

ORIGINAL ARTICLE

Ethnic differences in male reproductive hormones and relationships with adiposity and insulin resistance in older men

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Summary

Objectives To assess ethnic differences in male reproductive hormone levels and to determine whether any differences are explained by adiposity, insulin resistance (IR) or comorbidities in older men.

Design Multi-ethnic cross-sectional observational study.

Participants Community dwelling middle-aged and elderly men residing in the UK aged 40–84 years of South Asian (SA; $n = 180$), White European (WE; $n = 328$) or African Caribbean (AC; $n = 166$) origin.

Observations Measured testosterone (T), calculated free T (cFT), sex hormone-binding globulin and LH in SA, WE and AC men along with an assessment of body composition, IR, lifestyle factors and medical conditions.

Results Age-adjusted mean T and cFT levels were lower in SA men when compared to WE and AC men (mean (SEM) T: SA: 14.0 ± 0.4 ; WE: 17.1 ± 0.3 ; AC: 17.2 ± 0.5 nmol/l, $P < 0.001$; cFT: SA: 283 ± 7 ; WE: 313 ± 5 ; AC: 314 ± 8 pmol/l, $P < 0.006$). Compared to WE and AC men, SA men had higher levels of body fat, IR, comorbidities and diabetes. After adjusting for body fat, IR and other confounders, T levels in SA men

remained lower than in WE men ($P = 0.04$) but ethnic differences in cFT became nonsignificant. LH levels were higher in SA than WE men in age-adjusted and fully adjusted models.

Conclusions T and cFT are lower in SA men than in WE and AC men. Whether ethnic-specific reference ranges for T and cFT might be appropriate in clinical practice requires further investigation. Ethnic differences in cFT, but not T, appear to be, more readily, explained by ethnic differences in adiposity, thus providing insights into potential pathophysiological mechanisms.

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Introduction

Testosterone (T) is critically important to men's health and is routinely measured to detect gonadal dysfunction. There are limited data comparing male reproductive hormone levels (RH), such as T, free T, sex hormone-binding globulin (SHBG) and luteinizing hormone (LH) in different ethnic groups.

The largest previous study, involving 50 South Asian (SA) men, showed lower levels of total T (T) and calculated free T (cFT) in SA compared to White European (WE) and African Caribbean (AC) men.¹ However, in this study these ethnic differences did not persist after adjusting for age, insulin, SHBG, height and waist–hip ratio. The results of this study may have been influenced by the relatively small sample size, statistical power, assay precision (immunoassay used), nonstandardized time of serum sample collection and unmeasured confounders such as diabetes therapies.

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A large US-based study showed no ethnic differences in T or cFT between Caucasian ($n = 706$), African American ($n = 530$) and Hispanic ($n = 649$) men and that relationships between these hormones and components of the metabolic syndrome were similar across these groups.² Although this important study had several strengths, it was limited because SA men were not included, T was measured by radio-immunoassay and relationships between androgens and body mass index (BMI) were not assessed directly.

Ethnic differences in adiposity have been reported, such that SA men generally have more visceral fat compared to WE men.^{3,4} Obesity can cause low T via its negative impact on gonadotropin secretion,^{5–7} but the accompanying insulin resistance (IR) and decreased SHBG may also contribute to the low total T without similarly affecting FT.⁸ It is unclear whether or how ethnic differences in adiposity could impact on assessment of RH in the diagnosis of hypogonadism. Other factors could also contribute to ethnic differences in RH including diabetes and IR (more common in SA men)⁹ and diabetes therapies which can influence IR.¹⁰

Therefore, our aims were as follows: (i) to compare RH in SA, WE and AC men; (ii) to assess the influence of confounders, especially, body fat, IR and comorbidity burden, on any ethnic differences in RH; and (iii) to compare the relationship of RH with adiposity, as assessed by BMI, waist circumference (WC) or body fat (%) across ethnic groups.

Methods

Study design and participants

The study population consisted of unselected community dwelling middle-aged and elderly men (mean \pm SD age: 59 ± 12 years) living in Greater Manchester, UK of SA ($n = 180$), WE ($n = 328$) or AC ($n = 166$) origin participating in the Human Serum Metabolome (HUSERMET) study as previously described.¹¹ Briefly, SA and AC men were recruited from the Central Manchester community. The WE men were the Manchester participants of the European Male Ageing Study (EMAS), which is a population-based observational study of male ageing.^{12–14} Ethnicity was determined based on the self-report of the country of origin of three grandparents, similar to a previous study.¹ Ethnicity could not be determined in a small proportion of men ($n = 10$), and some men had a different ethnic background than SA, WE or AC ($n = 25$).

Inclusion and exclusion criteria

From the 709 men in HUSERMET, participants were excluded from this study if they reported use of medication affecting HPT-axis function, treatment for pituitary, testicular or adrenal disease ($n = 31$ men), or if their BMI exceeded 60 kg/m^2 ($n = 2$ AC men). In addition, men with other or uncertain ethnic backgrounds were excluded ($n = 34$ men) leaving 642 men for analysis (flow chart, Fig. 1).

Clinical, anthropometric and male reproductive hormone measurements

The presence of one or more comorbidities was determined based on self-reported presence or treatment for heart disease, stroke, hypertension, diabetes, bronchitis, cancer, kidney or liver disease. Poor health status was determined by how each participant rated his general health and included responses, such as 'fair' or 'poor'. Clinical symptoms were assessed according to definitions proposed in previous work from our research groups.¹⁴ Physical activity (PA) was assessed by self-report and included responses on the Physical Activity Scale For The Elderly (PASE).¹⁵ Smoking status was classified as never, ex- and current smoker. Frequent alcohol intake was considered as alcohol intake ≥ 5 days/week. Measurements of weight (kg), height (cm), waist (cm), calf circumference (cm) and body fat percentage (skin calipers; %) were made using standardized protocols described previously.^{12,13} Insulin resistance was determined by the Homeostasis Model Assessment of Insulin Resistance [HOMA-IR = fasting insulin (U/ml) \times fasting glucose (mmol/l)/22.5].¹⁶

Fasting venous blood samples were provided before 10:00 AM for T, SHBG, luteinizing hormone (LH) and albumin measurements. cFT was derived from T, SHBG and albumin measurements by the Vermeulen formula.^{17,18} T was analysed via liquid chromatography–tandem mass spectrometry (LC-MS/MS).¹⁹ SHBG and LH were determined via a modular E-170 immunoassay platform, as described previously.⁶ Intra- and Interassay coefficients of variation (CVs) for these assays were as follows: T, 4.0% and 5.6%; SHBG, 1.7% and 3.2%; LH, 1.9% and 3.0%, respectively. The lower limit of detection for the male RHs investigated was T (0.50 nmol/l; 0.14 $\mu\text{g/l}$), cFT (5.77 pmol/l; 1.7 ng/l), SHBG (8.2 nmol/l) and LH (0.38 U/l).

Statistical analysis

Clinical characteristics are presented as mean \pm SEM for continuous parameters or number (percentage) values for binary parameters. Between-group comparisons were assessed using analysis of variance for continuous variables and Z-test for categorical variables. Univariate differences in T and cFT levels across BMI categories within ethnic groups were assessed using analysis of variance.

Age-adjusted and multivariable-adjusted mean \pm SEM RH were compared between the three ethnic groups. Covariates included age, frequent alcohol intake, smoking (current vs never), body fat (%), IR and comorbidity burden (≥ 1 comorbidities). Between-group comparisons were assessed using analysis of covariance.

Linear regression was used to assess the relationships of RH (outcomes) with BMI, WC or body fat (%; predictors), and age-adjusted and fully adjusted beta coefficients (95% CI) were depicted in forest plots [covariates: age, frequent alcohol intake, smoking (current vs never), IR and comorbidity burden (≥ 1 comorbidities)].

Independent replication of the regression analyses was performed within the analytical sample via use of fivefold

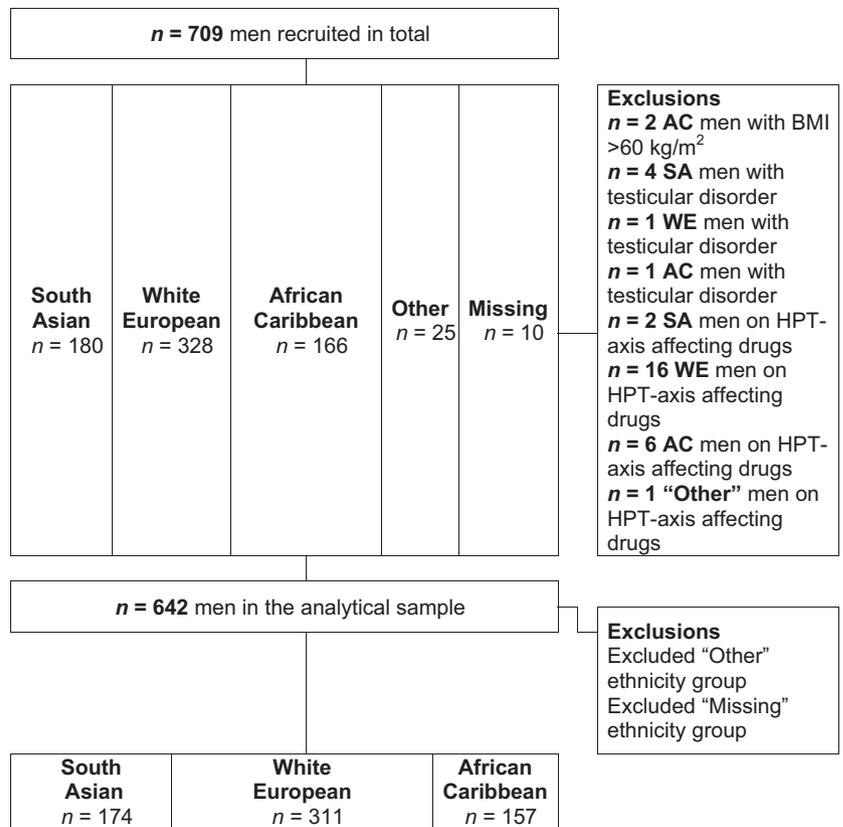


Fig. 1 Flowchart of participants. SA, South Asian; WE, White European; AC, African Caribbean.

cross-validation,²⁰ and results are depicted as mean squared error (MSE) values for each ethnic group. This allowed for an assessment of the robustness of observed relationships in the regression analyses. All analyses were performed using STATA 13 SE software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP.).

Results

Clinical characteristics

South Asian men vs White European men. Compared with WE men, SA men were younger, shorter, with a higher percentage of body fat (but similar BMI) and had lower calf circumference values (Table 1). The prevalence of current smokers was higher in SA men, while self-reported frequent alcohol intake and PA were lower. SA men had higher glucose and IR, a higher prevalence of self-reported diabetes, a higher prevalence of poor health status and a higher burden of comorbidities. T and SHBG were lower, but cFT and LH similar, in SA men, when compared to WE men. In addition, SA men had a higher prevalence of decreased libido, limited walking, inability to bend, fatigue, loss of energy and sadness, when compared to WE men (Table S1)

South Asian men vs African Caribbean men. Compared with AC men, SA men were older, shorter and also displayed a higher percentage of body fat but had similar BMI values (Table 1). In SA men, the prevalence of current smoking, frequent alcohol intake and PA was lower. SA men had higher glucose levels and

IR, more self-reported diabetes, poorer health status and a higher burden of comorbidities. T, cFT and SHBG were lower, but LH was similar in SA men, when compared to AC men. In addition, SA men had a higher prevalence of decreased vigorous activity, limited walking, inability to bend, fatigue and loss of energy, when compared to AC men (Table S1).

African Caribbean men vs White European men. Compared with AC men, WE men were older and had higher WC but similar BMI. They had higher calf circumference, a lower prevalence of current smoking and a higher prevalence of frequent alcohol intake (Table 1). WE men had a lower prevalence of diabetes and better health status. cFT was lower, and T, SHBG and LH were similar in WE men, when compared to AC men. In addition, WE men had a higher prevalence of decreased morning erections, erectile dysfunction and decreased vigorous activity, when compared to AC men (Table S1).

Univariate relationships between body mass index (BMI) categories and testosterone levels across ethnic groups. In SA men, levels of T did not differ across BMI categories, while in WE men T levels differed in lean vs overweight, lean vs obese and overweight vs obese men. In AC men, T levels differed in lean vs obese and overweight vs obese men.

In SA men, levels of cFT did not differ across BMI categories, while in WE men, cFT levels differed in lean vs overweight, lean vs obese and overweight vs obese men. In AC men, cFT levels differed in lean vs obese and overweight vs obese men (Table S2).

Table 1. Comparison of clinical characteristics of older South Asian, White European and African Caribbean men

Parameter	South Asian (SA) N = 174	White European (WE) N = 311	African Caribbean (AC) N = 157	P-value SA vs WE	P-value SA vs AC	P-value WE vs AC
Age, years	55.9 ± 0.8	63.8 ± 0.6	53.0 ± 0.8	<0.001	0.041	<0.001
Height, cm	170.2 ± 0.5	174.5 ± 0.4	173.5 ± 0.5	<0.001	<0.001	0.417
Body fat, %	32.6 ± 0.3	28.9 ± 0.3	27.9 ± 0.5	<0.001	<0.001	0.180
BMI, kg/m ²	27.7 ± 0.3	27.5 ± 0.2	27.2 ± 0.3	1.000	0.906	1.000
Waist circumference, cm	99.6 ± 0.8	98.5 ± 0.6	92.5 ± 0.9	0.788	<0.001	<0.001
Calf circumference, cm	32.8 ± 0.3	35.2 ± 0.2	33.0 ± 0.3	<0.001	1.000	<0.001
Smoking (current), n (%)	26 (29)	22 (7)	37 (41)	<0.050	>0.050	<0.050
Frequent alcohol intake, n (%)	2 (1)	81 (26)	28 (18)	<0.050	<0.050	>0.050
PASE score	160 ± 8	194 ± 5	224 ± 11	0.002	<0.001	0.016
Fasting plasma glucose, mmol/l	6.3 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	<0.001	<0.001	1.000
HOMA-IR	5.7 ± 0.6	4.2 ± 0.6	3.1 ± 0.3	0.851	0.031	0.877
Diabetes, n (%)	50 (33)	30 (10)	16 (11)	<0.050	<0.050	>0.050
Poor health, n (%)	51 (29)	30 (10)	28 (18)	<0.050	<0.050	<0.050
One or more chronic illness, n (%)	105 (60)	157 (51)	56 (36)	>0.050	<0.050	<0.050
Total testosterone, nmol/l	14.2 ± 0.4	16.8 ± 0.3	17.6 ± 0.5	<0.001	<0.001	0.460
Free testosterone, pmol/l	294 ± 8	296 ± 5	335 ± 8	1.000	0.001	<0.001
SHBG, nmol/l	32.5 ± 1.2	42.3 ± 1.0	38.6 ± 1.2	<0.001	0.003	0.066
LH, U/l	6.8 ± 0.3	6.1 ± 0.3	6.1 ± 0.2	0.263	0.520	1.000

Data are unadjusted mean ± SEM for continuous variables or number (percentage) for categorical variables.

P values were calculated using analyses of variance for continuous variables or Z-test for categorical variables.

N, sample size; BMI, body mass index; PASE, Physical Activity Scale for the Elderly; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; SHBG, sex hormone-binding globulin; LH, luteinizing hormone.

Bold values represent statistical significant P-values ($p < 0.05$).

Age-adjusted and multivariable-adjusted male reproductive hormone levels across ethnic groups

South Asian men vs White European men. Age-adjusted mean T, cFT and SHBG were lower, but LH levels were higher in SA men when compared to WE men (Table 2). After adjusting for age, frequent alcohol intake, smoking, %body fat, IR and comorbidities, T levels remained lower and LH remained higher in SA men compared to WE men (Table 2 and Table S3).

South Asian men vs African Caribbean men. Age-adjusted mean T, cFT and SHBG were lower in SA men when compared to AC men. However, these ethnic differences were no longer statistically significant in fully adjusted models (Table 2 and Table S3).

African Caribbean men vs White European men. Age-adjusted mean LH levels were higher in AC than in WE men, but all other RH were similar. The ethnic differences in LH levels were no longer statistically significant in fully adjusted models (Table 2 and Table S3).

Age-adjusted and multivariable-adjusted associations between measures of adiposity and male reproductive hormone levels within ethnic groups

Relationships between adiposity (BMI, WC or body fat) and RH differed within ethnic groups (Figs 2–4). In SA men, an age-adjusted and multivariable-adjusted analysis showed that BMI

was not related to RH. In SA men, an age-adjusted analysis showed that WC had a weak negative relationship with T and SHBG and that %body fat was negatively associated with T. However, in multivariable analysis, these age-adjusted estimates were no longer significant and only %body fat was negatively associated with LH levels (Fig. 4).

In contrast, in WE and AC men, BMI, WC and body fat showed strong negative relationships with T, cFT and SHBG in age-adjusted and fully adjusted models in which the covariates were age, frequent alcohol intake, smoking, IR and comorbidity burden.

Relationship of male reproductive hormone levels to insulin resistance within ethnic groups

In univariate analysis, HOMA-IR showed negative relationships with T and cFT in WE and in AC men, and a negative relationship with LH in WE men. In contrast, in SA men HOMA-IR was not related to any RH (Table S4).

In multivariable-adjusted models, HOMA-IR was not associated with any RH within any ethnic group [Table S4; covariates: age, alcohol intake, smoking status, comorbidity and adiposity (WC, BMI or %body fat)].

K-fold cross-validation to assess robustness of results

Fivefold cross-validation showed that our linear regression analyses had good internal consistency, with similar differences in MSE values across the folds and across the three ethnic groups (Tables S5, S6 and S7).

Table 2. Age-adjusted male reproductive hormone levels in older South Asian, White European and African Caribbean men

Parameter	White European (WE)		African Caribbean (AC)		Model 1 Includes age		Model 2 Includes age and % body fat		Model 3 Includes age and HOMA-IR		Model 4 Includes age, % body fat and HOMA-IR		Model 5 Includes age, alcohol intake, smoking, % body fat, HOMA-IR		Model 6 Includes age, alcohol intake, smoking, % body fat, HOMA-IR and comorbidity burden								
	N	Mean ± SEM	N	Mean ± SEM	SA vs WE	AC vs WE	SA vs WE	AC vs WE	SA vs WE	AC vs WE	SA vs WE	AC vs WE	SA vs WE	AC vs WE	SA vs WE	AC vs WE							
All men																							
Total testosterone, nmol/l	174	14.0 ± 0.4	311	17.1 ± 0.3	17.2 ± 0.5	<0.001	1.000	0.003	0.029	1.000	<0.001	<0.001	1.000	0.004	0.022	1.000	0.018	0.907	0.288	0.035	1.000	0.323	
Free testosterone, pmol/l	283	± 7	313	± 5	314 ± 8	0.002	1.000	0.262	0.510	1.000	0.004	0.011	1.000	0.204	0.470	1.000	0.212	1.000	0.395	0.283	1.000	0.428	
SHBG, nmol/l	342	± 1.2	39.8 ± 0.9	41.7 ± 1.3	0.001	<0.001	0.783	0.276	0.086	1.000	0.005	<0.001	0.425	0.404	0.061	0.813	1.000	1.000	1.000	1.000	1.000	1.000	1.000
LH, U/l	7.1	± 0.3	5.6 ± 0.2	6.8 ± 0.3	<0.001	1.000	0.014	<0.001	0.693	0.034	0.001	1.000	0.010	<0.001	0.591	0.028	0.008	0.387	0.492	0.014	0.470	0.540	

Data are age-adjusted mean ± SEM.

Group comparisons P values were calculated using analyses of covariance.

Multivariable-adjusted models. Model 1: adjusted for age; Model 2: adjusted for age and % body fat; Model 3: adjusted for age and HOMA-IR; Model 4: adjusted for age, % body fat and HOMA-IR; Model 5: adjusted for age, frequent alcohol intake, smoking (current), body fat (%) and HOMA-IR; Model 6: adjusted for age, frequent alcohol intake, smoking (current), body fat (%), HOMA-IR and comorbidity burden (≥1 comorbidities).

N, sample size; SHBG, sex hormone-binding globulin; LH, luteinizing hormone; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

Bold values represent statistical significant P-values (p < 0.05).

Italic values represent a trend for statistical significance (p < 0.010, but p ≥ 0.050)

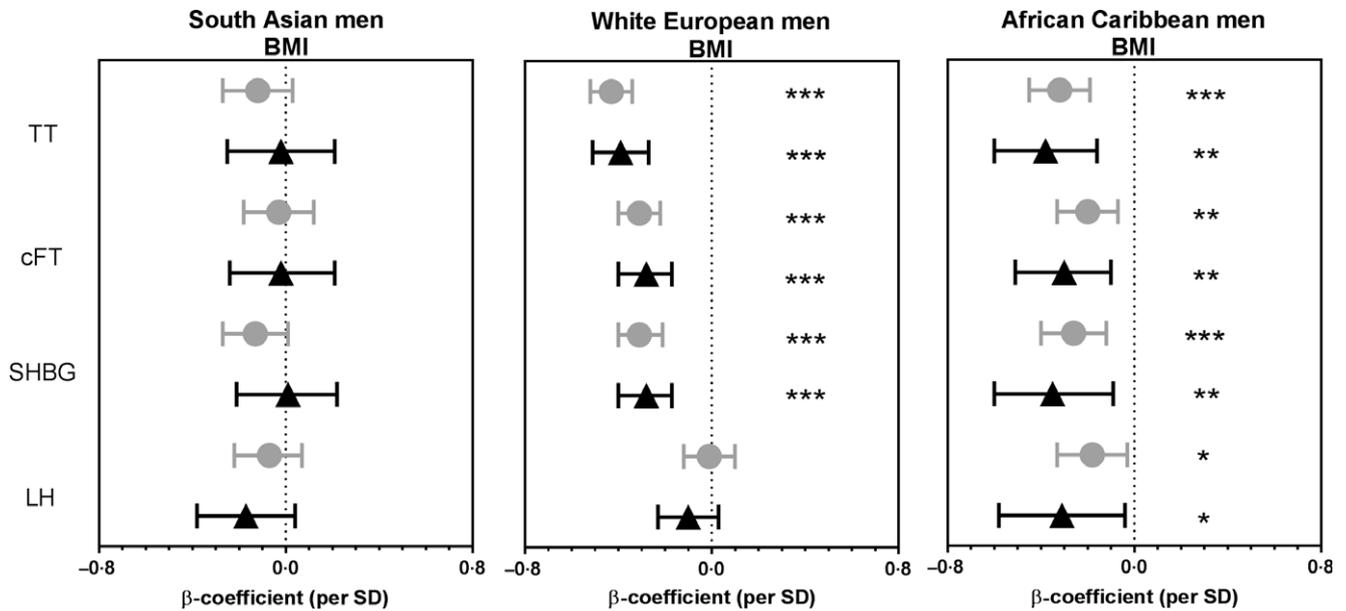


Fig. 2 Age-adjusted and multivariable-adjusted relationships between body mass index (BMI) and male reproductive hormone levels in older South Asian, White European and African Caribbean men. Circles represent beta-coefficients from age-adjusted linear regression models using BMI as a predictor, and triangles represent beta-coefficients from fully adjusted linear regression models using BMI as a predictor and study age, alcohol intake, smoking, HOMA-IR and comorbidity burden as covariates. The strength and directions of the associations are depicted by the beta-coefficients and the 95% confidence intervals, respectively. The statistical significance of the associations is provided by the stars, whereby * star indicates $P < 0.05$, ** indicate $P < 0.01$ and *** indicate $P < 0.001$.

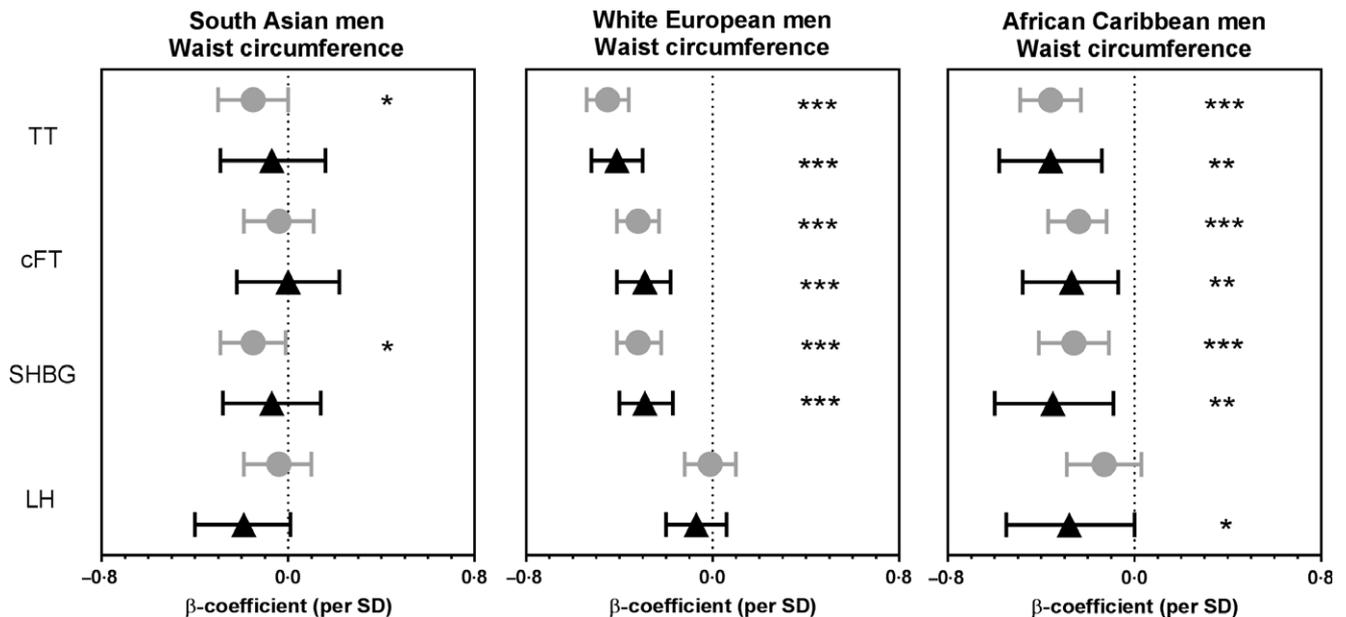


Fig. 3 Age-adjusted and multivariable-adjusted relationships between waist circumference and male reproductive hormone levels in older South Asian, White European and African Caribbean men. Circles represent beta-coefficients from age-adjusted linear regression models using waist circumference as a predictor, and triangles represent beta-coefficients from fully adjusted linear regression models using waist circumference as a predictor and study age, alcohol intake, smoking, HOMA-IR and comorbidity burden as covariates. The strength and directions of the associations are depicted by the beta-coefficients and the 95% confidence intervals, respectively. The statistical significance of the associations is provided by the stars, whereby * indicates $P < 0.05$, ** indicate $P < 0.01$ and *** indicate $P < 0.001$.

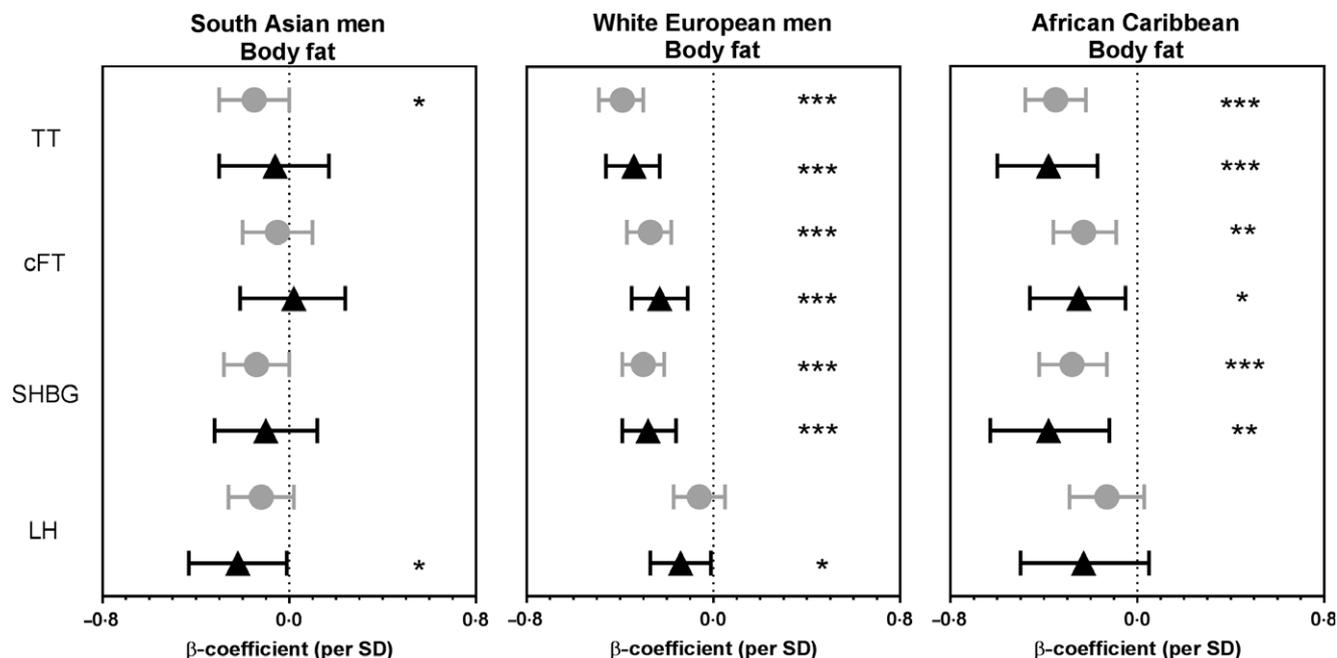


Fig. 4 Age-adjusted and multivariable-adjusted relationships between body fat (%) and male reproductive hormone levels in older South Asian, White European and African Caribbean men. Circles represent beta-coefficients from age-adjusted linear regression models using body fat (%) as a predictor, and triangles represent beta-coefficients from fully adjusted linear regression models using body fat (%) as a predictor and study age, alcohol intake, smoking, HOMA-IR and comorbidity burden as covariates. The strength and directions of the associations are depicted by the beta-coefficients and the 95% confidence intervals, respectively. The statistical significance of the associations is provided by the stars, whereby * indicates $P < 0.05$, ** indicate $P < 0.01$ and *** indicate $P < 0.001$.

Discussion

We have shown that (i) T and cFT levels were lower in SA men when compared to WE and AC men; (ii) T levels were lower and LH levels were higher in SA compared to WE men after adjusting for confounders; (iii) differences in cFT levels between SA and WE men could be explained by covariables (i.e. body fat); (iv) T and cFT levels were not strongly related to adiposity (%body fat, BMI and WC) in SA men while strong negative relationships were observed in WE and AC men. These findings have potential implications for the assessment of men with suspected hypogonadism and for understanding the mechanisms underlying these interethnic differences.

Prior studies

Prior data are limited when comparing ethnic differences in T in men, and to our knowledge, there are no data on ethnic associations with LH. Heald and co-workers studied 50 SA Pakistani men, 55 WE men and 75 AC men¹ and showed that T was lower in SA men compared to other ethnic groups but these ethnic differences disappeared after adjusting for insulin, SHBG, waist-hip ratio and height. Although this study was first to report these relationships, some limitations might have influenced the results: it had a relatively small sample size with limited ability to adjust for covariates, it used a T immunoassay with suboptimal performance at low T levels when compared to LC-MS/MS methods^{21,22}; it did not measure LH and their study did not report a standardized time at which serum samples were

collected. Addressing these limitations, our study shows that in SA men, the lower cFT, but not T levels, is explained by measured covariables (i.e. body fat). Additional analysis revealed that these results did not differ after excluding men who used diabetes medications (data not shown).

Giton and colleagues reported higher T levels in AC men compared to WE men.²³ We showed numerically higher T levels in AC compared to WE men but this difference was not statistically significant (Table 1) and T levels were virtually identical after age adjustment (Table 2). In addition, our study also reports cFT, and LH levels and our hormone data were adjusted for differences in IR and % body fat – variables which differed between AC and WE men and could explain between-group differences in T levels.

There are limited data describing relationships between adiposity and RH in different ethnic groups. Shamin and co-workers studied 200 young and middle-aged SA men (aged 30–50 years) in Pakistan and showed that T was inversely related to both BMI and WC in an unadjusted cross-sectional analysis.²⁴ In contrast to these findings, we observed no significant relationship between T and BMI or WC in older SA men (predominantly of Pakistani extraction) after adjusting for confounders. The most likely explanation for the discrepancies is that Shamin and co-workers presented the results of a univariate analysis whereas our analysis was multivariable-adjusted. This explanation is supported by the observation that in our age-adjusted analysis, WC was negatively related to T and the statistical significance was lost after further adjusting for additional covariates. Another potential explanation is that our SA men were older (mean age 56 vs

39 years) and the results could have been influenced by age-related loss of lean mass and increase in fat mass.^{4,25}

Physiological mechanisms underlying ethnic differences in male reproductive hormone levels

We have shown that ethnic differences in adiposity appear to explain ethnic differences in cFT but not the ethnic differences in T. From a physiological perspective, the relationships with cFT are likely to be more important because this is the active hormone at the cellular level.

We found that body fat is more strongly related to cFT levels in WE men than in SA men. The reason for this is unclear, but perhaps our measures of adiposity in SA men are less representative of metabolically active visceral fat than in other ethnic groups.²⁶

Our data suggest that IR-induced hypothalamic suppression of LH in SA men does not explain lower T levels in these men. As we have observed, higher levels of abdominal obesity are commonly seen in SA men, which is an indirect marker of visceral obesity and IR.⁴ IR is known to have a negative impact on hypothalamic/pituitary function.⁷ However, in SA men we observed higher LH levels rather than lower levels compared to WE men.

Another possibility is that the low T levels in SA men may be due to selective testicular resistance because of target organ resistance to insulin action – as suggested by a prior study showing that increasing IR is associated with decreased Leydig cell responsiveness to gonadotropin stimulation, which would lead to increased LH levels.²⁷

Comorbidity burden is another factor that could contribute to gonadal axis dysfunction in ageing men⁶ and may contribute to lower responsiveness of testicular Leydig cells to LH leading to low T.²⁸ However, we found that additional adjustment for comorbidity burden within our study did not explain the lower T observed in SA men.

Strengths and limitations

Our study has several strengths: first, the large sample size and comprehensive array of assessments allowed adjustment for several potential confounders – critically important because many of these variables are highly prevalent especially in SA men; second, we assessed LH in addition to T, cFT and SHBG adding insight into the feedback relationships in the HPT-axis; third, in a subset of participants, we included multiple measures of adiposity (BMI, WC or %body fat) and confirmed their correlation with dual-energy X-ray absorptiometry (DEXA) measurements of total body fat (Table S8); and finally, we used a highly accurate method (LC-MS/MS) to measure T, which has important advantages over the radio-immunoassay.^{21,22}

We acknowledge some limitations: first, this cross-sectional observational study was unable to assess temporal changes or implicate causality; second, hormones were measured on a single occasion; third, we were unable to assess testicular size; fourth, we were unable to assess the androgen receptor exon 1 CAG repeat length; fifth, we used an indirect measure of IR; sixth, oestradiol (E2) levels obtained from LC-MS/MS were not

available; and finally, we were unable to assess migratory status (previously related to T in SA men).²⁹

Summary and conclusions

We have shown that T and cFT levels were lower in SA men when compared to WE and AC men. After adjusting for confounders, T was lower and LH higher in SA compared to WE men. Differences in cFT levels between SA and WE men appear to be explained by body fat. T and cFT levels in SA men were only weakly related to adiposity in contrast to the strong negative relationships observed in WE and AC men.

These findings require replication in larger studies to determine whether ethnic-specific reference ranges for T, cFT and LH might be appropriate, especially for SA men. When interpreting low or borderline low T and cFT results, measures of adiposity, such as BMI, are more valuable in WE and AC than in SA men.

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Authors' contributions

RJAHE, FCWW and MKR wrote the analysis plan for the study. BGK provided laboratory measurements required to perform the analysis. RJAHE, AS and SRP performed data cleaning. RJAHE performed the analysis. PSG, AM, GT, RG, FCWW and MKR supervised the analysis. RJAHE wrote the manuscript. AS, SRP, TWO, GT, RG, FCWW and MKR supervised the writing process.

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Conflict of interest

Nothing to declare.

Disclosure statement

FCWW has acted as a consultant for Bayer-Schering, Eli Lilly and Besins Healthcare and also participated in advisory board meetings and lectured on their behalf. FCWW has received

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