**Differential associations of testosterone,
dihydrotestosterone and estradiol with physical, metabolic
and health-related factors in community-dwelling men aged
17-97 years from the Busselton Health Survey.**

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Title

Differential associations of testosterone, dihydrotestosterone and estradiol with physical, metabolic and health-related factors in community-dwelling men aged 17-97 years from the Busselton Health Survey.

Short title

Sex hormones in men aged 17-97 years

Authors

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Testosterone, dihydrotestosterone, estradiol, dyslipidemia, inflammation

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Abstract

Objectives

Lower testosterone (T) levels are associated with poorer health outcomes in older men, but associations in younger or middle-aged men are uncertain and data for dihydrotestosterone (DHT) and estradiol (E2) are limited. We assessed associations of circulating T, DHT and E2 with physical and health-related factors in a cohort comprising men aged 17-97 years.

Participants and methods

Serum from 2,143 community-dwelling men from the 1994/95 Busselton Health Survey was assayed for T, DHT and E2 using liquid chromatography-tandem mass spectrometry. Men receiving hormonal therapy, or with prostate cancer or orchidectomy were excluded.

Results

Of the men 43% had never smoked, 6.1% had diabetes and 16.8% cardiovascular disease (CVD). Mean ($\pm$ SD) age was 50.3 $\pm$ 17.0 years. Total T was moderately correlated with DHT ($r=0.56$), E2 ($r=0.35$) and sex hormone-binding globulin ($r=0.53$). In age, smoking, body mass index (BMI) and sex hormone-binding globulin (SHBG)-adjusted analyses, T was inversely associated with metabolic syndrome score, while DHT and E2 were not associated. In multivariable models, higher total T was associated with lower age, BMI and C-reactive protein, and with higher creatinine and haemoglobin, independently of SHBG. Higher DHT was associated with lower age, BMI and glucose level, and higher creatinine and haemoglobin. E2 was positively associated with age, BMI and haemoglobin.

Conclusions

In men spanning younger, middle and older ages, circulating androgens are more related to age and metabolic factors than CVD or chronic disease. Further investigation is required to clarify whether androgens and estrogens have contrasting roles as risk predictors for CVD.

Introduction

As men grow older circulating levels of testosterone (T) decline [1,2]. Lower levels of T are associated with poorer health outcomes in older men including frailty, cardiovascular disease and mortality [3-5]. However, whether low T reflects existing ill-health or contributes to the development of disease remains unclear [5]. This debate has intensified following the report that in healthy, predominantly middle-aged men without major comorbidities, T levels might be stable and not show age-associated differences [6]. Similar T levels have also been reported when healthy young men were compared with healthy elderly men [7]. Therefore, lower T might reflect the presence of ill-health accumulated during ageing, representing a biomarker rather than a causal contributor to health outcomes. Alternatively, associations of lower T with poorer outcomes might be present in older men but attenuated in younger or middle-aged men.

In the circulation, T is present bound with high affinity to sex hormone-binding globulin (SHBG) and with lower affinity to albumin with a small proportion unbound or free. In large studies free T is commonly calculated rather than measured directly [8]. The increase in SHBG concentrations during ageing results in a greater reduction in calculated free compared to total T levels in older men [7,9]. However biological significance of calculated free T and its utility in the prediction of health outcomes in ageing men is debated [10]. T is converted by 5 α -reductase into dihydrotestosterone (DHT), a more potent ligand for the androgen receptor (AR), and by aromatase into estradiol (E2) a ligand for the estrogen receptor (ER) α or ER β [11]. E2 levels have been associated with bone density in men [12]. Yet there are limited data comparing associations of T, DHT and E2 with physical, metabolic and other health-related factors in ageing men, which might clarify whether measuring levels of T

metabolites provides a more comprehensive assessment of the role of male sex hormones in health and disease.

Recently liquid chromatography-tandem mass spectrometry (LC-MS) has become established as the preferred methodology for assay of sex steroids, rather than immunoassays which exhibit loss of specificity and method-dependent bias [13]. We sought to test the hypothesis that T, DHT and E2 are differentially associated with physical, metabolic and other health-related factors in men spanning younger, middle and older ages by measuring these sex hormones using LC-MS in a large cohort of community-dwelling men.

Participants and Methods

Study population

Busselton is a coastal town in Western Australia, whose residents have participated in a series of health surveys from 1966 onwards. In 1994-1995, all surviving subjects of earlier Busselton surveys were invited to participate in a follow-up survey [14]. More than 90% of the Busselton population is of Caucasian ethnic origin. The present study is based on 2,143 men aged 17-97 years who attended the 1994/95 survey, who provided blood samples for analysis. The study was approved by the Human Research Ethics Committee of the University of Western Australia, and all participating men provided written informed consent.

Assessment of medical comorbidities

Methods used in the Busselton Health Survey have been described previously [14]. Men were asked to complete a comprehensive health and lifestyle questionnaire and underwent a physical assessment. Blood pressure was measured using a mercury sphygmomanometer

after five minutes at rest in a sitting position. Height was measured using a stadiometer and weight in light underclothes. Smoking, diabetes, and the use of antihypertensive or glucose-lowering medications were obtained via questionnaire. Medical history was assessed from questionnaire responses and also via the Western Australian Data Linkage System (WADLS), which captures electronically all admissions to public or private hospitals in Western Australia [15]. A history of cardiovascular disease (CVD), including coronary heart disease or stroke, was defined as any hospital admission with a primary or secondary diagnosis of CVD during the preceding 15 years prior to the survey. History of chronic obstructive pulmonary disease (COPD) and history of chronic renal disease (CRD) were similarly defined using hospital admissions history via WADLS. Metabolic syndrome scores were defined using the NCEP ATP III 2005 definition [16]. Hypertension was defined as systolic blood pressure ≥ 130 mmHg or diastolic blood pressure ≥ 85 mmHg or receipt of drug treatment for hypertension. Hyperglycemia was defined as fasting glucose ≥ 5.6 mmol/L. Hypertriglyceridemia was defined as triglycerides ≥ 1.7 mmol/L or receipt of fibrates or nicotinic acid. Low high density lipoprotein (HDL) cholesterol was defined as HDL ≤ 1.0 mmol/L. Central obesity was defined as waist circumference ≥ 102 cm. The Metabolic Syndrome score was the number of these five risk components meeting the defined criteria and a participant was regarded as having the Metabolic Syndrome if three or more criteria were met.

Laboratory assays

Blood samples were collected in the early morning after an overnight fast. Collected serum was stored at -70°C . Fasting serum cholesterol, HDL and triglycerides were determined at the time of the survey by standard enzymatic methods on a Hitachi 747 analyser (Roche Diagnostics, Australia). Glucose was measured using a hexokinase assay. Insulin was assayed

using a Tosoh A1A-600 immunoassay analyzer using a two-site immunoenzymometric assay. The between-run coefficient of variation (CV) was 5.6% at 14.3 mU/L. We measured C-reactive protein (CRP) using a particle-enhanced immunoturbidimetric assay on a Modular analyser (Roche Diagnostics, Germany). Inter-assay precision was 3.9% at 1.6 mg/L and 1.6% at 5 mg/L. Plasma adiponectin levels were measured by a commercially available quantitative sandwich enzyme immunoassay technique (R&D Systems Inc., Minneapolis, Minnesota, USA). The inter-assay coefficients of variation (CVs) ranged from 7.0 to 8.5%.

Serum T, DHT and E2 were quantified within a single LC-MS run without derivatization using atmospheric pressure photo-ionisation in positive mode for androgens and negative mode for estrogens, from 200 µL samples as previously described [17]. Between run imprecision for T was 8.6% at a concentration of 5.3 nmol/L and 7.9% at 26.9 nmol/L. For DHT between run imprecision was 11.3% at a concentration of 1.3 nmol/L and 9.1% at 5.3 nmol/L. For E2 between run imprecision was 14.5% at a concentration of 73 pmol/L and 9.9% at 279 pmol/L. LH was assayed using a 2 step non-competitive chemiluminometric immunoassay (Abbott Architect, Abbott Diagnostics, Australia) with between run imprecision of 5.6% at 4.8 IU/L. SHBG was assayed using a solid phase, two-site enzyme immunometric assay with chemiluminescent substrate (Immulite 2000XPi, Siemens Healthcare) with between run imprecision of 3.4% at 39.4 nmol/L. Free T was calculated using empirical formulae which provides closer concordance with measured free T compared with calculations based on equilibrium binding equations [8].

Statistical analyses

Descriptive statistics are presented as mean (SD). Linear regression models were used to examine the relationships between age, body mass index (BMI) and hormone variables

(Table 1), and the adjusted relationships between clinical and metabolic factors with hormone variables (Tables 2 and 3). Non-linearity in relationships was investigated using both the square and log transform of quantitative risk factors. To identify the important and independent correlates of each hormone variable we used a forwards stepwise selection procedure using $p < 0.01$ as the criterion for selection. The final fitted multivariable model from this procedure is presented (Table 4). Results from linear regression modelling are presented as the estimated difference in mean hormone level compared with reference category for categorical risk factors and for an increase of one SD in continuous risk factors together with the p-value for the association (Tables 2-4).

Results

Characteristics of the study population

There were 2,143 men who participated in the 1994/95 survey, who had provided blood samples for analysis. After excluding men reporting use of testosterone or hormonal therapy, or with a history of prostate cancer or orchidectomy ($n=48$) and restricting the analysis to men with complete data for key variables, there were 1,920 men in this analysis. The mean ($\pm$ SD) age was 50.3 ± 17.0 years. Of these men, 43% had never smoked, 18.6% had hypertension, 3.1% were on lipid lowering therapy, 6.1% had diabetes, 2.9% had chronic pulmonary disease, 0.2% chronic renal disease and 16.8% had a history of CVD at the time of blood sampling. The age distribution of these men and the mean and SD of BMI, T, calculated free T, DHT, E2, SHBG and LH overall and for each age decade are shown in Table 1. There was an even distribution of men across decades of age except for fewer men aged 80 years and above. The overall mean for BMI was 26.5 kg/m^2 and was highest in 50-69 year old men. Total and calculated free T levels were lower in older compared to younger men, while SHBG and LH levels were higher in older men.

TABLE 1

Associations of T with DHT, E2 and SHBG

Total T was moderately positively correlated with DHT ($r=0.56$, $p<0.001$), SHBG ($r=0.53$, $P<0.001$) and E2 ($r=0.35$, $p<0.001$) but not correlated with LH ($r=0.00$, $p=0.907$).

Age-adjusted associations of hormones with smoking and obesity

All hormone variables were significantly positively associated with current smoking after adjustment for age. Total T, calculated free T, DHT, SHBG and LH were significantly ($p<0.001$) negatively associated with BMI, waist-hip-ratio and waist circumference and the strength of the association (effect size per SD change in obesity measure) was very similar for BMI, waist-hip-ratio and waist circumference (data not shown). E2 had a significant positive relationship with BMI ($p=0.004$) and non-significant relationships with waist-hip-ratio ($p=0.481$) and waist circumference ($p=0.203$). Thus in further analyses all associations with clinical and metabolic variables were adjusted for age, smoking and BMI.

Age, smoking and BMI-adjusted associations of hormones with clinical and metabolic factors

After adjustment for age, smoking and BMI, total T was positively associated with HDL, haemoglobin and adiponectin, and negatively associated with lipid lowering medication, **hypertension, systolic BP**, glucose, insulin, triglycerides, CRP and ALT (see Table 2).

Associations of calculated free T and DHT were similar to those found with total T, except that neither was associated with ALT, DHT was not associated with lipid lowering medication, DHT was lower in men with diabetes ($p=0.012$) and cholesterol was marginally positively associated with calculated free T ($p=0.021$). Associations of SHBG were also

similar to those found with total T except that SHBG was not associated with lipid medications and haemoglobin, and was negatively associated with **diastolic as well as systolic BP and hypertension**. Of note, total and calculated free T, DHT and E2 were positively associated with HDL and haemoglobin and negatively associated with insulin, triglycerides and CRP. LH was negatively associated with haemoglobin ($p=0.023$) but was not associated with any other variable. Total and calculated free T, DHT and SHBG were strongly ($p<0.001$) negatively associated with the metabolic syndrome score whereas E2 was marginally associated with metabolic syndrome ($p=0.039$).

TABLE 2

Associations after additional adjustment for SHBG

Several associations with total and calculated free T, DHT and E2 were attenuated after further adjustment for SHBG (Table 3). Total and calculated free T remained negatively associated with lipid medications, glucose and CRP, and positively associated with HDL and haemoglobin ($p<0.05$). DHT remained negatively associated with diabetes, glucose and CRP and positively associated with haemoglobin. E2 remained negatively associated with CRP and positively associated with haemoglobin. Alcohol consumption became more strongly associated with total and calculated free T, and cholesterol and creatinine more strongly associated with total and calculated free T and DHT after adjustment for SHBG. After adjustment for SHBG, the metabolic syndrome score remained negatively associated with total T ($p=0.027$) and calculated free T ($p=0.027$) but was not associated with DHT or E2.

TABLE 3

Multivariable models

The final multivariable models from the forward stepwise selection procedure are shown in Table 4. Variables in these models are necessarily significant and independent determinants of the hormone variable but the variables not selected may still be related to hormone level (see Tables 2 and 3) with their capacity already conveyed through one or more of the selected variables with which they are correlated. The significant independent determinants of SHBG were age, smoking, chronic renal disease, HDL, and adiponectin (higher SHBG) and BMI, alcohol, insulin, triglycerides, CRP, creatinine (lower SHBG). The significant independent determinants of total and calculated free T were SHBG, alcohol consumption, creatinine and haemoglobin (higher T) and age, BMI, lipid medication and CRP (lower). The independent determinants of DHT were SHBG and haemoglobin (higher DHT) and age, BMI and glucose (lower). Age, BMI, SHBG and haemoglobin were independent determinants of E2, with the difference that higher BMI was associated with higher E2.

TABLE 4

Discussion

Higher total T was independently associated with younger age, reduced adiposity, alcohol consumption, absence of lipid lowering medication, haemoglobin and lower CRP. There were comparable associations of DHT with several of these factors, but DHT was independently associated with lower glucose and not with alcohol or lipid medication. E2 levels were associated with age and haemoglobin, with a positive association with BMI.

Age was an independent determinant of both total and calculated free T levels, even allowing for SHBG. Medical comorbidities including diabetes and CVD were not independently

associated with T; instead metabolic risk factors reflecting central adiposity and inflammation were associated with lower levels of total or calculated free T. These findings are consistent with previous reports spanning younger, middle-aged and older men where total T declined with age in both cross-sectional and longitudinal analyses after adjustment for factors such as BMI, waist hip ratio and physical activity [1,18,19]. Our results contrast with the report by Sartorius et al that serum total T did not vary with age in a cross-sectional analysis of 325 men aged 40 years and above who self-reported very good or excellent health [6]. Frost et al reported similar reference intervals for T in healthy young and elderly men, consistent with the presence of ill-health underlying lower total T in elderly men [7]. In our cohort of men spanning a wider age range with co-existing medical comorbidities, increasing age was an independent determinant of lower total and free T levels, which were not lower in the presence of diabetes, pulmonary, renal or cardiovascular disease. We did not assess self-reported very good or excellent health in our study; therefore it remains possible some men without specific medical conditions might have been unwell with low levels of T. However, our results are consistent with findings in older men which support the concept that lower T levels in men across a span of ages are at least in part an age-associated phenomenon [20].

Our results are consistent with previous studies which identified central adiposity assessed via BMI, waist circumference or fat mass as being closely associated with lower total T levels in men [21,22]. We found that while DHT and E2 levels appeared to be stable across decades of age, in the final multivariable model age was an independent determinant of these hormones. While age-associated changes in calculated free DHT have been reported to parallel trends seen with calculated free T, the calculation of free DHT based on immunoassay for total DHT and SHBG has not been well validated [23]. Previously Feldman et al measured DHT by radioimmunoassay in the Massachusetts Male Ageing Study [2]. In

that study the cross-sectional analysis of 1,709 men aged 40-70 years showed no association of DHT with age, but the longitudinal analysis found higher DHT levels in the subset of 1,156 men followed after 7-10 years. Our results contrast with previous reports of negative associations between E2 and age in cross-sectional studies using immunoassays [18,19], but are consistent with the cross-sectional analysis from the Framingham Heart Study of 1,461 men aged from 30 to 80+ years [24]. In that study E2 was measured by LC-MS, and was positively associated with age. Therefore, when measured accurately using LC-MS, E2 is positively associated with age and BMI, and does not share the associations of T with metabolic factors. Longitudinal studies are required to clarify the evolution of hormone profiles with ageing.

Strengths of our study are the large size of the cohort, its inclusion of men spanning younger, middle and older ages, extensive phenotyping and availability of physical, metabolic and laboratory variables for the analyses. We assayed total T, DHT and E2 using LC-MS which provides a more accurate measurement compared to immunoassays. We explored associations of total and calculated free T, DHT and E2 with variables of interest after systematically adjusting for age, smoking, BMI and SHBG, and then using a multivariable model to identify factors independently associated with hormone levels. Thus we were able to compare distributions of T, DHT and E2 across age groups from young to old, and contrast the associations of each hormone with a range of health-related factors in a community-based cohort of men spanning young to older ages. We acknowledge several limitations of this study. This is an observational study and we performed a cross-sectional analysis thus we cannot infer causation. While WADLS possesses accuracy comparable to adjudication of medical records [25], individuals who did not require hospitalisation might not be identified by this means and reliance on data linkage remains a limitation. The men were community-

dwelling survivors from previous surveys, thus a “healthy survivor” effect might be present making our findings more applicable to healthier older men. We used frozen serum aliquots retrieved from long term storage. Sex steroids are robust molecules with the concentrations in serum stable on assaying after 20 or more years including via LC-MS [26,27]. Even in the event of freezer failure, steroids are largely unaffected by multiple freeze-thaw cycles [28]. However, the same may not be true for proteins such as SHBG and LH. We did not have serial blood samples to assess longitudinal changes in hormones levels, instead we used a single blood sample albeit collected early in the morning to minimise the effect of circadian variation on hormone levels which may be preserved in healthy older men [29]. Additionally the Busselton cohort is almost entirely Caucasian in ethnicity thus care must be taken before extrapolating our findings to other populations.

Lower total T may be part cause and part consequence of central adiposity, and associated metabolic syndrome traits [30]. In our study T was inversely associated with metabolic syndrome score after adjusting for age, smoking, BMI and SHBG, while DHT and E2 were not associated. The contribution of DHT and E2 to cardiometabolic risk is less well understood. Suppression of DHT levels in men by administration of 5 α -reductase inhibitors such as dutasteride or finasteride does not affect bone turnover, lipid profiles or haemoglobin levels [31]. In an interventional study, the effect of exogenous T to increase fat-free mass in men with suppressed T production was preserved in the presence of 5 α -reductase inhibition [32]. Conversely, administration of high dose DHT to healthy men aged >50 years increased haemoglobin level, suppressed T, E2 and LH and decreased spinal bone mineral density most likely via the reduction in oestrogen exposure [33]. In our study, DHT shared several associations with metabolic risk factors in common with T, but its distinct and inverse

associations with diabetes and fasting glucose are consistent with an additive role as a biomarker for cardiometabolic risk.

Increased haematocrit can be a side effect of T or DHT therapy [33] thus the positive associations of T and DHT with haemoglobin are expected. Of note, E2 was positively associated with haemoglobin level. Further investigation would be needed to clarify whether E2 regulates haemoglobin levels in men distinct from androgens. In the final multivariable model T was inversely associated with CRP, consistent with previous studies [34]. However, DHT and E2 were not suggesting divergent associations of androgens and estrogens with inflammation. In the multivariable model, levels of T, DHT and E2 were not associated with current smoking.

Finkelstein et al reported that in men given depot goserelin acetate to suppress endogenous T and E2, compared to men given T without anastrozole, those additionally treated with the aromatase inhibitor anastrozole to fully suppress circulating E2 exhibited greater increases in subcutaneous and intraabdominal fat [35]. In our study E2 and BMI were positively correlated. Therefore, E2 may regulate as well as be regulated by adipose tissue mass. By contrast E2 does not appear to be associated with metabolic risk factors nor with the presence of metabolic syndrome in the manner of T. Thus any influence of E2 on cardiovascular risk may be limited compared to its role in other tissues such as bone [12].

In conclusion, in men spanning younger, middle and older ages circulating androgens are more related to metabolic risk factors than medical comorbidities. However, E2 does not exhibit corresponding associations with metabolic risk traits. Associations of sex hormones with age, metabolic factors and medical comorbidities may alter as men make the transition

from middle to older age. Further investigation is required to clarify the roles of DHT and E2 distinct from T as a risk predictor for the development of CVD in men spanning middle to older ages.

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Table 1. Distributions of testosterone (T), calculated free testosterone (cFT), dihydrotestosterone (DHT), estradiol (E2), sex hormone binding globulin (SHBG) and luteinizing hormone (LH) stratified according to decades of age in years. Data are shown as the number (N) of men per age group and mean (SD) values for each continuous variable.

Age (years)	N	BMI (kg/m ²)	T (nmol/L)	cFT (pmol/L)	DHT (nmol/L)	E2 (pmol/L)	SHBG (nmol/L)	LH (IU/L)
< 30.0	235	24.5 (3.2)	16.4 (5.0)	277 (67)	1.70 (0.69)	63.7 (32.9)	24.1 (9.5)	3.52 (1.36)
30.0 – 39.9	357	25.9 (3.1)	14.8 (4.6)	244 (56)	1.78 (0.75)	57.6 (30.4)	26.1 (11.9)	3.27 (1.83)
40.0 – 49.9	385	26.9 (3.3)	12.9 (4.8)	214 (59)	1.63 (0.73)	56.8 (27.3)	25.5 (11.0)	3.15 (1.62)
50.0 – 59.9	344	27.2 (3.5)	13.2 (4.7)	208 (53)	1.72 (0.74)	59.1 (27.6)	29.1 (11.2)	3.53 (1.93)
60.0 – 69.9	309	27.2 (3.6)	12.9 (4.8)	197 (55)	1.67 (0.69)	62.0 (32.0)	32.1 (11.4)	4.26 (2.87)
70.0 – 79.9	227	26.8 (3.1)	12.6 (4.6)	185 (51)	1.72 (0.69)	63.7 (32.3)	36.6 (13.5)	6.05 (5.05)
≥ 80.0	63	26.0 (4.0)	11.7 (4.0)	164 (48)	1.77 (0.68)	67.7 (30.2)	45.3 (15.6)	8.24 (7.89)
All	1,920	26.5 (3.5)	13.6 (4.9)	218 (64)	1.71 (0.72)	60.3 (30.3)	29.1 (12.6)	3.98 (3.13)
Overall p-value		<0.001	<0.001	<0.001	0.151	0.005	<0.001	<0.001
p-value for trend		<0.001	<0.001	<0.001	0.779	0.057	<0.001	<0.001

Table 2. Age-, smoking- and BMI-adjusted* associations between each potential risk factor and testosterone (T), calculated free testosterone (cFT), dihydrotestosterone (DHT), estradiol (E2), sex hormone binding globulin (SHBG) and luteinizing hormone (LH). Table shows estimated difference in mean hormone level compared with reference category for categorical risk factors or for an increase of one standard deviation in continuous risk factors, and the p-value for the association.

	T (nmol/L)		cFT (pmol/L)		DHT (nmol/L)		E2 (pmol/L)		SHBG (nmol/L)		LH (IU/L)	
	Est	p-value	Est	p-value	Est	p-value	Est	p-value	Est	p-value	Est	p-value
Exercise vigorous	0.047	0.839	2.120	0.461	-0.016	0.658	-2.314	0.151	-0.344	0.544	-0.048	0.752
Alcohol Non	0.000	0.390	0.000	0.022	0.000	0.564	0.000	0.491	0.000	0.244	0.000	0.666
Ex	-0.719		-5.196		-0.111		-0.178		-1.332		0.506	
Light	-0.256		2.395		-0.121		-1.435		-2.002		0.227	
Heavy	-0.020		8.830		-0.116		0.974		-2.498		0.207	
Diabetes	-0.877	0.050	-11.42	0.038	-0.175	0.012	0.045	0.988	-1.057	0.330	-0.151	0.606
Cardiovascular disease history	0.125	0.677	0.547	0.883	0.073	0.118	-0.647	0.753	-0.140	0.848	-0.083	0.674
Chronic pulmonary disease history	-1.192	0.069	-15.45	0.055	-0.197	0.054	-7.011	0.119	-2.069	0.194	0.138	0.748
Chronic renal disease history	2.271	0.384	19.92	0.534	0.060	0.882	13.40	0.444	7.991	0.206	0.101	0.952
Lipids medication	-1.480	0.017	-22.02	0.004	-0.139	0.148	-2.520	0.558	0.384	0.797	0.476	0.239
Hypertension	-0.676	0.033	-9.427	0.015	-0.086	0.082	2.393	0.273	-1.748	0.024	-0.027	0.897
Systolic BP (SD = 15.8 mmHg)	-0.244	0.041	-1.095	0.456	-0.017	0.349	0.753	0.361	-1.191	<0.001	-0.070	0.374
Diastolic BP (SD = 10.1 mmHg)	-0.177	0.118	-0.550	0.692	-0.001	0.935	-0.133	0.864	-0.884	0.001	-0.096	0.192
log Glucose (SD = 0.184 mmol/L)	-0.310	0.005	-4.225	0.002	-0.062	<0.001	-0.200	0.794	-0.449	0.090	-0.028	0.691
log Insulin (SD = 0.603 μ U/mL)	-0.793	<0.001	-6.172	<0.001	-0.096	<0.001	-2.295	0.006	-2.442	<0.001	-0.146	0.068
Cholesterol (SD = 1.04 mmol/L)	0.098	0.378	3.138	0.021	0.031	0.071	-1.470	0.055	-0.523	0.052	0.043	0.554
HDL (SD = 0.302 mmol/L)	0.828	<0.001	6.949	<0.001	0.083	<0.001	2.444	0.001	2.311	<0.001	0.059	0.406
Triglycerides (SD = 1.02 mmol/L)	-0.834	<0.001	-5.599	<0.001	-0.067	<0.001	-1.932	0.010	-2.761	<0.001	-0.064	0.378
Metabolic score 0	0.000	<0.001	0.000	<0.001	0.000	<0.001	0.000	0.039	0.000	<0.001	0.000	0.259

* For SHBG BMI adjustment includes BMI and BMI squared.

Table 3. Age-, smoking-, BMI- and SHBG-adjusted associations between each potential risk factor and testosterone (T), calculated free testosterone (cFT), dihydrotestosterone (DHT) and estradiol (E2). Table shows estimated difference in mean hormone level compared with reference category for categorical risk factors or for an increase of one standard deviation in continuous risk factors, and the p-value for the association.

	T (nmol/L)		cFT (pmol/L)		DHT (nmol/L)		E2 (pmol/L)	
	Est	p-value	Est	p-value	Est	p-value	Est	p-value
Exercise vigorous	0.162	0.373	2.824	0.301	-0.003	0.925	-1.968	0.214
Alcohol Non	0.000	0.006	0.000	0.002	0.000	0.761	0.000	0.352
Ex	-0.351		-2.937		-0.069		0.295	
Light	0.318		5.918		-0.056		-0.689	
Heavy	0.717		13.36		-0.032		2.065	
Diabetes	-0.611	0.080	-9.796	0.061	-0.145	0.020	0.324	0.915
Cardiovascular disease history	0.142	0.546	0.646	0.854	0.075	0.071	-0.529	0.793
Chronic pulmonary disease history	-0.814	0.111	-13.14	0.086	-0.154	0.090	-6.279	0.155
Chronic renal disease history	0.210	0.918	7.328	0.810	-0.176	0.626	9.075	0.597
Lipids medication	-1.548	0.001	-22.44	0.002	-0.147	0.085	-2.623	0.534
Hypertension	-0.350	0.157	-7.439	0.045	-0.049	0.267	3.320	0.121
Systolic BP (SD = 15.8 mmHg)	0.082	0.384	0.904	0.520	0.020	0.231	1.463	0.071
Diastolic BP (SD = 10.1 mmHg)	0.072	0.415	0.976	0.460	0.027	0.083	0.394	0.606
log Glucose (SD = 0.184 mmol/L)	-0.189	0.026	-3.492	0.006	-0.048	0.001	0.009	0.991
log Insulin (SD = 0.603 μ U/mL)	-0.177	0.068	-2.453	0.090	-0.026	0.124	-0.980	0.240
Cholesterol (SD = 1.04 mmol/L)	0.256	0.003	4.114	0.001	0.049	0.001	-1.158	0.123
HDL (SD = 0.302 mmol/L)	0.220	0.012	3.314	0.011	0.013	0.390	1.145	0.135
Triglycerides (SD = 1.02 mmol/L)	-0.125	0.160	-1.281	0.335	0.016	0.306	-0.428	0.574
Metabolic score 0	0.000	0.027	0.000	0.013	0.000	0.208	0.000	0.210
1	-0.017		-0.766		0.034		0.391	

2	-0.444		-5.648		-0.053		-4.018	
3+	-0.759		-13.18		-0.038		-0.768	
log CRP (SD = 1.21 mg/L)	-0.546	<0.001	-8.303	<0.001	-0.043	0.009	-2.060	0.010
Creatinine (SD = 17.1 µmol/L)	0.247	0.004	3.169	0.013	0.036	0.018	0.422	0.564
log ALT (SD = 0.462 U/L)	-0.149	0.103	-0.946	0.488	0.009	0.574	0.902	0.254
Haemoglobin (SD = 10.0 g/L)	0.554	<0.001	8.125	<0.001	0.070	<0.001	4.469	<0.001
log Adiponectin (SD = 0.653 mg/L)	0.041	0.632	0.757	0.558	0.004	0.777	-1.867	0.013

Table 4. Final multivariable models from forward stepwise selection procedure ($p < 0.01$ for entry) with age, smoking and BMI forced into model for SHBG and LH, and SHBG also forced into model for T, cFT, DHT and E2. Table shows estimated difference in mean hormone level compared with reference category for categorical risk factors or for an increase of one standard deviation in continuous risk factors, and the p-value for the association.

		T		cFT		DHT		E2		SHBG		LH	
		Est	p-value	Est	p-value	Est	p-value	Est	p-value	Est	p-value	Est	p-value
Agegroup	< 30.0	0.000	<0.001	0.000	<0.001	0.000	<0.001	0.000	0.001	0.000	<0.001	0.000	<0.001
	30.0 – 39.9	-1.759		-31.21		0.051		-9.536		3.973		3.829	
	40.0 – 49.9	-3.222		-55.31		-0.045		-11.14		4.982		4.760	
	50.0 – 59.9	-3.811		-66.35		-0.074		-11.49		9.132		8.729	
	60.0 – 69.9	-4.554		-77.78		-0.194		-9.763		12.16		11.69	
	70.0 – 79.9	-5.687		-91.40		-0.256		-8.638		15.89		15.42	
	≥ 80.0	-9.109		-129.5		-0.495		-6.991		23.59		24.02	
Smoking	Never	0.000	0.068	0.000	0.143	0.000	0.184	0.000	0.329	0.000	<0.001	0.000	0.003
	Ex	0.113		1.620		-0.044		1.892		0.304		-0.525	
	Current	0.559		7.148		0.032		2.653		3.568		2.064	
BMI (SD = 3.5 kg/m ²)		-0.442	<0.001	-6.439	<0.001	-0.038	0.020	3.689	<0.001	-13.23	<0.001	-4.016	<0.001
BMI ² (SD = 189 kg/m ²)										11.44	<0.001		
SHBG (SD = 12.6 nmol/L))		3.243	<0.001	19.81	<0.001	0.377	<0.001	7.231	<0.001				
Alcohol	Non	0.000	0.001	0.000	<0.001					0.000	<0.001		
	Ex	-0.411		-3.807						-1.210			
	Light	0.270		5.151						-3.314			
	Heavy	0.751		13.77						-4.771			
Chronic renal disease history										18.90	0.003		
Lipids medication		-1.489	0.002	-21.43	0.003								
log Glucose (SD = 0.184 mmol/L)						-0.046	0.002						

log Insulin (SD = 0.603 µU/mL)										-1.236	<0.001		
HDL (SD = 0.302 mmol/L)										1.256	<0.001		
Triglycerides (SD = 1.02 mmol/L)										-1.737	<0.001		
log CRP (SD = 1.21 mg/L)	-0.528	<0.001	-8.056	<0.001						-1.116	<0.001		
Creatinine (SD = 17.1 µmol/L)	0.317	<0.001	4.149	<0.001	0.039	0.010				-1.077	<0.001		
Haemoglobin (SD = 10.0 g/L)	0.557	<0.001	8.103	<0.001	0.071	<0.001	4.469	<0.001					
log Adiponectin (SD = 0.653 mg/L)										1.087	<0.001		