific structural requirement for biological, but not chemical, activity is now known to be dependent on the hepatic α -TTP, as discussed in the Section on Vitamin E Bioavailability. α -TTP maintains plasma, and indirectly tissue, α -tocopherol concentrations.

Molecular Function

In addition to antioxidant activity, there have been claims that there are specific α -tocopherol-dependent functions that normalize cellular functions, are involved in cell signaling or in gene regulation. However, most of the information in this area has been obtained from *in vitro* studies. Various studies *in vivo* have not lead to clear documentation that α -tocopherol has any specific molecular function in addition to its antioxidant activity.

Vitamin E Metabolism

α - and γ -Tocopherols, as well as α - and γ -tocotrienols, are metabolized to α - and γ -carboxyethyl hydroxychroman (γ -CEHCs) (2,5,7,8-tetramethyl- and 2,7,8-trimethyl-2-(2'-carboxyethyl)-6-hydroxychromans), respectively. About 1% of a dose of α -tocopherol or tocotrienol, or 5% of a dose of γ -tocopherol or tocotrienol is excreted in the urine as CEHCs. Vitamin E metabolism is a key pathway for the regulation of vitamin E status and essential for depleting the body of non- α -tocopherol forms (**Figure 3**).

Recommended Intake Levels

In 2000, the Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences published the DRIs for vitamin C, vitamin E, selenium, and the carotenoids. Their recommendations for vitamin E appear in **Table 1**.

The requirements for vitamin E intakes are based primarily on long-term (5–7 years) depletion and repletion studies in humans. Serum α -tocopherol concentrations and corresponding hydrogen peroxide-induced erythrocyte hemolysis were determined at various intervals. Serum concentrations necessary to prevent *in vitro* erythrocyte hemolysis in response to known levels of vitamin E intake in subjects who had undergone experimentally-induced vitamin E deficiency were used to determine estimated average requirements (EARs) for vitamin E. The recommended dietary allowances (RDAs) are levels that represent the daily α -tocopherol intakes required to ensure adequate nutrition in 95–97.5% of the population and are an overestimation of the level needed for most people in any given group.

Vitamin E Units

According to the US Pharmacopoeia (USP), 1 international unit (IU) of vitamin E equals 1 mg *all rac* α -tocopheryl acetate, 0.67 mg *RRR*- α -tocopherol, or 0.74 mg *RRR*- α -tocopheryl acetate. These conversions were estimated on the relative biologic activities of the various forms when tested in the rat assay for vitamin E deficiency, the fetal resorption assay. IUs are currently used in labeling vitamin E supplements and food fortificants. It should be noted that the current RDA does not use vitamin E USP units but rather the recommendation for adults is set at 15 mg of *RRR*- α -tocopherol or 2*R*- α -tocopherols. Most foods contain *RRR*- α -tocopherol naturally, but foods that have been fortified with vitamin E contain the synthetic form, for example, fortified breakfast cereals. If the amount of vitamin E on the label is given in IU, then the factors given in **Table 2** must be used to multiply times the IU to obtain the mg 2*R*- α -tocopherol.

Over-Dosage

The 2000 Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences recommended 1000 mg as an upper limit (UL) of all forms of α -tocopherol in supplements taken by adults 19 years and older, including pregnant and lactating women. UL were set for children and adolescents by adjusting the adult limit on the basis of relative

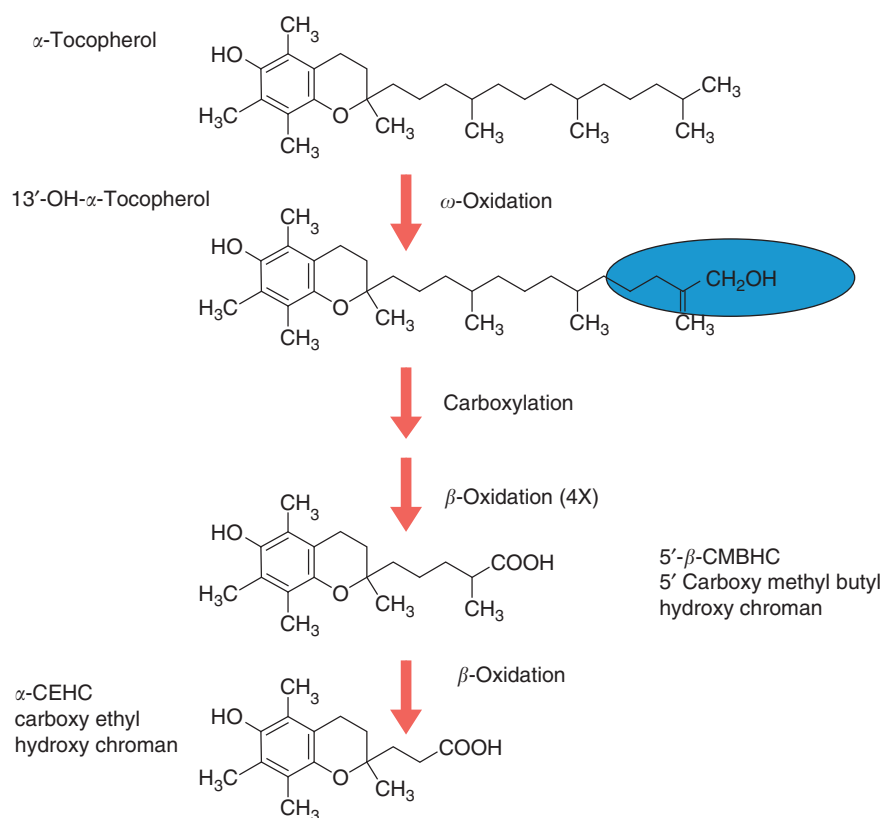


Figure 3 Shown is an example of α -tocopherol metabolism to α -CEHC. Vitamin E metabolism is a key pathway for the regulation vitamin E status and essential for depleting the body of non- α -tocopherol forms. The initial step is an ω -hydroxylation, then formation of the carboxyl group, followed by four rounds of β -oxidation to yield the products α -CMBHC and α -CEHC, which can be excreted in the bile or the urine.

Table 3 Upper limits (UL) for α -tocopherol intakes

Age (years)	UL (mg day ⁻¹)
1–3	200
4–8	300
9–13	600
14–18	800
> 19	1000

body weight. **Table 3** gives the α -tocopherol UL by age group. No UL was set for infants owing to lack of adequate data. The 2000 Food and Nutrition Board did recommend that food be the only source of vitamin E for infants. However, a UL of 21 mg d⁻¹ was suggested for premature infants with birth weights of 1.5 kg, based on the adult UL.

The vitamin E UL was set for supplements because it is almost impossible to consume enough α -tocopherol-containing foods to achieve a daily 1000-mg intake for prolonged periods of time. The UL was defined for all forms of α -tocopherol, not just the 2R-forms, because all of the forms in *all rac*- α -tocopherol are absorbed and delivered to the liver. The appropriate conversion factors are *different* from those shown in **Table 2**, and necessary to estimate the UL for supplements containing either *RRR*- or *all rac*- α -tocopherol supplements. The UL amounts given in IU are shown in **Table 4**. The UL for *RRR*- α -tocopherol is apparently higher because each capsule

Table 4 Upper limits (UL) reported in IU for α -tocopherol-containing supplements

	Number of IU that equal the UL
<i>all rac</i> - α -Tocopherol and esters	
<i>dl</i> - α -Tocopheryl acetate	1100
<i>dl</i> - α -Tocopheryl succinate	1100
<i>dl</i> - α -Tocopherol	1100
<i>RRR</i> - α -Tocopherol and esters	
D- α -Tocopheryl acetate	1500
D- α -Tocopheryl succinate	1500
D- α -Tocopherol	1500

of *RRR*- α -tocopherol contains less α -tocopherol than does one containing *all rac*- α -tocopherol.

A number of meta-analyses of vitamin E supplementation trials have been reported. Some report increased risk of mortality with vitamin E supplementation, whereas others do not. To date, there are no mechanistic studies demonstrating how vitamin E supplements might lead to increased risk of death.

Precautions and Adverse Reactions

High vitamin E intakes are associated with an increased tendency to bleed. It is not known if this is a result of decreased platelet aggregation caused by an inhibition of protein kinase

C by α -tocopherol, some other platelet related mechanism, or decreased clotting owing to a vitamin K and E interaction causing abnormal blood clotting.

Individuals who are deficient in vitamin K or who are on anticoagulant therapy are at increased risk of uncontrolled bleeding. Patients on anticoagulant therapy should be monitored when taking vitamin E supplements to ensure adequate vitamin K intakes.

Adverse Effects of Drugs on Vitamin E Status

Drugs intended to promote weight loss by impairing fat absorption, such as Orlistat or sucrose polyester, can also impair vitamin E and other fat-soluble vitamin absorption. Therefore, multivitamin supplementation is recommended. Vitamin supplements should be taken with meals at times other than when these drugs are taken to allow adequate absorption of the fat-soluble vitamins.

Vitamin E Bioavailability

Absorption and Plasma Transport

Intestinal absorption of vitamin E is dependent upon normal processes of fat absorption. Specifically, both biliary and pancreatic secretions are necessary for solubilization of vitamin E in mixed micelles containing bile acids, fatty acids, and monoglycerides. α -Tocopheryl acetates (or other esters) from vitamin E supplements, are hydrolyzed by pancreatic esterases to α -tocopherol before absorption. Following micellar uptake by enterocytes, vitamin E is incorporated into chylomicrons and secreted into the lymph. Once in the circulation, chylomicron triglycerides are hydrolyzed by lipoprotein lipase (Figure 4). During chylomicron catabolism in the circulation, vitamin E is nonspecifically transferred both to tissues and to other circulating lipoproteins.

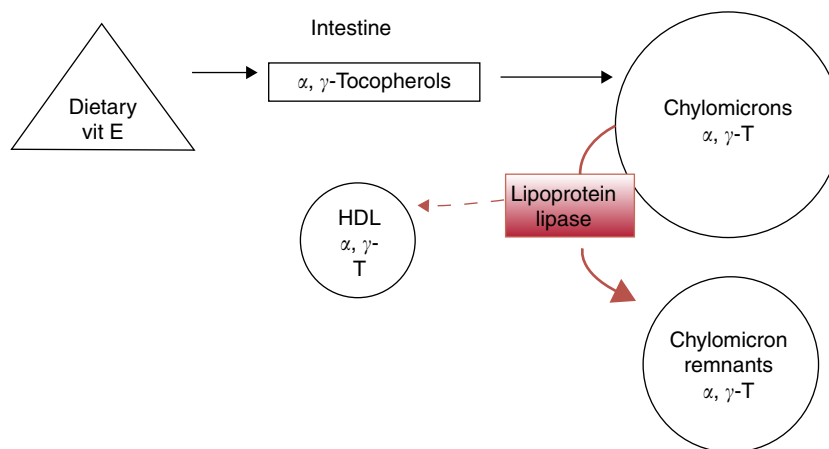


Figure 4 Dietary or supplemental vitamin E is dependent upon normal processes of fat absorption. Specifically, both biliary and pancreatic secretions are necessary for solubilization of vitamin E in mixed micelles containing bile acids and monoglycerides. α -Tocopheryl acetates (or other esters) from vitamin E supplements are hydrolyzed by pancreatic esterases to α -tocopherol prior to absorption. Following uptake of micelles by enterocytes, vitamin E is incorporated into chylomicrons and secreted into the lymph. Once in the circulation, chylomicron triglycerides are hydrolyzed by lipoprotein lipase. During chylomicron catabolism in the circulation, vitamin E is non-specifically transferred both to tissues and to other circulating lipoproteins, such as high-density lipoprotein (HDL).

It is not until the vitamin E-containing chylomicrons reach the liver that discrimination between the various dietary vitamin E forms occurs (Figure 5). The hepatic α -TTP preferentially facilitates secretion of α -tocopherol, specifically 2R- α -tocopherols, not other tocopherols or tocotrienols, from the liver into the plasma in very low-density lipoproteins (VLDLs). In the circulation, VLDLs are catabolized to low-density lipoproteins (LDL are also known as the bad cholesterol because high LDL levels are associated with increased heart disease). During this lipolytic process, all of the circulating lipoproteins become enriched with α -tocopherol.

There is no evidence that vitamin E is transported in the plasma by a specific-carrier protein, but rather is

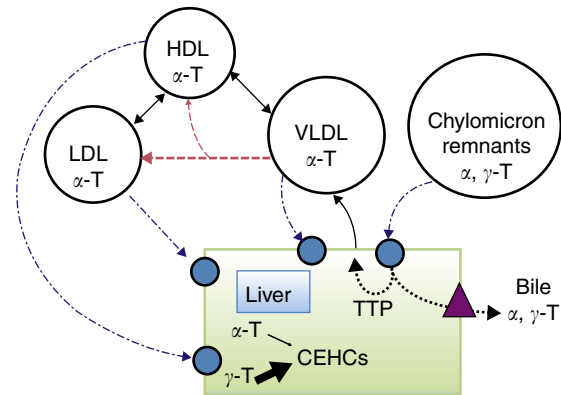


Figure 5 When the vitamin E-containing chylomicrons reach the liver, the hepatic α -TTP preferentially facilitates secretion of α -tocopherol, specifically 2R- α -tocopherols, not other tocopherols or tocotrienols, from the liver into the plasma. In the circulation, VLDLs are catabolized to low-density lipoproteins (LDL). During this lipolytic process, all of the circulating lipoproteins become enriched with α -tocopherol. Thus, the preference for α -tocopherol is dependent upon the hepatic secretion of α -tocopherol into plasma.

nonspecifically transported in lipoproteins. A variety of lipoprotein receptors and lipid transporters also facilitate vitamin E disposition. An advantage of vitamin E transport in lipoproteins is that easily oxidizable lipids are protected by the simultaneous transport of this lipid-soluble antioxidant. Similarly, delivery of vitamin E to tissues is dependent upon lipid and lipoprotein metabolism. Thus, as peroxidizable lipids are taken up by tissue, the tissues simultaneously acquire a lipid-soluble antioxidant.

Plasma Concentrations, Kinetics, and Tissue Delivery

Plasma α -tocopherol concentrations in normal humans range from 11 to 37 $\mu\text{mol l}^{-1}$. When plasma lipids are taken into account the lower limits of normal are 1.6 $\mu\text{mol } \alpha$ -tocopherol per mmol lipid or 2.5 $\mu\text{mol } \alpha$ -tocopherol per mmol cholesterol. α -Tocopherol is transported in plasma lipoproteins, so if lipid concentrations are extraordinarily high or low, then correction for lipid levels are helpful to determine adequacy of vitamin E status. Additionally, α -tocopherol concentrations in erythrocytes, adipose tissue, or even peripheral nerve have been used to assess vitamin E status.

The apparent half-life of *RRR*- α -tocopherol in plasma of normal subjects is approximately 48 h, whereas those of *SRR*- α -tocopherol or γ -tocopherol are only 15 h and the tocotrienols are less than 4 h.

Vitamin E is delivered to tissues by three mechanisms: transfer from triglyceride-rich lipoproteins during lipolysis, as a result of tissue lipoprotein uptake by various receptors that mediate lipoprotein uptake, and as a result of vitamin E exchange between lipoproteins and tissues. The regulation of tissue vitamin E is not well understood, but α -tocopherol is the predominant form in tissues as a result of its dominance in plasma.

Human Vitamin E Deficiency

Vitamin E deficiency was first described in children with fat malabsorption syndromes, principally abetalipoproteinemia, cystic fibrosis, and cholestatic liver disease. Subsequently, humans with severe vitamin E deficiency with no known defect in lipid or lipoprotein metabolism were described to have a defect in the α -TTP gene. This syndrome is called ataxia with vitamin E deficiency (AVED). The demonstration of vitamin E deficiency in AVED patients solidified the role of α -TTP as a critical determinant of plasma α -tocopherol concentrations. This protein is an important factor in the discrimination between α -tocopherol and other forms of vitamin E, as well as between natural and synthetic α -tocopherols.

Erythrocyte fragility, hemolysis, and anemia were described as vitamin E deficiency symptoms in various animals fed diets devoid of vitamin E. Additionally, studies in experimental animals have shown that a deficiency of both selenium (a required component of glutathione peroxidases) and vitamin E causes a more rapid and severe onset of debilitating deficiency symptom. Deficiency of both vitamins E and C should also cause more severe antioxidant deficiency symptoms, but most animals make their own vitamin C, so this

interaction has been difficult to demonstrate in animals. However, when guinea pigs were fed a vitamin E deficient diet and then made vitamin C deficient, the results were catastrophic, resulting in the animals' death within weeks of feeding the vitamin C deficient diet.

In contrast to experimental vitamin E deficiency in rodents, in humans the major vitamin E deficiency symptom is a peripheral neuropathy characterized by the degeneration of the large caliber axons in the sensory neurons.

Vitamin E deficiency occurs only rarely in humans and almost never as a result of inadequate vitamin E intakes, therefore, interactions with other nutrients have not been well studied. There have been reports of vitamin E deficiency symptoms in persons with protein-calorie malnutrition. Vitamin E deficiency does occur as a result of genetic abnormalities in α -TTP and as a result of various fat malabsorption syndromes. Vitamin E supplementation halts the progression of the neurologic abnormalities caused by inadequate nerve tissue α -tocopherol, and in some cases, has reversed them.

Patients with these disorders require daily pharmacologic vitamin E doses for life to overcome the mechanisms leading to deficiency. Generally, patients with AVED are suggested to consume 1000 mg *RRR*- α -tocopherol per day in divided doses, patients with abetalipoproteinemia 100 mg kg^{-1} body weight, cystic fibrosis 400 mg d^{-1} . However, patients with fat malabsorption owing to impaired biliary secretion generally do not absorb orally administered vitamin E. These patients are treated with special forms of vitamin E, such as α -tocopheryl polyethylene glycol succinate, that spontaneously form micelles, obviating the need for bile acids.

Chronic Disease Prevention

The frequency of human vitamin E deficiency is very rare. In individuals at risk, it is clear that vitamin E supplements should be recommended to prevent deficiency symptoms. What about vitamin E supplement use in normal individuals? Dietary changes such as decreasing fat intakes, substituting fat-free foods for fat-containing ones, and increased reliance of meals away from the home, have resulted in decreased consumption of α -tocopherol-containing foods. Therefore, intakes of the vitamin E RDA, 15 mg α -tocopherol, may be difficult. Special attention to consuming nuts, seeds, and whole grains will improve α -tocopherol intakes; alternatively multivitamin pills can be consumed.

Importantly, vitamin E's potential role in preventing or ameliorating chronic diseases associated with oxidative stress leads us to ask whether vitamin E supplements might be beneficial. For many vitamins, when excess amounts are consumed, they are excreted and provide no added benefits. Antioxidant nutrients may, however, be different. Heart disease and stroke, cancer, chronic inflammation, impaired immune function, Alzheimer's disease – a case can be made for the role of oxygen free radicals in the etiology of all of these disorders, and even in aging itself. Do antioxidant nutrients counteract the effects of free radicals and thereby ameliorate these disorders? And if so, do large antioxidant supplements have beneficial effects beyond required amounts? The 2000

DRI report on vitamin C, vitamin E, selenium, and carotenoids stated that there was insufficient proof to warrant advocating supplementation with antioxidants. But, they also stated that the hypothesis that antioxidant supplements might have beneficial effects was promising. This remains a very controversial area in vitamin E research.

One approach that has been successful in demonstrating vitamin E effectiveness in mitigating chronic disease risk is the identification of patients with increased levels of oxidative stress. One such strategy, called pharmacogenomics by its proponents, identified that diabetic patients with the haptoglobin 2-2 genotype is associated with increased risk of cardiovascular disease (CVD). These patients were found to benefit from vitamin E supplementation.

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Physiology and Health Effects

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Introduction

In 1922, Evans and Bishop discovered a fat-soluble dietary constituent that was essential for the prevention of fetal death and sterility in rats accidentally fed a diet containing rancid lard. This was originally called 'factor X' and 'antisterility factor' but was later named vitamin E. Subsequently, the multiple nature of the vitamin began to appear when two compounds with vitamin E activity were isolated and characterized from wheat germ oil. These compounds were designated α - and β -tocopherol, derived from the Greek 'tokos' for childbirth, 'phorein' meaning to bring forth, and 'ol' for the alcohol portion of the molecule. Later, two additional tocopherols, γ - and δ -tocopherol, as well as four tocotrienols were isolated from edible plant oils. After the initial discovery, more than 40 years passed before it was proved that vitamin E deficiency could cause disease in humans and was associated with antioxidant functions in cellular systems. It took another 25 years before the nonantioxidant properties of the vitamin were highlighted.

The present article reviews the chemistry of the tocopherols; their dietary sources, absorption, transport, and storage; and their metabolic function. In addition, the potential role of dietary or supplemental tocopherol intake in the prevention of chronic disease and possible mechanisms for observed protective effects are discussed. Finally, a summary of the assessment of tocopherol status in humans, intake requirements, and an overview of the safety of high intakes is also provided.

Chemistry

The chemistry of vitamin E is rather complex because there are eight structurally related forms – four tocopherols (α , β , γ , and δ) and four tocotrienols (α , β , γ , and δ) – that are synthesized from homogentisic acid and isopentenyl diphosphate in the plastid envelope of plants. The structures of α -, β -, γ -, and δ -tocopherols are shown in Figure 1. α -Tocopherol is methylated at C5, C7, and C8 on the chromanol ring, whereas the other homologs (β , γ , and δ) have different degrees of methylation (Figure 1). Tocopherols have a saturated phytyl side chain attached at C2 and have three chiral centers that are in the R configuration at positions C2, C4¹, and C8¹ in the naturally occurring forms, which are given the prefix 2R, 4¹R, and 8¹R (designated RRR). The members of the tocotrienols are unsaturated at C3¹, C7¹, and C11¹ in the isoprenoid side chain and possess one chiral center at C2 in addition to two sites of geometric isomerism at C3¹ and C7¹. Vitamin E biological activity is expressed as milligrams of RRR- α -tocopherol equivalents (α -TEs) whenever possible. The activity of RRR- α -tocopherol is 1. The activities of RRR- β -, RRR- γ -, and RRR- δ -tocopherol are 0.5, 0.1, and 0.03, respectively.

Dietary Sources

The composition and content of the different tocopherol components in plant tissue vary considerably, ranging from

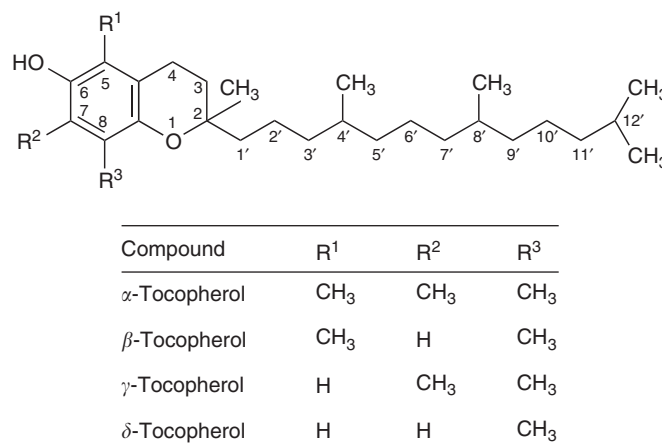


Figure 1 The four major forms of vitamin E (α -, β -, γ -, and δ -tocopherols) differ by the number and positions of methyl groups on the chromanol ring. In α -tocopherol, the most biologically active form, the chromanol ring is fully methylated. In β - and γ -tocopherols, the ring contains two methyl groups, whereas δ -tocopherol is methylated in one position. The corresponding tocotrienols have the same structural arrangement except for the presence of double bonds on the isoprenoid side chain of C3¹, C7¹, and C11¹.

extremely low levels found in potato tubers to high levels found in oil seeds. α -Tocopherol is the predominant form in photosynthetic tissues and is mainly localized in plastids. The particular enrichment in the chloroplast membranes is probably related to the ability of tocopherols to quench or to scavenge reactive oxygen species (ROS) and lipid peroxy radicals by physical or chemical means. In this way, the photosynthetic apparatus can be protected from oxygen toxicity and lipid peroxidation. In nonphotosynthetic tissues, γ -tocopherol frequently predominates and can be involved in the prevention of autoxidation of polyunsaturated fatty acids (PUFAs).

Most of the tocopherol content of wheat germ, sunflower, safflower, canola, and olive oils is in the form of α -tocopherol, and these oils contain approximately 1700, 500, 350, 200, and 120 mg α -TE kg⁻¹, respectively. Vegetable oils (e.g., corn, cottonseed, palm, soybean, and sesame) and nuts (e.g., Brazil nuts, pecans, and peanuts) are rich sources of γ -tocopherol. Corn and soybean oils contain 5–10 times as much γ -tocopherol as α -tocopherol-rich sources of γ -tocopherol, and each contains approximately 200 mg α -TE kg⁻¹. Because of the widespread use of these plant products, γ -tocopherol is considered to represent ~70% of the vitamin E consumed in the typical US diet. The level of vitamin E in nuts ranges from 7 mg α -TE kg⁻¹ in coconuts to 450 mg α -TE kg⁻¹ in almonds. Cereals are moderate sources of vitamin E, providing between 6 (barley) and 23 mg α -TE kg⁻¹ (rye). Fresh fruit and vegetables generally contain approximately 1–10 mg α -TE kg⁻¹. The concentration of vitamin E (α -tocopherol is the predominant form) in animal products is usually low, but these may be significant dietary sources because of their high consumption.

Mean dietary intakes of 6.3–13.0 mg α -TE day⁻¹ have been reported in various European and the US population studies. Data from the Third National Health and Nutrition Examination Survey (1988–94) in the US indicate a median total intake (including supplements) of α -TE of 12.9 mg day⁻¹ and a median intake from food only of 11.7 mg day⁻¹ in men aged 31–50 years. In women in this age range, the median total intake (including supplements) of α -TE was 9.1 mg day⁻¹ and the median intake from food only was 8.0 mg day⁻¹. In the US, fats and oils used in spreads, etc., contribute 20.2% of the total vitamin E intake; vegetables, 15.1%; meat, poultry, and fish, 12.6%; desserts, 9.9%; breakfast cereals, 9.3%; fruit, 5.3%; bread and grain products, 5.3%; dairy products, 4.5%; and mixed main dishes, 4.0%.

The North/South Ireland Food Consumption Survey, published in 2001, reported that the median daily intake of vitamin E from all sources was 6.3 mg in men and 6.0 mg in women aged 18–64 years. The largest contributors of vitamin E to the diet were vegetables and vegetable dishes (18.9%) and potatoes and potato products (12.4%), most likely as a result of the oils used in composite dishes. Nutritional supplements contributed 5.5% of the vitamin E intake in men and 11.9% in women overall. In the subgroup that regularly consumed nutritional supplements (23% of total), vitamin E was the nutrient most frequently obtained in supplemental form in men (78%) and women (73%). In these people, supplements made a larger contribution to total vitamin E intakes than did food.

Absorption Metabolism and Excretion

Because of its hydrophobicity, vitamin E requires special transport mechanisms in the aqueous environment of plasma, body fluids, and cells. In humans, vitamin E is taken up in the proximal part of the intestine depending on the amount of food lipids, bile, and pancreatic esterases that are present. It is emulsified together with the fat-soluble components of food. Lipolysis and emulsification of the formed lipid droplets then led to the spontaneous formation of mixed micelles, which are absorbed at the brush border membrane of the mucosa by passive diffusion. Both α - and γ -tocopherol and dietary fat are taken up without preference by the intestine and secreted in chylomicron particles together with triacylglycerol and cholesterol (Figure 2). The nearly identical incorporation of α - and γ -tocopherol in chylomicrons after supplementation with equal amounts of the two tocopherols indicates that their absorption is not selective (Figure 2). The chylomicrons are stored as secretory granula and eventually excreted by exocytosis to the lymphatic compartment, from which they reach the bloodstream via the *ductus thoracicus*. The exchange between the apolipoproteins of the chylomicrons (types AI, AII, and B₄₈) and high-density lipoprotein (HDL) (types C and E) triggers the intravascular degradation of the chylomicrons to remnants by the endothelial lipoprotein lipase (LPL) and is a prerequisite for the hepatic uptake of tocopherols (Figure 2). During LPL-mediated catabolism of chylomicron particles, some of the chylomicron-bound vitamin E appears to be transported and transferred to peripheral tissues, such as muscle, adipose, and brain (Figure 2). The formation of remnants favors the rapid uptake of the tocopherols via the hepatic receptors for apo-E and apo-B.

The chylomicron remnants are subsequently taken up by the liver, where α -tocopherol is preferentially incorporated into nascent very low-density lipoprotein (VLDL) by a specific 32-kDa α -tocopherol transfer protein (α -TTP), which enables further distribution of α -tocopherol to peripheral cells (Figure 2). α -TTP is mainly expressed in the liver, in some parts of the brain, in the retina, in low amounts in fibroblasts, and in the placenta. α -TTP possesses stereospecificity as well as regiospecificity toward the most abundant isomer of vitamin E, (RRR)- α -tocopherol. The sorting process does not tolerate alteration at C2. As a consequence of the selective transfer mechanism, major parts of the natural homologs and non-natural isomers of α -tocopherol are excluded from the plasma and secreted with the bile. Relative affinities of tocopherols for α -TTP are as follows: α -tocopherol, 100; β -tocopherol, 38; γ -tocopherol, 9; and δ -tocopherol, 2. A 75-kDa plasma phospholipid transfer protein (PLTP), which is known to catalyze the exchange of phospholipids and other amphipathic compounds between lipid structures, has been shown to facilitate the exchange of α -tocopherol from VLDL to HDL and low-density lipoprotein (LDL) for further delivery to tissues (Figure 2).

A family of cellular tocopherol-associated proteins (TAPs) with the ability to bind and redistribute α -tocopherol has been identified. TAPs bind to α -tocopherol but not to other isomers of tocopherol. Present in all cells, TAPs may be specifically involved in intracellular α -tocopherol movement, for example, between membrane compartments and plasma

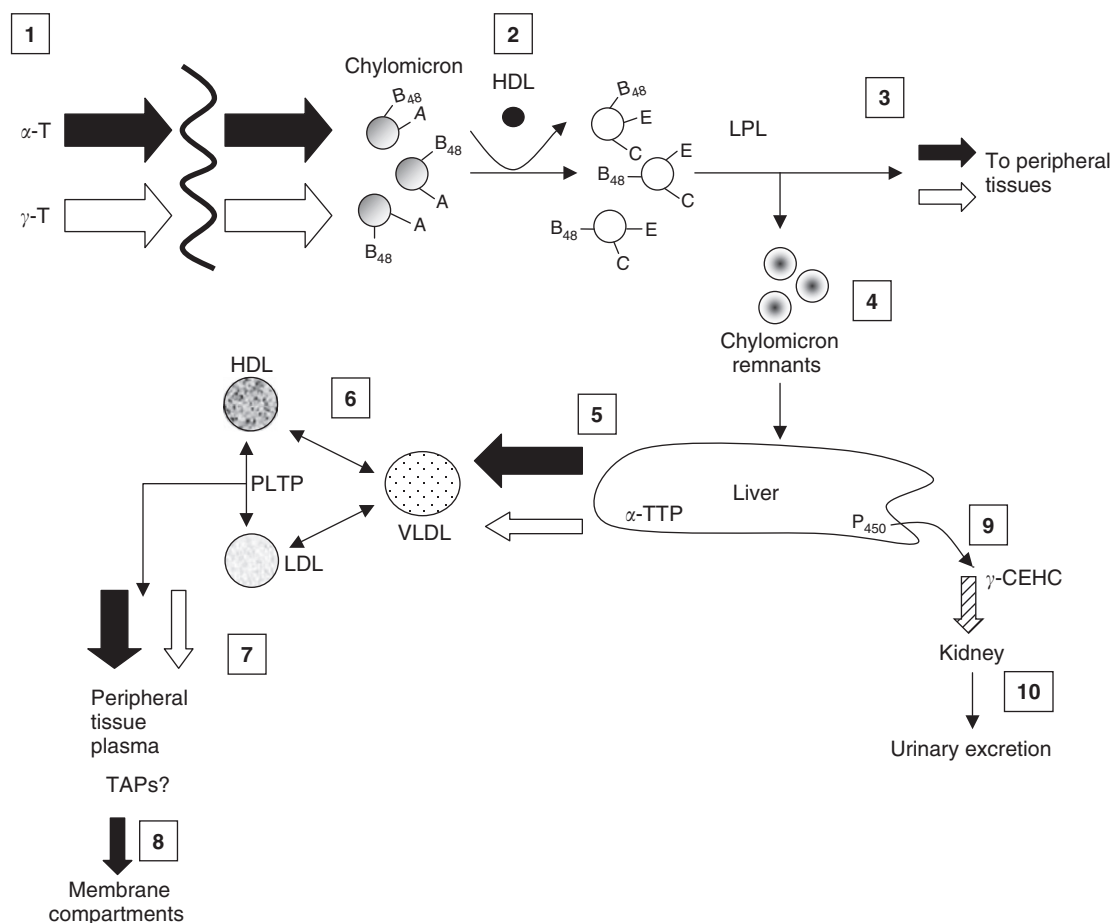


Figure 2 Absorption, transport, and metabolism of α -tocopherol (α -T) and γ -tocopherol (γ -T) in peripheral tissues. (1): Both α -T and γ -T are absorbed without preference by the intestine along with lipid and reassembled into chylomicrons. (2): Exchange between apolipoproteins of the chylomicrons (types AI, AII, and B₄₈) and HDL (types C and E) occurs. (3): Chylomicrons are degraded to remnants by LPL and some α -T and γ -T are transported to peripheral tissues. (4): The resulting chylomicron remnants are then taken up by the liver. (5): In the liver, most of the remaining α -T, but only a small fraction of γ -T, is reincorporated in nascent VLDLs by α -TTP. (6): PLTP facilitates the exchange of tocopherol between HDL and LDL for delivery to tissues. (7): Plasma tocopherols are delivered to tissues by LDL and HDL. (8): TAPs probably facilitate intracellular tocopherol transfer between membrane compartments. (9): Substantial amounts of γ -T are degraded by a cytochrome P-450-mediated reaction to 2,7,8-trimethyl-2-(β -carboxyethyl)-6-hydroxychroman (γ -CEHC). (10): γ -CEHC is excreted into urine. Adapted from Azzi A and Stocker A (2000) Vitamin E: Non-antioxidant roles. *Progress in Lipid Research* 39: 231–255, and Jiang Q, Christen S, Shigenaga MK, and Ames BN (2001) γ -Tocopherol, the major form of vitamin E in the US diet, deserves more attention. *American Journal of Clinical Nutrition* 74: 714–722, with permission from American Society for Nutrition.

membranes, or in optimizing the α -tocopherol content of membranes.

γ -Tocopherol appears to be mainly degraded to its hydrophilic 3'-carboxychromanol metabolite, 2,7,8-trimethyl-2-(β -carboxyethyl)-6-hydroxychroman (γ -CEHC) (Figure 3), and excreted in the urine. The mechanism of γ -tocopherol metabolism involves terminal cytochrome P-450 (CYP)-mediated ω -hydroxylation of the tocopherol phytol side chain, oxidation to the corresponding terminal carboxylic acid, and sequential removal of two- or three-carbon moieties by β -oxidation, ultimately yielding the hydrophilic 3'-carboxychromanol metabolite of the parent tocopherol that is excreted in the urine. Functional analysis of several recombinant human liver P-450 enzymes revealed that tocopherol ω -hydroxylase activity was associated only with the cytochrome P-450 isoform 4F2 (CYP4F2). Kinetic analysis of the tocopherol ω -hydroxylase activity in recombinant human

CYP4F2 microsomal systems revealed similar K_m values (37 and 21 μ M) but notably different V_{max} values (1.99 vs 0.16 nmol nmol⁻¹ of P-450 min⁻¹) for γ - and α -tocopherol, respectively. The data suggest a role for the CYP-mediated ω -hydroxylase pathway in the preferential physiological retention of α -tocopherol and elimination of γ -tocopherol. In nonsupplemented individuals, a substantial proportion of the estimated daily intake of γ -tocopherol is excreted in human urine as its γ -CEHC metabolite, but a much smaller proportion of α -tocopherol is excreted as 2,5,7,8-tetramethyl-2-(β -carboxyethyl)-6-hydroxychroman (α -CEHC) (Figure 3). α -CEHC is excreted in large amounts only when the daily intake of α -tocopherol exceeds 150 mg or plasma concentrations of α -tocopherol are above a threshold of 30–40 μ mol l⁻¹. Even then, urinary excretion of α -CEHC is lower than that of γ -CEHC.

It is likely that it is the capacity of α -TTP rather than the plasma α -tocopherol concentration that determines

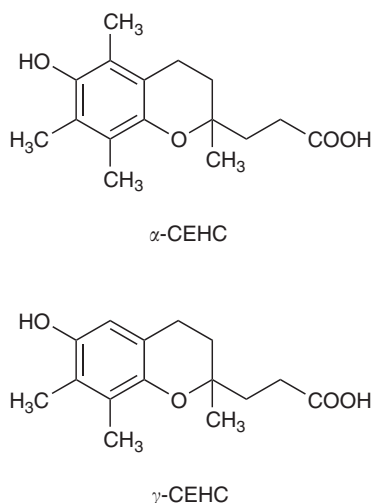


Figure 3 Chemical structures of α -CEHC and γ -CEHC.

α -tocopherol degradation. Overall, hepatic catabolism of γ -tocopherol appears to be responsible for the relatively low preservation of γ -tocopherol in plasma and tissues, whereas α -TTP-mediated α -tocopherol transfer plays a key role in the preferential enrichment of α -tocopherol in most tissues. Supplementation with α -tocopherol depletes plasma and tissue γ -tocopherol levels. This is likely due to the preferential affinity of α -TTP for α -tocopherol. However, the depletion of γ -tocopherol may also occur because an increase in α -tocopherol may further reduce the incorporation of γ -tocopherol into VLDL, which leaves more γ -tocopherol to be degraded by CYP. However, γ -tocopherol supplementation may spare α -tocopherol from being degraded.

Plasma (RRR)- α -tocopherol incorporation is a saturable process. Plasma concentrations of α -tocopherol reach a threshold of 30–40 $\mu\text{mol l}^{-1}$ despite supplementation with high levels (400 mg or greater) of (RRR)- α -tocopherol. Dose–response studies showed that the limitation in plasma α -tocopherol concentration appears to be a result of rapid replacement of circulating with newly absorbed α -tocopherol. Kinetic analysis has shown that the entire plasma pool of α -tocopherol is replaced daily. The highest concentrations of α -tocopherol in the body are in adipose tissues and adrenal glands. Adipose tissues are also a major store of the vitamin, followed by liver and skeletal muscle. The rate of uptake and turnover of α -tocopherol by different tissues varies greatly. Uptake is most rapid into lungs, liver, spleen, kidney, and red cells (in rats, $t_{1/2} < 15$ days) and slowest in brain, adipose tissues, and spinal cord ($t_{1/2} < 30$ days). Likewise, depletion of α -tocopherol from plasma and liver during times of dietary deficiency is rapid, whereas adipose tissue, brain, spinal cord, and neural tissues are much more difficult to deplete.

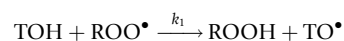
The major route for the elimination of tocopherol from the body is via the feces. Fecal tocopherol arises from incomplete absorption, secretion from mucosal cells, and biliary excretion. Excess α -tocopherol as well as forms of vitamin E not preferentially used, such as synthetic racemic isomer mixtures, or γ -tocopherol are eliminated during the process of nascent VLDL secretion in the liver and are probably excreted into bile. In addition to the urinary excretion of γ -tocopherol as

γ -CEHC, biliary excretion is an alternative route for elimination of excess γ -tocopherol. This is confirmed by the fact that the ratio of γ - to α -tocopherol in bile is sevenfold higher than in plasma.

Tocopherols as Antioxidants

Under normal physiological conditions, cellular systems are incessantly challenged by stressors arising from both internal and external sources. The most important potential stressors are reduced derivatives of oxygen, which are classified as ROS, and include the superoxide anion ($\text{O}_2^{\bullet-}$), hydroxyl radical (OH^{\bullet}), and oxygen-centered radicals of organic compounds (peroxyl (ROO^{\bullet}) and alkoxyl (RO^{\bullet})) together with other nonradical reactive compounds, such as hydrogen peroxide (H_2O_2). In addition, reactive nitrogen species such as nitric oxide (NO^{\bullet}), nitrogen dioxide (NO_2^{\bullet}), peroxynitrite (ONOO^-), and hypochlorous acid are involved.

Cellular systems have evolved as a powerful and complex antioxidant defense system to limit inappropriate exposure to these stressors. α -Tocopherol is quantitatively the most important chain-breaking antioxidant in plasma and biological membranes. The antioxidant activities of chain-breaking antioxidants are determined primarily by how rapidly they scavenge peroxyl radicals, thereby preventing the propagation of free radical reactions. When the chromanol phenolic group of α -tocopherol (TOH) encounters a ROO^{\bullet} it forms hydroperoxide (ROOH), and in the process a tocopheroxyl radical (TO^{\bullet}) is formed:



The rate constant (k_1) for hydrogen abstraction from α -tocopherol is $2.35 \times 10^6 \text{ M}^{-1} \text{ s}^{-1}$, which is higher than that for the other tocopherols and related phenols. Because the rate constant (k_2) for the chain propagation reaction between ROO^{\bullet} and an unsaturated fatty acid (RH) ($\text{ROO}^{\bullet} + \text{RH} \rightarrow \text{ROOH}$) is much lower than k_1 , at approximately $10^2 \text{ M}^{-1} \text{ s}^{-1}$ α -tocopherol outcompetes the propagation reaction and scavenges the $\text{ROO}^{\bullet} \sim 10^4$ times faster than RH reacts with ROO^{\bullet} . Thus, the kinetic properties of antioxidants, in particular α -tocopherol, require that only relatively small concentrations are required for them to be effective. The concentration of α -tocopherol in biological membranes is approximately 1 mol per 1000–2000 mol phospholipids (i.e., $\sim 1:10^3$). Ascorbic acid can reduce the tocopheroxyl radical (TO^{\bullet}) to its native state, and it has been concluded that part of the reason why low concentrations of α -tocopherol are such efficient antioxidants in biological systems is because of this capacity to be regenerated by intracellular reductants such as ascorbic acid.

The heterocyclic chromanol ring of α -tocopherol has an optimized structure for resonance stabilization of the unpaired electron of the α -tocopheroxyl radical, and the electron-donating substituents (e.g., the three methyl groups) increase this effect. Because γ -tocopherol lacks one of the electron-donating methyl groups on the chromanol ring, it is somewhat less potent in donating electrons than α -tocopherol and is thus a slightly less powerful antioxidant. However, the

unsubstituted C5 position on γ -tocopherol allows it to trap lipophilic electrophiles such as peroxynitrite, thereby protecting macromolecules from oxidation.

Vitamin E Deficiency

Vitamin E deficiency is seen rarely in humans. However, there may be a risk of vitamin E deficiency in premature infants because the placenta does not transfer α -tocopherol to the fetus in adequate amounts. When it occurs in older children and adults, it is usually a result of lipoprotein deficiencies or a lipid malabsorption syndrome. These include patients with abetalipoproteinemia or homozygous hypobetalipoproteinemia, those with cholestatic disease, and patients receiving total parenteral nutrition. There is also an extremely rare disorder in which primary vitamin E deficiency occurs in the absence of lipid malabsorption. This disorder is a rare autosomal recessive neurodegenerative disease caused by mutations in the gene for α -TTP. This disorder is known as ataxia with vitamin E deficiency (AVED). Patients with AVED have extraordinary low plasma vitamin E concentrations ($<5 \mu\text{g ml}^{-1}$) and have an onset between 4 and 18 years, with progressive development of peripheral neuropathy, spinocerebellar ataxia, dysarthria, the absence of deep tendon reflexes, and vibratory and proprioceptive sensory loss. Patients with an α -TTP defect have enhanced urinary excretion of α -CEHC despite having much lower plasma α -tocopherol concentrations than healthy subjects. Therapeutic and prophylactic vitamin E supplementation (up to 2000 mg day^{-1}) prevents the onset of the disease before irreversible neurological damage develops.

Tocopherols and Low-Density Lipoprotein Modification

The hypothesis that oxidative stress plays an important role in the pathogenesis of atherosclerosis is generally accepted. Substantial *in vitro* evidence indicates that oxidized LDL is the component central to the initiation and/or progression of atherogenesis at the molecular and cellular level. The typical LDL particle is not only rich in cholesterol, but also contains approximately 1300 molecules of RH, which are very sensitive to oxidation. Vitamin E, mainly α -tocopherol, is quantitatively the most important lipophilic antioxidant present in LDL particles. On average, each LDL particle is protected by $\sim 6 \text{ mol } \alpha$ -tocopherol (range, 3–15 mol), 1 mol of γ -tocopherol, and small amounts of carotenoids.

All major cells of the artery wall, such as monocyte macrophages, endothelial cells, and smooth muscle cells, can modify LDL oxidatively *in vitro*. Monocytes have been shown to induce peroxidation of lipids such as those in LDL by the generation of reactive species, including superoxide anion, hydrogen peroxide, and hydroxyl radicals. Other oxidants have been implicated, including 15-lipoxygenase, myeloperoxidase-generated hypochlorous acid, and reactive nitrogen species such as peroxynitrite. *In vivo*, oxidized LDL particles are recognized by macrophage scavenger receptors and taken up by macrophages, forming lipid-laden foam cells in the fatty streak lesions. The free radical oxidation of LDL results in

numerous structural changes that all depend on a common event – the peroxidation of PUFAs in the LDL particle.

In vitro studies have indicated that increasing the vitamin E content of LDL particles increases their resistance to oxidation and decreases their uptake by macrophages. Vitamin E supplementation has also been reported to suppress macrophage uptake of oxidized LDL in human arterial lesions and decrease urinary F_2 -isoprostane (a 'footprint' of free radical-mediated oxidation of arachidonic acid) concentrations. Reactive nitrogen species are also implicated in aortic oxidation of LDL and therefore potentially in atherosclerosis. Because of the nonsubstituted 5-position, γ -tocopherol reacts with peroxynitrite and other electrophilic mutagens generated during inflammation and forms a stable carbon-centered adduct, 5-nitro- γ -tocopherol. This mechanism of protecting LDL may be significant when γ -tocopherol constitutes a major portion of vitamin E in the diet. It is worth noting that the ability of γ -tocopherol to attenuate oxidative damage produced by these reactive species may prevent or delay the progression of other diseases as well as cardiovascular disease (CVD), in which inflammation plays a role, such as cancer, rheumatoid arthritis, inflammatory bowel disease, and neurodegenerative disorders. In addition, γ -CEHC has natriuretic activity and functions in the kidney to control sodium excretion, and it regulates the body's extracellular fluid volume, an important determinant in hypertension and congestive heart failure.

Tocopherols and Other Metabolic Functions

Vitamin E, in addition to having a protective role in the oxidative modification of LDL, may affect or limit the progression of atherosclerosis and a number of other conditions in ways that are unrelated to its antioxidant activity. Some of these effects appear to stem from the ability of α -tocopherol, at physiological concentrations of vitamin E, to activate protein phosphatase 2 A, which inhibits the activity of protein kinase C (PKC), a biological indicator of inflammation, by dephosphorylating the protein. PKC is an important element in the signal transduction cascade mediated by growth factors, such as platelet-derived growth factors, which are necessary for the progression and completion of the cell proliferation cycle.

The cellular effects of α -tocopherol-mediated inhibition of PKC depend on the cell type in question, but the cumulative effect is highly protective against the progression of atherosclerosis. PKC inhibition results in reduced smooth muscle cell proliferation, inhibition of platelet aggregation, and thus delayed intra-arterial thrombus formation. Endothelial cell function is preserved by the downregulation of adhesion molecule (ICAM-1 and VCAM-1) expression (possibly by downregulation of nuclear factor- κB) and hence prevention of monocyte and neutrophil adhesion, which is an important early event in the initiation of fatty streak formation and atherogenesis. In addition, PKC inhibition in monocytes reduces the production of ROS by impairment of NADPH-oxidase assembly, which may help to reduce LDL oxidation.

The release of proinflammatory cytokines in monocytes, such as interleukin- 1β and tumor necrosis factor- α , is impeded by α -tocopherol-mediated inhibition of the 5-lipoxygenase pathway, and production of eicosanoids, such as

prostaglandin E₂ and thromboxane A₂, is impeded by γ -tocopherol-mediated inhibition of the cyclooxygenase pathway. Lower circulating levels of inflammatory mediators, which are aggregatory and vasoconstrictive, as well as inhibition of monocyte chemoattractant protein-1 (MCP-1) production, reduces the attraction of monocytes to inflammatory sites at the arterial wall and prevents the formation of foam cells. Furthermore, α -tocopherol increases production of prostacyclin, which has antiaggregatory and vasodilatory properties, thereby reducing the risk of a coronary event. There is evidence that in a formed atherosclerotic plaque, vitamin E may have a stabilizing effect and prevent its rupture and subsequent clot formation. This may be an important contributor to the prevention of heart disease because plaque types that are most subject to rupture present the greatest threat.

Nitric oxide (NO) produced by NO synthase in the endothelium is important in the maintenance of vascular tone; it suppresses the expression of proinflammatory cytokines, adhesion molecules, and MCP-1. It also inhibits platelet adhesion, maintains the integrity of the arterial wall, and acts as an antioxidant. Vitamin E can reduce the inhibition of NO synthase by ROS, thus maintaining NO production, either through its antioxidant activity or perhaps by suppressing PKC activity in smooth muscle.

Tocopherols and Cardiovascular Disease – Epidemiological Evidence

The effects of dietary vitamin E have been examined in several studies, many of which have reported a clear association between the reduction in the relative risk of CVD and high intake or supplement of vitamin E, although some have shown no such association. The vitamin substudy of the WHO/MONICA Project showed that in European populations whose classical risk factors for CVD were very similar, the sevenfold differences in CVD mortality could be explained at least to approximately 60% by differences in the plasma levels of vitamin E and up to 90% by the combination of vitamins E, A, and C. The Edinburgh Case Control Study and Basel Prospective Study consistently revealed an increased risk of ischemic heart disease and stroke for low plasma levels of vitamin E. However, other European population studies have not found an association between blood levels of vitamin E and end points of CVD. In the EURAMIC study, the adipose levels of vitamin E did not correlate with the relative risk of myocardial infarction.

A number of prospective studies have examined the association between vitamin E intake and risk of coronary heart disease (CHD). The Nurses' Health Study, conducted on 87 245 women, showed a 34% reduction in CHD in women who had consumed vitamin E supplements containing more than 67 mg α -TE daily for more than 2 years. However, there was no significant effect of vitamin E obtained from food sources. The Established Populations for Epidemiologic Studies of the Elderly trials showed that the use of vitamin E supplements significantly decreased risks for all-cause-mortality and mortality from heart disease. Another prospective study, performed in Canada, reported a consistent

inverse association between CVD and vitamin E supplement usage. The Health Professionals Study, conducted on 39 910 men aged 40–75 years, also showed that dietary intakes of vitamin E were not significantly correlated with reduced risk of CHD or death. A protective effect was seen in those who took 67–160 mg supplemental α -TE daily for more than 2 years. In contrast, the Iowa Women's Health Study reported that dietary vitamin E (mainly γ -tocopherol) was inversely associated with the risk of death from CVD. This association was particularly striking in the subgroup of women who did not consume vitamin supplements. There was little evidence that the intake of vitamin E from supplements (mainly α -tocopherol) was associated with a decreased risk of death from CVD. The reasons for the differences between dietary and supplemental vitamin E are not clear. However, some epidemiological studies point to the potential importance of γ -tocopherol in preventing heart disease. High dietary intake of nuts, an excellent source of γ -tocopherol, lowered serum cholesterol, improved plasma lipid profiles, and was inversely associated with the risk of death from heart disease.

The ability of α -tocopherol supplementation to prevent cardiovascular events in different populations was tested in four larger prospective clinical trials: the α -Tocopherol, β -Carotene Cancer Prevention (ATBC) study, the Cambridge Heart Antioxidant Study (CHAOS), the Gruppo Italiano per lo studio della Sopravvivenza nell'Infarto Miocardico (GISSI) trial, and the Heart Outcome Prevention Evaluation (HOPE) study. In addition, at least two smaller prospective clinical trials have been completed: the Secondary Prevention with Antioxidants of Cardiovascular Disease in Endstage Renal Disease (SPACE) study and the Antioxidant Supplementation in Atherosclerosis Prevention Study (ASAP).

In the ATBC study, the subjects who were supplemented with 50 mg all *rac*- α -tocopheryl acetate day⁻¹ for 5–8 years had only a moderately lower incidence (4%) of angina pectoris than did the control subjects, and among male smokers, cardiovascular mortality did not differ significantly between those who received supplementation and those who did not. However, subjects who received supplementation had a significantly higher incidence of hemorrhagic stroke than did the control subjects. Note that the ATBC study was not designed to investigate CVD development. The results of the CHAOS trial, the first prospective trial with CVD as an end point, were encouraging. The risks of nonfatal myocardial infarction declined 77% and total (fatal plus nonfatal) myocardial infarction declined 47% when patients with established coronary artery disease were treated with 268 or 536 – mg α -TE daily for approximately 500 days. The GISSI study showed that feeding 211 mg α -TE day⁻¹ for 3.5 years did not significantly reduce the rate of all-cause death, nonfatal myocardial infarction, or nonfatal stroke. However, in a later four-way reanalysis in which each individual variable was considered as an end point, there were significantly fewer (20%) cardiovascular deaths in the α -tocopherol group than in the control group. The HOPE study reported that vitamin E (400 IU (268 mg) day⁻¹ RRR- α -tocopherol) treatment of CVD patients had no effect on reducing the primary end points, which included nonfatal myocardial infarction, stroke, and cardiovascular death. In the SPACE trial, hemodialysis patients with preexisting CVD received 536 mg (RRR)- α -tocopherol or placebo day⁻¹. Patients

who received vitamin E had a striking 54% reduction in cardiac events compared with control subjects.

In the ASAP study, men and women (all subjects had hypercholesterolemia at entry) were given vitamin E (91 mg twice daily), slow-release vitamin C (250 mg twice daily), a combination of both, or placebo for 3 years. The progression of atherosclerosis (the mean intima-media thickness of the common carotid artery measured) was significantly retarded only in the men who smoked and took both vitamins. It is important to note that, in general, women develop fewer cardiovascular events than do men. Thus, women may profit less from vitamin E treatment than men. In studies in which many women are enrolled, the low incidence of CVD may weaken the statistical power of the overall trial.

Tocopherols and Cancer – Epidemiological Evidence

Clinical and epidemiological data, together with evidence from experimental models, support a role for the involvement of free radicals throughout the cancer process. Attempts to prevent cancer using vitamin E are based on the rationale that oncogenesis results from free radicals attacking DNA. As an antioxidant, vitamin E may inhibit cancer formation by scavenging ROS or reactive nitrogen species. Several studies of oral, pharyngeal, and cervical cancer found a relationship between vitamin E status and cancer risk. The evidence for stomach and pancreatic cancers has not been consistent, and no association with breast cancer has been found.

The Linxian, China, intervention trial provided evidence that nutritional supplementation may lower the risk of certain cancers. A modest but significant reduction in cancer mortality was observed in a general population trial in those receiving daily (for 5.25 years) a combination of β -carotene (15 mg), vitamin E (30 mg), and selenium (50 μ g). The subjects who received this mixture had a 13% lower incidence of cancer and a 10% lower mortality from stomach and esophageal cancer than did the subjects who did not receive the mixture. In the ATBC study, male smokers who took vitamin E supplements had a 34% lower incidence of prostate cancer and 41% lower mortality from prostate cancer than did those who did not take the supplements. In the US, in a nested case-control study conducted to examine the association of α -tocopherol, γ -tocopherol, and selenium with the incidence of prostate cancer, a striking fivefold reduction in risk was observed for the men in the highest quintile of γ -tocopherol compared with those in the lowest. Overall, evidence for the protection from cancer by vitamin E is not compelling.

Tocopherols and Other Diseases – Epidemiological Evidence

Vitamin E appears to act as an immunosuppressant due to its ability to suppress both humoral and cellular immune responses. Tocopherol supplementation significantly enhances lymphocyte proliferation, interleukin-2 production, and delayed-type hypersensitivity skin response and decreases prostaglandin E_2 production by inhibiting cyclooxygenase activity. There appears to be compelling evidence that

intervention with dietary antioxidants, such as vitamin E, may help maintain the well-preserved immune function of 'very healthy' elderly, restore the age-related decrease in immune function, and reduce the risk of several age-associated chronic diseases. Epidemiological evidence suggests an association between the incidence of cataract and vitamin E status. In a prospective study, the sum of serum α - and γ -tocopherol, but neither tocopherol alone, was inversely associated with the incidence of age-related nuclear cataracts.

Among the most common neurologic diseases are neurodegenerative disorders such as Alzheimer's and Parkinson's disease, which may be caused by oxidative stress and mitochondrial dysfunction leading to progressive neural death. An increasing number of studies show that antioxidants (vitamin E and polyphenols) can block neuronal death *in vitro*. In a 2-year, double-blind, placebo-controlled, randomized trial of patients with moderately severe impairment from Alzheimer's disease, treatment with 1340 mg day⁻¹ α -TE significantly slowed the progression of the disease. Clinical treatment of Alzheimer's patients with large doses of vitamin E (670 mg α -TE twice daily) is one of the key therapeutic guidelines of the American Academy of Neurology. In a multicentre, double-blind trial, vitamin E (1340 mg α -TE day⁻¹) was not beneficial in slowing functional decline or ameliorating the clinical features of Parkinson's disease. Administration of vitamin E significantly relieved symptoms in patients suffering from several types of acute or chronic inflammatory conditions, such as acute arthritis, rheumatoid arthritis, and osteoarthritis.

Vitamin E Status and Requirements

Interest in the role of vitamin E in disease prevention has encouraged the search for reliable indices of vitamin E status. Most studies in human subjects make use of static biomarkers of status, usually α -tocopherol concentrations in plasma, serum, erythrocytes, lymphocytes, platelets, lipoproteins, adipose tissues, buccal mucosal cells, LDL, and the α -tocopherol: γ -tocopherol ratio in serum or plasma. Other markers of vitamin E status include susceptibility of erythrocyte or plasma LDL to oxidation, breath hydrocarbon exhalation, and the concentration 16.2 μ mol⁻¹ are normally regarded as indicating a deficient, low, and acceptable vitamin E status, respectively. It is recommended that plasma or serum α -tocopherol concentrations be lipid-corrected (i.e., expressed relative to either the sum of cholesterol and triacylglycerol or cholesterol alone). For convenience, α -tocopherol:cholesterol is the simplest to obtain and probably the most useful, with values below 2.2 μ mol α -tocopherol mmol⁻¹ cholesterol indicating a risk or deficiency and an optimal 5.2. It has been estimated that an average daily dietary intake of 15–30 mg α -tocopherol would be required to maintain this plasma level, an amount that could be obtained from dietary sources if a concerted effort were made to eat foods rich in vitamin E.

The US Institute of Medicine Food and Nutrition Board set an estimated average requirement (EAR) of 12 mg α -tocopherol for adults >19 years on the criterion of vitamin E intakes that were sufficient to prevent hydrogen peroxide-induced hemolysis in men. The same value was set for men and women on the basis that although body weight is smaller

on average in women than men, fat mass as a percentage of body weight is higher on average in women. Because information is not available on the standard deviation of the requirement for vitamin E, the recommended dietary allowance was established for men and women as the EAR (12 mg) plus twice the coefficient of variation (assumed to be 10%), rounded up, giving a value of 15 mg day⁻¹. In Europe, the Scientific Committee for Food did not set a population reference intake (PRI) for vitamin E on the basis that there is no evidence for deficiency from low intakes, and the frequency of distribution of intakes is skewed to the right, making it difficult to set a PRI that is not inappropriately high, especially for those with a low consumption of PUFA, whose requirements are lower than those with a high consumption of PUFA.

It has been suggested that the optimum concentration of α -tocopherol in plasma for protection against CVD and cancer is $>30 \mu\text{mol l}^{-1}$, given normal plasma lipid levels and in conjunction with a plasma vitamin C concentration $>50 \mu\text{mol l}^{-1}$ and a β -carotene level $>0.4 \mu\text{mol l}^{-1}$. This has not been proven in large-scale human intervention trials, but even in the absence of conclusive evidence for a prophylactic effect of vitamin E on chronic disease prevention, some experts believe that a recommendation of a daily intake of 87–100 mg α -tocopherol is justifiable based on current evidence. Realistically, these levels can be achieved only by using nutritional supplements. The tolerable upper intake level for vitamin E is 1000 mg day⁻¹, based on studies showing hemorrhagic toxicity in rats, in the absence of human dose–response data.

See also: Antioxidants. Fats and Oils. Fatty Acids: Health Effects of Omega-6 Polyunsaturated Fatty Acids. Lipoproteins. Nuts and Seeds. Omega-3 Polyunsaturated Fatty Acids: Nutrigenetic and Nutrigenomic Aspects in the Determination of Dietary Requirements, Development, and Chronic Diseases

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Introduction

The discovery of vitamin K as an essential nutrient arose in the late 1920s from Henrik Dam's studies of sterol metabolism. He observed that chicks fed with a fat-free diet developed subcutaneous hemorrhages and anemia. A lipid extract of liver or of certain plant tissues was curative, and by 1935 he claimed the discovery of a new vitamin in these extracts that he named 'vitamin K' from the German word 'Koagulation.' By the late 1930s, two chemically similar forms of the vitamins from different sources were recognized, namely phyloquinone or K₁ and menaquinones or K₂, which had been isolated from alfalfa and from putrefied fish meal, respectively (Figure 1). Phyloquinone, with its saturated phytyl side chain, is found in plant tissues, especially in green leafy ones, where it acts as a component of the electron transport chain. The menaquinones, or MK-n, by contrast, comprise a broad family of representatives that have a variable length, unsaturated side chain, and are composed of one or more (sequential) isoprene units in place of the saturated phytyl side chain. These menaquinones can be produced by certain types of bacteria, both in the large bowel of animals and at other locations where they may contribute to human food sources of menaquinones. The specific menaquinone with the same side chain length as phyloquinone is called menaquinone-4 (MK-4), or menatetranone, which is produced commercially as human medication, especially in Japan. Menadione, a water-soluble form of the vitamin that has a single methyl group in place of the side chain, can be converted to MK-4 *in vivo*, and is used in animal feeds, but it is not used in humans because of its toxicity at high doses. Phyloquinone is converted to MK-4 through synthesis within the tissue, or by

transport to the tissue through circulation. Menadione is the intermediate vitamin K metabolite in this conversion. There is an evidence that UbiA prenyltransferase domain-containing protein1 (UBIAD1) is a key enzyme for MK-4 biosynthesis, and possesses side chain cleavage and prenylation activities. Most bacterially synthesized menaquinones have longer side chains, typically 7–9 isoprene units and up to 13, which are indicated by 'n' in the MK-n shorthand notation.

Food Sources, Absorption, and Catabolism

Food sources of phyloquinone (Table 1) include green leafy vegetables as the major quantitative source. The longer-chain menaquinones, MK 7–9, are typically obtained from foods, such as cheeses or Japanese 'natto' (fermented bean curd), in which bacterial fermentation has occurred. Smaller amounts of both phyloquinone and menaquinones are obtained from liver and other animal-derived foods. Phyloquinone-rich plant oils when hydrogenated form 2',3'-dihydrophyloquinone, which differs from the parent form by saturation of the 2',3' double bond on the side chain. Dihydrophyloquinone is most prevalent in margarines, prepared foods, and infant formulas. With a general trend toward a reduction in the USA in hydrogenation of plants oils, it is expected that there will be an overall reduction in dihydrophyloquinone in the USA food supply.

Phyloquinone is highly lipophilic; however, at low concentrations it is transported by a saturable, energy-dependent transport system across the gut wall, mainly in the upper small intestine. Phyloquinone in foods consisting of plant tissues is much less readily bioavailable for absorption than the pure

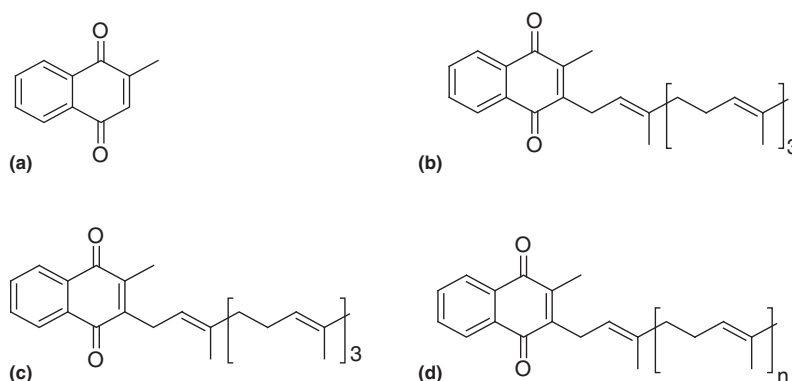


Figure 1 Chemical structures of: (a) menadione, (b) phyloquinone, (c) MK-4, and (d) menaquinones, where n represents the number of repeating isoprene units.

Table 1 Phylloquinone concentrations (μg per measure) in common foods^a

Food Item	Common measure	Content ^b
<i>Vegetables</i>		
Collards, frozen, cooked	1 Cup	1059
Spinach, cooked	1 Cup	888
Brussels sprouts, cooked	1 Cup	300
Corn, sweet, cooked	1 Ear	97.2
Broccoli, raw	1 Cup	89.4
Cucumbers, raw	1 Large	49.4
Celery, raw	1 Cup	35.2
Lettuce, green leaf, raw	1 Cup	35
Asparagus, boiled	4 Spears	30.4
Carrots, raw	1 Cup	14.5
<i>Protein sources</i>		
Soybeans, mature cooked	1 Cup	33
Egg, whole, cooked	1 Large	2.6
Beef, cooked	3 oz	1.9
Milk, whole, 3.25% fat	1 Cup	0.5
Fish, salmon, smoked	3 oz	0.1
<i>Prepared foods</i>		
Pizza, cheese	1 Serving	4.2
Apple pie	1 Piece	4.1
Salad dressings	1 Tbsp	2.2
Potato chips	1 oz	2.0
Cheese, cheddar	1 oz	0.8
<i>Fats and oils</i>		
Margarine, regular	1 Tbsp	13.2
Olive oil, cooking	1 Tbsp	8.1
Soybean oil, cooking	1 Tbsp	3.4
Butter, salted	1 Tbsp	1.0
<i>Fruits</i>		
Blackberries, raw	1 Cup	28.5
Apples, with skin	1 Apple	3.0
Pineapple, raw	1 Cup	1.1
Bananas, raw	1 Banana	0.6
Strawberries, raw	1 Strawberry	0.3

^aUSA Department of Agriculture's National Nutritional Database for Standard Reference, Release 22 (<http://www.nal.usda.gov/fnic/foodcomp>).

^bThe unit is μg phylloquinone per measure.

vitamin since it is tightly bound to the thylakoid membranes of the chloroplasts, and the absorption of vitamin K from plant foods is considerably improved by including additional fat in the meal. Its absorption also depends on the stimulation of bile salt and pancreatic lipase secretions. The long-chain menaquinones, which are even more lipophilic, are only passively absorbed and are much less bioavailable for absorption than phylloquinone. Current understanding of the relative bioavailability and bioactivity of the different forms and food sources of vitamin K is limited and needs more research.

The triglyceride-rich lipoproteins (TRL) are the major carriers of phylloquinone in the circulation, with lesser but significant amounts carried by low- and high-density lipoproteins (LDL and HDL). Long-chain menaquinones are mainly transported by LDL. Plasma vitamin K concentrations,

which are typically in the low nanomolar range in humans are much lower than for the other fat-soluble vitamins (A, D, and E), and they are strongly correlated with the triglyceride content of the plasma. Based on early isotopic work in humans, the turnover of phylloquinone results in *ca.* 40–50% of the exchangeable body pool being transferred via the bile into the feces and 20% being excreted into the urine, the latter including the excretion of oxidized products that become conjugated as glucuronides. Through the use of ¹³C-labeled vegetables, it has been determined that phylloquinone has a plasma half-life of 8.8 h, and a tissue half-time of 215 h. No comparable studies using stable isotopes have been reported for any of the menaquinones.

Human urinary metabolites of phylloquinone comprise of glucuronide conjugates of carboxylic aglycone moieties, including two side chain-shortened carboxylic acids. These metabolites respond to dietary manipulation of phylloquinone and 2',3'-dihydrophylloquinone, which suggests utility as biological markers of vitamin K dietary exposure and status.

The enzymatic pathway for the catabolism of vitamin K has not been studied in detail. Vitamin K upregulates CYP3A isoforms by binding to and activating the steroid and xenobiotic receptor (SXR), also known as the pregnane X receptor (PXR). The SXR is a nuclear receptor which acts as a sensor for the presence of many drugs such as phenobarbital, taxol, rifampicin, and others, and initiates the detoxification process. SXR is involved in the transcriptional regulation of enzymes, such as cytochrome P-450s, which through transporter molecules clear these substances from the body.

Vitamin K-Dependent (VKD) Proteins

Vitamin K acts as the essential recycling cofactor (or cosubstrate) for all vitamin K-dependent protein carboxylation and Gla-forming reactions (**Figure 2**). In its dihydro or quinol form, the vitamin reacts with molecular oxygen, thereby creating a highly reactive, high-energy carbanion at the glutamate (Glu) site for insertion of carbon dioxide, creating a new Gla residue. This vitamin K quinol oxidation step provides the essential energy for the endothermic carboxylation step. The other product of the reaction is the vitamin K epoxide, comprising a three-membered carbon–oxygen ring. Since the oxidized vitamin needs to be recycled back to the quinol form before the next protein carboxylation cycle, a two-stage reduction process ensues, forming first vitamin K quinone and then the original quinol (**Figure 2**). Both of these reduction steps can be catalyzed by the enzyme vitamin K epoxide reductase, which is linked to a dithiol–disulfide reducing couple and which is highly sensitive to inhibition by the coumarin class of drugs, of which warfarin is the best known and most commonly used. The reduction of the intermediate vitamin K quinone to its quinol form can also be catalyzed by another, NAD(P)H-dependent, quinone reductase that is warfarin resistant, and for this reason the inhibition of carboxylation by warfarin can be reversed or antagonized by large doses of vitamin K provided exogenously in its normal quinone form.

The VKD coagulation proteins, factors II, VII, IX, and X, and proteins C, S, and Z, are synthesized in the liver.

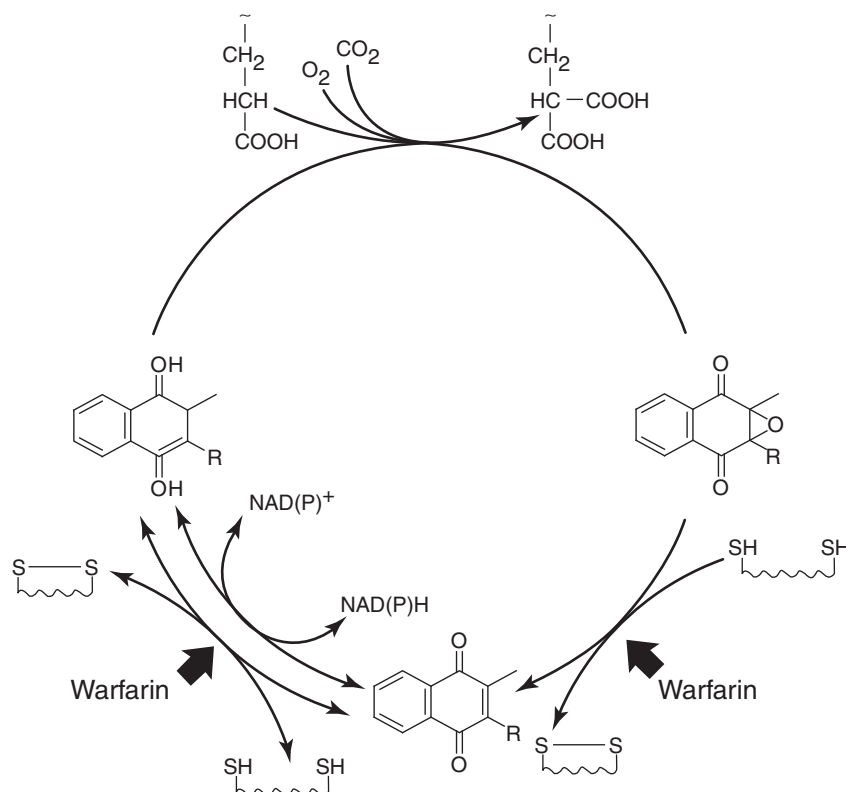


Figure 2 Tissue recycling of vitamin K. Vitamin K epoxide formed in the carboxylation reaction is reduced to the quinone form of the vitamin by a warfarin-sensitive enzyme – the vitamin K epoxide reductase. This reaction is driven by a reduced dithiol. The naphthoquinone form of the vitamin can be reduced either by the same warfarin-sensitive dithiol-driven reductase or by one or more of the hepatic NADH or NADPH-linked quinone reductases that are less sensitive to warfarin.

The extrahepatic VKD proteins include osteocalcin (OC), matrix Gla protein (MGP), Gla-rich protein (GRP), proline-rich Gla proteins, periostin, periostinlike factor (PLF), TGFBI, and growth arrest specific 6 (Gas6). Undercarboxylation of these proteins has been linked to a variety of age-associated conditions, including loss of bone mineral density, increase in fracture risk, osteoarthritis, arterial calcification, insulin resistance, and inflammation.

Vitamin K and Coagulation

The physiological function that led to the discovery of vitamin K, and its confirmation as an essential vitamin for higher vertebrates, was its unique role in the blood clotting cascade. This cascade comprises of a complex series of linked pro-enzyme-to-enzyme conversions, which leads eventually to a fibrin clot. Central to this process is the activation of Gla residues by calcium in some of the members of the cascade series: factors VII, IX, and X and factor II (prothrombin). In addition, there is an inhibitory level of control by proteins C, S, and possibly Z. All seven of these Gla proteins have Gla clusters that interact specifically with calcium so as to alter their polypeptide conformations and to permit their interaction with other members of the coagulation cascade (by exposing a phospholipid-binding domain) and hence leading either to activation or to inhibition of individual components.

Vitamin K and Mineralization

Besides these coagulation factors, there are other extrahepatic VKD proteins associated with bone metabolism and soft tissue calcification.

OC is synthesized specifically by osteoblasts and odontoblasts, and it accounts for *ca.* 15–20% of the noncollagen protein of the bone matrix. Approximately 20% is secreted into blood plasma during bone formation. Circulating OC has frequently been measured as an index of bone-forming (osteoblastic) activity, and is present in increased amounts during growth and among individuals with certain bone diseases. It is a small protein, MW 5700, with just three Gla residues. Unlike the blood coagulation Gla proteins, which are almost completely carboxylated in people neither severely vitamin K deficient nor treated with vitamin K antagonist, circulating OC is partially undercarboxylated in most humans, as measured by assays that depend on the affinity of the undercarboxylated form for hydroxyapatite or a specific ELISA assay for the undercarboxylated form. Since vitamin K supplements can reduce its degree of undercarboxylation, it has been proposed as a highly sensitive functional test of vitamin K status in man.

Despite the growing level of interest in its practical use as a status index, our understanding of the essential function of OC remains incomplete. Its affinity for calcium is less strong than that of the larger Gla proteins, but it binds avidly to

hydroxyapatite. Transgenic mice that lack the gene for OC have increased bone mass, despite an increased number of osteoclasts. In humans, however, undercarboxylation of OC, especially in postmenopausal women has been linked to low vitamin K intakes, reduced bone mineral density, and increased risk of fracture. Intervention with high dose MK-4, mainly in Japan, has been reported to improve bone mineral density and decrease fracture risk, although more recent data report has no beneficial effect of MK-4 supplementation on fracture reduction above that achieved from calcium supplementation alone. Randomized clinical trials testing the efficacy of phylloquinone in reducing bone loss in otherwise healthy elderly adults have generally not produced measurable beneficial effects. It is plausible that the associations between high vitamin K intakes and low hip fracture risk are reflective of a healthy diet and lifestyle, and not indicative of causality.

More recently, OC has been reported to be involved in glucose metabolism. Primarily based on data from *in vitro* studies, the uncarboxylated form of OC has been reported to influence β -cell function, insulin sensitivity, adiponectin production, energy expenditure, and adiposity, thus assuming the role of a hormone. The human data are generally limited to small observational studies, and to date are too equivocal to draw conclusions on OC's role in regulation of insulin sensitivity.

The second VKD protein, MGP, having five Gla residues conferring high affinity for calcium and phosphate is a strong inhibitor of calcification and of recognized importance for vascular health. Its synthesis is modulated by 1,25-dihydroxy vitamin D and retinoic acid. Mice lacking the gene for MGP quickly developed calcified arteries and died of aortic rupture before 2 months of age. For this reason, MGP is believed to antagonize the pathological calcification of soft tissues, and thus to protect them. The absence of MGP also led to inappropriate calcification of growth plate cartilage, reduced growth, osteopenia, and fracture in the MGP gene knockout mice. In humans, defects in the MGP gene are associated with Keutel's syndrome and chondroplasia punctata, in which cartilage calcification is abnormal. Similar abnormalities have been observed in infants whose mothers were treated with warfarin during the first trimester of pregnancy. Low vitamin K intake was associated with atherosclerotic calcification of the aorta in postmenopausal women. Also, circulating MGP levels were found to be raised in severe atherosclerosis and in type 1 diabetes in humans. Measurement of total MGP is not a marker specific to vitamin K activity because vitamin K only influences carboxylation of the protein, and not synthesis. Current immunoassays measuring different elements of MGP carboxylation are in the process of being validated in different populations. More recently, minor alleles of the MGP gene have been reported to confer an increased risk for coronary artery calcification and osteoarthritis.

The third bone-associated Gla protein, protein S, is also involved with blood clotting. It is synthesized by osteoblastlike and osteblastoma cells in culture, and it has been detected in bone matrix. It is also synthesized by hepatocytes, megakaryocytes, and endothelial cells. Children with an in-born deficiency of protein S developed osteopenia and bone lesions; however, its precise functional role is unknown.

Gla-rich protein, GRP, is expressed in most tissues of species studied and, in particular, those containing cartilage and

bone cells. GRP contains 16 Gla residues, which is the highest number of Gla residues in any known VKD protein. The high number of Gla residues indicates that this protein has high potential for calcium binding, suggesting its role as a physiological calcium modulator in the extracellular domain.

Periostin and PLF are novel γ -carboxylated proteins. Although known for more than a decade, it is only within the last few years that periostin was identified as a VKD protein. Periostin is highly expressed in bone extracellular matrix and is produced by mesenchymal stromal cells. Periostin is a necessary factor for tissue development, maturation, and repair. PLF, one of the naturally occurring splice variants from the periostin locus, is primarily expressed in the periosteum during the early adaptive stage of remodeling.

Gas6 and Other VKD Gla Proteins

Gas6 is associated with the central nervous system. It acts as a ligand for a number of receptor protein kinases; it potentiates the growth of vascular smooth muscle cells, Schwann cells, and the neurons that synthesize gonadotropin-releasing hormones; and it can prevent apoptotic cell death. Knockout mice in which three Gas6 receptors were mutated had major neurological and spermatogenic abnormalities. There is interest in potential roles for Gas6 in Alzheimer's disease and Parkinson's disease. Clearly, these properties and emerging roles have helped to confirm the growing suspicion that VKD proteins possess key functions beyond blood clotting and even bone remodeling.

There are several other Gla proteins from a variety of tissues that are even less well characterized. TGFBI is an extracellular matrix protein only recently recognized to be γ -carboxylated. Knockdown of TGFBI causes mitotic spindle abnormalities and centrosome duplication. Genomic instability is associated with increased cancer risk and also correlates with the risk of metastasis. TGFBI promotes microtubule stability and down-regulates genes related with tumor growth.

Proline-rich Gla proteins PRGP-1 and PRGP-2 are found predominantly in the spinal cord and thyroid gland, respectively, but their functions are unknown. Gla proteins occur in most vertebrates and also in molluscs, so their evolutionary appearance in the animal kingdom is probably quite ancient in origin.

Potential Non-Gla Functions of Vitamin K

Vitamin K is thought to be involved in sphingolipid metabolism in certain bacteria by modulating serine palmitoyl transferase, and warfarin treatment decreased brain concentrations of sulfatides and galactocerebroside sulfotransferase activity in animals, which was reversible by vitamin K (either phylloquinone or MK-4). Therefore, it is now thought that vitamin K may be involved in sphingolipid metabolism.

Most recently, vitamin K, in the forms of both phylloquinone and MK-4, has also been reported in both *in vivo* models and in human studies to modulate proinflammatory cytokine production independent of its role as a cofactor. The mechanism underlying the potential influence of vitamin K on

inflammatory cytokine production is unclear, although it may be through the regulation of the transcription factor NF- κ B.

There are several functions of MK-4 that are shared by the isolated geranyl-geraniol side chain, which involve the induction of apoptosis of osteoclasts and of certain cancer cells in culture. Depriving certain tumors of vitamin K, both *in vitro* and *in vivo*, seemed to inhibit their growth and metastasis. In contrast, patients at risk of hepatocellular carcinoma had lower incidence of the disease when given high doses of MK-4 compared to those not receiving MK-4. Data from observational studies also suggest that high intakes of long-chain menaquinones may be associated with lower incidence of certain cancers. However, these studies were not designed to test the hypothesis that vitamin K forms reduce cancer incidences, hence should be considered hypothesis-generating at this time. Recent studies have suggested that MK-4, in particular, has a transcriptional regulatory function. MK-4 promotes extracellular matrix formation by activating SXR to upregulate tsukushi (TSK), which plays a role in collagen accumulation. MK-4 and its isolated geranyl-geraniol side chain were also able to suppress the synthesis of prostaglandin E₂, which is a potent bone resorption catalyst. These observations have led to speculations that (i) some of the menaquinones may possess some functions that are not shared by phyloquinone, and (ii) there may be implications for cell proliferation and for cancer risk from variations in the supply of vitamin K. Because phyloquinone is converted to MK-4 in some but not all tissues, it is highly likely that MK-4 has unique roles independent of its function as an enzyme cofactor. However, it is also plausible that phyloquinone is the optimal dietary form because it can be converted to MK-4 in tissues where presumably needed.

Status, Requirements, and Recommended Intakes

A published 'normal' range for plasma phyloquinone, the predominant circulating form of vitamin K among individuals not taking dietary supplements in the US, is 0.25–2.7 nmol l⁻¹, corresponding to approximate average daily intakes of 100 µg per day in men and 80 µg per day in women. As noted earlier, the phyloquinone content of plasma has a short half-life and is strongly correlated with plasma triglycerides. It also has wide intra-individual and inter-individual margins of variation. It is therefore not ideal as a long-term index of status. Alternatives include functional indices such as PIVKA-II (Protein Induced in Vitamin K Antagonism or Absence-Factor II, which is only sensitive to marginal deficiency), and under-carboxylated osteocalcin (ucOC) (which is the most sensitive functional indicator). These functional indices are not totally specific for vitamin K deficiency, although ucOC (for which monoclonal antibodies now exist) does appear to possess reasonably good specificity. Unfortunately, the different commercial kit assays measure different epitopes of OC, which makes harmonization difficult. More recently, use of urinary metabolites has been proposed as a non-invasive, yet sensitive measure of vitamin K status. Unfortunately none of these biomarkers have physiological outcomes on which to base dietary requirements. For example, although one can increase the amount of the OC that is carboxylated through

dietary and supplementary vitamin K, there is no consistent concomitant change in bone turnover markers or bone mineral density. PIVKA-II can be indicative of clotting disorders if elevated. However dietary restriction of vitamin K is not known to induce elevated PIVKA-II levels commensurate with abnormal coagulation.

In the US, the Food and Nutrition Board of the National Academy of Sciences has defined an Adequate Intake (AI) of phyloquinone of 90 µg per day for adult women and 120 µg per day for adult men, with proportionately smaller values for children. The guidelines in the UK are 1 µg per kg body weight per day, and the AI in Japan is set at 75 µg per day for adult men, 60 µg per day for women, aged 19–29 years, and 65 µg per day for women 30 years and over. The true requirement based on the role of extrahepatic VKD proteins is still unknown. Since vitamin K is thought to have a wide range of functions in the body in addition to blood clotting, and some of these may have long-term health implications, research on requirements and optimal intakes, with multiple end points, is needed. Metabolic and health-related differences between the menaquinones and phyloquinone also need to be defined.

Population Groups at Risk of Vitamin K Deficiency

Because of the minimal extent of transfer of vitamin K across the placenta, the fetus and newborn infant have much lower circulating vitamin K than adults (typically 30-fold lower). Although low vitamin K levels have not been found to affect the developing fetus in a functionally deleterious way, it is clear that the newborn, and especially the solely breast-fed infant, is at a higher risk of functional deficiency than older infants and adults. In a minority of cases, this can lead to life-threatening or long-term damage associated with intracranial bleeding. Vitamin K deficiency bleeding (VKDB) is divided into three different types depending on their time of presentation. Early VKDB occurs within the first day of life and is generally associated with maternal use of warfarin or anti-coagulants that interfere with the vitamin K cycle. Classic VKDB occurs within the first week of life and is usually idiopathic or associated with breastfeeding. Late VKDB occurs after the first week of life and peaks at 3–8 weeks of life. The late form is usually secondary to an underlying disorder such as malabsorption, chronic diarrhea, liver disease, or undiagnosed cholestasis. Breastfeeding is thought to put infants at a higher risk of classic or late VKDB due to low-milk intakes associated with difficulty in establishing adequate lactation coupled with low vitamin K content of breast milk. In Western countries, since the 1950s, it has been routine practice to give prophylactic phyloquinone in a 1 or 2 mg dose at birth, and this has been found to considerably reduce the risk of VKDB. Recent data suggest that lower doses are as effective in reducing PIVKA-II levels associated with vitamin K deficiency as the current doses (0.5–1 mg phyloquinone), but may result in less accumulation of unmetabolized vitamin K. The consequences of unmetabolized vitamin K measured among children receiving the higher therapeutic prophylactic doses are currently unknown. An intramuscular depot dose was found to be highly effective; however, a study in the UK in the 1990s suggested a possible link with childhood cancer.

Despite little subsequent support for this contraindication, the adverse publicity led to a shift in practice toward oral dosing. An oral micellar preparation containing glycholate and lecithin has been developed that has improved absorption characteristics. Another approach toward the prevention of VKDB is through modern commercial formulas, which typically contain 50–125 µg phyloquinone l⁻¹ compared to an average intake of 2 µg per day through breast milk.

Antibiotic-treated patients may be at increased risk of developing vitamin K deficiency. Some antibiotics may reduce the production of usable menaquinones by gut bacteria; others, such as cephalosporins, may also exert vitamin K epoxide reductase inhibitory effects. Vitamins A and E in large doses may increase the risk of vitamin K deficiency and its sequelae in susceptible people. Thus, in one study, patients receiving anticoagulant drugs exhibited a further reduction of prothrombin levels if they were given 400 IU α -tocopherol per day for 4 weeks. The microsomal vitamin K-dependent carboxylase enzyme was found to be inhibited by α -tocopheryl quinone and, to a lesser extent, by α -tocopherol. It is also inhibited by other oxygen free radical antagonists. Control of blood clotting with warfarin-type drugs thus requires control of intakes of vitamins A and E as well as vitamin K so as to achieve consistent results.

Currently the elderly are at the greatest risk of very low vitamin K intakes. The health consequences of these low intakes are currently unknown, but are the focus of active research.

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See also: Aging. Amino Acids: Chemistry and Classification. Bioavailability. Nutritional Aspects of Bone. Vitamin E: Metabolism and Requirements



WEIGHT MANAGEMENT

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Approaches

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Weight loss and weight loss maintenance require a decrease in energy intake (diet), an increase in energy expenditure (exercise and physical activity), or both. Dietary management should encourage healthy eating, that is, an appropriately balanced intake of macro- and micronutrients. For most obese individuals, this will entail not just a decrease in total energy intake, but specifically a decrease in fat intake, together with an increase in complex carbohydrates, fruit, and vegetables. Myriad diets have been popularized as a means of reducing energy intake, but few are recommended as meeting the overall nutritional needs of an obese individual, and many are so restrictive that they clearly could not be followed for more than a few weeks. Increasing exercise and physical activity has benefits beyond those that result from the relatively modest amounts of extra energy expended. These include a beneficial protection from excessive loss of lean body tissue during dieting, improved fitness and psychological health, and a greater likelihood of long-term weight maintenance. Diet and exercise are core components of behavioral treatments; such treatments, based on learning theories, also aim to help individuals become aware of the behaviors that have led to their weight gain, and to develop strategies to alter them. Weight loss can be achieved successfully with all strategies; behavioral therapies that include a strong focus on increasing exercise and activity seem to offer the best chances of long-term success.

The Concept of Desirable Weight

Body weight reflects the additive mass of the various tissues that make up the organism and is a function of energy and nutrient balance over a prolonged period. Positive energy balance will result in weight gain (mainly from deposition of lipid in adipose tissue), whereas prolonged undernutrition will lead to weight loss. For most of human history, the dominant disorder of body weight has been thinness. Thinness, whether from malnutrition or disease, was associated with illness and was often a prelude to death; in societies where food supplies are scarce or seasonal, a high body weight may be seen as a desirable sign of health and probably wealth. In contrast, in developed societies where levels of activity are low and food is plentiful, the growing prevalence of overweight and obesity has been clearly linked to illness and premature mortality. The concept of a desirable weight at which health is optimal and the risk of disease minimal has not been easy to define, largely because of the effects of many other factors such as age, sex, social status, and smoking.

Dietary Management

Dietary management of obesity aims to reduce fat stores by changing eating habits to reduce energy intake below that

required for weight maintenance. The term 'reducing diet' has been coined to describe such diets used to treat the obese. Because many obese individuals may eat a nutritionally inadequate (apart from energy) diet, it is important that advice on energy restriction is accompanied by the prescription of a 'healthy' diet that contains adequate protein, vitamins, calcium, trace elements, and a desirable ratio of complex carbohydrate to fat. Weight loss *per se* is of no medical benefit unless it is maintained, and this will require the obese individual to adhere to a permanent change in eating habits. Many think of a 'diet' as a temporary change in eating habits (often extreme or quirky), a view encouraged by many of the diet books that hold out the promise of easy and instant success. It is essential that the concept of a long-term change in dietary habits be accepted at the start of treatment.

The energy value of weight gained or lost is approximately 31 MJ kg^{-1} ($7500 \text{ kcal kg}^{-1}$) because it is composed approximately of three parts fat to one part lean. Thus, a daily energy deficit of 2.1 MJ (500 kcal) will produce a weight loss of approximately 2 kg per month. For the average man or woman,

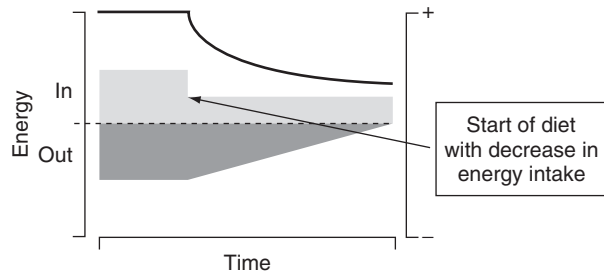


Figure 1 Fall in body weight (solid line) resulting from a fixed decrease in energy intake. Note that the rate of weight loss slows as the gap between energy expenditure (darker shading) and energy intake (lighter shading) narrows.

this represents a 20–30% reduction in energy intake, although for the obese the percentage reduction will be smaller. Thus, the severely obese, for example, with a body mass index (BMI) of 35 or more, will need to follow an energy-restricted diet for months rather than weeks to reverse their obesity. As weight is lost, energy requirements fall, in part because of the reduced energetic mass of the person, and also because of adaptive changes in energy expenditure. For this reason, the rate of weight loss will eventually slow and reach a plateau for any fixed level of dietary energy restriction (**Figure 1**).

Myriad diets have been popularized and promoted directly to the public, reflecting every possible permutation of increasing or decreasing the major macronutrients. Fashion and commercialism have dictated many of them. **Table 1** shows the variety of diets that have been suggested, and used, for treating obesity. Many of these diets fail to focus on long-term dietary change, and the quirkiness of many makes it unlikely that they would be followed for long.

Current ideas on a reasonable reducing diet are that it should contain at least 100 g carbohydrate to prevent glycogen depletion and ketosis. High-carbohydrate diets are composed of complex carbohydrates and are thus of low-energy density, which may aid management of hunger. Because high-carbohydrate diets are low in fat, they have the theoretical advantage of directly reducing the risk of cardiovascular disease. The energetic efficiency with which carbohydrate is converted and stored as fat is lower than that of dietary fat, providing a further advantage. Protein intake must be adequate to maintain lean body mass. Although there is an inevitable fall with weight loss, 0.8 g per kg of body weight per day + 1.75 g per 100 cal deficit of protein (approximately 44 g daily for women and 56 g daily for men) should be consumed, and fat restricted to less than 30% of total energy. The diet should contain recommended daily intakes of vitamins, minerals, and electrolytes, if necessary by supplementation; 20–30 g daily of fiber should also be consumed.

Table 1 Types of diet used for treating obesity

Generic name for diet	Typical dietetic modification	Popular example of diet
Starvation diet	Less than 1.2 MJ (300 kcal) per day	Grapefruit and black coffee
Very low-energy (protein-sparing) diets ^a	Approximately 2 MJ (500 kcal) per day with $>50 \text{ g}$ high-quality protein; usually liquid	Cambridge diet modifast
Low-energy diet ^a	$5\text{--}7.5 \text{ MJ}$ ($1200\text{--}1800 \text{ kcal}$) per day often from menus, recipes	Weight watchers TOPS
Fixed-energy deficit diet ^a	Nutritionally balanced, individually tailored to produce fixed-energy deficit (e.g., 2 MJ or $500 \text{ kcal day}^{-1}$) based on measured or predicted energy needs	Prescribed by dietician
High-protein diet	Greater than 40% protein, thus low in carbohydrate and fat	Scarsdale medical diet
Low-protein diet		Beverly hills diet
High-fat diet	Restricted carbohydrate and protein	Drinking man's diet
Low-fat diet ^a	Restrict fat to $<20\%$ energy	Prescribed by dietician Pritikin diet
High-carbohydrate diet	Effectively low fat, may be high in fiber	F-plan diet
Low-carbohydrate diet	Limits carbohydrate to maximum $<50 \text{ g}$ daily	Yudkin diet
Macronutrient choice ^a	Choice from lists of macronutrients to encourage intake of foods high in complex carbohydrates	No counting diet
Meal replacement	Liquid formula meals of approximately 1.7 MJ (400 kcal) to replace 1–2 meals daily	Slim fast
Fad diets	Varied; e.g., food combining diets that require macronutrients to be eaten separately and separated by time	Hay diet

^aDiets considered medically reasonable under defined circumstances.

Many diets prescribe an energy intake that is based on a generalized rather than an individualized assessment of energy needs. The common prescription of 4.2–5.0 MJ (1000–1200 kcal) daily may be problematic and inappropriate. Weight loss in men will be faster and greater compared with women of equal BMI because of the relatively greater metabolic rate per kilogram of body weight of men. The very obese, whose daily energy requirements can be as high as 12.6 MJ (3000 kcal), may lose weight at an excessive rate and develop symptoms of ketosis, postural hypotension, or excessive hunger. Many obese patients fail to register or admit to the amount of food they consume and claim that such a diet is more than their habitual intake.

One principle of energy prescription that has proved easy to administer and successful in outcome is to calculate energy requirements from standard formulae (Table 2) and prescribe a diet that provides a fixed-energy deficit of 2.1 MJ (500 kcal). Compliance and weight loss were better with this approach than with a fixed 5 MJ (1200 kcal) diet.

A diametrically opposite approach is the use of very low-energy liquid diets. These were originally developed in the 1960s to provide a nutritionally complete intake in terms of protein, vitamins, and micronutrients, but provide as little as 1.4 MJ (350 kcal) daily. The inclusion of sufficient high-quality protein was designed to prevent the excessive loss of lean body mass seen with starvation or other ketotic diets, hence the alternative term 'protein-sparing modified fast.' Appropriately selected, well-motivated patients are highly compliant with such diets, and their weight loss can be very rapid. Paradoxically, perhaps, patients seem to find it easier to mount levels of near-total restraint than more moderate restriction. It appears that withdrawing all solid or 'proper' food helps the patient to define himself or herself as 'not eating,' in the same way that some quitting smokers find it easier to abstain completely from cigarettes rather than to cut down.

In the 1970s, a commercial very low-energy diet formulation (the Last Chance Diet) was marketed and was associated with a number of deaths from cardiac arrhythmia. This diet was deficient in essential amino acids and in minerals such as magnesium and potassium. It was withdrawn. In the 1980s newer, better-formulated diets were commercially marketed. Concerns about their inappropriate use by already slim women, often with an eating disorder, forced governmental health agencies to issue guidelines on their use. In the USA, a task force recommended that such diets contain at least

3.3 MJ (800 kcal), be supervised by experienced physicians, and be used only by those with a BMI more than 30, for less than 16 weeks. In the UK, a report from the Committee on Medical Aspects of Food Policy suggested such diets should provide a minimum of 1.7 MJ (400 kcal) and 40 g protein daily for women, and 2.1 MJ (500 kcal) and 50 g protein daily for men and tall women. They were recommended for use only by those with a BMI more than 25 and under medical supervision, for no longer than 4 weeks. The drawback of such diets is that unless they are combined with, or followed by, some other treatment (pharmacological or behavioral), weight regain, often soon and rapid, is almost universal.

More recently, low-energy liquid diets of approximately 3 MJ (750 kcal) daily have been popularized, often as part of an overall behavior modification program (see later), or in the form of sachets intended to be used as meal replacements. Both approaches have been shown to have potential for success in short-term studies lasting up to 1 year.

Exercise and Physical Activity

The term 'physical activity' refers to bodily movement produced by skeletal muscle that results in energy expenditure; it thus includes activities of daily living, as well as leisure activity from sport and exercise. The term 'exercise' refers to planned or structured bodily movements, usually undertaken in leisure time in order to improve fitness (e.g., aerobics), whereas 'sport' is physical activity usually in structured competitive situations (e.g., football). Physical activity at recommended levels (moderate intensity for 30 min for 5 days each week) is associated with many health benefits; these include lower all-cause mortality rates; fewer cardiovascular events, such as myocardial infarction and stroke; and a lower incidence of metabolic disorders, including noninsulin-dependent diabetes mellitus and osteoporosis. Levels of activity have been falling in westernized societies largely because of a decrease in physical activity at work (from increasing mechanization) and increasingly sedentary leisure-time pursuits (such as television viewing). The Allied Dunbar National Fitness Survey of UK showed that 70% of the population are insufficiently active, and a separate UK government survey showed that one in three adults could be classified as sedentary, that is, taking less than half an hour of continuous moderate-intensity physical activity each week (Figure 2).

Table 2 Formula for estimating RMR for men and women

Age (years)	RMR
Men	
18–30	$0.063 \times \text{weight in kilogram} + 2.896 \text{ MJ daily}$
31–60	$0.048 \times \text{weight in kilogram} + 3.653 \text{ MJ daily}$
>60	$0.049 \times \text{weight in kilogram} + 2.459 \text{ MJ daily}$
Women	
18–30	$0.062 \times \text{weight in kilogram} + 2.036 \text{ MJ daily}$
31–60	$0.034 \times \text{weight in kilogram} + 3.538 \text{ MJ daily}$
>60	$0.038 \times \text{weight in kilogram} + 2.755 \text{ MJ daily}$

The energy expenditure greater than 24 h can be estimated by multiplying by a factor related to activity levels (1.3, sedentary; 1.5, moderate activity; 1.8, physically very active).

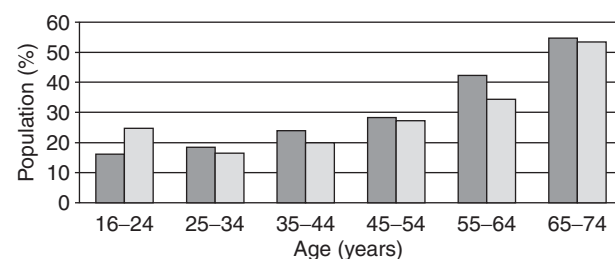


Figure 2 Percentage of adults in England by age and sex (1990–91) with a sedentary lifestyle; dark bars, men; light bars, women. Reproduced from Fentem P and Walker A (1995) Setting targets for England: Challenging, measurable and achievable. In: Killoran A (ed.) *Moving on: International Perspectives on Promoting Physical Activity*. London: Health Education Authority.

Both cross-sectional data and prospective studies confirm an inverse relationship between physical activity and weight gain. The finding that in many countries such as the UK, average-energy intake has fallen over the time that obesity has been increasing, emphasizes the importance of inactivity as a cause of obesity. These secular changes of inactivity are most marked in children who now spend much of their leisure time watching television or in other sedentary pursuits. Health authorities in many countries now advocate an increase in physical activity as a means of preventing obesity and improving health and fitness. Although there is agreement that such measures may be useful in preventing obesity, the role of exercise in treating obesity is less clear. Potential mechanisms linking exercise and activity with weight loss and weight loss maintenance are shown in Figure 3. Like dietary change, increasing time spent on exercise and activity can be seen as part of a generalized behavioral change, which can be self-reinforcing.

Exercise and activity raise energy expenditure over and above the resting metabolic rate (RMR). Under some circumstances, such as prolonged vigorous exercise in trained individuals, rates of energy expenditure remain elevated for some time after the cessation of exercise. Logically, therefore, exercise should be a useful way to treat obesity. However, the amounts of exercise-induced energy expenditure are small in comparison with potential changes in energy intake.

The energy cost of activity and exercise can be expressed as a multiple of RMR, termed an MET; the term 'physical activity level' represents the total daily energy expenditure divided by the resting energy expenditure; it typically averages 1.5. The energy costs of walking are approximately 2.0 MET – for a 70-kg individual, this is approximately 0.5 MJ h^{-1} (120 kcal h^{-1}) – whereas gentle running costs are approximately 8 MET or 2 MJ h^{-1} (480 kcal h^{-1}). A moderately fit individual would only be able to maintain a level of exercise of 7 MET for approximately 30 min, representing an additional energy expenditure of approximately 1.5 MJ (360 kcal) resulting, if

energy intake were maintained, in a weight loss of approximately 0.3 kg per week.

Energy expenditure remains above baseline for some time after exercise has stopped; this is termed 'postexercise energy expenditure.' The effect is small and only produced by very high levels of activity, capable of achievement only by elite athletes. The mechanism for this effect is unknown. Moderate-intensity exercise programs, of the sort prescribed to the obese, are unlikely to raise energy expenditure by more than approximately 0.2 MJ (50 kcal) per exercise session.

Regular exercise does, however, elevate long-term energy expenditure by its effect on altering body composition. RMR is proportional to the fat-free mass (FFM). Exercise increases muscle development and bone mass, so directly raising metabolic rate. The purpose of weight loss is to reduce fat mass, with as little loss of FFM as possible. The loss of fat to meet the extra-energy requirements of regular exercise will decrease the ratio of fat-to-FFM and thus indirectly favor an increase in RMR for any given body weight. These effects are modest and mainly only seen from the sort of high-intensity exercise achieved by athletes. Even endurance-level training over periods of up to 12 weeks increases nonexercising daily energy expenditure by less than 0.8 MJ (190 kcal).

The effects of exercise are thus quantitatively small. The relatively small potential for exercise to reduce body weight is borne out by the results of trials of exercise in obesity treatment, which suggest that exercise programs achieve weight losses of less than 0.1 kg week^{-1} , and that total weight loss averages approximately 3 kg . In one meta-analysis of five controlled trials of exercise without dietary restriction, mean weight loss in 95 men was 2.6 kg over 30 weeks, compared with a gain of 0.4 kg in the control group.

Programs that combine dietary and exercise interventions can be more successful, but it is often difficult to separate the effects of one from the other. To explore the effect of exercise on the composition of weight loss during dieting, Garrow analyzed data from 21 randomized, controlled studies. All trials that

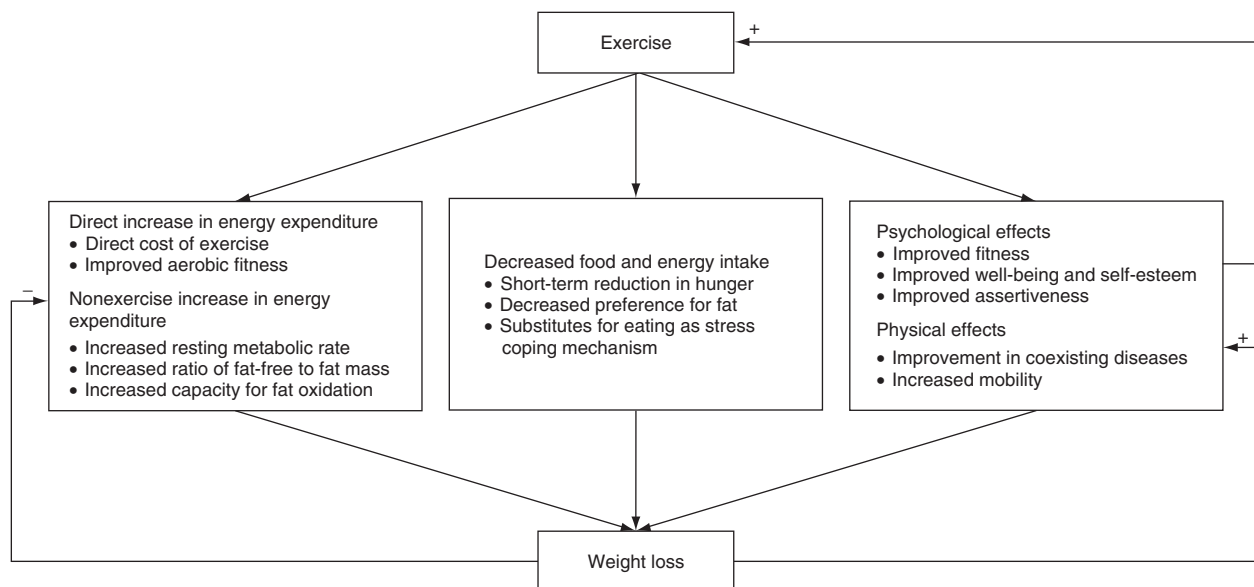


Figure 3 Mechanisms linking exercise with weight loss and weight loss maintenance.

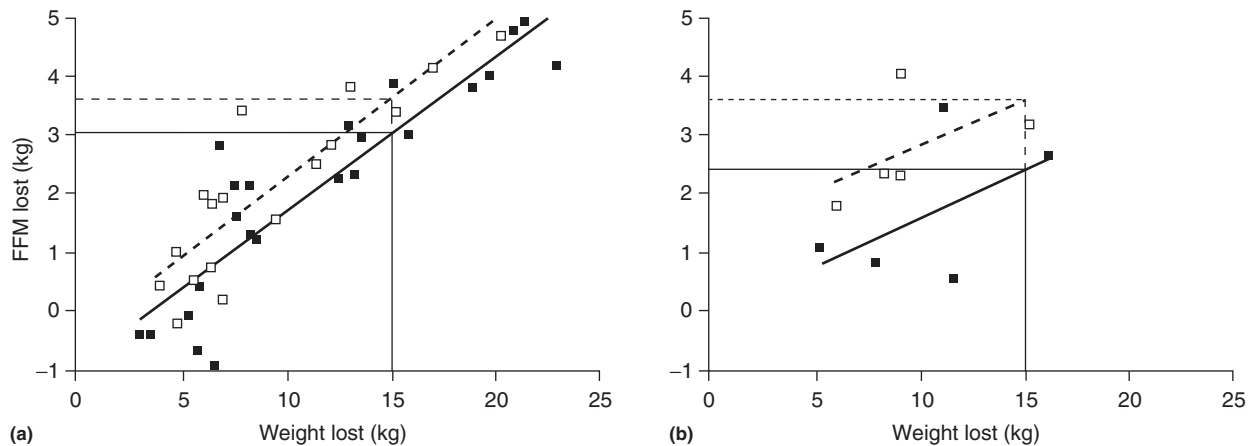


Figure 4 Relationship of total weight loss to FFM loss in (a) women and (b) men undertaking a diet with exercise (solid squares, solid line) or without exercise (open squares, broken line). Reproduced from 21 randomized controlled studies, collated by Garrow JS and Summerbell CD (1995) Meta-analysis: Effect of exercise, with or without dieting, on the body composition of overweight subjects. *European Journal of Clinical Nutrition* 49: 1–10, with permission from Nature.

combined exercise and diet and included information about weight and FFM loss were included (Figure 4). A small reduction in the percentage of FFM lost is observed if exercise is included with the dietetic intervention. Thus, for example, in a woman losing 15 kg, exercise would reduce her FFM loss from 3.6 kg (24%) to 3.0 kg (20%). Similar but quantitatively greater benefits are seen in men: For a 15-kg weight loss, exercise reduced FFM loss from 3.6 kg (24%) to 2.5 kg (17%).

Activity and exercise are strong predictors for successful weight loss maintenance. A number of studies have shown that obese women who have lost weight and continue to undertake regular exercise are three to four times more likely to maintain their weight loss over a follow-up period of 2–3 years. The amount of exercise also correlates with the degree of success. In one study of approximately a 100 obese men and women who had lost approximately 27 kg, those with high levels of exercise were maintaining an average of 18 kg loss at 3 years, compared with 9 kg in the moderate exercise group and no weight loss in the nonexercisers. The importance of exercise and weight loss maintenance is demonstrated by a 2-year study of obese subjects treated by either diet, exercise, or a combination of the two. Weight loss in the diet group at 1 year was 6.8 kg, in the exercise group 2.9 kg, and 8.9 kg in the combination treatment group. However, after 2 years, the groups that had included exercise were maintaining losses of 2.2–2.7 kg, whereas those on diet alone had only managed to maintain a 0.9-kg loss. Similar findings have been seen in dieters from commercial slimming groups.

Behavioral Modification

Behavioral modification is seen as the cornerstone of any treatment program that seeks to empower and enable obese individuals to make voluntary changes in lifestyle. Any therapy relies to a greater or lesser extent on such a principle. For example, treating hypertension should be an apparently straightforward clinical management issue, but patient non-compliance with medication is common. The skilled clinician

will often include the principles of behavior therapy in consultations to help the patient understand and put into practice the new 'lifestyle' of taking their drugs regularly. The approach in obesity is firmly based on theories of learning and relies on the concept that behaviors associated with weight gain and weight maintenance are to a significant extent learned and subject to modification. Such a behavioral theory is not undermined by the knowledge that genetic and environmental factors are also important in determining the predisposition to obesity. A prerequisite for successful behavior change is that the individual must be 'ready' and motivated to change. It is a common practice to assess this aspect of 'readiness' before enrolling patients in behavioral programs, and a number of standardized and validated questionnaires are available. Because behavioral programs are intensive of therapist time, patients are often treated in groups, often with manuals, which allow for individual study. These groups are usually 'closed,' that is, a small group of patients start the program simultaneously and go through it together. This contrasts with many commercial diet groups, in which patients are free to join or leave at any time. More recently computer-aided interventions have been developed, but as yet results are not promising.

The components of a typical behavior modification program are shown in Table 3. For each area, patients need to learn the underlying concepts, recognize the importance to their own situation, and practice strategies to change their behavior. The results of a large number of programs have been published, either as audit outcome or as comparative trials. Programs vary in duration from 12 to 52 weeks (there has been a trend since the 1970s to lengthen treatment time). Dropout rates are clearly biased by selection procedures, but are typically 10–20%. Weight loss during treatment is typically 10–15% of initial weight, at a rate of about 0.5 kg week⁻¹. To strengthen the impact of the intervention on weight loss, many programs have included a period of time on very low-energy or liquid-based diets. This approach of a complete withdrawal for a time from established (abnormal) eating habits can be usefully integrated into a model of behavior

Table 3 The components of a typical behavior modification program

Domain	Intervention strategy	Example
Self-monitoring	Food intake diaries Exercise and activity Weight change	Food diaries Activity logs Regular weighing and recording on weight charts
Nutrition	Nutrition knowledge Healthy eating	Energy, macronutrients, and understanding food labeling Low fat, high complex carbohydrate, and adequate fruit and vegetable intake
Exercise and activity	Increasing daily energy-using activities Decreasing sedentariness Formal exercise	Using stairs and not escalators Decrease television viewing Group workouts at sports centers
Goal setting	Realistic rates of weight loss Realistic target weight Weight maintenance	Aim for 0.5–1.0 kg weekly 10% weight loss as initial goal
Problem solving	Identifying conflicts with aims Interpersonal conflicts Stimulus control and negative feelings	Holidays, parties, restaurant meals The unhelpful relative or friend Hunger on returning home from work
Cognitive change	Modifying thoughts about and responses to food cues Self-esteem and assertiveness training Preventing relapse	Good and bad foods; food as a reward; coping with 'highly desirable' foods Recognizing and exerting choice Acceptance of occasional small weight gains

change, and is well and positively tolerated by obese patients. Although data suggest that the greater weight loss induced by very low-energy diets has little effect on the long-term results in terms of weight loss maintenance, these diets do represent a practical and pragmatic initial approach to treating patients in a group, especially when many individuals within such a group may resist the idea that they are able to lose weight on conventional reduced-energy diets.

Research is now directed toward finding ways of improving the results of such programs in terms of long-term weight loss maintenance. An increased focus on weight-maintaining behavior rather than weight loss, a stronger emphasis on increasing activity and exercise, and better relapse strategies are being evaluated. Targeting the needs of specific subgroups, for example, those with binge-eating disorders or dysfunctional family circumstances, is another way in which behavioral therapy may be improved.

See also: Eating Disorders: Bulimia Nervosa. Energy Expenditure: Indirect Calorimetry. Obesity: Childhood Obesity; Definition, Etiology, and Assessment; Prevention; Treatment. Starvation and Fasting: Biochemical Aspects

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Weight Cycling/Weight Change

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Is it better to have lost and re-gained than never to have lost at all? (Weight cycling refrain, loosely adapted from Alfred, Lord Tennyson)

Weight Cycling – A Health Risk?

The term weight cycling is used in the fields of nutrition and obesity research to refer to losses and subsequent regains of body weight typically, but not exclusively, occurring in association with dieting. Interest in this phenomenon was initially based on the observation that conventional weight loss programs are often unsuccessful in the long term. Dieting recidivism thus sets the stage for weight cycling, popularly referred to as yo-yo dieting, whereby dieters undergo multiple cycles of weight loss and regain in pursuit of their ideal body weights.

There seems to be little disagreement that weight cycling is one of the most difficult therapeutic aspects in the management of obesity. Most of the obese subjects seeking treatment have previously experienced cycles of weight loss and regain. Examples of weight cycling in two case studies are shown in **Figure 1**. These figures illustrate several phenomena that are important when considering weight cycling in humans: Weight losses and subsequent regains occur in conjunction with intentional weight loss dieting; however, weight changes vary in magnitude, and important fluctuations may be missed if weight measurements are taken at infrequent time intervals. Moreover, these two cases illustrate the common observation that intentional weight losses are frequently followed by regains in excess of the original body weight, and that true weight stability may be difficult to achieve.

Dieting to control body weight is not confined to overweight individuals, but has been widely reported even among men and women who have never been overweight. As described by Jeffery in 1984, 72.5% and 43.7% of surveyed women and men, respectively, had dieted to lose weight; even among women who had never been overweight, the majority reported having been on weight-loss diets. Although it is traditionally assumed that adherence to weight-reduction diets is beneficial to health, the high rates of dieting and weight regain, among the nonobese as well as the obese, have naturally created concern regarding potentially negative health consequences. However, weight loss can be intentional or unintentional, and the most recent generation of research on weight cycling has specifically focused on intentional weight loss as the risk factor of relevance to the weight-cycling debate, as reviewed by Simonsen.

In this article, some of the main points of this debate will be highlighted. The first epidemiological studies suggesting health implications of weight cycling were reviewed by Lissner and Brownell, when the majority of available observational evidence indicated adverse consequences. Subsequently, this topic became a source of considerable controversy, and a number of

investigators continued to examine this issue, focusing on possible effects of weight cycling on metabolism, chronic disease, and mental health. This article will offer an overview of knowledge in this area, together with some methodological controversies surrounding existing research on weight cycling.

The Metabolic Hypothesis

Given the fact that most people who lose weight are unable to sustain their losses, a ‘metabolic’ hypothesis was formulated. It was proposed that if weight loss dieting caused permanent decreases in metabolic rate, the weight would be easily regained and every subsequent weight loss attempt would be more difficult. In 1994, the National Task Force on the Prevention and Treatment of Obesity in the US reviewed the evidence and reported an overall lack of support for the hypothesis that weight cycling promoted obesity, increased body fat, or had permanent effects on metabolism. This report also concluded that the majority of available data in animals did not independently link weight cycling to any parameter of energy balance (food intake, body composition, or energy expenditure). This conclusion was supported by studies in humans, using a variety of designs, which failed to document irreversible effects of weight loss on metabolic rate, body composition, or adipose tissue distribution after regain. Also of interest in this context is the observation by Field in 2001 that weight-cycling women, in spite of their regains of lost weight, tended to gain less weight over time than their non-weight-cycling peers. In contrast, Van Wye reported that a history of weight cycling in healthy subjects did not seem to modify the risk of long-term weight gain in men and was associated with marginally more weight gain in women.

Weight Cycling and Mortality

Although most studies did not bear out the original idea that weight cycling alters metabolic rate, the possibility that weight fluctuation predicts chronic disease and death has been more difficult to discount. A number of prospective epidemiological studies have shown that an individual's own variations in body weight over time, a proxy for weight cycling, can statistically predict subsequent risk of mortality and disease. Positive associations have been reported between body weight fluctuation and all-cause mortality in several but not all such studies, as reviewed by the National Task Force on the Prevention and Treatment of Obesity in 1994. These findings are often expressed in terms of relative risk estimates, which represent the mortality rates in a weight-fluctuating group compared with the rates in a weight-stable group. The relative risk estimates for all-cause mortality have been reported to be as high as 2, indicating a double excess mortality risk in the weight-fluctuating

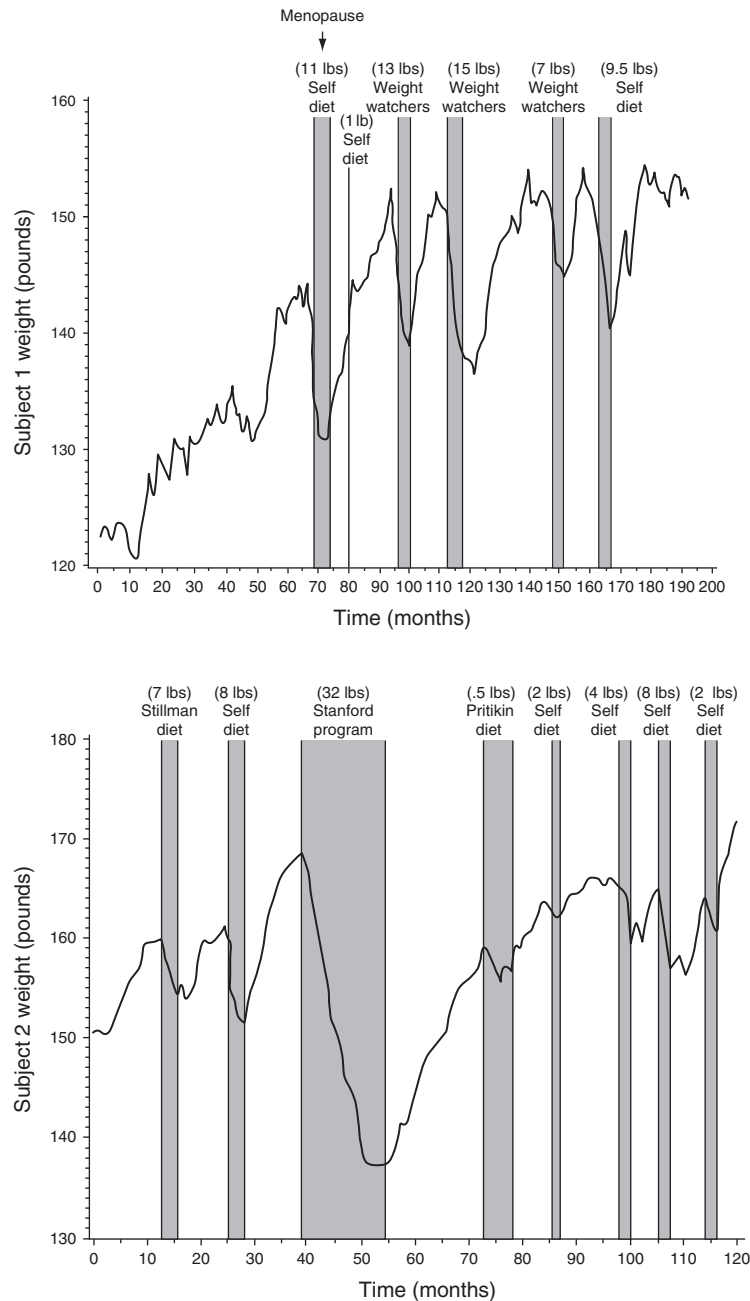


Figure 1 Monthly body weights (average of daily weights) of two female subjects, self-monitored over time. Reproduced from Black DR, Pack DJ, and Hovell MF (1991) A time-series analysis of longitudinal weight changes in two adult women. *International Journal of Obesity* 15: 623–633, with permission from Nature.

individuals. Some investigators have reported that significant associations are restricted to certain types of individual, that is, nonobese or nonsmoking subgroups. For instance, data from the Multiple Risk Factors Intervention Trial (1993) concluded that any adverse effects of weight fluctuation were occurring in relatively normal-weight subjects.

By way of example, results are shown from a reanalysis from a longitudinal population study of Swedish women, started in 1968 when subjects were 38–60 years old. Women were weighed on three occasions over a 12-year observation period, based on which four subcategories of weight change

could be created: stable, weight gain, weight loss, and weight cycle. Specifically, these categories were defined as: (1) women whose weights remained stable within plus or minus 3 kg; those who; (2) gained or; (3) lost at least 3 kg between the first and last observation; and (iv) those who had lost and then regained at least 3 kg, or gained then lost 3 kg, without an overall change of more than 3 kg from the first to final observation. When these groups were followed up for an additional 20 years, it was found that the weight-loss group and weight-cycling group both had approximately double risk of mortality, compared with the weight-stable women (Figure 2).

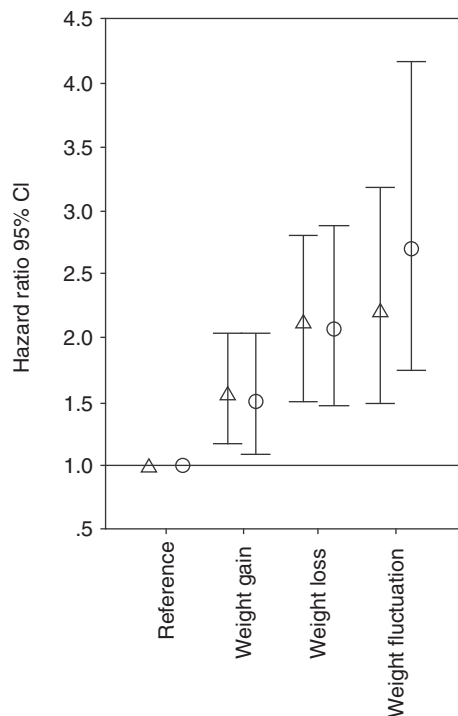


Figure 2 Relative risks of 20-year all-cause mortality, in relation to previous 12-year weight changes in the Prospective Population Study of Women in Göteborg. Triangular symbols refer to risks of total mortality, adjusted for age and final body mass index in 800 women. Circular symbols display results after excluding 99 prevalent cases of coronary heart disease, diabetes, or cancer during 12 years of weight observation.

It may be argued that the weight losses and subsequent gains may not be voluntary but rather reflect preexisting diseases. However, after exclusion of women with prevalent or incident cancer, diabetes, or cardiovascular disease during the entire period of weight observations, the excess mortality in the fluctuating group was not attenuated, suggesting that morbidity from these conditions was not the underlying cause of the fluctuations or reason for the association. These findings and similar observations in a number of other populations have not been adequately explained by biologically plausible mechanisms.

A systematic literature review focusing on intentional weight loss in healthy men and women, rather than weight cycling per se, identified nine studies that could address this issue. The review yielded no consistent evidence that intentional weight loss either increased or decreased mortality. Simonsen concluded that it is still not possible to make secure recommendations that intentional weight loss will increase longevity. This is in contrast to research on severely obese bariatric surgery patients showing improved longevity following elective weight-loss surgery, as reviewed in a recent meta-analysis by Pontiroli. Other factors may influence the associations between intentional weight loss and survival. The age at which weight fluctuations/cycles occurs may be of some relevance as weight loss among the younger adults may be less hazardous than in old age, when even apparently successful weight loss may in fact result from underlying disease.

Similarly, weight gain early in adulthood may be more deleterious to overall health and mortality than weight gain later on. However, more research is needed to examine the influence of timing on the relation between weight variations and later disease and death.

Weight Cycling and Cardiovascular Disease

Most investigators have considered it more informative to focus the association between weight fluctuation and specific diseases, and causes of death, and have frequently observed positive associations between weight fluctuation and cardiovascular disease endpoints. However, as reviewed by Lissner and Brownell, the results were not always in agreement; data from the Framingham study showed excess cardiovascular disease among male and female weight-fluctuating individuals, whereas in men from the Baltimore Longitudinal Study on Aging there was no association between weight fluctuation and coronary heart disease. Two additional studies in male populations are illustrated in Figure 3. Both show a pattern of elevated risk of mortality from cardiovascular or coronary heart disease among weight-cycling men, and consistently lowest risk associated with stable body weight, based on which Jeffery in 1996 concluded that “stable weight over time is associated with best health. All patterns of weight change other than stable weight – gains, losses, and both combined – appear to be associated with increased mortality risk”

It has been hypothesized that some of the observed associations between weight fluctuation and cardiovascular disease might be explained by changes in cardiovascular risk factors occurring during weight gain that are not fully reversible with weight loss. This possibility has been explored using longitudinal data on body weight and risk factors that are concurrently measured on multiple occasions. A systematic review of these studies by the National Task force on the Prevention and Treatment of Obesity (1994) revealed no consistent associations between weight fluctuation and concomitant increases in traditional cardiovascular disease risk factors such as blood pressure and serum cholesterol.

In particular, hypertension has been examined as an endpoint in a number of studies with somewhat mixed results. Using data from the Nurses’ Health Study II, Field and coworkers in 1999 reported that intentional weight cycling was not associated with significant excess risk of development of hypertension. In contrast, a retrospective study by Guagnano indicated that a positive history of weight cycling among obese women as well as the sum of weight regained increased the likelihood of being hypertensive. Interestingly, Stevens reported a possible beneficial effect of one weight cycle: In this blood pressure reduction trial, weight returned to baseline levels after 3 years, although blood pressure remained well below control levels. Control subjects in this study experienced a net weight gain, and one interpretation of this study is that a weight cycle did not predispose further hypertension, but rather, seemed to deter further weight gains at control levels, as also observed in the Nurses’ Health Study II (2001). However, intentional weight loss may not have persisting beneficial effects on blood pressure, as shown by the Swedish

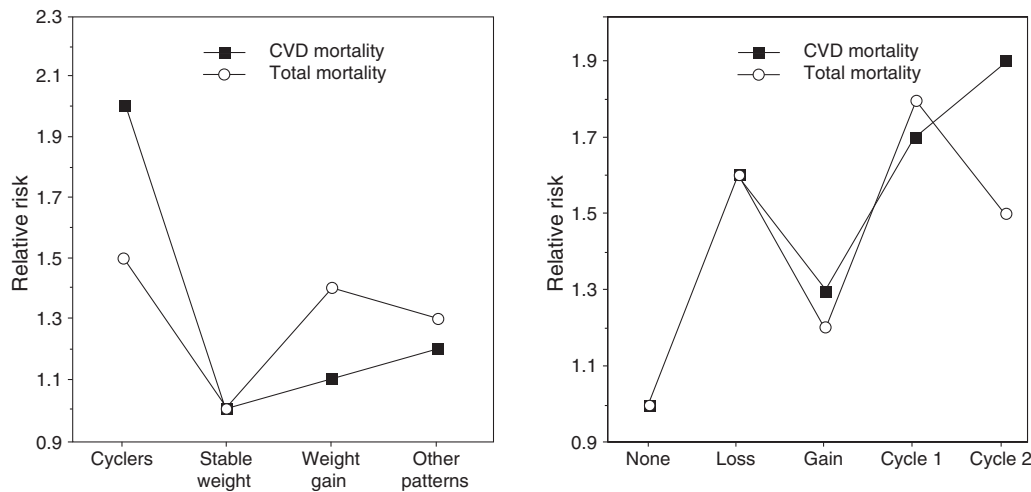


Figure 3 Total and cardiovascular disease mortality in male subjects with different weight change patterns. On the left, data from the Chicago Western Electric Study (Hamm, 1989) and on the right data from MRFIT (Blair, 1991).

Obesity Study (2000) where a large weight loss induced by gastric surgery and maintained over 8 years had no effect on the 8-year incidence of hypertension. In contrast, the beneficial effect on blood pressure was of a transitory nature, with dramatic initial reductions in both systolic and diastolic blood pressure during the initial 12 months of weight loss, and although an 18- to 30-kg weight loss was maintained over the subsequent 7 years, both the systolic and diastolic blood pressure of the surgically treated group increased. After 8 years there was no difference in systolic blood pressure between the surgically treated and the control groups, and diastolic blood pressure ended up higher in the surgical group than in the controls.

Other Health Outcomes: Cancer and Diabetes

Although several of the original weight-cycling studies also tested associations between weight cycling and cancer, cancer endpoints have typically not followed the same patterns as cardiovascular disease. It has been observed by Trentham-Dietz in 2000 that temporary weight cycling (weight loss followed by weight gain) was not associated with increased risk of postmenopausal breast cancer.

A number of studies have examined associations between weight cycling and diabetes, and yielded little evidence of a relation. In 1997, a study by Podar monitored glucose tolerance and weight fluctuations in obese patients, and reported no deterioration directly associated with weight cycling. In the Nurses' Health Study in 2004, no association was found between weight fluctuation and diabetes incidence. Interestingly, the Diabetes Prevention Program Research Group in 2002 found that the diabetes reduction achieved for more than 4 years with a lifestyle intervention was not diminished with the gradual regaining of more than half of the weight lost. This observation is an indication that a period of weight reduction may exert a net benefit for diabetes, even if weight is subsequently regained.

Psychological Consequences

It has often been assumed that the experience of dieting followed by involuntary regain of the lost weight must take a psychological toll, independent of any medical consequences of weight fluctuation. The possible psychological effects of weight cycling among obese persons were the topic of a literature review by Foster, who reported that weight cycling was not associated with depression, other psychopathology, or depressogenic cognitive styles. However, it was observed that weight cycling was associated with decreased perceptions of health and well-being, decreased eating self-efficacy, and weak increases in binge eating severity. Subsequently, Friedman concluded that an individual's perception of being a weight cyclist may be more related to psychological problems than the actual number of pounds lost and regained over time. In 2000, the National Task Force on the Prevention and Treatment of Obesity concluded that concerns that dieting induces eating disorders or other psychological dysfunction in overweight and obese adults are generally not supported by empirical studies. A more recent prospective study of weight changes in relation to women's self-rated health found that that weight gain was associated with significant worsening of self-rated health but that weight loss did not result in an improvement in the same index. The observation that initially poor self-rated health actually predicted more weight gain suggests that causality is not simple, but that the association is more likely to be bidirectional.

Methodological Issues

Although a number of studies have shown that increased weight fluctuations are associated with subsequent occurrence of adverse health outcomes, a number of methodological problems make interpretation of these findings difficult. For example, weight gain, weight loss, and weight cycling are often considered separately, but it is almost impossible to determine

their degree of overlap in observational studies. An individual who is observed to be systematically gaining weight at two points in time may experience a number of unmeasured fluctuations in the interim. However, as both loss and gain have been shown to increase risk of dying prematurely, it is possible that the increased mortality risk associated with weight cycling depends on the cumulated risk contributions from repeated weight loss and gain. The statistical complexities in defining weight cycling were reviewed in 1994 by the National Task Force on the Prevention and Treatment of Obesity.

A common observation surrounding this type of research is that different studies may be measuring quite different kinds of weight change – voluntary and involuntary. Involuntary changes may reflect serious underlying illness, depression, and other nondieting phenomena. However, intentional dieting may also occur by a variety of dietary methods, some of which are more detrimental to health than others, as discussed by French in 1993. The issue of volition may shed light on the problem, but the specific impact of previous and current illness on epidemiological associations between weight cycling and longevity is still not fully understood. Other covarying factors besides intentionality of weight change and underlying illness may be producing artifactual associations in observational studies. These include aging, smoking and other lifestyle choices, degree and regional pattern of adiposity, and psychological factors. In epidemiological analyses, various types of statistical corrections can be made for potential confounding factors of this type, although adjustment may be incomplete. Finally, biological plausibility is always an issue to consider when reviewing any epidemiological evidence and has been a particular concern when considering the observations of excess risk in association with weight fluctuations.

Conclusions

As summarized in [Table 1](#), many – but not all – epidemiological studies indicate that men and women undergoing body weight fluctuations are at higher risk of mortality and/or cardiovascular disease than individuals experiencing less fluctuation, but the lack of biologically plausible mechanisms to explain these associations has limited the conclusions that can be drawn. Moreover, the evidence for effects on psycho-

logical and other health endpoints is inconclusive. Uncontrolled confounding from disease states resulting in a loss–gain or gain–loss pattern must be considered a plausible explanation for some of these findings, underscoring the difficulty of using observational data to study weight cycling. Some studies are suggesting that even limited periods of weight reduction may be beneficial in the long run, whereas others are not. Experimental data and intervention studies are required for confirmation of the weight-cycling hypothesis. The published observational studies of subjects whose weight changes are known to be caused by dieting have been important contributions to the critical discussion of the weight-cycling phenomenon, but additional studies are needed in which weight fluctuations are assessed in a more controlled manner.

Regarding the hypothesis that dieting exacerbates the problem of obesity and weight gain, most studies have failed to demonstrate that weight fluctuation per se depresses metabolic rate. Available research on the effects of weight cycling on both metabolism and disease thus provides little basis to discourage overweight patients from losing weight. Nevertheless, one of the conclusions of the 1994 report from the National Task Force on the Prevention and Treatment of Obesity was that individuals who are not obese and who have no risk factors for obesity-related illness should not undertake weight loss efforts. The only uncontroversial message of the weight-cycling research is that overweight individuals need to be counseled in skills to maintain weight loss, and that relapse prevention should be a more central focus of weight loss programs.

In conclusion, although this research has drawn attention to the necessity of developing improved behavioral and nutritional strategies for sustaining weight reductions and thus preventing weight cycling, the evidence relating weight cycling to adverse health outcomes must be considered equivocal. Regarding the hypothesis that weight cycling exacerbates weight problems, a 1995 opinion survey of obesity researchers conducted by Bray concluded that weight cycling was not considered a very important cause of obesity, and little convincing evidence has emerged in subsequent years to change that conclusion. With regard to the question in the ‘weight cycling refrain’ regarding possible consequences of weight cycling, the current knowledge would suggest that it is probably not worse to have lost and regained than never to have lost at all. However, there remain some curious and persistent

Table 1 Summary of hypothesized weight cycling effects and selected sources of evidence

<i>Hypothesized adverse health consequences of weight cycling</i>		<i>Comments (with suggested reading)</i>
Psychological consequences		Not supported by evidence (reviewed by the US National Task Force, 2000)
Metabolic rate and body composition		Not supported by evidence (reviewed by the US National Task Force, 1994)
All-cause mortality		Supported by most prospective studies, for example, Hamm, 1989; Lissner, 1991; Blair, 1993 (reviewed in Obesity 1992, and by the US National Task Force, 1994)
Cardiovascular disease		Supported by most prospective studies, for example, Hamm, 1989; Lissner, 1991; Blair, 1993 (reviewed in Obesity 1992 and the US National Task Force, 1994)
Diabetes		Not supported by epidemiological studies (Field, 2004). Mixed evidence from clinical setting (Guagnano, 2000)
Cancer		Not supported by epidemiological studies (Lissner, 1991; Trentham-Dietz, 2000)

results in the experimental as well as epidemiological literature suggesting that we do not completely understand the phenomenon.

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Weight Maintenance

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The majority of adults, more than 65%, in the USA are overweight or obese. Obesity negatively impacts on health outcomes and health care costs, and thus there is heightened awareness of the importance of achieving and maintaining a healthy weight. The goal of this article is to review information obtained from observational and experimental studies on successful weight loss maintenance. A definition and the prevalence of successful weight loss maintenance are provided, and then theories regarding why weight loss maintenance may be difficult are discussed. Next, factors important for successful weight loss maintenance, identified in research examining weight loss maintenance, obtained from the National Weight Control Registry (NWCR) and from randomized controlled trials examining long-term weight loss and weight loss maintenance, are described. The article concludes with general recommendations for achieving successful weight loss maintenance.

Definition of Successful Weight Loss Maintenance

There is no universally accepted definition of successful weight loss maintenance. One definition, proposed in the “Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults,” developed by an expert panel convened by the National Obesity Education Initiative of the National Heart, Lung, and Blood Institute (NHLBI), in cooperation with the National Institute of Diabetes and Digestive and Kidney Diseases in 1995, is that successful weight loss maintenance is a weight regain of <3 kg (6.6 lb) in 2 years, following a 10% reduction in body weight. The criterion of 10% weight loss is recommended because weight losses of this magnitude have been shown to have positive health consequences. The definition assumes that the weight loss is intentional.

Data on Prevalence of Long-term Maintenance of Weight Loss

Most information about long-term maintenance of weight loss comes from obesity treatment studies. A review of published lifestyle intervention trials, which are intervention trials that contain a diet and leisure-time activity goal and use behavior modification strategies to help participants meet these goals, resulted in a mean weight loss of -7.9 kg (-8.5% of initial body weight) from baseline to 6 months. At 6 months, weight loss generally plateaued and remained fairly stable from 6 to 12 months. Weight regain started after 12 months, with approximately a -4 kg (-4% of initial body weight) weight loss maintained at 36 and 48 months.

A study using data from the 1999 to 2006 National Health and Nutrition Examination Survey found that among US adults who reported ever being overweight or obese at some point in their life, 36.5% and 17.3% were able to maintain a weight loss of at least 5% and 10%, respectively, for at least 1 year. This study did not differentiate between intentional and unintentional weight losses.

Why is Weight Loss Maintenance Difficult?

Maintenance of long-term weight loss may be difficult due to a combination of physiological, environmental, and psychological factors. Proposed physiological factors contributing to weight regain include reduced resting metabolic rate, and insulin and leptin resistance. Environmental factors may affect energy balance by promoting either increased intake and/or reduced energy expenditure, causing weight regain to occur. The strong impact that environmental cues have on energy intake and expenditure has recently been acknowledged as Americans are now described to be living in an ‘obesogenic environment’. The psychological self-control needed to override these environmental cues may be difficult for most people to sustain over long periods. Finally, during obesity treatment, weight loss itself can provide reinforcement for adherence to eating and activity prescriptions that promote weight loss. During weight loss maintenance, weight loss no longer occurs; therefore, there is less reinforcement of healthy eating and activity behaviors, causing motivation for sustaining these behaviors to decrease.

Research Investigating Successful Weight Loss Maintenance

To increase the prevalence of successful weight loss maintenance, two types of research investigating weight loss maintenance have been conducted: observational and experimental. In observational research, successful weight loss maintainers are identified and information about how they maintain their weight loss is collected. With experimental research, variables that are believed to affect weight status are manipulated and weight change over time is measured.

The National Weight Control Registry

The largest observational study of successful weight loss maintainers is The NWCR. The NWCR, which was started in 1993, is a registry of approximately 5000 self-selected individuals at least 18 years of age who have lost at least 30 pounds and kept it off at least 1 year. On average, these

participants have lost more than 60 pounds and kept it off more than 5.5 years. The registry members are primarily female (77%) and Caucasian (95%).

Although there is marked heterogeneity in the approaches used for weight loss, there appear to be some common themes for weight loss maintenance. The first common element is consumption of a low-calorie, low-fat diet. Registry participants are consuming an average of 1381 kcal day⁻¹, with 24% of calories from fat, 19% from protein, and 56% from carbohydrates. Registry members also report having a highly structured diet: they regularly consume breakfast, have a consistent diet across weekdays and weekends, limit the variety of foods consumed, and they report consuming close to five eating occasions, meals and snacks, per day.

These long-term changes in diet in registry members are accompanied by sustained increases in physical activity (PA). Women in the registry report 2532 kcal week⁻¹ of PA and men report 2903 kcal week⁻¹. This would be equivalent to 1 h day⁻¹ of brisk activity. Almost 70% of registry members engage in walking for PA, and 36.3% report walking plus engaging in another form of PA. More than 85% of registry members use PA to help maintain weight loss.

The final characteristic of registry members is that they weigh themselves regularly. More than 35% weigh themselves daily and an additional 40% weigh themselves at least once a week. Registry members who decreased their frequency of self-weighing in their first year in the registry regained significantly more weight than members whose frequency of self-weighing stayed the same or increased in their first year in the registry. Frequent monitoring of weight may allow individuals to quickly catch small weight gains and institute corrective actions in regards to energy balance behaviors. See [Table 1](#) for a summary of the strategies that registry members have reported as being helpful for successful weight loss maintenance.

Experimental Studies Examining Weight Loss Maintenance

Randomized controlled trials evaluating specific treatment components also assist with understanding factors that are important to weight loss maintenance. These trials are stronger scientifically because participants are randomly assigned to treatment conditions and all aspects of the intervention are kept constant except the factor under investigation, providing greater ability to determine cause and effect relationships. However, these studies are limited by their short duration (typically 1–2 years) and their relatively small sample size (usually 100–200 participants).

Table 1 Strategies used by successful weight loss maintainers in the NWCR

Area	Strategy
Diet	1. Consuming a low-calorie, low-fat diet 2. Having a highly structured diet
Physical activity	1. Engaging in at least 1 h of moderate-intensity physical activity per day
Behavioral tools	1. Self-monitoring of weight

In experimental studies of weight loss maintenance, the primary focus is usually on overall weight loss (from baseline to the end of the study), usually defined as long-term weight loss; rather than on maintenance of weight loss from the end of the initial treatment (typically 6 months) to study end. Overall weight loss is selected as the variable of interest because it is most strongly associated with health impact.

Several strategies have been tested in experimental studies to improve weight loss maintenance. These strategies include focusing on energy balance, in which changes in diet and/or PA are used to create larger energy deficits that produce greater weight loss, or focusing on intensifying behavioral components of interventions so that skills necessary for sustaining weight loss can be maintained over a longer period.

Energy Balance

To produce weight loss, it is necessary to modify energy balance by eating less and/or exercising more. A substantial body of research suggests that the combination of diet plus exercise is most effective for long-term maintenance of weight loss.

Diet

Within the context of diet, weight loss researchers have focused primarily on the level of caloric restriction and the degree of structure in the diet. More recent research has investigated the influence of macronutrient composition of the diet and energy density on weight loss and weight loss maintenance.

The traditional diet associated with lifestyle interventions is a low-calorie, low-fat, balanced diet. Participants are instructed to eat 1000–1500 kcal day⁻¹ (low-calorie diet – LCD) depending on their initial body weight and to reduce dietary fat to 20–30% of calories. There are no specific foods that are required or prohibited, but consumption of complex carbohydrates and guidelines based on MyPyramid are stressed. Participants are instructed to self-monitor the calories and fat grams in all foods they consume. Daily self-monitoring of eating is recommended, as adherence to self-monitoring has been shown to be one of the best predictors of maintenance of weight loss.

Very Low-Calorie Diets

Very low-calorie diets (VLCDs) are dietary regimens typically administered in a medically supervised setting, providing less than 800 kcal day⁻¹ usually in the form of meal replacements (specially formulated liquid formulas or bars) or lean meats, fish, or poultry. These diets are designed to provide a high percentage of protein to lessen lean muscle mass losses during rapid weight loss. VLCDs have been shown to produce excellent initial weight losses (–20 kg at 12 weeks); this effect is partly due to the degree of caloric restriction and partly due to decreased dietary variety and the use of portion-controlled foods in these regimens. Given the large initial weight loss produced by VLCDs, it was hoped that combining these diets with behavioral approaches would maximize long-term weight loss. Although VLCDs improve initial weight loss, they do not appear to produce better long-term weight loss than LCDs. Owing to the lack of significant differences in long-term outcomes between VLCDs and LCDs, NHLBI does not support use of VLCDs over LCDs composed of conventional foods.

Structured LCDs

Long-term weight loss maintenance requires continual adherence to reduced energy intake. One approach that has been used to improve long-term adherence to reduced energy intake is to increase the structure of the diet. Several studies have investigated different ways to increase the structure of LCDs, predominantly by limiting food choices and controlling portion sizes consumed.

A study examined whether providing food to participants improved long-term weight loss during a standard behavioral intervention using an LCD. Participants were provided all of the food they should eat for five breakfasts and dinners each week for 18 months. Participants receiving the food provisions had greater weight loss throughout the trial (18 months: -6.4 kg vs. -4.1 kg) than those participants receiving a standard intervention, although both groups had identical calorie goals (1000 – 1500 kcal day⁻¹). However, even with the greater dietary structure, participants still regained weight during the maintenance phase.

Structure in the diet, by decreasing food choices, can also be increased by providing structured meal plans and detailed grocery lists. One investigation that provided meal plans and grocery lists along with a standard intervention showed greater weight loss than the standard intervention alone. The weight losses achieved with the meal plans were similar to those achieved with food provisions.

Using portion-controlled foods available in the marketplace, such as frozen entrees and meal-replacement products, such as Slim-Fast, also increases dietary structure. When an LCD composed of conventional foods was compared with an LCD using two Slim-Fast meal replacements, two Slim-Fast snack bars, and a healthy dinner, the diet using the Slim-Fast portion-controlled foods produced better weight loss at 3 months (-7.1 kg vs. -1.3 kg). For the next 24 months, both groups were instructed to consume one Slim-Fast meal replacement and snack bar per day. At 27 months, the Slim-Fast group still had better weight loss (-10.4 kg vs. -7.7 kg), and the greater weight loss was maintained at 4 years (-9.5 kg vs. -4.1 kg) in those participants available for the follow-up.

Diet Composition

Although reduced energy intake is necessary for weight loss, the composition of the diet is also believed to impact on weight loss and weight loss maintenance. As the macronutrients have differing satiating qualities, composition of the diet may influence how quickly hunger and fullness develop, which can help with reducing energy intake. However, a lifestyle intervention trial testing the effects of a reduced energy prescription with differing amounts of percent energy from carbohydrate, protein, and fat found that at both 6 and 24 months, all dietary prescriptions produced similar levels of weight loss.

Reducing the energy density (kilocalories/gram weight of food) of the diet may also help individuals maintain long-term decreases in energy intake. Reducing dietary energy density is considered to be a beneficial strategy because it allows for a greater volume of food to be consumed without increases in caloric content, which promotes satiation. A year-long trial was conducted to examine the effects of two strategies to reduce dietary energy density on weight loss in obese

women. Over 1 year, the condition that reduced fat and consumed water-rich foods had a lower energy density, reported less hunger, and lost more weight (-7.9 kg vs. -6.4 kg) than the condition that reduced fat only.

Physical Activity

Correlational studies suggest that PA is the single best predictor of long-term weight loss maintenance. Physical activity is important because it increases energy expenditure, but it may also reduce hunger and improve mood. The American College of Sports Medicine emphasizes that there is a dose-response relationship with PA and weight loss, such that with greater amounts of PA, greater weight loss occurs.

In lifestyle intervention trials, PA is usually prescribed at a level of 1000 kcal week⁻¹ or 150 min of moderate-intense activity/week; however, the ideal amount of PA for weight loss maintenance is still unknown. Studies have shown that high levels of PA, up to 200 – 300 min/week⁻¹, may be helpful for long-term weight loss maintenance. A study compared the effect of a standard activity recommendation (1000 kcal week⁻¹) versus a higher PA prescription (2500 kcal week⁻¹, equivalent to walking 75 min for 5 days per week) in a lifestyle intervention. The condition with the higher PA prescription had greater long-term weight loss at 18 months (-6.7 kg vs. -4.1 kg). However, even with the higher exercise prescriptions, participants still regained weight during the maintenance phase.

Strategies for Improving Maintenance of PA

One of the most challenging problems with PA in weight control programs is adherence to activity prescriptions. One way to increase activity adherence is to prescribe activity in short bouts (40 min day⁻¹ in four 10 -min bouts) rather than in long bouts (40 min day⁻¹ in one bout). Although short bouts of exercise improved initial adoption of exercise, short-term bouts did not appear to increase PA adherence or weight loss at 12 and 18 months. Participants have also been provided with personal trainers, supervised walks, home exercise equipment, and financial incentives to improve PA adherence. Although personal trainers and financial incentives did increase attendance at exercise sessions, it neither improved total exercise achieved nor weight loss at 18 months. Providing participants with home exercise equipment has been shown to improve both adherence to PA and weight loss. This suggests that home exercise equipment and other approaches that make exercise more convenient may facilitate long-term adherence, and consequently weight loss maintenance.

Sedentary Activities

In addition to promotion of increased PA, decreasing time spent in sedentary activities is receiving more attention as a potential strategy in promoting weight loss and weight loss maintenance. For the average American adult, time spent in sedentary activities has greatly increased, with the largest contributor being television watching. It is believed that sedentary activities may influence weight in two ways. First, sedentary activities may compete with time for being physically active. Second, television watching often cues eating, and thus may encourage excessive energy intake. Although the research is clear that reducing sedentary activities is an effective weight

control strategy in children, few studies have examined this relationship in adults.

Intensifying the Behavioral Component

Although the research described previously focused on ways to enhance negative energy balance through modifications in diet and PA, other investigations have examined ways to intensify behavioral components in weight loss or weight loss maintenance interventions. These strategies include extending professional contact, use of technology, increasing social support, enhancing motivation using incentives, and use of systems-level interventions.

Extending Professional Contact

The maximum weight loss in a behavioral weight loss intervention is typically attained at 6 months, which also represents the end of the weekly phase of therapy and the start of the less-intense maintenance phase. Weight regain is commonly assumed to be due to a failure to continue practicing effective behavioral techniques when treatment transitions. One way to sustain behavioral strategies is to lengthen treatment or to continue to provide some form of professional contact during the maintenance phase.

Lengthening the initial phase of treatment has been shown to increase initial weight loss. For example, when behavioral treatments of identical content, differing only in length of treatment (20 vs. 40 weeks) are compared, the two programs produce similar weight losses at 20 weeks (-9.5 kg), but the extended treatment produces greater weight loss at 40 weeks (-13.6 kg vs. -6.4 kg). Based on this, several investigators have tried to develop year-long programs, with weekly meetings throughout. Weight losses at the end of the year were 10–14 kg, but attendance became quite poor toward the end of the program and the cost-effectiveness of such long-term weekly programs was questioned. Thus, investigators have considered how best to provide contact after the end of the initial 6 months of weekly contact.

One of the first methods used to extend professional contact during the maintenance phase was using booster sessions. Booster sessions take place on a fairly infrequent basis after treatment, with an increasing interval of time between sessions to fade professional contact (e.g., meeting at months 1, 3, 6, and 12). Booster contacts have yielded inconsistent results. This finding and the fact that better maintenance of weight loss occurs when participants continue to be seen biweekly suggest that patients need a fairly high level of contact during maintenance. Studies using biweekly maintenance programs have found better weight loss maintenance at 18-month (87% vs. 33%) follow-ups, as compared with a control intervention receiving no maintenance component.

Technology

As technology has become more sophisticated, it has been used to help with extending contact, predominantly through the use of the Internet, and to increase ease of self-monitoring weight, dietary intake, and PA. A review of weight loss web-based interventions for adults found that trials examining in-person weight loss interventions followed by an Internet-

based maintenance intervention were more successful at producing long-term weight loss than trials that only used Internet-based interventions, with no in-person component. However, as studies to date investigating the use of the Internet in delivering intervention have been highly variable in terms of their methodology, strong conclusions regarding the efficacy of the Internet-based interventions on weight loss and weight loss maintenance are not able to be drawn.

Using devices, such as a personal digital assistant (PDA), are also believed to potentially increase adherence to self-monitoring during a lifestyle intervention. Electronic devices, as compared with written records, may make self-monitoring less tedious and can provide real-time feedback to aid with motivation and support changes in energy balance behaviors. One trial examined the use of a PDA with dietary and exercise software as compared with a paper diary during a lifestyle intervention. At 6 months, the condition that monitored with the PDA and received feedback on goals had a greater proportion of participants reach $>5\%$ weight loss as compared with the paper diary condition (63% vs. 46%). Self-monitoring adherence was also greater with the use of the PDA. As a whole, studies investigating the use of technology suggest that it may be helpful for improving weight loss maintenance, but more research is needed.

Social Support

Another approach for providing long-term support is involving friends and family of participants in the treatment program. Spouses have been included in treatment, but the effects have been mixed. A meta-analysis of the spouse support literature showed a small positive effect through 2–3 months of follow-up. A more recent study examined the effectiveness of natural social support (participants were recruited with three other friends and family members who were all losing weight in the same program) and experimentally created social support (through the use of intragroup activities and intergroup competitions) during a standard behavioral weight loss intervention. Sixty-six percent of participants recruited with a friend and given the social support intervention retained their weight loss in full from month 4 to month 10, compared with 24% of individuals recruited alone and given the standard behavioral intervention, without any social support intervention.

Peer support can also be developed among group members in the same weight loss intervention. Support from other members of the group may explain the finding that group treatment tends to be more successful than individual therapy.

Incentives for Weight Loss and Weight Loss Maintenance

Behavioral interventions used in obesity treatments focus on changing antecedents and consequences of behaviors that influence energy balance. Behaviors that produce negative energy balance, and consequential weight loss, can be reinforced, thereby increasing the likelihood that these behaviors will continue. A systematic review of randomized controlled trials investigating the use of financial incentives in obesity interventions found no significant effect of the use of incentives on weight loss at 12 and 18 months. The review concluded by suggesting future research in this area should target a better understanding of the amount, frequency, and

Table 2 Helpful strategies for successful weight loss maintenance

Area	Strategy
Diet	<ol style="list-style-type: none"> 1. Consume an LCD (~ 1500 kcal day⁻¹) 2. Consume a low-fat (<30% kcal from fat) diet 3. To assist with consuming an LCD, increase structure in the diet using: <ol style="list-style-type: none"> a. Food provisions b. Meal plans c. Meal replacements 4. Lowering the energy density of the diet may be helpful
Physical activity	<ol style="list-style-type: none"> 1. Be active at a moderate-intensity level for at least 200 min week⁻¹ 2. To assist with achieving 200 min week⁻¹ <ol style="list-style-type: none"> a. Have exercise equipment available at home b. Allow activity to accumulate during the day (in at least 10-min bouts)
Self-monitoring	<ol style="list-style-type: none"> 1. Record body weight at least once per week and not more than once per day
Professional contact	<ol style="list-style-type: none"> 1. Extend professional contact beyond initial treatment, occurring at least on a biweekly basis
Technology	<ol style="list-style-type: none"> 1. Technology may be helpful for extending professional contact, as well as increasing the ease of self-monitoring energy balance behaviors
Social support	<ol style="list-style-type: none"> 1. Include support from other individuals also working toward weight loss or weight loss maintenance <ol style="list-style-type: none"> a. Can be from family, friends, or other members of treatment group

type of incentive that could be motivating to improve long-term weight loss outcomes.

Systems-Level Programs

As little progress has been made in reducing the prevalence of obesity, more emphasis is being placed on developing interventions that can help change energy balance behaviors within the larger context in which people live. Thus, these interventions would help change organizations (i.e., worksites and schools), communities, and policy, such that it makes it easier for individuals to engage in healthy eating and leisure-time activity behaviors. As this is a more recent area of research in weight management, it is not clear yet how best to develop these types of interventions, and how effective they are on long-term weight management.

Conclusion

Successful weight loss maintenance can be challenging. However, information obtained from the NWCR, a registry of long-term successful weight loss maintainers, and from experimental studies, which have examined different approaches to improve weight loss maintenance, indicates that there are several strategies that may assist with long-term weight loss maintenance. These strategies are described in **Table 2**. Most notably, it is vital to recognize that for successful weight loss maintenance, individuals have to continue to consume fewer calories and to engage in a greater level of PA than what they did before weight loss, otherwise they will return to a state of positive energy balance, in which weight (re)gain occurs.

Experimental studies show that at least during the weight loss phase, a structured LCD improves long-term weight loss. Being physically active, for at least 200 min week⁻¹, also seems to aid in successful weight loss maintenance. In formalized

behavioral programs, a strategy that improves weight loss maintenance is extending professional contact beyond the initial 6 months of weekly treatments. The use of new technologies, such as the Internet and PDAs, are currently being investigated and show promise for improving weight loss maintenance. Including peer support, during both the weight loss and weight maintenance phases of treatment, can also improve long-term weight loss. Interventions that more broadly influence the overall environment in which people live may help with improving weight management.

To date, studies suggest that effective long-term treatment of obesity may require several different strategies, implemented over an extended period of time. Although these tactics improve long-term weight loss, many patients still regain weight after the initial 6-month treatment. Consequently, further research on improving successful weight loss maintenance is needed.

See also: Appetite: Psychobiological and Behavioral Aspects. Energy: Balance. Physical Activity: Beneficial Effects. Weight Management: Approaches

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WHOLE GRAINS

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Glossary

Bran Outer protective coat of a cereal grain which protects the seed from the environment. Rich in dietary fiber, micronutrients, and phytochemicals. 5–15% of grain weight depending on species.

Endosperm Energy source for the growing seed. Contains carbohydrate in the form of starches and other oligosaccharides such as fructans. Protein is found in the extracellular matrix. Source of B-vitamins. 60–85% of grain weight depending on species.

Germ The embryo of the seed, rich in protein, oils, fat soluble vitamins, micronutrients, and phytochemicals. 2–4% of grain weight depending on species.

Phytochemicals Non-nutrient bioactive chemicals naturally found in fruits, vegetables, nuts, legumes, and grains that may have a positive impact on health.

Whole grains Intact, ground, cracked, or flaked kernel after removal of inedible parts such as the hull and the husk. The starchy endosperm, bran, and germ are present in the same relative proportions as they were in the intact kernel.

Introduction

It is universally accepted that eating wholegrains is beneficial to health. Wholegrains are nutrient-dense foods which, in addition to being dominant sources of carbohydrate and protein in the diet, contain a wealth of beneficial nutrients and phytochemicals that can be lost during milling. Consuming wholegrain foods prepared from these flours is, therefore, recommended by government and other health promotion agencies. Evidence for the health benefits of whole grain comes from a range of sources, mostly based on observational or epidemiological studies and a growing number of intervention studies. This evidence will be reviewed in this article, focusing on the relationship between whole grain intake and reduced risk of the common causes of death, cardiovascular disease, type 2 diabetes, and cancer. Possible mechanisms of action including effects on inflammatory status, body weight and body fatness, delivery of antioxidants and phytochemicals in the diet, and delivery of dietary fiber and fermentable carbohydrates to the large intestine will be described.

Definition of Wholegrains

Cereal wholegrains are the seeds of the *Poaceae* (or *Gramineae*) family of grasses which are staple foods in the diets of many. Worldwide, the dominant grains consumed include wheat, rice, maize, barley, rye, and oats. Other minor grains common in some countries include millet, sorghum, teff, triticale, and wild rice (**Table 1**). The ‘pseudocereals’ amaranth, quinoa, and buckwheat are included in this category because structurally they are very similar to the cereal grasses and are used in similar ways as foods. Various definitions of whole grain have

been suggested, largely based on a definition proposed by the AACC International; “whole grains consist of the intact, ground, cracked or flaked caryopsis (kernel) after the removal of inedible parts such as the hull and husk. The principal anatomical components – the starchy endosperm, germ, and bran – are present in the same relative proportions as they exist in the intact kernel.” The definitions recognize that in modern milling processes the anatomical components of the grain are fractionated during milling but can be recombined to produce wholemeal flour; there is no evidence that such recombined flours are nutritionally different than traditional stone-ground flours where the grains are crushed without separation of the component fractions.

Structural Components and Composition of Grains

Cereal grains have three principle anatomical components each related to their function within the seed. Within each component there is additional complexity which brings with it unique nutrient profiles (**Figure 1**). The bran is often referred to as a single component of the grain, but as **Figure 1** shows, there are several distinct layers including the pericarp and aleurone layers each further subdivided. Modern milling techniques can be used to sequentially remove these layers each of which provides a range of nutrients to the overall profile, particularly dietary fiber(s), some vitamins and minerals, and phytochemicals.

Bran

The function of the bran is to protect the seed and the tough outer layers provide a physical barrier to the external

environment. Removal of the bran is a vigorous abrasive process known as de-hulling, pearling, or peeling, and millers can adapt the severity of the abrasion to remove more or less of the layers. For some cereal grains, notably barley, the degree

Table 1 Common grains included in the whole grain category

Cereal type	Scientific name
<i>Cereal</i>	
Wheat, including spelt, emmer, faro, einkorn, kamut, durums	<i>Triticum</i> spp
Rice	<i>Oryza</i> spp
Maize (corn)	<i>Zea mays</i>
Barley, including hull-less or naked varieties but excluding pearled barley	<i>Hordeum</i> spp
Rye, including hull-less or naked varieties	<i>Secale</i> spp
Oats	<i>Avena</i> spp
Millet	<i>Brachiaria</i> spp; <i>Pennisetum</i> spp; <i>Panicum</i> spp; <i>Setaria</i> spp; <i>Paspalum</i> spp; <i>Eleusine</i> spp; <i>Echinochloa</i> spp
Sorghum	<i>Sorghum</i> spp
Teff	<i>Eragrostis</i> spp
Triticale	<i>Triticale</i> spp
Canary seed	<i>Phalaris arundinacea</i> and <i>P. Canariensis</i>
Job's tears	<i>Coix lacryma-jobi</i>
Fonio, Black fonio and Asian Millet	<i>Digitaria</i> spp
Wild rice	<i>Zizania aquatica</i>
<i>Pseudocereals</i>	
Amaranth	<i>Amaranthus cordatus</i>
Quinoa	<i>Chenopodium quinoa</i> Willd
Buckwheat	<i>Fagopyrum</i> spp

of abrasion required to remove the inedible fraction of the grain and the extent of removal is such that the resultant grain can no longer be considered a 'whole grain' using the definition above. Nutritionally, the principle nutrient in bran is dietary fiber and this is predominantly of the insoluble type although there can be significant quantities of soluble fibers such as arabinoxylan found in wheat and rye, and β -glucan which is found in particularly high concentrations in oats and barley. The bran layers are also a principle source of phytochemicals and contribute to the antioxidant potential of grain.

Endosperm

Quantitatively, the biggest part of the grain is the endosperm which comprises from approximately 60% up to almost 85% of the cereal grain dry weight, depending on species. The endosperm provides the necessary energy for the growing embryo. Nutritionally, the endosperm is mainly carbohydrate in the form of starches and other oligosaccharides such as fructans. Protein is found in the extracellular matrix, and there are some B vitamins in particular pantothenic acid and riboflavin.

Germ

The germ is the smallest fraction of the grain at approximately 2.5% of the grain weight. The germ is the plant embryo which would form the new plant if germinated and is characterized by a high lipid and protein content. The germ is also a rich source of minerals, particularly potassium, calcium, magnesium, and zinc, and both water- and fat-soluble vitamins including vitamin A, tocopherols, and tocotrienols. The oil content of the germ is prone to oxidation which reduces the storage time for wholegrain flours.

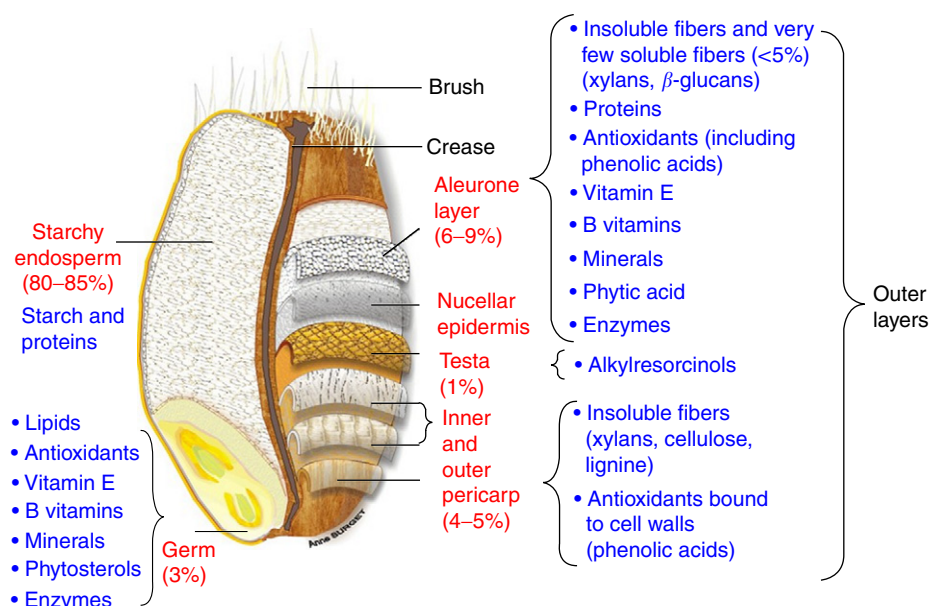


Figure 1 Anatomical structure of a whole wheat grain. Reproduced with permission from Surget A and Barron C (2005) Histologie du grain de blé. *Industrie des Céréales* 145: 3–7, and Hemery Y, Rouau X, Lullien-Pellerin V, Barron C, and Abecassis J (2007) Dry processes to develop wheat fractions and products with enhanced nutritional quality. *Journal of Cereal Science* 46(3): 327–347.

Wholegrain Foods and Health Claims

Intact wholegrains must be processed before consumption. Mostly this involves milling, but less destructive processes such as rolling, flaking, and cracking are also used. Intact wholegrains are used in small quantities in some mueslis or when added as texture in some breads. Processing of the grains increases digestibility and improves texture, flavor, and cooking characteristics of the products. This, together with the fractionation and reconstitution procedures during milling will, inevitably, result in changes to the gross morphology of the product and most likely the chemical composition. The metabolic consequences of consuming wholegrain foods prepared from crushed and minimally processed flours compared with heavily processed reconstituted flours have not been investigated. One characteristic known to change is the glycemic index (GI) which is higher in foods prepared from finely ground flours.

Currently, there is no universal definition for wholegrain foods although this is important in advising the consumer of the healthfulness of whole grain. The US Food & Drug Administration (FDA) defined a wholegrain food as a product containing >51% whole grain by weight per reference amount customarily consumed (RACC) per day. In Europe, industry has mostly used a similar definition although there is no legislative control. The FDA allowed the first health claim for wholegrain foods which could be used on foods which met this criterion. The 1999 claim was modified in 2003 and is based on the following authoritative statement: "Diets rich in whole grain foods and other plant foods and low in total fat, saturated fat, and cholesterol may reduce the risk of heart disease and some cancers." Similar claims in the UK and Europe followed although the claims in Europe are similarly cumbersome. For example, the Swedish claim certified by the Swedish Nutrition Foundation states: "A healthy lifestyle and a well balanced diet rich in wholegrain products reduces the risk for (coronary) heart disease. The product X is rich in wholegrains (contains Y% of wholegrain)." In 2010, the European Food Safety Authority did not approve a whole grain health claim on the grounds that whole grain was 'insufficiently characterized' meaning that, after a period of grace, health claims on wholegrain foods will no longer be allowed in European-Zone countries.

Recommendations for Whole Grain Consumption and Consumption Patterns

Currently, specific recommendations for whole grain consumption exist only in the USA and Denmark. The US Dietary Guidelines 2010 recommend that at least half of total grain-food intake should be as wholegrains, which for those over 9 years of age is a *minimum* of approximately 3 ounce-equivalents or 48 g per day⁻¹. In Denmark, where there is already a stronger tradition in eating wholegrain foods, the target level for consumption is 75 g per 10 MJ energy consumed. In industrialized countries, average whole grain consumption is much lower than these recommendations. In the USA, for example, Americans eat less than 1 ounce-equivalent of wholegrains per day. For the majority of other countries, with

the exception of Scandinavian, Nordic, and some Eastern European Countries, wholegrain consumption is of a similar magnitude. Where specific guidelines on amounts are not specified but food agencies recommend consumers to 'choose wholegrains whenever possible' such as the UK, Switzerland, and Australasia, consumption is also very low. In the UK, for example, data from the National Diet and Nutrition Survey showed that median whole grain intake was less than one serving per day, over 97% of adults did not achieve 48 g day⁻¹ and more than 30% of individuals consumed no whole grain at all. Measurement of whole grain intake at the population level is required both for development of public health initiatives and for exploring the consequences of different levels of whole grain intake. However, methods for measuring wholegrain intake are varied and often imprecise. For example, in many earlier studies, dark bread was assumed to be wholegrain bread which may not be the case. The studies which collected data in the early 1990s were not originally designed to investigate wholegrain intake (as the food category was not at that time the focus for research interest), and most relied on the use of food frequency questionnaires to collect data on dietary intake. This makes comparison between the studies difficult although the estimates of intake between the highest and lowest wholegrain consumers are reasonably similar. Some studies have reported intake in grams of wholegrain per day, whereas others have reported intake as servings, requiring additional assumptions on definitions of servings. There are relatively few studies where the whole grain content of foods has been systematically calculated based on recipes and manufacturer information and then applied to population survey data with accurate measures of dietary intake. In many studies, a cut-off of 25% whole grain has been used as an inclusion criterion, compared with the 51% advocated by the FDA. In the UK, data from the National Diet and Nutrition Survey for 2000–2001 using a lower cut-off of 10% whole grain to define wholegrain foods showed that 27% of wholegrain intake was for foods with less than 51% wholegrain.

Wholegrains and Reduction in Disease Risk

Cardiovascular Disease

Deaths from cardiovascular diseases (CVD) account for approximately one-third of deaths globally and morbidities associated with CVD account for significant proportions of health care costs. The inverse relationship between increased wholegrain intake and reduced risk of CVD has been documented in numerous observational studies for all different types of CVD. Many of these data come from very large observational and cohort studies carried out in North America and Europe. When analyzing these data it is important to note that, almost without exception in these studies, higher wholegrain intake is associated with many other indicators of a healthy lifestyle, such as lower smoking status and alcohol intake, higher physical activity, and use of supplements and a generally higher household income. Therefore, there is some suggestion that the relationships observed with whole grain intake are merely indicators of a generally healthier

lifestyle. These confounding factors can be mitigated in multivariate analyses which should be used for data evaluation. Even with these considerations, however, the inverse relationships remain significant and in many cases are paralleled by similar relationships for cereal but not total dietary fiber intake and positive relationships between refined grain intake and increased risk of CVD. Data from observational studies have been subjected to meta-analyses and these show significant and marked reductions in risk of the order of 20–30% reductions in CVD risk between groups with the lowest and highest quintiles of whole grain intake. **Figure 2** summarizes some of these data using the most adjusted models of analysis for some of the large cohort studies investigating CVD incidence as endpoints. The range of reductions in either hazards ratio or relative risk of incident CVD is large at between 18% and 52%. The highest figure is for a study with a smaller number of subjects (535 adult men and women) than the other studies which are based on the largest US cohort studies (Health Professionals Follow-up ($n=42\,850$); Nurses' Health Study (NHS) ($n=75\,521$); Iowa Women's Health Study ($n=34\,491$); and Atherosclerosis Risk in Communities Study ($n=15\,972$)) so should be treated with caution. Functional measures of cardiac health are few, but in one longitudinal study, smaller reductions in minimum coronary artery diameter and a trend for lower progression in mean percent stenosis with higher whole grain intake have been reported.

Type 2 Diabetes

The incidence of type 2 diabetes has increased dramatically in line with the rise in obesity worldwide, and consequences of the epidemic represent a major challenge for health services. The etiology of the disease is complex, including interactions between genes, diet, and lifestyle. The underlying pathology is the development of insulin resistance which has been linked to increased intake of refined carbohydrates along with decreased intake of fiber. Many of the studies to show the relationship between wholegrain intake and CVD risk have been used to explore similar relationships for type 2 diabetes. In these observational studies, whole grain intake is also shown to be inversely related to risk of type 2 diabetes and is also associated with lower concentrations of glucose and insulin where these measures are available. One of the largest combined datasets investigating incidence of type 2 diabetes in relation to whole grain intake is research based on 12–18 years of follow-up of women from NHS I and II. 6486 cases of type 2 diabetes were recorded from 73 327 subjects in NHSI and 88 410 subjects in NHSII. Wholegrain intake in the lowest and highest quintiles of intake was 3.7 and 31.2 g day^{-1} for NHSI and 6.2 and 39.9 g day^{-1} for NHSII, respectively. In a multivariate analysis including BMI, the RR for developing type 2 diabetes for the highest quintile of wholegrain intake compared with the lowest quintile of intake was 0.75 (95% CI $0.68, 0.83$) for NHSI and 0.86 (95% CI $0.72, 1.02$) for NHSII. Addition of

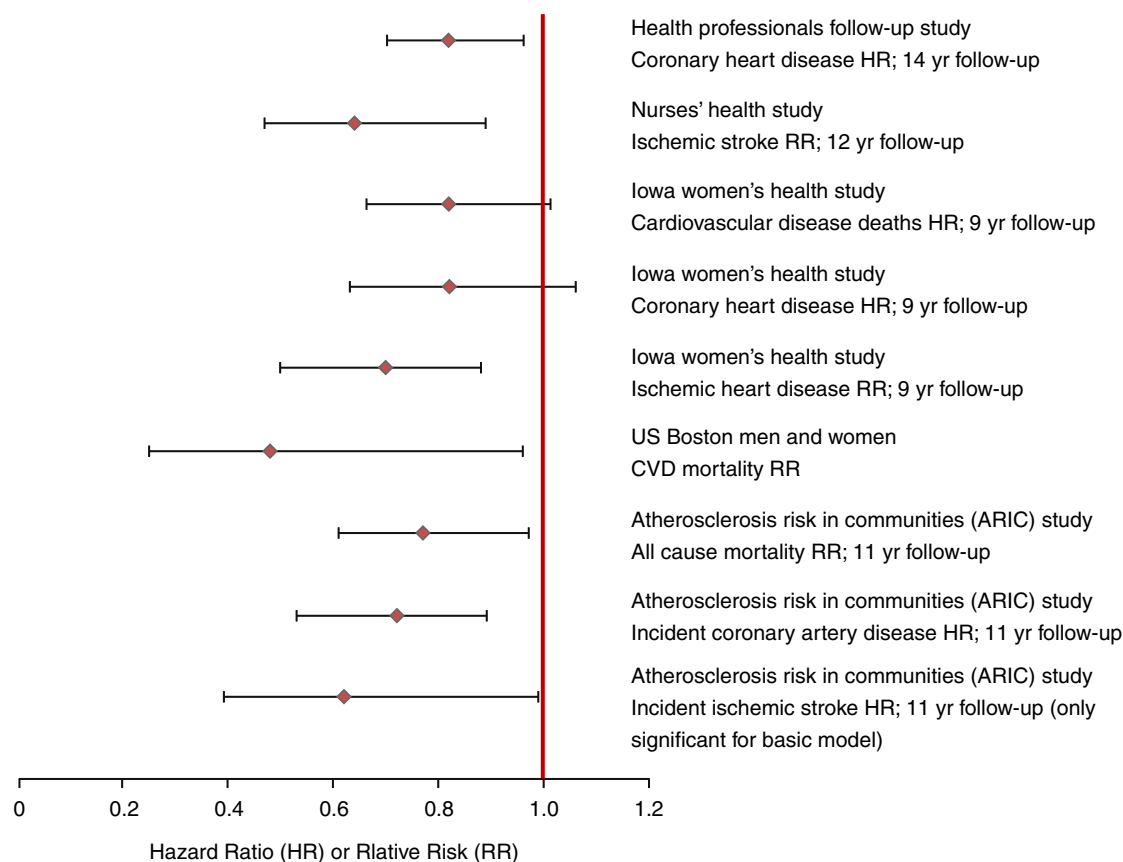


Figure 2 Selection of large-scale observational studies showing a significant ($p<0.05$) benefit of increased whole grain intake on CVD risk. All studies compare the highest versus the lowest whole grain consumers and except where noted are for the most adjusted models.

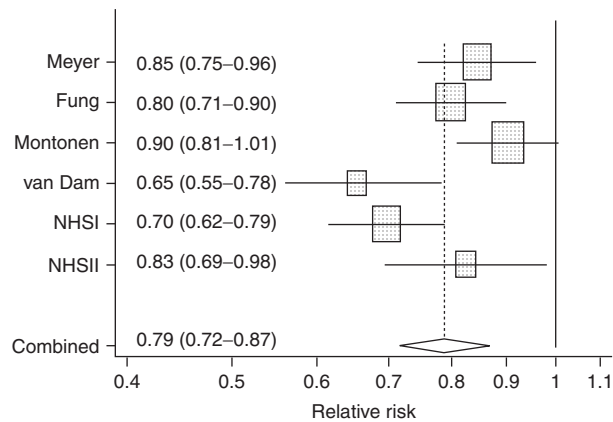


Figure 3 Forest plot showing the multivariate-adjusted RR of type 2 diabetes for a two-servings-per-day increment in whole grain intake for individual cohort studies and all studies combined bars and the diamond indicate 95% CIs. The size of the squares corresponds to the weight of the study in the meta-analysis (De Munter *et al.*, 2007).

data from a further four cohort studies gave a total of 286 125 participants with 10 944 cases of type 2 diabetes; from this full dataset, it was calculated that a two-serving-per-day increment in wholegrain intake was associated with a 21% (95% CI 13, 28) decrease in risk of type 2 diabetes after adjustment for potential confounders and BMI (Figure 3).

Intervention studies which have investigated the effect of wholegrain consumption on measures of insulin resistance/sensitivity have given conflicting results. In a very well-designed randomized, nonblinded, 6-week, cross-over dietary intervention with 34 participants, markers of both inflammation and insulin sensitivity were not affected by dietary whole grain compared with the refined grain period. In this study, peripheral insulin sensitivity was determined by the euglycemic hyperinsulinemic glucose clamp method which, although time consuming, is considered the gold standard for this type of measurement. The data showed a 7% reduction in insulin sensitivity in the whole grain group (i.e., insulin resistance increased) although this was not statistically significant. In contrast, in the Multi-Ethnic Study of Atherosclerosis (MESA) study, there was a 9% reduction in insulin resistance with higher whole grain consumption calculated from fasting glucose and insulin concentrations using the homeostatic (HOMA) model of insulin resistance. In another study, employing a tightly controlled dietary regime in a metabolic feeding study with hyperinsulinemic overweight male subjects, insulin sensitivity, again measured by glucose clamp was significantly improved.

Evidence that genetic variability can influence the response to wholegrain consumption is demonstrated in a subset of diabetic subjects compared with control subjects in the European Prospective Investigations into Cancer (EPIC)-Potsdam study. Single-nucleotide polymorphisms in the transcription factor-7-like 2 encoding gene (TCF7L2) were determined for the participants. The TCF7L2 gene is associated with an early insulin secretory defect and has been consistently attributed to the risk of developing type 2 diabetes through the rs7903146 risk-conferring recessive allele (indicated as the T polymorphism compared with the dominant

allele C). For the CC genotype, the hazards ratio for 50 g day⁻¹ intake of whole grain for development of type 2 diabetes was 0.86 (95% CI 0.75, 0.99), whereas for the CT and TT genotypes combined the hazards ratio was significantly higher at 1.08 (95% CI 0.96, 1.23). Thus, the beneficial effect of wholegrains in reducing risk of type 2 diabetes was completely attenuated in those carrying the rs7903146 T allele.

Cancer

The protective influence of wholegrains on many cancer sites has been well reported in the literature. Early data were assessed in a meta-analysis and there is a growing body of more recent evidence to support its conclusions. The original meta-analysis collected data from observational studies in the USA (12 studies), Italy (16 studies), and central Europe (12 studies) published between 1984 and 1997. The analysis has some shortcomings, for example whole grain consumption was assessed against mentions of wholegrain foods in broadly descriptive terms. Nevertheless, there were 51 mentions of wholegrains in 40 studies included in the analysis and in 46 of these wholegrains were shown to be protective. When some of the studies were removed from the dataset to remove data with reporting flaws this still left 45 mentions, of which 43 were protective. The associations were strongest for cancers of the gastrointestinal tract as would be expected for these high-fiber foods. For example, for individual cancer types, the odds ratios for disease risk compared between low and high whole grain intake were <1.0 in nine out of 10 studies of colorectal cancers and polyps and all mentions of gastric and other digestive tract cancers. Strong associations were also demonstrated for hormone-related cancers and pancreatic cancer, and a total of eight other cancers. The pooled odds ratio averaged across all studies was 0.66 (95% CI 0.60, 0.72). Data from some of the more recent studies are shown in Table 2.

There are considerable data from case-control studies which show a positive association between wholegrain consumption and colorectal cancer risk; however, evidence from prospective studies is limited and contrasting with studies showing both protective and no association between wholegrain intake and risk. Because incidence of colorectal cancer has been shown to be associated with dietary fiber intake, this diversity of results is surprising as wholegrains make a significant contribution to fiber intake. This lack of association may be due to the timescale of some of the studies and the often small number of incident cases identified in the follow-up period. A recent, large, Danish study of men and women from the Diet, Cancer, and Health cohort investigated incident cases of colon cancer and rectal cancer over a median follow-up of 10.6 years. Associations between total and individual whole grain product consumption was examined using data from 461 incident cases of colon cancer and 283 incident cases of rectal cancer among 26 630 men and 29 189 women. Overall, higher total whole grain intake was associated with lower incident relative risk (IRR) of colon cancer and rectal cancer in men. The IRR per 50 g of whole grain intake from the fully adjusted model were 0.85 (95% CI 0.77, 0.94) for colon cancer and 0.90 (95% CI 0.80, 1.01) for rectal cancer. For colon cancer in men, wholegrain wheat bread was the only

Table 2 Observational studies showing a benefit of increased whole grain consumption on cancer risk. All are significant ($p < 0.05$) unless stated for fully adjusted model

Study	Whole grain intake range for comparison	Outcome	Source
National Institutes of Health and AARP Diet and Health Study (291 988 men and 197 623 women) 5-year follow-up	0.3 and 1.3 servings per 1000 kcal day ⁻¹	Colon cancer: RR 0.86 (95% CI 0.75, 0.99) Distal colon cancer: RR 0.85 (95% CI 0.69, 1.06) Rectal cancer: RR 0.64 (95% CI 0.51, 0.81)	Schatzkin A, Mouw T, Park Y, <i>et al.</i> (2007) Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIH-AARP Diet and Health Study. <i>American Journal of Clinical Nutrition</i> 85: 1353–1360.
National Institutes of Health and AARP Diet and Health Study (293 703 men and 198 618 women) 5 year follow-up	0.2 and 1.4 servings per 1000 kcal per day ⁻¹	Small intestinal cancer: RR 0.59 (95% CI 0.33, 1.05) $p = 0.06$	Schatzkin A, Park Y, Leitzmann MF, Hollenbeck AR and Cross AJ (2008) Prospective study of dietary fiber, whole grain foods, and small intestinal cancer. <i>Gastroenterology</i> 135: 1163–1167.
Swedish Mammography Cohort (61 433 women over 40 years) 14.6 year follow-up	1.5 and ≥ 4.5 servings per day	Colon cancer: RR 0.65 (0.45, 0.94)	Larsson SC, Giovannucci E, Bergkvist L and Wolk A (2005) Whole grain consumption and risk of colorectal cancer: A population-based cohort of 60 000 women. <i>British Journal of Cancer</i> 92: 1803–1807.
Danish Diet, Cancer and Health cohort (26 630 men and 29 189 women) 10.6 year follow-up	< 75 g day ⁻¹ and > 160 g day ⁻¹	Colon cancer: Incident rate ratio 0.61 (95% CI 0.43, 0.86)	Egeberg R, Olsen A, Loft S, Christensen J, Johnsen NF, Overvad K and Tjønneland A (2010) Intake of wholegrain products and risk of colorectal cancers in the Diet, Cancer and Health cohort study. <i>British Journal of Cancer</i> 103: 730–734.
Population-based cohort study 156 cases 349 controls	No or rare and > 10 times per week	Esophageal cancer: OR 0.30 (95% CI 0.1, 0.6) Total oral cancers: OR 0.5 (95% CI 0.3, 1.0)	Levi F, Pasche C, Lucchini F, Chatenoud L, Jacobs DR and La Vecchia C (2000) Refined and whole grain cereals and the risk of oral, oesophageal and laryngeal cancer. <i>European Journal of Clinical Nutrition</i> 54: 487–489.
Population-based case-cohort study 532 incident cases 1701 controls	Never or < 1 serving per day and > 2 servings per day	Pancreatic cancer: OR 0.60 (95% CI 0.31, 1.2)	Chan JM, Wang F and Holly EA (2007) Whole grains and risk of pancreatic cancer in a large population-based case-control study in the San Francisco Bay Area, California. <i>American Journal of Epidemiology</i> 166: 1174–1185.
Health Professionals Follow-up Study (49 934 men) > 2 years follow-up	3 and 53 g day ⁻¹	Prostate cancer: RR 1.13 (95% CI 1.03, 1.24) Prostate cancer PSA screened: RR 1.03 (95% CI 0.91, 1.17)	Nimptsch K, Kenfield S, Jensen MK, <i>et al.</i> (2011) Dietary glycemic index, glycemic load, insulin index, fiber and whole-grain intake in relation to risk of prostate cancer. <i>Cancer Causes & Control</i> 22: 51–61.
Danish Diet, Cancer and Health cohort (25 278 postmenopausal women) 9.6 year follow-up	72 and 163 g day ⁻¹	Total breast cancer: RR 1.03 (95% CI 0.85, 1.24)	Egeberg R, Olsen A, Loft S, Christensen J, Johnsen NF, Overvad K and Tjønneland A (2009) Intake of whole grain products and risk of breast cancer by hormone receptor status and histology among postmenopausal women. <i>International Journal of Cancer</i> 124: 745–750.
Population-based cohort study 310 incident cases 353 controls	< 8.9 g day ⁻¹ and > 47.1 g day ⁻¹	Breast cancer: OR 0.57 (95% CI 0.34, 0.95)	Adzersen KH, Jess P, Freivogel KW, Gerhard I and Bastert G (2003) Raw and cooked vegetables, fruits, selected micronutrients, and breast cancer risk: A case-control study in Germany. <i>Nutrition and Cancer</i> 46: 131–137.

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wholegrain food where the IRR per 25 g of wholegrain wheat bread intake was significantly reduced with higher consumption, IRR 0.89 (95% CI 0.82, 0.97), although there was a trend for rye bread. This response is interesting because the intake of rye bread was approximately twice that of the wheat bread (63 g day⁻¹ compared with 31 g day⁻¹) and rye was shown to reduce colon cancer risk in women from the

Swedish Mammography Cohort. In the Danish study, there were no consistent associations between total or individual wholegrain food consumption and colon or rectal cancer risk in women which, although this has been seen in some (but not all) studies, was unexpected.

There have been several studies on breast cancer risk and here the data are very variable. Some studies show small,

but modest, benefits of wholegrain consumption, but others show either no benefit or even negative effects of whole grain. Data from the Iowa Women's Health Study suggested that the multivariate-adjusted risk of incident breast cancer was 20% higher for women who had the highest wholegrain intake compared with those with the lowest wholegrain intake. However, when the data were further examined, it appeared that there was no increase in breast cancer incidence in women who had not undergone screening mammography before the follow up suggesting that increasing whole grain intake was associated with more health-conscious behaviors such as attending for cancer screening thus increasing detection rate in whole grain consumers. Recent data from the Danish Diet, Cancer and Health cohort of postmenopausal women also failed to show any association between whole grain intake and breast cancer risk including all estrogen and progesterone receptor disease subtypes. However, in this study whole grain intake was very high; even in the lowest quintile of intake, it was in excess of 70 g of wholegrain food per day which is much higher than intakes reported in other studies. This may suggest a threshold effect for wholegrain benefits which is exceeded in this population.

Wholegrains and Potential Mechanisms of Action

Wholegrains and Inflammatory Status

It is now recognized that chronic subclinical inflammation is central in the progression of many diseases such as CVD and type 2 diabetes. In several cross-sectional studies, the relationship between wholegrain consumption and markers of inflammatory status such as high-sensitivity C-reactive protein (hsCRP), tumor necrosis factor receptor-2 (TNF-R2), fibrinogen, plasminogen activator inhibitor-1 (PAI-1), and interleukin-6 (IL-6) has been investigated. For some of these, inverse relationships have been observed – i.e., increased whole grain consumption is associated with lowered inflammatory marker concentrations. In one weight loss intervention, subjects on a calorie-restricted diet containing wholegrains had lower CRP concentrations than those who only ate refined grain foods. In another study, healthy premenopausal women who ate small amounts of wholegrain (but still less than one serving per day) for 2 years, concentrations of hsCRP were on average 11.5% lower than those who ate no whole grain at all. For women eating one or more servings of wholegrain the reduction in hsCRP concentrations was on average 12.3% lower. These observations, however, have not been found in all intervention studies and the cause and effect relationships are not completely understood although it has been suggested that the responses may be mediated through changes in body weight and adiposity seen in those consuming wholegrains. Improved glucose control has also been suggested as wholegrains may have a slower rate of stomach emptying and decrease carbohydrate digestion. Thus, wholegrain consumption may reduce the time spent in postprandial hyperglycemia reducing the formation of advanced glycation end products which are known to induce oxidative stress and inflammation.

Wholegrains, Body Weight, and Body Fatness

In several, but not all, observational studies, increased wholegrain consumption is associated with lower body mass, BMI, and waist circumference (a measure of abdominal or central obesity). Longitudinal data investigating weight gain over time are available from some of the larger North American cohort studies. For example, women in the NHS were followed from 1984 to 1996 and their habitual diet was assessed every 2 years by a food frequency questionnaire from which the women were divided into quintiles of whole grain intake. Women consuming more wholegrains at baseline weighed less than women consuming fewer wholegrain servings. Women from all quintiles of wholegrain consumption gained weight over the 12 year follow-up period. However, the women who increased their intake of wholegrains during the study gained significantly less weight during the study period. The same trend was found for women who increased their intake of dietary fiber over the follow-up period, suggesting that the fiber content of wholegrain foods may be partly responsible for the protective effect against weight gain. Similar results were found in men in the Health Professionals Follow-up Study. Over an 8-year follow-up period, as with the NHS, all participants tended to gain weight. Wholegrain intake was inversely associated with weight gain, after adjusting for potential confounding variables. Both cereal fiber and fruit fiber (but not vegetable fiber) were also inversely associated with weight gain. Added bran resulted in a dose-dependent reduction in weight gain but added germ did not. Indirect evidence of the benefits of wholegrain foods as part of a healthy food pattern is also seen in the EPIC-Potsdam cohort of German subjects. In this cohort, annual weight gain over a 4-year follow-up was lower with increasing food pattern (more healthy) score; subjects scoring high for the healthy food pattern maintained their weight or gained significantly less weight over time compared with those with a lower (unhealthy) score. Longitudinal changes in overweight and obesity are shown in UK NDNS data. In the 1986/87 survey, whole grain intake was inversely associated with the percentage of men with a BMI $>30 \text{ kg m}^{-2}$, independent of other factors. However, intake was not associated with body weight or prevalence of overweight. There were no corresponding associations seen in women. In subjects from the 2000/01 survey, whole grain intake was not associated with body weight, BMI, or waist circumference in men or women. This suggests some dissociation of the relationship between whole grain intake and body weight, but in the 2000/01 survey whole grain intake was significantly lower than in 1986/87 (14 (IQR 0.36) g day⁻¹ vs 16 (IQR 0.45) g day⁻¹) with significantly more adults consuming no wholegrains in the later survey (29% vs 25%).

Data from the Framingham Heart Study were used to assess the relationship between wholegrain consumption and body fat distribution. The results showed that those from the highest wholegrain consumption group had less subcutaneous abdominal fat (fat under their skin) and less visceral fat (fat around their organs). In contrast, those from the highest refined grain consumption group had more of both types of abdominal fat, especially visceral fat. In a further study involving 434 free-living older adults (60–80 years), percentage

body fat and percentage abdominal fat mass were measured by dual-energy X-ray absorptiometry (DEXA) scanning. After adjustment for covariates, whole grain intake was inversely associated with BMI (-1 kg m^{-2} differences between lowest and highest quartiles of whole grain intake), percentage body fat (-2.4%), and percentage abdominal fat mass (-3.6%). Refined grain intake was not associated with any measure of body fat distribution. There were inverse associations between cereal fiber and BMI (-1.2 kg m^{-2}), percentage body fat (-3.2%), and percentage abdominal fat mass (-5%). Thus, cereal fiber, particularly from whole-grain sources, was associated with improved measures of body fatness in these older adults. Body fatness is a risk factor for several chronic diseases. In particular, visceral fat mass is a key indicator of metabolic syndrome, so reduction in visceral fat results in a significant improvement in cardiovascular risk factors. This may be one reason for the health benefits seen for consuming whole grain.

Wholegrains and Blood Pressure

There are a small number of observational studies in which an inverse relationship between hypertension and whole grain intake has been reported. For incident hypertension in men, a relative risk of 0.81 (95% CI: 0.75, 0.87) for those in the highest (median intake 46 g whole grain per day) compared with the lowest (median intake 3 g whole grain per day) quintiles of whole grain intake has been reported. This study, interestingly, was also able to differentiate bran and germ intakes; for the former the relative risk across quintiles of intake was 0.85 (95% CI: 0.78, 0.92) but there was no reduction in risk based on germ intake alone although this may be due to difficulties in quantifying germ intake at the low levels seen for this component of the grain. The effects of whole grain consumption on blood pressure have also been measured in a number of intervention studies with varying results. In two studies from the UK, both randomized control trials with more than 200 healthy volunteers, one showed that 12 weeks consuming three servings per day of wholegrain wheat or a 50:50 mix of whole grain wheat and whole grain oats caused a significant reduction of 6 mmHg in systolic blood pressure and 3 mmHg in pulse pressure. In the second study, however, volunteers consumed either three servings of mixed wholegrains per day for 16 weeks or three servings per day for 8 weeks followed by six servings per day for a further 8 weeks, and blood pressure was not affected in either group. Several studies have used subjects with dyslipidemia, again with varying results. For example, both systolic and diastolic blood pressure were reduced when subjects following a controlled Step I diet for 2 weeks replaced approximately 20% of energy with whole wheat/brown rice, barley, or half wheat-rice/half barley, for 5 weeks compared with a control group consuming no whole grain.

Wholegrains, Antioxidants, and Phytochemicals

Whole grain cereals contain many potentially protective compounds which are lost during the milling process. These include fermentable nonfermentable carbohydrates (dietary

fiber), phytochemicals, polyphenolics, and antioxidants. Many of these compounds are found in the bran and germ portions of the grain so are readily lost during even the mildest abrasion. The phytochemicals, polyphenolics, and antioxidants have received particular attention because there are plausible explanations why their consumption may be beneficial for health. For example, antioxidants may have potential to reduce inflammatory responses; phytoestrogens can interact with estrogen receptors and may affect susceptibility to hormone-related cancers.

Phytochemicals are bioactive non-nutrient plant compounds in fruits, vegetables, wholegrains, and other plant foods that have been associated with reduced risk of major chronic diseases and the most studied phytochemicals found in grains are the phenolics. Phenolics are compounds with one or more aromatic rings and one or more hydroxyl groups such as phenolic acids, plant lignans, alkylresorcinols, and flavonoids. Phenolics exist in cereal grains in two forms: soluble free and insoluble bound; the latter include compounds that are esterified to macromolecules and as a result may escape digestion in the upper gastrointestinal tract. It has been suggested that as much as 74% of phenolic compounds in wheat, maize, oats, and barley are found in the insoluble bound fraction. Phenolic compounds mainly exist as glycosides linked to various sugar moieties or as other complexes linked to organic acids, amines, lipids, carbohydrates, and other phenols. There are numerous publications on the phenolics content of cereals and often these may underestimate the true content because they only extract the soluble fraction. These fractions may be released during processing of the grain and be available in the upper part of the intestine or they may be released during bacterial fermentation in the large intestine. Thus, there is potential for local effects in different parts of the gut or systemic effects once the compounds are absorbed. Phenolics and other 'antioxidant' compounds found in cereal grains are able to act as antioxidants because they can donate hydrogen atoms to free radicals. In principle, the higher the amount of phenolics and other antioxidant compounds in cereal grains, the greater their potential antioxidant capacity, and this has been well documented *in vitro* using a variety of assays. However, their potential to affect antioxidant status *in vivo* is rather less certain, and the mechanisms by which antioxidants that cross the intestinal barrier protect the body remain uncertain. Acute feeding studies have shown that ferulic acid, a phenolic found in high concentrations in many cereals, can be absorbed into the blood stream from where it is rapidly excreted. However, in a 2-week randomized cross-over study where men consumed eight servings and women six servings of whole or refined grain foods, antioxidant capacity measured using a range of different methods including markers of oxidative damage did not change.

Lignans are a group of dietary phytoestrogen compounds found in the bran of cereals and some other seeds, legumes, and fruits. The common plant lignans in the human diet include secoisolariciresinol, matairesinol, lariciresinol, pinoresinol, and syringaresinol. Lignans are metabolized by bacteria in the intestine to produce the mammalian lignans, enterodiol, and enterolactone. In several studies, plasma concentrations of enterodiol and enterolactone are positively

correlated with wholegrain intake, and in a Finnish study were inversely correlated with markers of CVD risk and all cause death. These compounds have strong antioxidant activity *in vitro* and weak estrogenic activity that may account for their biological effects on health.

Whole Grain, Dietary Fiber, and Fermentable Carbohydrates

Wholegrains are good sources of dietary fiber, and in observational studies dietary fiber intake is correlated with whole grain intake. The range of complex carbohydrates and non-starch polysaccharides derived from cereal grains include cellulose, hemicellulose, arabinoxylans, lignin, fructans, such as inulin, and resistant starch. In many of the observational studies described above, health benefits associated with increased wholegrain intake are similarly correlated with cereal fiber intake. The modes of action of wholegrain dietary fiber encompass many physico-chemical and physiological processes all of which can be linked to health benefits ascribed to increased intake of wholegrains. These include direct effects on digesta characteristics such as viscosity, flow rate/transit time, and bulking. These changes then affect digestive processes such as reducing stomach emptying, reducing rates of carbohydrate and lipid digestion, blunting the postprandial glucose and insulin response, lowering glycemic index/glycemic load, binding of bile acids and interrupting the enterohepatic circulation of cholesterol, and delivering fermentable carbohydrate to the large intestine. The potential prebiotic effects of nondigestible oligosaccharides, such as fructans and arabinoxylan fractions, have also been suggested.

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Z

ZINC

Contents

Deficiency Disorders and Prevention Programs **Physiology, Dietary Sources, and Requirements**

Deficiency Disorders and Prevention Programs

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Glossary

Bioavailability The proportion of the nutrient content of a food or meal that is absorbed and retained.

Phytate : zinc molar ratio The molar ratio between phytate (molecular weight 660 g mol^{-1}) and zinc (molecular weight 65.4 g mol^{-1}) and is used to estimate the bioavailability of zinc in food and mixed diets.

Phytic acid Myo-inositol hexaphosphate, the principal storage form of phosphorus in many plants and the major inhibitor of zinc absorption (also described as phytate).

Zinc A trace element essential for the human body as it required for the activity of > 100 enzymes involved in most major metabolic pathways.

Zinc deficiency Lack of sufficient zinc to meet the physiological requirement.

Causes of Zinc Deficiency

As with other micronutrient deficiencies, four main factors are responsible for the development of zinc deficiency in lower-income countries: inadequate dietary zinc intake; poor zinc absorption from high-phytate, plant-based diets; disease states that either induce excessive losses or impair utilization of zinc; and physiological states that increase zinc requirements, such as the periods of rapid growth during childhood and pregnancy.

Adequate zinc nutrition is essential for human health because of zinc's critical structural and functional roles in multiple enzyme systems that are involved in gene expression, cell division and growth, and immunologic and reproductive functions.

Inadequate Dietary Zinc Intake

Inadequate dietary intake of absorbable zinc is one of the major causes of zinc deficiency. Animal-source foods, in particular shellfish, small whole fish, beef, and organ meats such as liver and kidney, are rich sources of zinc. Plant-source foods, such as most fruits and vegetables including green leaves, and starchy roots and tubers, have relatively low zinc content. Although whole grains, nuts, and legumes have moderate to high zinc content, these foods also contain large quantities of phytate (phytic acid or myo-inositol hexaphosphate), the most potent identified dietary inhibitor of zinc absorption.

Plants synthesize phytate, which occurs in highest concentrations in seeds and to a lesser extent in vegetative plant

parts. Phytate forms chelates with zinc and other minerals, making these minerals less available for absorption. The inhibitory effect of phytate on zinc absorption appears to follow a dose-dependent response, and the phytate : zinc molar ratio can be used to estimate the proportion of absorbable zinc. The phytate : zinc molar ratio of foods or diets is calculated as follows, where 660 equals the molecular weight of phytate and 65.4 the molecular weight of zinc:

$$\frac{\text{mg phytate}/660}{\text{mg zinc}/65.4}$$

The zinc and phytate content, and the phytate : zinc molar ratio in some foods are shown in **Table 1**. If information on the phytate content of the diet cannot be calculated, then diets can be categorized as having low or average zinc

Table 1 The average content of zinc and phytate, and the phytate : zinc molar ratio in uncooked foods

<i>Food</i>	<i>Zinc (mg per 100 g)</i>	<i>Phytate (mg per 100 g)</i>	<i>Phytate : zinc molar ratio</i>
<i>Cereals</i>			
Corn	1.8	800	44
Pasta	0.7	282	40
Rice (milled)	1.1	352	32
Wheat or whole- wheat bread	2.9	845	29
White bread	0.9	30	3
<i>Nuts and legumes</i>			
Lentils/mung beans	1.3	358	27
Peanuts	3.3	1760	53
Peas	2.9	1154	39
Red beans	2.9	1629	56
<i>Roots and tubers</i>			
Cassava	0.3	54	18
Potato	0.3	81	27
Sweet potato	0.5	50	10
<i>Vegetables</i>			
Cabbage	0.1	0	—
Green leaves	0.2	42	21
Onion	0.2	0	—
Tomato	0.1	6	6
<i>Fruits</i>			
Banana	0.2	0	—
Coconut	1.1	324	29
Orange	0.1	0	—
Mango	0.0	20	—
<i>Animal-source foods</i>			
Beef	3.0	0	—
Chicken	1.3	0	—
Eggs	1.1	0	—
Fish	0.5	0	—
Milk	0.4	0	—
Pork	1.9	0	—

bioavailability based on certain dietary characteristics. For example, unrefined cereal and/or legume-based diets generally have phytate : zinc ratios > 18, which is associated with relatively low zinc bioavailability. In contrast, mixed diets containing higher amounts of animal-source foods and less plant-source foods, or refined plant-based diets generally have phytate : zinc ratios between 4 and 18, which are associated with higher zinc bioavailability.

Other Causes of Zinc Deficiency

Under normal physiological conditions, zinc is secreted into the intestine in large quantities together with digestive juices, but is largely reabsorbed. Diarrhea may not only lead to a reduced absorption of dietary zinc during the episode due to decreased intestinal transit time, but may also cause an increase in the loss of endogenous zinc. Given the important role of the intestine in regulating dietary zinc absorption, and the secretion and reabsorption of endogenous zinc during digestion, conditions that affect the health or integrity of the intestine, such as tropical enteropathy, could interfere with the adequate maintenance of zinc balance. The contribution of these conditions to zinc deficiency in lower-income countries requires investigation.

High Physiological Requirements

In accordance with age and physiological status, some population groups have increased daily physiological requirements for absorbed zinc. During growth and pregnancy, the incorporation of zinc into newly synthesized tissue requires relatively larger amounts of zinc daily. Similarly, the amount of zinc transferred from mother to infant in breastmilk must be added to the lactating women's physiological requirement for absorbed zinc. These increased needs for zinc add to the challenge of acquiring sufficient amounts of absorbable zinc from the food supply. Those groups with higher zinc requirements and who are thus at elevated risk of zinc deficiency include: infants (particularly those born prematurely), young children, children recovering from severe malnutrition or diarrhea, adolescents, and pregnant and lactating women. Some evidence exists for the occurrence of zinc deficiency among each of these groups in lower-income country settings.

The elderly may also be at elevated risk of zinc deficiency, due to a decline in zinc intakes and possibly a reduction in the absorption of dietary zinc. However, evidence for zinc deficiency among the elderly has thus far only been reported from industrialized countries; studies are needed among elderly populations in lower-income countries.

Prevalence of Zinc Deficiency in Developing Countries

Guidelines on the assessment of population zinc status were recently published following a consensus conference convened by the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), and the International Zinc Nutrition

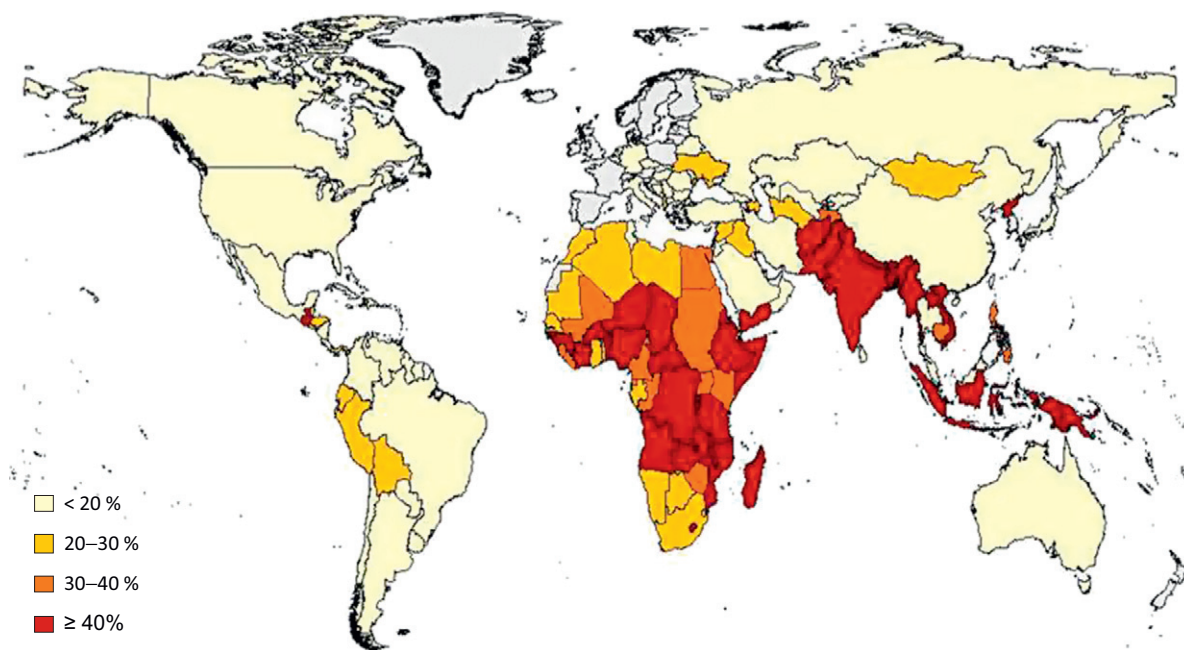


Figure 1 Prevalence of nutritional stunting in children under 5 years of age. Data derived from WHO or most recent Demographic Health Surveys. <http://www.izincg.org/stunning>

Consultative Group (IZiNCG). The three main types of zinc status assessment that were considered included biochemical, dietary, and functional methods. Because so little information is available from nationally representative surveys on the prevalence of low serum zinc concentration or inadequate dietary zinc intake, current estimates of the extent of risk of zinc deficiency must rely on the prevalence of stunting among children less than 5 years of age. Approximately 30% of children less than 5 years of age worldwide are stunted (height-for-age Z-score < -2 SD with respect to the distribution of the reference population data). WHO recommends a prevalence of stunting greater than 20% of the population to indicate a public health concern. The highest prevalence rates of stunting ($>30\%$) are observed in countries in sub-Saharan Africa, South Asia, Southeast Asia, and Central America. Intermediate prevalence rates (20–30%) are found in the Andean countries, some Central American countries, Southern Africa, and some countries in North Asia. As zinc deficiency is not the only factor affecting children's growth, assessment of dietary zinc intake and serum zinc levels should be used to confirm the risk of zinc deficiency in these countries. (Figure 1)

Consequences of Zinc Deficiency in Developing Countries: Evidence Derived from Zinc Supplementation Trials

In the context of developing country settings, present knowledge on the health consequences of zinc deficiency has been almost entirely derived from community-based trials of zinc supplementation among populations at possible risk of zinc deficiency. In these trials, individuals in the study population are randomly allocated to receive either a zinc supplement,

usually in the form of tablets or syrups, or the same supplement format without zinc (i.e., placebo). The condition under study is then monitored for a given period (typically for 2 months to 1 year), and the occurrence of or change in the condition is compared between the zinc-supplemented group and the corresponding control group. Given that several other nutritional and environmental factors can influence the health conditions hypothesized to occur with zinc deficiency, such studies have been essential in demonstrating unequivocally the causal role of zinc deficiency in these conditions among human populations. The following section provides an overview of the population groups at elevated risk of zinc deficiency, and the health consequences associated with zinc deficiency, as concluded from these studies.

Child Growth

Zinc plays an important role in child growth. Several mechanisms may be involved, including the role of zinc in the transcription and translation of genetic material and perhaps more importantly, the regulatory role of zinc in the endocrine system, which controls growth (i.e., the growth hormone-somatomedin axis). Specifically, zinc status is associated with the concentration of circulating insulin-like growth factor-1, the principal growth factor that controls early childhood growth. Among populations where growth restriction occurs, both height and weight gain have improved following supplemental zinc. Stimulation of linear growth appears to be the primary response, while the increase in body weight likely reflects the synthesis of lean tissue, such as bone, cartilage, and muscle, associated with linear growth. This is evident because, in general, weight does not increase independently of improved height in response to zinc supplementation.

According to a recent meta-analysis including 37 studies in infants, preschool, and older prepubertal children, zinc supplementation produces a small, but significant increase in linear growth and weight gain. Zinc deficiency has been demonstrated to be an important limiting factor to the growth of children across a wide range of geographical settings in developing regions. However, it should be noted that not all the studies have demonstrated a significant, positive effect of zinc on growth. Possible explanations for this include: the prevalence or severity of growth stunting in the study communities was low, zinc status was adequate, or deficiencies of other growth-limiting nutrients coexisted thus preventing a positive effect of zinc on growth. The latter situation may also explain the observation in some studies of a transient effect of zinc on growth.

Low-birth-weight infants (<2.5 kg) may have additional needs for zinc, presumably to facilitate their rapid postnatal catch-up growth. Some benefits of supplemental zinc to growth have been observed among low-birth-weight infants in the first 6 months of life.

Severely malnourished infants and children have exhibited improved rates of weight gain, height gain, or synthesis of lean tissue when supplemental zinc has been included in their usual rehabilitation treatment regimen. In these recovering children, zinc has been shown to augment the deposition of lean tissue by increasing protein synthesis. Thus, the inclusion of zinc among other micronutrients is recommended by WHO in the treatment of severely acute malnourished children.

Morbidity and Mortality

Zinc is involved in many aspects of the immune system, contributing both to specific and nonspecific immune functions. Zinc has an important role in both the prevention and treatment of diarrhea, which may be mediated both through functions in immune competence and maintenance of the integrity of the intestine. Preventive zinc supplementation reduces the incidence of diarrhea by approximately 20% among children in lower-income countries, although the current evidence indicates that this beneficial effect of zinc may be limited to children greater than 12 months of age. Zinc also has therapeutic benefits for recovery from diarrheal infections. Overall, supplemental zinc provided to children during either acute or persistent diarrhea leads to a reduction in the duration and possibly the severity of the episode. It has been recommended that zinc should be used in the management of acute diarrhea, in conjunction with oral rehydration salt solution. Zinc deficiency appears to also be associated with an increased incidence of pneumonia. Present evidence from studies that diagnosed acute lower respiratory tract infections (ALRI) based on counting respiratory rate or a physician's examination indicates that preventive zinc supplementation reduces the incidence of pneumonia and ALRI in children by about 21%. Some studies also suggest that zinc supplementation reduces the incidence of malaria, although this remains uncertain because of the limited amount of evidence that is currently available. Based on recent reviews on the impact of zinc on mortality, zinc supplementation reduces the overall mortality rate in children by 6–9%. Similarly to the impact of

zinc on diarrhea incidence, the benefits seem to be limited to children above 12 months of age in which the mortality reduction is approximately 18%.

Pregnancy

Zinc requirements during pregnancy have been estimated from the zinc content of accrued tissues during pregnancy. In addition to the zinc transferred to the fetus, zinc is deposited in the placenta, amniotic fluid and uterine, and mammary tissue. The estimated total additional zinc needed for pregnancy is approximately 100 mg. Few firm conclusions can be made as to the consequences of zinc deficiency during pregnancy on maternal, fetal, and infant health. Observational studies in human populations suggest that maternal zinc deficiency during pregnancy may cause adverse pregnancy outcomes for the mother and the fetus. However, results from zinc supplementation trials have been inconsistent and therefore difficult to interpret. This may be partly attributed to an inadequate study design or failure to consider the zinc status of the women studied. Nevertheless, a recent meta-analysis of supplementation trials indicates a 14% reduction in premature delivery among zinc-supplemented women.

Zinc Intervention Strategies

There are numerous zinc intervention strategies available and all have their strengths and weaknesses. While some approaches are considered short-term solutions, others may require a longer period until successful and effective implementation is achieved. In some cases, a combination of these intervention strategies may be needed to ensure the prevention of zinc deficiency in the most vulnerable population groups.

Preventive Zinc Supplementation

As described above, most of the evidences on the beneficial effects of zinc are derived from randomized controlled trials of preventive zinc supplementation and benefits include improved growth, reduced incidence of diarrhea, ALRI, and reduced all-cause mortality. Evidence to date shows no significant adverse effects on indicators of iron and copper status. It has been stated previously that zinc needs to be provided on a daily basis for an extended period of time, although weekly supplementation may also be beneficial. IZiNCG recommends a daily dose of 5 mg zinc for young children. Zinc supplementation is a short-term strategy that relies heavily on the availability of zinc supplements and individual compliance. The challenges for scaling up zinc supplementation programs are similar to those faced by other programs that attempt to procure and distribute nutritional supplements or medicines in lower-income countries. Several existing delivery platforms have been identified, which could be used for delivering preventive zinc supplements with or without other micronutrients. As these programs are being developed, monitoring and evaluation should be included to allow the required assessment of the program's effectiveness.

Therapeutic Zinc Supplementation in the Treatment of Diarrhea

WHO and UNICEF recommend that zinc supplementation should be included as a component in the treatment of all cases of diarrhea. It is recommended to provide children with 20 mg per day of supplemental zinc for 10–14 days (10 mg per day for infants under six months old) along with oral rehydration salt solution and continued feeding. The aim is that the recommendations become routine practice both in the home and health-care facility and that caretakers will act quickly at the first sign of diarrhea. Efforts are underway to reinforce national diarrhea programs and scale up the inclusion of therapeutic zinc supplementation in many countries.

A recent analysis confirmed that there is strong evidence that therapeutic zinc supplementation during diarrhea decreases the duration of diarrheal episodes by ~0.5 day. To what extent the dose of supplemental zinc provided during diarrhea episodes can prevent zinc deficiency is uncertain. Although it is believed that therapeutic zinc supplementation has the potential to prevent future diarrhea episodes following diarrhea treatment, present evidence is weak.

Food Fortification with Zinc

Food fortification is increasingly recognized as an effective approach to improve population's micronutrient status. Available absorption studies clearly show that zinc fortification can increase dietary zinc intake and total daily zinc absorption. Most studies also indicate that adding zinc to food does not adversely affect the sensory properties of the food or the absorption of other micronutrients, such as iron. However, despite the positive effect of zinc fortification on total zinc absorption, the impact as a public health intervention remains unknown.

There are different types of food fortification: foods fortified that are widely consumed by the general population (mass fortification), foods fortified for specific population subgroups, such as complementary foods for young children or rations for displaced populations (targeted fortification), and foods fortified voluntarily by the manufacturers and available in the market place (market-driven fortification).

Mass Fortification of Staple Foods

As described above, mass fortification is the addition of one or more micronutrients to foods commonly consumed by the general public, such as cereals, condiments and milk. Zinc fortification of cereal food staples (wheat flour, maize flour, or rice) is not yet widely practiced, but many new flour fortification programs are beginning to include zinc. Currently, in four countries—Indonesia, Mexico, Jordan, and South Africa—fortification of wheat flour with zinc is mandatory. Thirteen countries include zinc in voluntary wheat flour fortification programs, and five countries have recently proposed new programs that would include zinc. WHO recently adopted guidelines for fortification of wheat flour with zinc. The recommended levels range from 30 to 100 parts per million (ppm) depending on the extraction of the wheat flour and the estimated per capita flour consumption, and the amount of zinc and phytate in the rest of the diet. Although several

fortification compounds are generally recognized as safe (GRAS) by the US Food and Drug Administration, zinc oxide is most widely used in food fortification due to its low cost.

Targeted Fortification

Infants from 6 to 24 months of age in lower-income countries are especially vulnerable to deficiencies of zinc and iron because: (1) rapid rate of growth during this period imposes relatively high requirements of these nutrients, (2) breastmilk intakes decrease with age, and the zinc and iron contents of human milk decline to low levels in the second semester postpartum, and (3) most home-available complementary foods are limited in the amounts and bioavailability of these nutrients. Reviews of the theoretical nutrient requirements during the period of complementary feeding indicate that it is very difficult for infants in lower-income countries to meet their requirements for zinc and iron from home-available preparations. One approach is to fortify complementary foods to help meet the physiological needs of young children. However, recent analyses of commercially available fortified complementary foods revealed that the zinc content is in many cases insufficient. Various strategies for 'home' fortification, or point-of-use fortification (POUF), have been developed to ensure adequate micronutrient intakes by infants and young children. These types of products include micronutrient powders, crushable tablets, and lipid-based nutrient supplements, which are added to the complementary food at the time of consumption. To date, there is only inconsistent evidence that zinc fortified complementary foods or POUF can improve zinc status, as measured by biochemical or functional indicators. Whether this is due to the low bioavailability of zinc from cereal-based complementary foods (due to the high phytic acid concentration) or due to other reasons is uncertain.

Dietary Diversification and Modification

The most desirable approach to eliminate zinc deficiency will be to ensure access to diets with adequate zinc content and bioavailability. Dietary diversification and modification have the potential to prevent deficiencies of zinc and other micronutrients. The best strategy for enhancing the zinc content of household diets is to promote the consumption of meat, poultry and fish, all good sources of readily-available zinc. This is because beef, pork, lamb, and liver have a higher content of readily absorbed zinc (3.0 to 6.8 mg of zinc/100 g) than poultry (~1.1 to 2.7 mg of zinc/100 g), eggs (~1.0 to 1.3 mg of zinc/100 g), dairy products (~0.3 to 1.0 mg of zinc/100 g), or finfish (~0.3 to 0.7 mg of zinc/100 g for flesh only; ~3.2 mg of zinc/100 g for whole, soft-boned fish with bones).

Several household food preparation and processing methods can be used to reduce the phytate content of diets based on cereals and legumes. These methods are based on the enzymatic hydrolysis of phytic acid to lower inositol phosphates that occurs during germination and fermentation. A combination of dietary strategies involving increased consumption of animal-source foods and phytate reduction is the preferred method to enhance both the content and bioavailability of zinc in the diets in rural areas of lower-income countries. To be effective, such strategies must be

integrated with ongoing national agriculture, food, nutrition, and health education programs and implemented using a participatory approach to ensure their acceptability, adoption, and sustainability.

Promotion and support of appropriate breastfeeding practices should also be considered among the recommended dietary strategies to enhance the zinc status of infants and young children, for two reasons: breastmilk is an important source of bioavailable zinc, and breastfeeding protects against diarrhea, which causes excessive zinc losses. Public health programs targeting young children should consider the promotion of the three key recommendations: (1) early initiation of breastfeeding, (2) exclusive breastfeeding to 6 months, and (3) continued breastfeeding to 24 months.

Biofortification

Biofortification is an agricultural strategy that aims to increase the content of selected micronutrients, including zinc, in staple foods such as rice, wheat, maize, pearl millet, and others. Biofortification of staple foods can be achieved through the following processes: conventional breeding, by selecting for genotypes with the highest micronutrient content observed for that crop; use of genetic modifications, such as gene insertions or induced mutations; and use of agronomic practices, such as applications of zinc-containing fertilizers. When consumed, biofortified staple foods would lead to improved adequacy of zinc intakes and hence a reduced risk of dietary zinc deficiency, among those who currently have high rates of inadequate intakes. Although the feasibility and efficacy of biofortification to prevent zinc deficiency still needs to be evaluated through efficacy trials, theoretical analyses indicate that it has a potential to improve zinc intakes in adults and children.

See also: Bioavailability. Biofortification. Breast Feeding. Diarrheal Diseases. Food Fortification: Programs. Growth and Development: Physiological Aspects. Nutritional Problems of Pre-School Children. Supplementation: Programmatic Issues

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Physiology, Dietary Sources, and Requirements

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Glossary

Acrodermatitis enteropathica A genetic disorder leading to zinc deficiency, associated with mutations in the ZIP4 transporter and impaired zinc absorption.

Metallothionein A small, cysteine-rich zinc binding protein that modulates intracellular zinc homeostasis and acts as an antioxidant.

MTF-1 A zinc-regulated transcription factor.

ZIP transporters A family of zinc transporters responsible for moving zinc into the cytoplasm. Also known as SLC39.

ZnT transporters A family of zinc transporters responsible for moving zinc out of the cytoplasm. Also known as SLC30.

Introduction

Zinc is moderately abundant in nature, ranking 23rd of the elements. Of the trace elements in the body, it is second only to iron, but unlike iron, it has only a single redox state. Together with its size and charge characteristics, this has led to its widespread use within proteins of the body. The number of zinc proteins is unknown but growing, including several hundred enzymes and many more nuclear proteins that regulate gene expression. Additional proteins are responsible for zinc homeostasis. Binding sites and functions of zinc within some of these proteins are well understood, but for others this is less clear. In particular, the links between these biochemical roles of zinc within proteins and its physiological functions are often obscure. The range of physiological functions of zinc is broad and can be observed in all tissues of the body. In general, zinc is required for DNA synthesis, cell division and growth, for protein synthesis and macronutrient metabolism, and for the development and function of most body systems. Lack of an appropriate assessment tool makes it difficult to estimate deficiency prevalence, but undiagnosed marginal zinc deficiency may be a concern.

History of Zinc as a Nutrient

The essentiality of zinc for bacterial growth has been known for almost 150 years. Later, it was shown to be required by plants and then in 1934 for the rat. Because of its broad distribution in the food supply, human zinc deficiency was initially thought to be unlikely. However, in the early 1960s Prasad and others in Iran described a syndrome of dwarfism and lack of sexual development in teenage boys and young adults. The Iranian young men consumed a diet based on unleavened bread with very little animal protein and also ate large amounts of clay (geophagia). They were anemic and responded to treatment with ferrous sulfate, coupled with a more balanced diet including animal protein. The other symptoms also resolved but it seemed unlikely that lack of iron itself was responsible. Prasad then moved to Egypt, where he encountered a similar syndrome. These patients were not geophagic, but ate mostly bread and beans and also were infested with schistosomiasis and

hookworm. Zinc deficiency was documented in these individuals and treatment with zinc was shown to be more effective at increasing growth rates than either iron or a diet with animal protein. Thus dietary zinc deficiency was demonstrated, presumably due to impaired absorption because of the high fiber and phytate contents of the diet.

Chemistry of Zinc

The conjunction of chemical properties of zinc underlies its biological significance. It is a relatively small ion (atomic number = 30) and carries a positive charge of two. It attracts electrons as a strong Lewis acid and this property can be important in its catalytic functions. It has relatively flexible coordination geometry and while binding its ligands with high affinity, exhibits rapid rates of exchange that can facilitate chemical reactions and biological processes. Its single redox state, in contrast to iron or copper, eliminates danger of oxidative damage. Although other trace elements share some of these properties, none share them all. This is what makes zinc so valuable for protein structure and function.

Zinc in Foods

Zinc is associated with proteins in the body and is found associated with proteins in food. Protein rich foods tend to be good sources (Table 1). However, there is great variability, ranging from egg whites, which have almost no zinc, up to oysters at 400 mg kg⁻¹. Legumes and grains have moderate zinc content, though refinement results in large losses, whereas fruits and vegetables are poor sources. Zinc bioavailability is quite variable, due to other food components eaten at the same time. A principal concern is phytate, which renders zinc unavailable. On the other hand, animal proteins appear to enhance zinc absorption, perhaps because amino acids derived from them keep zinc in a soluble form. Enriched breakfast cereals can make significant contributions to zinc intake.

Table 1 Dietary sources of zinc

Food	Zinc content mg kg ⁻¹ raw weight
Oysters	400
Beef, lean	50
Pork	26
Chicken	
Breast	8
Leg	18
Salmon	4
Egg	
Whole	11
White	0.3
Milk	
Whole	4
Cheese	
Cheddar	31
Wheat	
Whole flour	29
White flour	7
Rice	
Brown	20
Polished	12
Breakfast cereals, fortified	100–500
Kidney beans	27
Lentils	36
Potatoes	3
Broccoli	4
Apples	0.4

Control of Zinc Homeostasis

The size and charge characteristics of zinc mandate the use of carriers to traverse biological membranes. Two families of transporters have been described and partially characterized. The ZIP family (also called Solute Carrier 39; SLC39), functions to move zinc into the cytoplasm, either from outside of the cell or from subcellular compartments. The second group of transporters, the ZnT family (SLC30), is responsible for zinc egress from the cytoplasm. Fourteen transporters belonging to the ZIP and 10 belonging to the ZnT family have been identified. Cellular zinc homeostasis is tightly regulated by these transporters in normal and pathological states. For example, the normally zinc-rich prostate exhibits zinc loss with malignancy that is associated with the down regulation of ZIP transporters. ZnT-1 is localized to plasma membranes and functions as a cellular efflux protein. ZnT-2 transports zinc into storage vesicles under conditions of high cellular zinc. Collectively, the ZIP and ZnT proteins are likely to

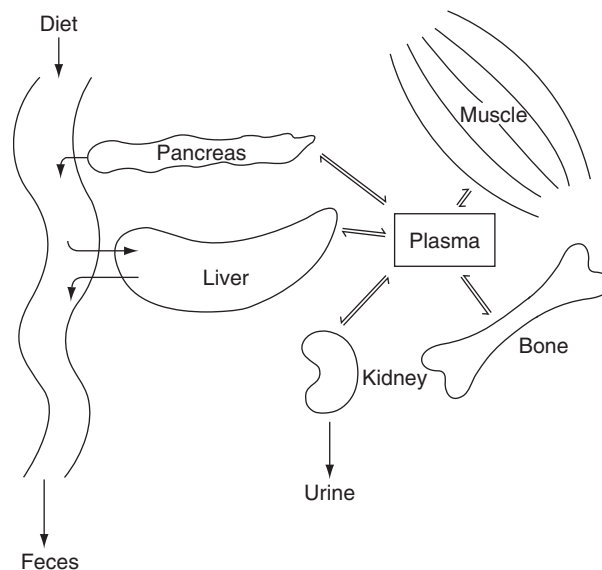


Figure 1 Whole body zinc homeostasis. Zinc in the intestine comes from the diet and endogenous secretions. A portion is absorbed, but much is lost in the feces, which constitute the major site of excretion. Absorbed zinc passes through the liver and then to the general circulation. Zinc is distributed throughout the body with muscle and bone constituting the largest pools. A minor but controlled amount of zinc is lost in the urine.

underlie the homeostatic control of zinc distribution around the body.

Zinc Absorption

The absorption, distribution, and excretion of zinc are shown in **Figure 1**. Overall, approximately 20–40% of consumed zinc is absorbed, depending on bioavailability within the particular food source. Zinc is absorbed by both saturable and non-saturable processes, with the greatest rates of absorption occurring in the jejunum. Absorption is adjusted to meet needs, being proportionately increased in deficiency states and reduced when intake is high. Zinc status is reflected by the intestinal concentration of the zinc binding protein, metallothionein (MT). MT may trap zinc within the epithelial cells, causing it to be lost to feces as the cells are sloughed off. This may be part of the explanation of how zinc absorption is adjusted to meet needs. Acrodermatitis enteropathica is an autosomal recessive condition of zinc malabsorption, which can lead to severe deficiency. Gene mutations that lead to this condition have been identified in *ZIP4*, which encodes one of the zinc transporters. This protein has been localized to the apical membrane of intestinal epithelial cells and, given the severity of symptoms associated with its inactivation, appears to be necessary for normal zinc absorption. The zinc efflux protein ZnT-1 is found at the basolateral membrane and so likely promotes passage of zinc out of the intestine. Acrodermatitis enteropathica can be treated with large doses of zinc, supporting the existence of paracellular transport at high intake levels. A large amount of zinc is secreted into the gut from the pancreas and intestine (**Figure 1**). Malabsorption

syndromes can lead to a failure to reabsorb these endogenous secretions and a rapid loss of body zinc.

Transport and Distribution

The zinc plasma pool is relatively small, representing only approximately 0.1% of total body zinc. It circulates bound to albumin and α -2-macroglobulin, with approximately 3% complexed with amino acids. Approximately five-fold greater amounts of zinc are found in whole blood, with erythrocytes accounting for approximately 75% of the total. However, approximately 85% of erythrocyte zinc is complexed within carbonic anhydrase and therefore does not exchange easily. Egress of zinc from the circulation across endothelial cells and into tissues of the body is not well understood. Uptake in association with albumin has been suggested but members of the ZIP family of transporters are likely to play a role here. The tissue distribution of zinc is relatively uniform. All cells require the mineral and no cell stores it. The concentration of zinc in the adult human is approximately $0.5 \mu\text{mol g}^{-1}$, giving a total body content of approximately 2 g. More than half is found in skeletal muscle and approximately 30% in bone. The bone pool appears to be more labile than the muscle pool and this has been used as an index of zinc status in experimental animals. Liver represents another labile pool. It receives dietary zinc from the portal circulation and contains approximately 5% of body zinc. At the cellular level, approximately 30–40% zinc is present in the nucleus whereas, 50% of zinc is distributed among the cytoplasm, organelles, and specialized vesicles. The remaining zinc is associated with membranes.

Excretion

Zinc is lost from the body primarily through the feces (Figure 1). Feces contain unabsorbed dietary zinc, zinc contained within intestinal epithelial cells, which have been sloughed off, and endogenous secretions into the gut from the pancreas, the gall bladder, and the cells lining the gastrointestinal tract. The endogenous secretions and the extent to which they are reabsorbed can be controlled and represent an important homeostatic mechanism for regulating zinc status. Zinc losses in urine are relatively minor, but do respond to extremes of intake to help maintain homeostasis. Shed skin cells, sweat, hair, menstrual blood, and semen represent additional routes of loss.

Zinc Biochemistry

Zinc homeostasis and action involves an intimate association of the mineral with proteins. These include membrane transporters responsible for the absorption of zinc in the gut and its passage into and out of cells and subcellular organelles, transport and delivery proteins, both in the circulation and within cells, sensing proteins that adjust homeostasis and function according to zinc availability and then a large range of proteins to which zinc is ultimately delivered. Two major classes of these latter proteins are the enzymes and transcription factors.

Homeostasis

The interaction of zinc with its transporters has not been well characterized, though transmembrane domains have been identified thought to be responsible for the transport function. Free concentrations of zinc within the cell appear to be extremely low and may not constitute a sufficient pool for the supply of zinc to its protein ligands. This implies the existence of delivery proteins, a role suggested for MT, which has been shown to transfer zinc to apoenzymes *in vitro*. MT is a small protein that is unusually cysteine-rich and can bind seven atoms of zinc. It may influence the subcellular distribution and availability of zinc, because its own distribution varies. For example, the nuclear content of MT varies with the cell cycle. MT expression is regulated by zinc and also by a range of other signals including glucocorticoids, interleukins, and cAMP. In addition, its zinc binding activity is influenced by the cellular redox state. For example, an increase in the glutathione disulfide/glutathione ratio results in release of zinc from MT and thus its availability for other proteins.

Investigation of the mechanism whereby zinc regulates the expression of MT led to the discovery of the single protein known to act as a zinc sensor within mammalian cells, MTF-1 (metal response element (MRE)-binding transcription factor-1). MTF-1 binds to MREs in the promoter region of MT and other genes and regulates their expression. The ability of MTF-1 to localize to the nucleus and bind to its target genes is dependent on its zinc content. Thus, an increase in cellular zinc concentrations results in greater MTF-1 activity and elevated expression of its target genes. In addition to MT, which will bind more zinc, these include ZnT-1, which will transport zinc out of the cell. Both actions serve to buffer the increase in cytosolic zinc (Figure 2).

Zinc Enzymes

The three-dimensional structures of more than 200 zinc-containing enzymes have now been characterized, and many more have been identified. All six International Union of Biochemistry classes are represented. In some cases (e.g., carbonic anhydrase), zinc is a direct participant in the catalytic function of the enzyme. The zinc atom is coordinated by three amino acids from the enzyme and a molecule of water at the active site. Other enzymes (e.g., protein kinase C) have structural zinc sites, where the metal binds four amino acids within the protein and ensures appropriate folding for bioactivity. For nitric oxide synthase, zinc has been found to serve a bridging function between two separate polypeptides, stabilizing a biologically active larger complex. A selection of zinc enzymes is listed in Table 2 and help to illustrate the wide variety of metabolic functions requiring zinc.

Zinc Transcription Factors

There are many zinc enzymes but even more transcription factors utilize zinc. Variable numbers of zinc atoms are each coordinated by four cysteine or histidine residues to stabilize a DNA binding structure. A search of the human genome has revealed over 1000 genes (approximately 3% of those identified) containing these characteristic zinc finger domains. An

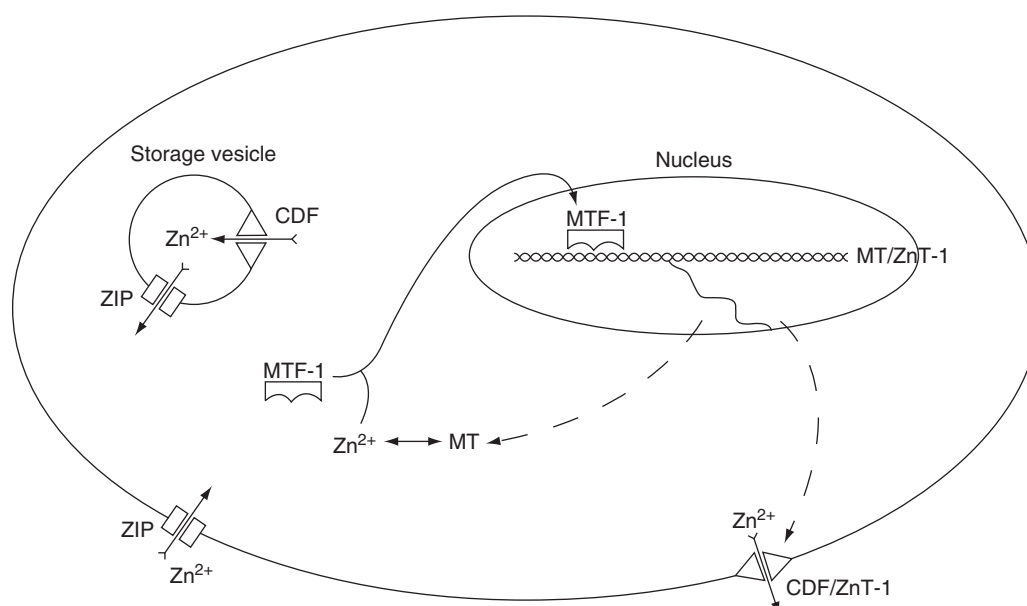


Figure 2 Cellular zinc homeostasis. Zinc is delivered to the cytoplasm from either the extracellular space or vesicles within the cell by members of the ZIP family of transporters. A rise in cellular zinc results in activation and nuclear translocation of MTF-1. In the nucleus, MTF-1 regulates transcription of a set of target genes including MT and ZnT-1. MT will bind zinc and ZnT-1 transports zinc out across the plasma membrane. MT may govern delivery of zinc to other proteins within the cell. Other members of the ZnT family transport zinc into vesicles.

Table 2 Examples of mammalian zinc-dependent enzymes

Enzyme	Function
RNA polymerase	Transcription, synthesis of mRNA
Carboxypeptidase A	Protein digestion in intestine
Protein kinase C	Signal transduction
Carbonic anhydrase	Respiration/buffering/hydration of CO_2
Cytochrome <i>c</i> oxidase	Respiration/electron transport chain
Alcohol dehydrogenase	Ethanol metabolism
Superoxide dismutase	Inactivation of free radicals
Nitric oxide synthase	Signaling, vasodilation
Angiotensin converting enzyme	Blood pressure regulation/activation of angiotensinogen

important class of zinc finger transcription factors is the steroid/thyroid receptor superfamily, responsible for mediating the biological response to a wide range of hormonal and metabolic signals, including retinoic acid and vitamin D. These all have nine conserved cysteine residues in the DNA binding region, eight of which are coordinated by two atoms of zinc. Loss of zinc from these sites would interrupt biological function but it is not clear that this ever happens in a physiological context. Gene expression profiling has been used to assess the genome-wide response to changing zinc availability in different tissues, including intestine, liver, and cells of the immune system. The gene products identified as zinc-sensitive by these approaches amount to approximately 5% of the expressed genes within a tissue. MTF-1 is likely to mediate some but not all of these changes and other transcription factors whose activity is dependent on zinc may soon be found.

Zinc Physiology

The enormous range of biochemical roles for zinc predicts broad effects on physiological function. The physiological roles of zinc may be further extended by secondary effects mediated by altered food intake and effects on the function of other nutrients. Although the physiological roles for zinc are well described, it is important to note that the connections between the biochemistry and physiology of zinc remain unclear. Thus the specific zinc-sensitive biochemical step leading to altered physiology is often unknown. The broad spread of zinc through the body at the organ, cellular and even protein levels suggests that the function of most systems is dependent on zinc. Its physiological roles become manifest under the circumstance of deficiency and that framework will be used to discuss principal functions here.

Growth

The requirement of zinc for growth of numerous organisms ranging from bacteria to humans is well established. Growth failure is an early consequence of zinc deficiency in experimental animals. Numerous processes seem to contribute to the growth failure. Experiments with animals have shown that zinc deficiency leads to a drop in food intake, though the use of pair-fed controls demonstrates clear effects of zinc deficiency beyond feeding behavior. Multiple effects of zinc deficiency on the somatotrophic axis have been demonstrated, notably a reduction in circulating concentrations of insulin-like growth factor-1 (IGF-1). Again, this is only part of the story because administering exogenous IGF-1 does not restore the growth failure of zinc deficiency. Growth of cultured cells

is dependent on media zinc. DNA synthesis is interrupted and production of thymidine kinase mRNA is diminished by removal of zinc, but again this is only a partial explanation. The IGF-1 signaling pathway within cells also seems to be affected. Zinc is also required for wound healing, presumably due to related processes.

Immune Function

The immune system is particularly sensitive to zinc deficiency. Lymphopenia and thymic atrophy are observed and both cell-mediated and antibody-mediated responses are reduced. As with growth, multiple mechanisms appear to be at play. In addition to generalized effects on DNA synthesis, zinc deficiency appears to induce apoptosis, resulting in a loss of B and T cell precursors within the bone marrow. Thymulin, a zinc-dependent enzyme that stimulates the development of T cells within the thymus, may be involved. Production of cytokines by mononuclear cells is also reduced by zinc deficiency. These effects can be of clinical significance. Infections occur more frequently in individuals with acrodermatitis enteropathica and reduced immune function is accompanied by zinc deficiency in several other conditions, including sickle cell anemia and various gastro-intestinal disorders.

Zinc has been used therapeutically in individuals with immune-compromised diseases. In the United States, zinc lozenges have become popular as a treatment for the common cold. Zinc supplementation has also been used as a supporting therapy for patients with HIV and AIDS, herpes simplex viruses, and hepatitis C infections. A shortening of cold duration, improved phagocytosis and increase in the number of Th cells, and a reduced frequency of opportunistic infections was observed in patients with HIV infection. However, results from controlled trials of zinc treatment with these diseases have been variable. Treatment effectiveness may depend on initial zinc status, with greater success being seen in individuals with marginal, undetected zinc deficiency.

Reproduction

The original description of zinc deficiency in humans included lack of pubertal development. Spermatogenesis is a zinc-dependent process. Seminal fluid is particularly rich in zinc and the sperm appears to accumulate zinc from this source before ejaculation. Zinc is also crucial for normal fetal development and deficiency leads to abnormalities in humans and animals. Maternal zinc deficiency has also been linked with pregnancy-associated morbidity, including preterm delivery.

Nervous System

The brain is one of the sites shown to be particularly sensitive to zinc deficiency during fetal development, with neural tube defects and other disorders being found. Although this work was performed with animals, a similar relationship appears likely with humans. Zinc is distributed throughout the brain, but greater concentrations are found within the hippocampus. Here a brain-specific transporter, ZnT-3, concentrates zinc in vesicles within glutamatergic neurons. It is co-secreted with

the neurotransmitter and appears to serve as a modulator of neurotransmission. Very high concentrations of zinc ($> 100 \mu\text{M}$) are found within the synaptic cleft during this process. In addition, brain injury, resulting from ischemia or trauma causes the release of massive amounts of zinc, which is thought to be responsible for the resultant cell death.

Antioxidant Defense System

Although not an antioxidant itself, there are several ways in which zinc participates in the antioxidant defense system of the body, with important implications for health. It can bind to thiol groups in proteins, making them less susceptible to oxidation. By displacing redox-reactive metals such as iron and copper from both proteins and lipids it can reduce metal-induced hydroxyl radical formation and thus protect the macromolecules. Copper/zinc superoxide dismutase is an important antioxidant enzyme, which contains zinc and whose activity is impaired in the deficient state. Zinc depletion affects the activity of reactive oxygen species (ROS) and reactive nitrogen species (RNS) scavengers such as glutathione, ascorbic acid, uric acid, and α -tocopherol. The role of zinc in inducing MT has already been mentioned and this protein scavenges hydroxyl radicals. Increased oxidative stress results in the release of zinc from MT, presumably making it more available for other proteins. However, although zinc deficiency has been linked to greater oxidative stress, excess zinc can also lead to the same condition. So although MTs possess intrinsic antioxidant properties, there is evidence that in some circumstances, MTs may also serve as a source of injurious zinc release. Recent findings also suggest that nitrosative stress can act as a critical trigger for zinc mobilization. Nitric oxide (NO) or peroxy nitrite ($-\text{ONOO}$) interact with MT and promote zinc release both *in vitro* and *in vivo*.

The likelihood of increased oxidative stress under conditions of zinc deficiency suggests a potential anticarcinogenic role for this mineral. Indeed, zinc deficiency has been shown to result in DNA damage. The tumor suppressor gene, p53, which is frequently mutated in human cancers, is a zinc-containing transcription factor whose expression is also dependent on zinc. Dysregulation of cellular zinc homeostasis is linked not only to oxidative stress but also mitochondrial dysfunction and activation of apoptotic pathways.

Macronutrient Metabolism

Many of the enzymes of intermediary metabolism contain zinc and deficiency affects all macronutrients. Protein synthesis, as well as DNA and RNA synthesis requires zinc. Insulin is secreted from the pancreas and circulates in association with zinc. This secretion is diminished under conditions of deficiency, leading to impaired glucose metabolism. Lipid metabolism is also affected, with zinc deficiency associated with reductions in circulating high-density lipoprotein.

Human Zinc Deficiency

In addition to dietary inadequacy, there are several other routes that lead to zinc deficiency. Acrodermatitis enter-

pathica, the genetic disorder of zinc malabsorption has already been mentioned. Other, more generalized malabsorption syndromes (e.g., celiac disease) can also lead to zinc deficiency. Deficiency has also resulted from inappropriate intravenous feeding and use of chelation therapy. Children are likely to be particularly at risk for zinc deficiency, because of its involvement in growth.

Mild

Given the difficulty of assessing marginal impairments in zinc status, effects of deficiency can often only be verified by a response to treatment. Growth provides a good example of this. Children in Denver, Colorado who were of low height for their age showed increased growth rates in response to zinc supplementation, whereas zinc had no effect in children of normal height. In addition to growth, improvements in immune function, taste and smell acuity, and reproductive function have been noted with zinc supplementation.

Severe

Severe human zinc deficiency has been well characterized by the original descriptions in the Middle East and in patients with acrodermatitis enteropathica. The symptoms of mild deficiency are continued and exaggerated. Thus stunting can be extreme and is accompanied by delayed sexual maturation and impotence. Characteristic skin lesions are found, originating around the mouth and nose but becoming widespread as deficiency develops. Diarrhea is also present. Deficits in taste and smell are accompanied by anorexia and other behavioral changes, including increased irritability and impaired cognitive function. Eye pathologies similar to those seen with vitamin A deficiency are observed.

Zinc Toxicity

Toxicity of zinc from food sources has not been reported and seems unlikely because absorption is homeostatically regulated. Acute gastro-intestinal symptoms and headaches from ingestion of amounts approximately 10–20-fold higher than the recommended intakes have been described. Chronic ingestion of these large amounts has been shown to impair immune response and lipoprotein metabolism. However, the

key danger from excessive zinc intake is reduced copper status. This is likely due to a zinc-induced blockage of copper absorption and in fact is clinically useful in individuals with Wilson's disease, a condition of copper toxicity. In the United States, an Upper Limit of 40 mg day⁻¹ has been set for adults, based on the threat to copper status. The popularity of zinc lozenges for treatment of the common cold could lead to circumstances where this intake could be exceeded. Thus use of these treatments should be limited in duration.

Assessment

The prevalence of marginal zinc deficiency in human populations is unknown because of the lack of a good means for assessing zinc status. Measurement of plasma zinc is straightforward, but it does not serve as a reliable indicator of status. Plasma zinc is a quantitatively minor pool that can be easily influenced by minor shifts in tissue zinc. Plasma concentrations do not fall with dietary intake, except at very low intakes. Plasma zinc can also be affected by other factors unrelated to zinc status (e.g., time of day, stress, infection). Cellular components of blood can be assayed, but erythrocyte concentrations of zinc are maintained in deficient states and variable results have been found with leukocytes. Hair zinc concentrations may reflect available zinc but will also be dependent on rate of hair growth.

Several different zinc-dependent enzymes have been investigated as potential markers of zinc status but none has proved reliable. MT in blood cells has been suggested as a useful indicator of zinc status, either assayed at the protein or mRNA levels. MT expression is likely to be regulated by factors other than zinc and therefore may lack the specificity required of a good indicator. The gene array approaches that have recently been used to determine the global effects of zinc deficiency within a tissue would appear to offer hope for the identification of an appropriate functional marker of zinc status.

Recommended Intakes

In the absence of a reliable index of zinc status, both the US Food and Nutrition Board and the Food and Agriculture

Table 3 Recommended intakes for zinc

Age group		US and Canadian recommended dietary allowance	FAO/WHO reference nutrient intake		
			Bioavailability		
			High	Moderate	Low
Children (1–3 years)		3	2.4	4.1	8.3
Adolescents (14–18 years)	Female	9	4.3	7.2	14.4
	Male	11	5.1	8.6	17.1
Adults (> 19 years)	Female	8	3.0	4.9	9.8
	Male	11	4.2	7.0	14.0
Pregnant women	3rd trimester	11	6.0	10.0	20.0
Lactating women	0–3 months	12	5.8	9.5	19.0

Organization/World Health Organization (FAO/WHO) expert committee used the factorial method to estimate human zinc requirements. Under this approach, routes of zinc loss from the body are estimated and summed. The requirement is then set as the amount of dietary zinc required to make up for these losses, making appropriate assumptions about bioavailability. For children, an additional amount is added to account for the zinc requirements for growth.

As shown in **Table 3**, the FAO/WHO give three sets of recommendations, depending on the zinc bioavailability of the diet. The US figures fall between those given for moderate and low availability diets. Both groups also set Upper Limits for intake, based largely on the risk of impairing copper status. These values are similar (40 mg US, 45 mg FAO/WHO, for adults).

See also: Antioxidants. Bioavailability. Children: Nutritional Requirements. Cofactors: Inorganic. Copper. Cytokines: Nutritional Aspects. Inborn Errors of Metabolism: Classification and Biochemical Aspects. Nutrient–Gene Interactions: Health Implications; Molecular Aspects. Zinc: Deficiency Disorders and Prevention Programs

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Notes

Cross-references terms in *italics> are general cross-references, or refer to subentry terms within the main entry (the main entry is not repeated to save space). Readers are also advised to refer to the end of each article for additional cross-references – not all of these cross-references have been included in the index cross-references.*

The index is arranged in set-put style with a maximum of four levels of heading. Major discussion of a subject is indicated by bold page numbers. Page numbers suffixed by *T* and *F* refer to Tables and Figures respectively. *vs.* indicates a comparison.

Where index subentries and sub-subentries pertaining to a subject have the same page number, they have been listed to indicate the comprehensives of the text.

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