The Aging Male

ISSN: 1368-5538 (Print) 1473-0790 (Online) Journal homepage: www.tandfonline.com/journals/itam20

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To cite this article: T. V. Rajan, Jane Kerstetter, Richard Feinn & Anne Kenny (2014) Evidence for low androgenicity among Indian (South Asian) men, The Aging Male, 17:1, 30-34, DOI: 10.3109/13685538.2013.832192

To link to this article: https://doi.org/10.3109/13685538.2013.832192

Published online: 11 Nov 2013.

The AGING MALE

http://informahealthcare.com/tam
ISSN: 1368-5538 (print), 1473-0790 (electronic)

Aging Male, 2014; 17(1): 30–34
 DOI: 10.3109/13685538.2013.832192

informa healthcare

ORIGINAL ARTICLE

Evidence for low androgenicity among Indian (South Asian) men

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Abstract

There are increasing data indicating profound ethnic differences in the levels of virilization of males [1–4]. It is well understood that the intensity of testosterone-mediated effects is modulated by sex hormone binding globulin (SHBG) [5] and the CAG repeat lengths in the androgen receptor (*AR*) gene [6]. We determined the serum testosterone, estradiol and SHBG levels and average CAG repeat lengths among a group of healthy older Indian men living in Connecticut, USA and compared these parameters with those of a reference group of white Caucasian men. We also compared various parameters that represent the end-manifestations of testosterone activity – serum prostate-specific antigen (PSA) levels, lean body mass, skeletal mineralization and visceral fat. Our data suggest that men from the Indian subcontinent are smaller, manifest lower levels of circulating free testosterone, lower mean PSA levels and lean body mass, but are comparable to white Caucasian men in terms of SHBG, estradiol, levels of visceral fat and CAG repeat length. These data suggest that Indian men manifest a lower level of virilization compared to white Caucasian males and that this might be due to lower mean circulating testosterone levels rather than higher *AR* CAG repeat length or SHBG.

Keywords

Androgen, South Asian men, body composition, androgen receptor, secondary sexual characteristics

History

Received 15 February 2013
Revised 24 July 2013
Accepted 3 August 2013
Published online 11 November 2013

Introduction

Testosterone (T) and its active metabolite dihydrotestosterone (DHT) are the limiting factors that determine the level of virilization [7]. These two compounds exert effects with differing potency on almost every tissue in the body, but most prominently on the prostate, skeletal muscle, bone, certain hair follicles and the secondary sex organs. In the absence of testosterone, virilization of the indeterminate mammalian fetus does not take place, and development proceeds along the default female pathway [8].

The intensity of activity of T and DHT at the end-organ level is modulated by several factors [9]. Not all of the testosterone in circulation is available to exert its effects. A circulating protein of hepatic origin, sex hormone binding globulin (SHBG), binds to testosterone with high affinity and prevents it from interacting with cells in target organs [5]. Changes in SHBG levels alter testosterone activity in complicated ways. Part of the complexity lies in the fact that SHBG binds not only to testosterone but also to female sex hormones, albeit with lower affinity [10], and that the ratio of testosterone to estrogen may affect physiology and disease [11]. In the presence of saturating amounts of testosterone, SHBG is fully bound to testosterone, making estrogen more effective. Within the cell, the structure of the androgen receptor (AR) itself modulates the efficacy of the hormone [6]. Receptors bearing a large number of glutamine repeats at the N-terminus, encoded by the population-polymorphic CAG repeat in the first exon of the *AR* gene, transduce the effects of testosterone at lowered efficiency. The normal range of glutamine residues appears to be between 19 and 37. Individuals who bear an *AR* gene with greater than 37 CAG repeats of the first exon (therefore encoding more than 37 glutamine residues near the N. terminus), suffer from the Kennedy syndrome or X-linked spinobulbar muscular atrophy [12]. Within the normal range of glutamine residue numbers, patients with high numbers tend to be less virilized for a given circulating androgen level. Therefore, even with the same level of circulating testosterone, the final level of virilization achieved can be substantially different among different individuals.

There are substantial data to indicate that males belonging to different ethnic groups differ in the level of virilization, even though there may be similarities in circulating testosterone levels [1–3,13]. These differences are of more than just academic interest. Subjects with high androgenic activity appeared to be more prone to prostatic hypertrophy as well

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(more controversially) to prostatic cancer [14]. At the other end of the scale, patients with lower levels of androgenization appeared to be more prone to bone loss as they age, as well as to sarcopenia [7,15]. Furthermore, they respond to more readily to contraceptive agents with azoospermia [16].

We have recently shown that Indian males living in the United States appear to be at higher risk for fragility bone fractures than their white Caucasian counterparts [17]. In this study, we investigated whether some of these differences in bone health between these two groups may be due to differences in levels of virilization. We report in this study in that the two groups are comparable in many parameters we analyzed. However, the mean skeletal muscle mass, bone density and serum prostate-specific antigen (PSA) levels are lower in Asian Indian males than in their white Caucasian counterparts, indicating lower androgenic effect in these tissues. Indian men have lower free testosterone, in agreement with previous data from England on Pakistani males in comparison with white counterparts [1,4].

Materials and methods

Study subjects

Subjects were recruited through circulars and advertisements approved by the Institutional Review Board. Subjects were also recruited with the help of Asian Indian cultural associations in Connecticut (Connecticut Tamil Sangam, Connecticut Valley Hindu Temple Society, Middletown, CT, and Sneha, Inc.). Subjects were excluded if they had a history of any major illness likely to affect bone health, chronic renal failure, parathyroid disorders, inflammatory arthritis or used any drugs that affect bone health such as anti-epileptics, steroids or bisphosphonates. The ''Asian Indian'' cohort had to have both parents to be of Asian Indian descent to be included in the study. The study protocol was approved by the Institutional Review Board at the University of Connecticut Health Center, Farmington, CT, and written informed consent was obtained from all subjects. Caucasians were identified by self-reporting.

The Institutional Review Board at the University of Connecticut Health Center approved the study, and all men gave written informed consent prior to evaluation. Men from the central Connecticut region were recruited for a longitudinal study of the use of testosterone in older men. Baseline information was used for this evaluation. Mailings were sent to men over 60 years of age from databases of men who had a history of a hip fracture and a general mailing to recruit men over 60 years of age for those without a history of fracture. Men with a femoral fracture were included if the fracture was non-traumatic and had occurred in the preceding 3 years. Exclusion criteria were use of any androgens, estrogens or other antiresorptive agents, consumption of greater than three alcoholic beverages per day and advanced cancer or chemotherapy. Ninety-one men were enrolled in the study: 42 men with a history of a hip fracture in the preceding 3 years (FX), 33 men with no fracture but femoral neck bone mineral density (FN BMD) t score <-2.0 (OP) and 16 men with FN BMD >-1.0 controls.

Subjects underwent a general physical exam. A fasting blood sample was drawn and all biochemical tests were performed in the Clinical Laboratory Improvement Amendments (CLIA) certified clinical laboratory of the John Dempsey Hospital of the UConn Health Center.

Participants in the study underwent medical history, physical exam and measurement of FN BMD. The coefficient of variation of BMD measurement (Lunar DPX-L, Madison, WI) of the proximal femur was <1%. Timed single leg stance was also included. Muscle strength and power of the lower extremities was measured using the Keiser leg press (Keiser, Fresno, CA). Hand grip strength is available in a subset of the population, measured using a Jamar dynamometer (Lafayette Instrument Company, Lafayette, IN).

Biochemical measurements

Blood and urine samples were collected between 0700 and 0900 h after a 10–12 h fast. Urine and serum were divided into 0.5 ml aliquots and stored at $-70\,^{\circ}$C. DNA was extracted from whole blood using the Puregene Genomic DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Purified DNA was stored at $-70\,^{\circ}$C. Forward primer was 5′-end labeled with γ-^{32}P-ATP using T4 polynucleotide kinase and then used in a subsequent polymerase chain reaction (PCR) to amplify the CAG containing region. Genomic DNA (100 ng) was amplified in 10 µl using 25 nM labeled forward primer + 250 nM unlabeled forward primer (CACCTCCCG GCGCCA) and 250 nM reverse primer (AGAACCATCCTCA CCCTGCTG) using the Thermal Ace Kit (Invitrogen, Carlsbad, CA). Denaturation was at 98 °C for 30 s and extension at 62 °C for 30 s for 40 cycles. PCR products were mixed with a denaturing electrophoresis loading buffer and electrophoresed on a 6% denaturing polyacrylamide sequencing gel along with samples of known CAG repeat number previously determined by direct DNA sequencing in order to determine CAG repeat numbers for study subjects. The gel was dried and exposed to x-ray film for 24 h.

Free testosterone was measured using an enzyme-linked immunoassay (EIA) and estradiol by radio immunoassay (RIA) (Beckman Coulter, Fullerton, CA). Free testosterone had a sensitivity level of 0.19 pg/mL, intraassay CV% of 6.6 and interassay CV% of 2.6. Estradiol had a sensitivity of 2.2 pg/mL, intraassay CV% of 7.5 and interassay CV% of 9.4. SHBG measurements were performed using a Immulite 1000 by solid-phase chemiluminescent immunometric assay (Siemens Medical Solutions Diagnostics, Los Angeles, CA) with a sensitivity of 0.2 nmol/L, intraassay CV% of 2.9 and interassay CV% of 8.7 [18]. PSA levels were measured by two-site immunoenzymatic (''sandwich'') assay (Access Immunoassay Systems, Chaska, MN) with an intra-assay variability of <6.5%.

All biochemical assays were performed in CLIA-approved clinical laboratories.

Statistical analysis

Descriptive statistics for both groups (Indian and Caucasian) were calculated, and groups were compared using independent sample T-tests and Levene's test for equality of variances for biochemical and standard dual energy x-ray absorptiometry (DXA) measurements. Correlations between

biochemical tests and BMD data were analyzed within each group and for the entire study population. All data were analyzed using IBM SPSS version 19.0 statistical software (Armonk, New York).

Results

Basic demographic parameters

We recruited 60 men of Indian extraction and 60 age-matched white Caucasians. The two groups studied are similar in terms of their age distribution (Table 1). However, there are substantial differences in other parameters. The Indian men are shorter (mean height 167.9 cm compared to the mean height of 177.2 cm for the white Caucasian group; 95% CI −11.71 to −7.05; $p<0.05$), smaller (mean of 71.0 kg compared to 85.5 kg) and have a lower body mass index (BMI; 25.14 instead of 27.15).

Biochemical parameters

Of the biochemical parameters relevant to the research question – whether Indian men were less androgenized than their white Caucasian counterparts, the most striking finding was a substantial deficit in mean free testosterone levels (17.3 pmol/l ml. compared to 21.5 pmol/l) (Table 2). Parameters that impact testosterone availability (SHBG levels; 41.00 nM/l in Indian males compared to 45.4 nM/l among Caucasian males) or activity (*AR* CAG repeat length of 22.30 compared to 22.37) were comparable. Estradiol levels were also comparable between the two groups (86.7 pmol/L in comparison to 94.7 pmol/L).

Table 1. Basic demographic parameters of study subjects (mean ± SD).

Variable	Indian ($n=60$)	White ($n=60$)	Sig.
Age in years	64.28 ± 8.3	62.73 ± 8.1	0.304
Height (cm)	167.9 ± 5.9	177.3 ± 7.0	<0.001
Weight (kg)	70.99 ± 10.2	85.45 ± 14.7	<0.001
BMI (kg/m^2)	25.12 ± 2.9	27.15 ± 4.1	0.002

BMI: body mass index.

Table 2. Comparisons of hormone levels and number of CAG repeats between Indian and white Caucasian males (mean ± SD).

Values	Indian	White	Sig.
SHBG (nmol/L)	40.83 ± 16.4	45.40 ± 18.0	0.148
Free T (pmol/L)	17.3 ± 6.4	21.5 ± 11.2	0.014
Estradiol (pmol/L)	86.7 ± 21.6	94.7 ± 25.7	0.067
Estradiol/SHBG	2.44 ± 1.11	2.58 ± 1.68	0.585
#CAG repeats	22.30 ± 3.7	22.37 ± 3.1	0.916
Free testosterone/ estradiol/SHBG ratio	7.96 ± 3.69	9.70 ± 4.41	0.021

SHBG: sex hormone binding globulin, free T: free testosterone.

Markers of virilization

Of the secondary sexual characteristics we measured, the most remarkable finding was the overall reduction in skeletal mass in the Indian group. Appendicular and truncal skeletal mass were significantly lower in Indian men in comparison to their Western white Caucasian counterparts (see Table 3).

PSA levels, a reflection of overall prostate mass, were lower in Indian men. In calculating mean PSA levels, we eliminated all individuals whose PSA values were higher than 4 ng/ml, as these were potentially individuals with prostatic malignancy. Of those whose PSA levels were less than 4 ng/ml, the mean PSA level was 1.22 ng/ml (±0.79) in Indian men compared to 1.61 ng/ml (±0.88) in white Caucasian men. This difference was significant at the 0.05 level ($p=0.029$).

Previous studies have shown that migrant Indian men have a higher level of adiposity than non-Hispanic Caucasian men, particularly in central body fat. In our study, however, the overall adiposity level, as well as specific regional adiposity was not measurably different between Indians and white men (Table 4).

Discussion

People from the Indian subcontinent are relatively recent immigrants to the United States. Initial cohorts arrived in this country in the 1960s, following the liberalization of immigration policies. Because they represent a relatively small minority in the United States, basic normative metabolic values are not widely available. This communication represents an early cross-sectional description of androgenicity among the males of this population.

We chose to examine the status of the virilization among males for one specific reason. Data from the Indian subcontinent suggest that this ethnic group is particularly vulnerable to chronic ''degenerative'' diseases such as type-II diabetes and cardiovascular disease [19,20]. Indeed, the Indian subcontinent represents the largest global burden of type-II

Table 4. Comparisons of total and regional body fat in Indian and white Caucasian males.

Fat mass by region	Indian	White	T (df), sig	95%CI
Legs fat (kg)	6.05	6.87	−1.59 (118) $p=0.114$	−1.84 to 0.20
Arms fat (kg)	1.84	1.98	−0.99 (118) $p=0.320$	−0.43 to 0.14
Trunk fat (kg)[1]	13.45	14.77	−1.45 (106) $p=0.151$	−3.13 to 0.49
Total body fat (kg)	22.10	24.36	−1.47 (118) $p=0.144$	−5.31 to 0.79

Table 3. Comparisons of skeletal muscle mass between Indian and white Caucasian males.

Skeletal muscle mass by region	Indian	White	Unadjusted Sig.	Adjusted Sig.*
Appendicular skeletal mass (kg)	20.70 ± 2.8	26.29 ± 3.5	<0.001	<0.001
Legs lean muscle (kg)	15.10 ± 2.0	19.22 ± 2.6	<0.001	<0.001
Arms lean muscle (kg)	5.60 ± 0.8	7.10 ± 1.0	<0.001	<0.001
Trunk lean muscle (kg)	21.81 ± 2.2	27.22 ± 3.9	<0.001	<0.001
Total body lean muscle (kg)	46.21 ± 4.9	57.63 ± 7.3	<0.001	<0.001

*Adjusted for BMI (body mass index).

diabetes and myocardial disease, despite the widespread impression that India is a country where infectious disease predominates as the public health preoccupation [19]. There are substantial data to indicate that variations in androgen levels and activity may underlie some of the variations in the prevalence of these chronic diseases among ethnic populations [15]. For this reason, we felt that the evaluating the average levels of sex steroids as well as some modifying factors might be of some value.

Clearly, the single most important determinant of the virilization is testosterone. Our data suggest that total testosterone levels are substantially lower in Indian men in comparison to age matched white male counterparts (20% lower). Serum estradiol levels, however, were comparable between these two groups. Interestingly, comparisons of African-American males and white Caucasian counterparts reveal that while serum testosterone levels are higher in African-American men, the differences were mitigated when controlling for body composition and lifestyle factors, whereas serum estradiol levels remain higher in African-American males even after the anthropometric and environmental adjustments [3].

While it is true that serum testosterone is the primary determinant of the virilization, many other factors affect the activity level of this hormone. Chief among this is SHBG [10]. SHBG may have an action on atherogenesis independent of serum testosterone levels [21,22]. Data in the literature indicate that higher levels of SHBG are associated with lower levels of systemic atherosclerosis [23]. Comparison of SHBG between Indian males and their white Caucasian counterparts do not reveal a statistically significant difference.

Another parameter apart from serum testosterone that has a profound level effect on the virilization is the CAG repeat length in the first exon of the *AR* gene. The longer the CAG repeat length, the less active the AR protein is in causing androgen mediated gene transcription [24,25]. Individuals with long CAG repeats are therefore less androgenized for a given serum testosterone concentration then individuals with a short CAG repeat. We surmised that Indian men may resemble their Asian counterparts in having a relatively long CAG repeat in comparison with either white Caucasian males or African-Americans. Instead, the mean *AR* CAG repeat length among Indian men was comparable to that in their white Caucasian counterparts.

The low mean testosterone level in Indian men appears to result in systemic parameters reflective of lower virilization. Chief among this is the substantially lower stature of Indian men, their lower mean weight as well as lower mean BMI. The chief determinant of the lower body weight may be the lower skeletal muscle mass of Indian men in comparison to their white Caucasian counterparts. We measured the skeletal muscle mass in most body regions using DXA and found that skeletal muscle mass was lower in Indian men in all regions. In contrast, the absolute fat mass was comparable.

Apart from skeletal muscle, another organ whose growth is heavily influenced by androgenic activity is the prostate. PSA levels are a surrogate marker of prostate mass; therefore, we evaluated PSA levels among Indian men and find that the mean PSA level in this group is lower than that in their white Caucasian counterparts.

Our data suggest that Indian men are less virilized than their white Caucasian counterparts. This appears not to be due to higher levels of circulating estrogen or SHBG or a longer *AR* CAG repeat length. In fact, the differences can be due solely to lower circulating testosterone levels. Our data in this regard are consistent with those of from the UK, who demonstrated that another population from the Indian subcontinent (Pakistani men) also demonstrate lower circulating testosterone levels [1].

To our knowledge, this is the first demonstration of lower circulating testosterone levels among Indian men living in the United States. The limitations of our study are that our population is derived entirely from Connecticut, and further from the relatively high social economic status (SES) group in the state. A vast majority of our subjects are physicians, computer programmers or engineers. This may or may not skew the data. However, our data may be useful as a beginning evaluation of the basic biochemical parameters of this small, but important ethnic group.

Acknowledgements

We would like to thank Lisa Godin for her assistance with manuscript preparation.

Declaration of interest

The authors report no declarations of interest. This work was funded by the Patrick and Catherine Donaghue Research Foundation.

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