Serum testosterone is inversely, and sex hormone-binding globulin directly, associated with all-cause mortality in men.

Bu B. Yeap PhD[1,2], Ross J. Marriott PhD[3], Leen Antonio PhD[4], Yi X. Chan MBBS[1,2], Suchitra Raj MBBS[2], Girish Dwivedi PhD[1,5], Christopher M. Reid PhD[6], Bradley D. Anawalt MD[7], Shalender Bhasin MD[8], Adrian S. Dobs MD[9], Graeme J. Hankey MD[1], Alvin M. Matsumoto MD[7,10], Paul E. Norman DS[1], Terence W. O'Neill MD[11], Claes Ohlsson PhD[12], Eric S. Orwoll MD[13], Dirk Vanderschueren PhD[4], Gary A. Wittert MD[14], Frederick C.W. Wu MD[15], Kevin Murray PhD[3].

[1]Medical School, University of Western Australia, Perth, Australia

[2]Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth, Australia

[3]School of Population and Global Health, University of Western Australia, Perth, Australia

[4]Laboratory of Clinical and Experimental Endocrinology, KU Leuven, Leuven, Belgium

[5]Harry Perkins Institute of Medical Research and Fiona Stanley Hospital, Perth, Australia

[6]School of Public Health, Curtin University, Perth, Australia

[7]Department of Medicine, University of Washington School of Medicine, Seattle, United States

[8]Brigham and Women's Hospital; and Harvard Medical School, Boston, United States

[9]Division of Endocrinology, Johns Hopkins University School of Medicine, Baltimore, United States

[10]Geriatric Research, Education and Clinical Center, VA Puget Sound Health Care System, Seattle, United States

[11]Manchester Institute for Collaborative Research on Ageing, University of Manchester, Manchester, United Kingdom

[12]Centre for Bone and Arthritis Research, Department of Internal Medicine and Clinical Nutrition, Sahlgrenska Academy, University of Gothenburg; and Region Vastra Gotaland, Sahlgrenska University Hospital, Gothenburg, Sweden

[13]Oregon Health and Science University, Portland, United States

jc.2020-02472

[14]Freemasons Centre for Men's Health and Wellbeing, School of Medicine, University of Adelaide, Adelaide, Australia

[15]Division of Endocrinology, Diabetes & Gastroenterology, School of Medical Sciences, University of Manchester, Manchester, United Kingdom

Corresponding author (to whom requests for reprints should be addressed)

Bu B. Yeap

Professor, Medical School, M582, University of Western Australia, 35 Stirling Highway, Crawley 6009, Western Australia, Australia

Email: bu.yeap@uwa.edu.au

Telephone: +61 8 6151 1148

Funding information

This work was funded by a Western Australian Health Translation Network (WAHTN) Medical Research Future Fund Rapid Applied Research Translation Grant. The funding source had no role in the conduct of the study, nor the analysis or interpretation of the results, nor the preparation and submission of the manuscript.

Disclosure summary

The authors have no conflicts of interest to declare in relation to this work.

Abstract

Context

Serum testosterone concentrations decline with age, while serum sex hormone-binding globulin (SHBG) concentrations increase.

Objective

To analyse associations of baseline serum testosterone and SHBG concentrations, and calculated free testosterone (cFT) values, with all-cause and cause-specific mortality in men.

Design, setting and participants

The UK Biobank prospective cohort study of community-dwelling men 40-69 years-old, followed for 11 years.

Main outcome measures

All-cause, atherosclerotic cardiovascular disease (CVD) and cancer-related mortality. Cox proportional hazards regression was performed, adjusting for age, waist circumference, medical conditions and other covariates. Models for testosterone included SHBG, and vice versa.

Results

In complete case analysis of 149,436 men with 10,053 deaths (1,925 CVD and 4,927 cancer-related), men with lower testosterone had higher mortality from any cause (lowest vs highest quintile, Q1 vs Q5, fully-adjusted hazard ratio [HR]=1.14, 95% confidence interval [CI]=1.06-1.22, overall trend $P<0.001$), and cancer (HR=1.20, CI=1.09-1.33, $P<0.001$), with no association for CVD deaths. Similar results were seen for cFT. Men with lower SHBG had lower mortality from any cause (Q1 vs Q5, HR=0.68, CI=0.63-0.73, $P<0.001$), CVD (HR=0.70, CI=0.59-0.83, $P<0.001$), and cancer (HR=0.80, CI=0.72-0.89, $P<0.001$). A multiply-imputed dataset (N=208,425, 15,914 deaths, 3,128 CVD and 7,468 cancer-related) and analysis excluding deaths within first two years (9,261, 1,734 and 4,534 events) yielded similar results.

Conclusions

Lower serum testosterone is independently associated with higher all-cause and cancer-related, but not CVD-related, mortality in middle-aged to older men. Lower SHBG is independently associated with lower all-cause, CVD-related and cancer-related mortality. Confirmation and determination of causality requires mechanistic studies and prospective trials.

Keywords

Testosterone, sex hormone-binding globulin, mortality, cardiovascular disease, cancer

Introduction

As men grow older, serum testosterone concentrations decline, while concentrations of its main binding protein, sex hormone-binding globulin (SHBG), increase.[1] Obesity and medical comorbidities contribute to the decline in circulating testosterone.[2,3] Obesity, particularly central adiposity and insulin resistance, are associated with lower, and liver or thyroid disease with higher SHBG concentrations.[4,5]

Previous studies have reported no associations of testosterone concentrations with mortality,[6-10] or associated lower testosterone with higher all-cause mortality.[11-17] Similarly, associations of testosterone concentrations with cardiovascular disease (CVD)-related deaths are inconsistent: some studies reported no associations,[6,8,10,13,17,18] others inverse associations.[11,12,14,15,19] Cancer is another major cause of deaths. Testosterone concentrations have been inversely associated with cancer mortality in some studies,[11,16] positively associated in one,[22] and not associated in others.[12,19] Several cohort studies have reported no association of SHBG concentrations with mortality, nor with deaths from CVD.[6,8,17-20] Other studies in middle-aged and older men,[21,22] and men with diabetes,[23-25] associated higher SHBG concentrations with mortality. In addition to inconsistent results, the heterogeneity of these studies with respect to geography, participant selection and covariates included in different analytical models, adds further uncertainty to the findings.

To accommodate the relationship between serum testosterone and SHBG, free testosterone is commonly calculated from (total) testosterone and SHBG using formulae based on mass action equations (calculated free testosterone, cFT).[26,27] Some studies reported similar findings for cFT and (total) testosterone concentrations with respect to mortality in men,[10,16,19] whereas some reported associations of low cFT but not (total) testosterone with all-cause[7,9] or CVD-related mortality.[22] Thus, studies of cFT and mortality risk in men have reported inconsistent results, and it remains unclear whether cFT offers additional information over testosterone alone for mortality-related outcomes.

A sufficiently large dataset with a correspondingly large number of outcome events would clarify the associations of serum testosterone and SHBG with mortality, enabling more precise estimates of effect sizes. Analysis of deaths from any cause, deaths from CVD, and also cancer-related deaths could be performed. Associations of serum testosterone and SHBG could also be compared with associations of cFT. The United Kingdom (UK) Biobank, with a large number of men from a broadly-based community-dwelling population who were prospectively followed for outcome events, is ideally suited to this purpose.[28]

We aimed to elucidate the associations of circulating testosterone and SHBG, and cFT, with overall mortality, and CVD and cancer-related deaths, in a large cohort of community-dwelling men aged 40-69 years from the UK Biobank. We tested the hypotheses that (i) lower testosterone and lower SHBG are independently associated with higher mortality in men, after adjusting for potential confounders, and (ii) cFT provides additional information beyond that of testosterone and SHBG as predictors of mortality risk in men.

Participants and methods

The UK Biobank

Over 500,000 participants aged 40-69 years were recruited across 22 assessment centres in the UK during 2006-10.[28] Detailed characterisation was undertaken using self-completed questionnaires, brief interviews, physical and functional measures, and blood collection. UK Biobank was approved by the North West Multi-Centre Research Ethics Committee (reference 06/MRE08/65), and all participants provided informed consent. This analysis was approved by the UK Biobank.

Variables of interest

Exposures

Exposures of interest were baseline serum testosterone and SHBG concentrations, and cFT values. Serum samples were prepared and stored at -80 C until assayed for testosterone and sex hormone-binding globulin (SHBG) in the UK Biobank central laboratory.[29,30] Serum total testosterone was assayed using a competitive binding chemiluminescent immunoassay, analytical range 0.35-55.5 nmol/L (10-1,599 ng/dL, DXI 800, Beckman Coulter, UK). Coefficients of variation were 8.3%, 3.7% and 4.2% for testosterone concentrations in low, medium and high ranges (1.0-2.2, 13.4-22.8 and 29.3-49.4 nmol/L, or 29-63, 386-657 and 844-1,424 ng/dL, respectively). Serum SHBG was assayed using a two-step sandwich chemiluminescent immunoassay, analytical range 0.33-242 nmol/L (DXI 800, Beckman Coulter, UK). Coefficients of variation were 5.7%, 5.3% and 5.2% for SHBG in low, medium and high ranges (15.0-27.7, 31.9-55.5 and 56.3-87.8 nmol/L). Free testosterone was calculated (cFT) using the Vermeulen method, from total testosterone and SHBG, with fixed albumin concentration (42 g/L).[26]

Study outcomes

Three different outcomes were investigated: deaths from any cause, deaths from atherosclerotic CVD; and deaths from cancer. Incident events (deaths and cause-specific deaths) were identified for participants from the time of recruitment (March 2006-October 2010) until 30 April 2020, the latest date to which mortality data from all UK Biobank sources were complete and available.[31] Primary cause of death ICD-10 codes were obtained from a central registry.[32] Men who died of atherosclerotic cardiovascular disease (CVD) were categorised as such if the primary cause of death was due to angina pectoris, myocardial infarction, other acute ischaemic heart disease, chronic ischaemic heart disease, dilated cardiomyopathy, cardiac arrest, heart failure, haemorrhagic or ischaemic stroke, atherosclerosis, or aortic aneurysm and dissection (Table S1[33]). Men who died of cancer were categorised as such if the primary cause of death was due to cancer (Table S1[33]). For

analyses of CVD deaths and cancer deaths, individuals were censored at the earliest of their date of death (for analyses of cause-specific deaths; if not attributed to that cause) or end of follow-up. The primary cause of death, as the full set of diagnosis codes used (International Classification of Disease, ICD-9 and ICD-10), is provided (Table S1[33]).

Covariates

Participants' age, ethnicity, living with partner, qualifications, alcohol consumption, dietary patterns, physical activity and smoking status were obtained from self-report. (South) Asian refers to men of India, Pakistan, Bangladesh and Sri Lanka extraction. Qualifications were categorised as below A-levels (high school), completed A-levels, completed college/university, or completed other professional qualification (not school/college/university). Alcohol consumption, diet and physical activity were categorised.[33] Height and weight were obtained by physical examination. Body mass index (BMI) was calculated (weight divided by the square of height, kg/m^2).

Prevalent medical conditions were determined using available variables from self-report, physical examination, blood chemistry, and linked general practice records and medical datasets (surgical codes, hospital admission diagnosis codes, and cancer registry). Medications were determined from self-report. Prevalent CVD (defined as prior myocardial infarction, stroke or heart failure), angina, atrial fibrillation, Chronic Obstructive Pulmonary Disease (COPD), human immunodeficiency viruses (HIV), and liver disease were determined from self-report or previous hospital admission diagnoses. Diabetes, dementia, renal impairment and hypertension were defined from combinations of self-report, medications, physical measures and biochemical data.[33] Thyroid disease was determined by use of anti-thyroid, thyroxine, or liothyronine medications, prior hospital admission diagnosis, or self-report condition. Hyperlipidemia was categorised by use of lipid-lowering medications. Other medication variables included baseline use of anticonvulsant, glucocorticoids, and opioid

medications, and number of medications taken.[33] The full set of hospital admission and diagnosis codes used is provided (Table S1[33]).

Statistical analyses

Participant characteristics were described by counts and percentages (categorical variables) or medians and interquartile ranges (IQRs; continuous variables). Kaplan-Meier survival plots were constructed according to quintiles of testosterone, SHBG and cFT values, and follow-up times calculated. Cox proportional hazards models were fitted, with separate analyses done for each exposure (testosterone, SHBG, and cFT) and outcome. Site was modelled as a stratified factor, representing geographic variation for male UK Biobank participants. For the main analyses, participants with missing covariate data were excluded. Analyses were repeated for multiply-imputed datasets, and also after excluding deaths occurring in the first two years of follow-up, to infer the potential effects of missing data and reverse causation on results.[33]

Each analysis involved fitting an unadjusted model, as well as two multivariable models, which included additional covariates, to adjust for known risk factors and possible confounders. Multivariable model 1 included lifestyle and demographic variables (age, alcohol consumption, BMI, cholesterol, diet, ethnicity, living with partner, physical activity, qualifications, smoking status, waist circumference), prevalent conditions (angina, atrial fibrillation, COPD, dementia, diabetes, HIV, hypertension, liver disease, renal impairment, thyroid disease, and medication used at baseline (anticonvulsants, lipid, glucocorticoids, opioids, total number of medications). The number of medications used at baseline was included as a proxy for comorbidity status. In analyses of CVD deaths, prevalent CVD was included as an additional term in the multivariable models. In analyses of cancer deaths, prevalent cancer was included as an additional term in the multivariable models.

Multivariable model 2 included all the above, and for testosterone analyses included SHBG as an additional covariate, and for SHBG analyses included testosterone.

To account for non-linearity, adjusted analyses were performed modelling continuous explanatory variables using restricted cubic splines.[33] Validity of the proportional hazards assumption was confirmed using Schoenfeld plots. Hazard ratios (HRs) calculated from each of the fitted models, relative to a reference value at the median of the fifth quintile were plotted against the exposure variable, with 95% confidence intervals (CIs). HRs were also calculated for median values within each quintile of the exposure, relative to this reference value, and tabulated with 95% CIs.[33] The statistical significance of associations with each of the three separate outcomes was evaluated using likelihood ratio tests, using a Bonferroni-corrected threshold of $P<0.017$ ($\alpha=0.05 \div 3$). All analyses were conducted in R version 4.0.2.[34]

Results

Study cohort

UK Biobank recruited 229,122 men aged 40-69 years from 2006-2010. Excluding men who were infertile or with prior pituitary disease, orchidectomy, hospitalisation for androgenital/testicular disorders, or taking androgen, anti-androgen, estrogen, anti-estrogen, progesterone or 5α-reductase medications, left 224,266. Further exclusions due to missing hormone measurements (15,841 and 31,389 men with missing testosterone and SHBG values, respectively) and other covariates, left 149,436 for analyses (Figure S1[33]). The median follow-up time was 11.3 years (IQR: 10.6-11.9) for all-cause deaths, 11.2 years (IQR: 10.5-11.9) for CVD deaths, and 11.2 years (IQR: 10.5-11.9) for cancer deaths.

Participant characteristics

Men were predominantly white (95.3%), living with a partner (78.3%), on a "low red meat" diet (80.5%), with median age 58.0 years, BMI 27.1 kg/m^2, and testosterone 11.7 nmol/L (Table 1). 7,800 (5.2%) had prevalent CVD, and 9,070 (6.1%) a cancer history. During follow-up, 10,053 (6.7%) died,

of which 1,925 had the cause of death attributed to atherosclerotic CVD, and 4,927 to cancer. Men with prevalent CVD were older, had higher BMI and waist circumference, fewer were living with partner, had lower educational attainment, were less physically active, more likely to have smoked, had lower cholesterol but more were on lipid-lowering medication, had more prevalent health conditions, took more medications, and had lower testosterone, and higher SHBG, compared to men without. Men with a history of cancer at baseline were older, slightly less physically active, more likely to have smoked, had more prevalent health conditions, took more medications, and had lower testosterone and higher SHBG, compared to men without (Table 1).

Associations of serum testosterone with mortality

Survival plots showed shorter average times to death from any cause, CVD and cancer in men with serum testosterone in the lowest quintile (Figure S2[33]).

In univariable analysis, there was a U-shaped association of serum testosterone with all-cause mortality, while men with lower serum testosterone had higher CVD and cancer-related mortality (Figure S3[33]). Multivariable model 1 accentuated the U-shaped association of serum testosterone with mortality, abrogated the association of lower testosterone with CVD deaths, and left the association with cancer deaths largely unchanged (Figure S4[33]). Multivariable model 2 (including SHBG), flattened the U-shaped association with all-cause mortality, showed no association with CVD deaths, and associated lower testosterone with cancer deaths (Figure 1).

Associations of testosterone in quintiles with all-cause mortality are tabulated (Table 2). In univariable analysis, HR for the lowest vs highest quintile (Q1 vs Q5) was 1.21, CI=1.15-1.27, overall trend *P*<0.001). In multivariable model 1, HR for Q1-4 were all significantly <1.00, with Q2 the lowest (HR=0.82, CI=0.78-0.87, trend *P*<0.001). In multivariable model 2 (including SHBG), only Q1 was associated with significantly higher all-cause mortality (HR=1.14, CI=1.06-1.22, trend *P*<0.001).

Univariable analysis associated lower serum testosterone with CVD deaths (HR=1.33, CI=1.18-1.50, trend *P*<0.001) (Table 2). In multivariable model 1, HR for Q1-4 were all <1.00 (overall trend not significant, *P*=0.056). In multivariable model 2 (including SHBG), there was no association of testosterone with CVD deaths (Q1 vs Q5, HR=1.01, CI=0.86-1.18, trend *P*=0.500). Univariable analysis associated lower testosterone with cancer mortality (Table 2). The association was attenuated in multivariable model 1, and restored in multivariable model 2 (Q1 vs Q5, HR=1.20, CI=1.09-1.33, trend *P*<0.001).

A multiply-imputed dataset (N=208,425, 15,914 deaths, 3,128 CVD and 7,468 cancer-related) showed similar results (Table S2[33]). Analyses excluding deaths within first two years (N=149,185, 9,261 deaths, 1,734 CVD-related and 4,534 cancer-related) showed similar results (Table S3[33]).

Associations of serum SHBG with mortality

Survival plots showed that the lower the quintile of serum SHBG, the longer the average time to death from any cause or from cancer (Figure S5[33]). Men with SHBG in the lowest quintile had the longest average time to CVD death, and those with SHBG in the highest quintile the shortest.

Univariable analysis showed linear associations of serum SHBG with all-cause, CVD and cancer mortality (Figure S6[33]). In multivariable model 1, the slopes of the regression lines for all-cause and CVD deaths were shallower, with a suggestion of a U-shaped association with cancer deaths (Figure S7[33]). In multivariable model 2 (including testosterone) linear associations of serum SHBG with all-cause, CVD and cancer mortality were present (Figure 2).

Analysis of serum SHBG in quintiles showed robust linear associations with all-cause and CVD mortality (Table 3). In univariable analysis, HR for all-cause mortality was lower in stepwise fashion

from Q4 to Q1 (Q1 vs Q5, HR=0.51, CI=0.48-0.54, trend *P*<0.001). Results for both multivariable models 1 and 2 were similar (Q1 vs Q5, multivariable model 2 HR=0.68, CI=0.63-0.73, trend *P*<0.001). Similar results were seen for CVD deaths (multivariable model 2, HR=0.70, CI=0.59-0.83, trend *P*<0.001). For cancer deaths the linear association was less apparent in multivariable model 1, but was robust in multivariable model 2 (Q1 vs Q5, HR=0.80, CI=0.72-0.89, trend *P*<0.001).

A multiply-imputed dataset (Table S4[33]) and analyses excluding deaths within first two years (Table S5[33]) showed similar results.

Associations of cFT values with mortality

Survival plots of cFT showed the lower the quintile of cFT, the shorter the average time to death from any cause, CVD or cancer (Figure S8[33]).

In univariable analyses, stepwise increases in risk of all-cause, CVD and cancer mortality were seen decreasing quintiles of cFT (Figure S9[33]). In multivariable analyses (model 1), lower cFT was associated with higher all-cause and cancer-related mortality, but not with CVD deaths (Figure 3), results resembling those seen with testosterone (Figure 1).

Univariable analyses showed stepwise increases in HR for all-cause, CVD and cancer-related mortality for decreasing quintiles of cFT (Table 4). However, these were substantially attenuated in multivariable analysis (multivariable model 1), leaving only Q1 vs Q5 with increased HR for all-cause (HR=1.13, CI=1.06-1.20, trend *P*<0.001) and cancer-related mortality (HR=1.17, CI=1.07-1.28, trend *P*<0.001). There was no association of cFT with CVD deaths (HR=1.03, CI=0.89-1.18, trend *P*=0.143) in multivariable analysis.

Discussion

This large cohort study of middle-aged and older men demonstrates that serum testosterone and cFT are inversely associated with overall and cancer-related, but not CVD-related mortality. The study also demonstrates direct relationships between serum SHBG and overall, CVD-related and cancer-related mortality.

Previous studies of testosterone and mortality have been smaller, generally analysing between one to five thousand men, with several hundred to a thousand or more deaths.[6-18] A nested case-control analyses drawn from a cohort of 11,606 men involved 825 men who died (369 CVD-related, 304 cancer-related) and 1,489 controls.[11] The largest previous cohort analysis involved 5,350 men, with 1,533 deaths (428 CVD-related, 480 from cancer-related).[19] Our analysis of 149,436 men from the UK Biobank, with 10,053 deaths (1,925 CVD-related, 4,927 cancer-related) provided unprecedented scope to clarify the associations of testosterone and SHBG with all-cause, CVD and cancer mortality, and to establish more precise estimates of effect sizes.

Additionally, previous multivariable models typically adjusted for between five to ten covariates,[7,9,10,12-15,19,22] while more extensive models included 11 to 18 covariates.[6,11,18,23] We adjusted for 26 covariates including age, and not only BMI but also waist circumference. Importantly, and unlike previous studies, we included adjustment for specific factors that raise SHBG, such as thyroid and liver disease and anticonvulsant medications.[5]

In unadjusted analyses, men with lower serum testosterone had higher risks of death from any cause, CVD and cancer. However, in multivariable analyses including SHBG, only men with the lowest testosterone concentrations had higher all-cause and cancer-related, but not CVD-related, mortality. Previous studies including SHBG in the model found either inverse associations,[11,18] or no association[9] of testosterone with all-cause mortality. Other studies adjusting for risk factors and

comorbidities, not including SHBG, reported inverse[12,13,15,16] or neutral[6,7,19,22] associations of testosterone with all-cause mortality. In our fully-adjusted analysis, the inverse association of testosterone with all-cause mortality was independent of SHBG. The association was statistically robust, but largely limited to men with testosterone in the lowest quintile, who had a modest (14%) increase in risk.

Several previous studies reported no associations of testosterone with CVD-related mortality,[6,8,10,13,17,18] whereas others reported inverse associations of testosterone with CVD deaths.[11,12,14,15,19] After adjusting for covariates including SHBG, we found no evidence that testosterone was associated with risk of CVD death. The HR was 1.01, with narrow confidence intervals (0.86-1.18), arguing against any substantive association of testosterone with this outcome.

Two previous studies suggested a relationship between lower testosterone concentrations and cancer-related mortality,[11,16] whereas another study associated higher testosterone with deaths from lung cancer,[22] and other studies have reported no association of testosterone with cancer deaths.[12,19] We found a robust inverse association, which was limited to men with serum testosterone in the lowest quintile, who had a modest (20%) increase in risk of dying from cancer.

Associations of cFT with all-cause, CVD and cancer-related mortality were seen in univariable analyses. However, in multivariable analyses these were attenuated: men with the lowest cFT values had higher all-cause and cancer-related, but not CVD-related mortality. Results for cFT, adjusted for covariates, did not provide additional information beyond analysis of testosterone and SHBG for mortality outcomes.

Lower SHBG concentrations are associated with obesity and insulin resistance.[4,5] However, we found serum SHBG was associated with all-cause mortality, directly and in a linear fashion, independently

of testosterone. There was a 32% lower risk for men with serum SHBG in the lowest compared to highest quintile, with a narrow confidence interval (CI=0.63-0.73). Previous studies in middle-aged and older men have reported no association of SHBG with all-cause mortality in multivariate analyses not including testosterone in the model,[6,8,20] or including testosterone in the model.[17] Two studies associated higher SHBG with all-cause mortality, without testosterone in the model,[22] and with testosterone in the model.[21] Studies of men with type 2 diabetes have associated higher SHBG with mortality.[23-25] Our analysis adjusted for factors that influence SHBG, and for testosterone, clarifies that lower SHBG concentrations are independently associated with lower mortality in a general population of middle- and older-aged men. The association is robust, and is linear, spanning the range of SHBG values.

Several previous studies have not associated SHBG with CVD deaths.[6,8,17-19] One study associated lower SHBG with lower CVD mortality,[9] and another with higher CVD mortality.[20] Our study provides robust evidence that lower SHBG is associated with lower risk of CVD death in middle- and older-aged men, independently of other covariates including testosterone. Men with serum SHBG in the lowest compared to highest quintile with a 30% lower risk, with a narrow confidence interval (CI=0.59-0.83). Furthermore, we found that lower SHBG was associated with lower cancer mortality, in a linear fashion, independently of other covariates including testosterone. This contrasts with previous studies which reported no association of SHBG with cancer mortality.[6,9,16,19,21]

Mechanisms by which circulating testosterone and SHBG might independently influence overall, CVD-related and cancer-related mortality, are outside the scope of this study. Testosterone is the major male sex hormone with multiple physiological actions.[35] While a direct influence of lower circulating testosterone concentrations is conceivable, lower testosterone concentrations may also be a marker for the presence of obesity or medical comorbidities.[2,3] Age, waist circumference and other covariates were adjusted for in the analysis. Further study is needed to determine whether

lower testosterone is a causal contributor to, rather than a biomarker for, poorer health outcomes in ageing men. SHBG is the major binding protein for testosterone in the circulation, with the liver its main site of production.[35] SHBG concentrations are reduced in the setting of obesity and insulin resistance, and increased by thyroid hormone excess, or in the presence of liver disease.[4,5] Thus, these covariates were adjusted for in the multivariable analyses. Whether SHBG modulates mortality risk either directly or indirectly via binding of circulating sex hormones, beyond a role as biomarker for underlying illnesses, remains to be ascertained.

Strengths of this study include the size of the cohort, the duration of follow-up, the large numbers of events observed for each of the outcomes, and adjustment for multiple covariates in the analyses. In addition to BMI, we adjusted for waist circumference as a measure of central adiposity. We adjusted for covariates influencing SHBG concentrations, including thyroid and liver disease. These enabled associations of testosterone and SHBG with mortality outcomes to be clarified with greater precision, making the absence as well as presence of associations interpretable as robust findings.

Limitations of this study include its observational nature, thus causality cannot be determined. Data on many variables was based on self-report, and also collated from medical datasets, with some missing data. However, results from the multiply-imputed datasets were similar to the complete-case analysis. Outcome events were obtained from registry data, not adjudicated. The proportion of CVD-related deaths was lower than cancer-related deaths, in keeping with previous UK Biobank studies.[36-38] Nevertheless, any omissions or inaccuracies would likely be random, and not expected to bias the results. There may be residual confounding from unmeasured variables, but our analysis involved a large number of covariates covering possible confounders. We did not have access to independent measures of aging other than chronological age, to examine as covariates. Serum testosterone was measured in a single baseline sample. The immunoassay used slightly underestimates testosterone concentrations compared to mass spectrometry,[39] however, the rank

order of testosterone values should be consistent. Calculating cFT has limitations and different methods are used, we employed the most widely-used Vermeulen formula.[26,27] Participants in UK Biobank may be healthier than the general UK population.[40] Thus further work is needed to determine the applicability of our findings to other populations.

In conclusion, men with testosterone levels in the lowest quintile, have higher risks of dying from any cause and cancer, but not from CVD. Men with lower SHBG, across the range of SHBG values, have lower risks of dying from any cause, CVD and cancer. Assessing both testosterone and SHBG provides information on key health outcomes in men, warranting further studies to explore causality and potential underlying mechanisms.

Acknowledgements

The authors wish to thank all the participants and staff involved with the UK Biobank, and the management of the UK Biobank, for the opportunity to perform this analysis.

Data availability

Datasets analysed during the current study are not publicly available but are available from the UK Biobank on reasonable request.

Author contributions

BBY, RM and KM led the project, all authors approved the analysis plan, RM and KM performed the statistical analyses, and BBY drafted the manuscript. All authors contributed to interpretation of the results and revision of the manuscript for important intellectual content, and approved its submission.

References

1. Feldman HA, Longcope C, Derby CA, Johannes CB, Araujo AB, Coviello AD, Bremner WJ, McKinlay JB. Age trends in the level of serum testosterone and other hormones in middle-aged men: longitudinal results from the Massachusetts Male Aging Study. J Clin Endocrinol Metab 2002; 87: 589–598.
2. Camacho EM, Huhtaniemi IT, O'Neill TW, Finn JD, Pye SR, Lee DM, Tajar A, Bartfai G, Boonen S, Casanueva FF, Forti G, Giwercman A, Han TS, Kula K, Keevil B, Lean ME, Pendleton N, Punab M, Vanderschueren D, Wu FCW, EMAS Group. Age-associated changes in hypothalamic-pituitary-testicular function in middle-aged and older men are modified by weight change and lifestyle factors: longitudinal results from the European Male Ageing Study. Eur J Endocrinol 2013; 168: 445–455.
3. Shi Z, Araujo AB, Martin S, O'Loughlin P, Wittert GA. Longitudinal changes in testosterone over five years in community-dwelling men. J Clin Endocrinol Metab 2013; 98: 3289–3297.
4. Gyawali P, Martin SA, Heilbronn LK, Vincent AD, Jenkins AJ, Januszewski AS, Taylor AW, Adams RJT, O'Loughlin PD, Wittert GA. Cross-sectional and longitudinal determinants of serum sex hormone binding globulin (SHBG) in a cohort of community-dwelling men. PLoS One 2018; 13: e0200078.
5. Bhasin S, Brito JP, Cunningham GR, Hayes FJ, Hodis HN, Matsumoto AM, Snyder PJ, Swerdloff RS, Wu FC, Yialamas MA. Testosterone therapy in men with hypogonadism: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2018; 103: 1715–1744.
6. Araujo AB, Kupelian V, Page ST, Handelsman DJ, Bremner WJ, McKinlay JB. Sex steroids and all-cause and cause-specific mortality in men. Arch Intern Med 2007; 167: 1252–1260.

7. Vikan T, Schirmer H, Njolstad I, Svartberg J. Endogenous sex hormones and the prospective association with cardiovascular disease and mortality in men: the Tromso study. Eur J Endocrinol 2009; 161: 435–442.
8. Chan YX, Knuiman MW, Hung J, Divitini ML, Beilby JP, Handelsman DJ, Beilin J, McQuillan B, Yeap BB. Neutral associations of testosterone, dihydrotestosterone and estradiol with fatal and non-fatal cardiovascular events, and mortality in men aged 17-97 years. Clin Endocrinol 2016; 85: 575–582.
9. Menke A, Guallar E, Rohrmann S, Nelson WG, Rifai N, Kanarek N, Feinleib M, Michos ED, Dobs A, Platz EA. Sex steroid concentrations and risk of death in US men. Am J Epidemiol 2010; 171: 583–592.
10. Shores MM, Biggs ML, Arnold AM, Smith NL, Longstreth WT Jr, Kizer JR, Hirsch CH, Cappola AR, Matsumoto AM. Testosterone, dihydrotestosterone, and incident cardiovascular disease and mortality in the cardiovascular health study. J Clin Endocrinol Metab 2014; 99: 2061–2068.
11. Khaw K-T, Dowsett M, Folkerd E, Bingham S, Wareham N, Lutben R, Welch A, Day N. Endogenous testosterone and mortality due to all causes, cardiovascular disease, and cancer in men. European Prospective Investigation into Cancer in Norfolk (EPIC-Norfolk) prospective population study. Circulation 2007; 116: 2694–2701.
12. Laughlin GA, Barrett-Connor E, Bergstrom J. Low serum testosterone and mortality in older men. J Clin Endocrinol Metab 2008; 93: 68–75.
13. Tivesten A, Vandenput L, Labrie F, Karlsson MK, Ljunggren O, Mellstrom D, Ohlsson C. Low serum testosterone and estradiol predict mortality in elderly men. J Clin Endocrinol Metab 2009; 94: 2482–2488.
14. Haring R, Volzke H, Steveling A, Krebs A, Felix SB, Schofl C, Dorr M, Nauck M, Wallaschofski H. Low serum testosterone levels are associated with increased risk of

mortality in a population-based cohort of men aged 20-79. Eur Heart J 2010; 31: 1494–1501.

15. Pye SR, Huhtaniemi IT, Finn JD, Lee DM, O'Neill TW, Tajar A, Bartfai G, Boonen S, Casanueva FF, Forti G, Giwercman A, Han TS, Kula K, Lean ME, Pendleton N, Punab M, Rutter MK, Vanderschueren D, Wu FC, EMAS Study Group. Late-onset hypogonadism and mortality in aging men. J Clin Endocrinol Metab 2014; 99: 1357–1366.
16. Hsu B, Cumming RG, Naganathan V, Blyth FM, Le Couteur DG, Hirani V, Waite LM, Seibel MJ, Handelsman DJ. Temporal changes in androgens and estrogens are associated with all-cause and cause-specific mortality in older men. J Clin Endocrinol Metab 2016; 101: 2201–2210.
17. Yeap BB, Alfonso H, Chubb SAP, Handelsman DJ, Hankey GJ, Almeida OP, Golledge J, Norman PE, Flicker L. In older men an optimal plasma testosterone is associated with reduced all-cause mortality and higher dihydrotestosterone with reduced ischemic heart disease mortality, while estradiol levels do not predict mortality. *J Clin Endocrinol Metab* 2014; **99**: E9–E18.
18. Gyawali P, Martin SA, Heilbronn LK, Vincent AD, Jenkins AJ, Januszewski AS, Adams RJT, O'Loughlin PD, Wittert GA. Higher serum sex hormone-binding globulin levels are associated with incident cardiovascular disease in men. J Clin Endocrinol Metab 2019; 104: 6301–6315.
19. Holmboe SA, Vradi E, Jensen TK, Linneberg A, Husemoen LLN, Scheike T, Skakkebaek NE, Juul A, Andersson A-M. The association of reproductive hormone levels and all-cause, cancer, and cardiovascular disease mortality in men. J Clin Endocrinol Metab 2015; 100: 4472–4480.

20. Kalme T, Seppala M, Qiao Q, Koistinen R, Nissinen A, Harrela M, Loukovaara M, Leinonen P, Tuomilehto J. Sex hormone-binding globulin and insulin-like growth factor-binding protein-1 as indicators of metabolic syndrome, cardiovascular risk, and mortality in elderly men. J Clin Endocrinol Metab 2005; 90: 1550–1556.
21. Schederecker F, Cecil A, Prehn C, Nano J, Koenig W, Adamski J, Zeller T, Peters A, Thorand B. Sex hormone-binding globulin, androgens and mortality: the KORA-F4 cohort study. Endocr Connect 2020; 9: 326–336.
22. Hyde Z, Norman PE, Flicker L, Hankey GJ, Almeida OP, McCaul KA, Chubb SAP, Yeap BB. Low free testosterone predicts mortality from cardiovascular disease but not other causes. J Clin Endocrinol Metab 2012; 97: 179–189.
23. Wang A, Arver S, Boman K, Gerstein HC, Lee SF, Hess S, Ryden L, Mellbin LG. Testosterone, sex hormone-binding globulin and risk of cardiovascular events: a report from the Outcome Reduction with an Initial Glargine Intervention trial. Eur J Prev Cardiol 2019; 26: 847–854.
24. Tint AN, Hoermann R, Wong H, Ekinci EI, MacIsaac RJ, Jerums G, Zajac JD, Grossmann M. Association of sex hormone-binding globulin and free testosterone with mortality in men with type 2 diabetes mellitus. Eur J Endocrinol 2016; 174: 59–68.
25. Ramachandran S, Strange RC, Fryer AA, Saad F, Hackett GI. The association of sex hormone-binding globulin with mortality is mediated by age and testosterone in men with type 2 diabetes. Andrology 2018; 6: 846-853.
26. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999; 84: 3666–3672.
27. Ly LP, Sartorius G, Hull L, Leung A, Swerdloff RS, Wang C, Handelsman DJ. Accuracy of calculated free testosterone formulae in men. Clin Endocrinol 2010; 73: 382–388.

28. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. UK Biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 2015; 12: e1001779.
29. UK Biobank. Biomarker assay quality procedures: approaches used to minimise systematic and random errors (and the wider epidemiological implications). Version 1.2, date 2 April 2019, 1–15. http://www.ukbiobank.ac.uk/ (accessed 31 January 2020).
30. Fry D, Almond R, Moffat S, Gordon M, Singh P. UK Biobank Biomarker Project. Companion document to accompany serum biomarker data. Version 1.0, date 11 March 2019, 1–16. http://www.ukbiobank.ac.uk/ (accessed 31 January 2020).
31. UK Biobank. Data providers and dates of availability. http://biobank.ndph.ox.ac.uk/showcase/exinfo.cgi?src=Data_providers_and_dates (accessed 17 July, 2020).
32. UK Biobank. Mortality data: linkage to death registries. http://www.ukbiobank.ac.uk/ June 2020 (accessed 17 July, 2020).
33. Yeap BB, Marriott RJ, Antonio L, Chan YX, Raj S, Dwivedi G, Reid CM, Anawalt BD, Bhasin S, Dobs AS, Hankey GJ, Matsumoto AM, Norman PE, O'Neill TW, Ohlsson C, Orwoll ES, Vanderschueren D, Wittert GA, Wu FCW, Murray K. Supplement to "Serum testosterone is inversely, and sex hormone-binding globulin directly, associated with all-cause mortality in men". University of Western Australia Research Repository. Deposited 27 August 2020. https://api.research-repository.uwa.edu.au/portalfiles/portal/90052682/Yeap_et_al_T_SHBG_vs_mortality_Supplement_27_Aug_2020.pdf.

34. R Core Team R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2020. URL https://www.R-project.org/.
35. Handelsman DJ. Androgen physiology, pharmacology and abuse. Endotext [Internet] Editors: Feingold KR. Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, Hershman JM, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE, New M, Perreault L, Purnell J, Rebar R, Singer F, Trence DL, Vinik A, Wilson DP. South Dartmouth (MA): MDText.com, Inc.; 2000-. 2016.
36. Yates T, Zaccardi F, Dhalwani NN, Davies MJ, Bakrania K, Celis-Morales CA, Gill JMR, Franks PW, Khunti K. Association of walking pace and handgrip strength with all-cause, cardiovascular, and cancer mortality: a UK Biobank observational study. Eur Heart J 2017; 38: 3232-3240.
37. Celis-Morales CA, Lyall DM, Steell L, Gray SR, Iliodromiti S, Anderson J, Mackay DF, Welsh P, Yates T, Jill P. Sattar N, Gill JMR. Associations of discretionary screen time with mortality, cardiovascular disease and cancer are attenuated by strength, fitness and physical activity: findings from the UK Biobank study. BMC Med 2018; 16: 77.
38. Fan X, Wang J, Song M, Giovannucci EL, Ma H, Jin G, Hu Z, Shen H, Hang D. Vitamin D status and risk of all-cause and cause-specific mortality in a large cohort: results from the UK Biobank. J Clin Endocrinol Metab 2020; in press. doi: 10.1210/clinem/dgaa432.
39. Dittadi R, Matteucci M, Meneghetti E, Ndreu R. Reassessment of the Access Testosterone chemiluminescence assay and comparison with the LC-MS method. *J Clin Lab Anal* 2018; **32**: e22286.
40. Fry A, Littlejohns TJ, Sudlow C, et al. Comparison of sociodemographic and health-related characteristics of UK Biobank participants with those of the general population. *Am J Epidemiol* 2017; **186**: 1026–1034.

Figure legends

Figure 1

Multivariable model showing effect of baseline serum testosterone on risk of A: death from any cause, B: CVD death, C: cancer death, adjusted for risk factors and potential confounders, and for SHBG. The horizontal dashed line is at the reference hazard (median of the fifth quintile). Shaded areas are the 95% confidence intervals. Horizontal plot axes are truncated to exclude values greater than four standard deviations from the mean (<0.2% of data). The vertical dashed lines are at medians for quintiles of testosterone, as they relate to hazard ratios presented in Table 2. To convert testosterone from nmol/L to ng/dL, divide by 0.0347.

Figure 2

Multivariable model showing effect of baseline serum SHBG on risk of A: death from any cause, B: CVD death, C: cancer death, adjusted for risk factors and potential confounders, and for testosterone. The horizontal dashed line is at the reference hazard (median of the fifth quintile). Shaded areas are the 95% confidence intervals. Horizontal plot axes are truncated to exclude values greater than four standard deviations from the mean (<0.4% of data). The vertical dashed lines are at medians for quintiles of SHBG, as they relate to hazard ratios presented in Table 3.

Figure 3

Multivariable model showing effect of baseline calculated free testosterone (cFT) value on risk of A: death from any cause, B: CVD death, C: cancer death, adjusted for risk factors and potential confounders. The horizontal dashed line is at the reference hazard (median of the fifth quintile). Shaded areas are the 95% confidence intervals. The vertical dashed lines are at medians for quintiles of cFT, as they relate to hazard ratios presented in Table 4.

Table 1. Baseline characteristics of UK Biobank men, stratified according to prevalent CVD and cancer at baseline, and for the cohort as a whole (complete-cases**).

***Characteristic**[§]*	**All**** *(n=149,436)*	**CVD** *(n =7,800)*	**No CVD** *(n=141,636)*	**Cancer** *(n=9,070)*	**No Cancer** *(n=140,366)*
Sociodemographic & Lifestyle					
Age (whole years)	58.0 (50.0-63.0)	63.0 (58.0-66.0)	57.0 (49.0-63.0)	63.0 (58.0-66.0)	57.0 (49.0-63.0)
BMI (kg/m^2)	27.1 (24.9-29.8)	28.4 (25.9-31.3)	27.1 (24.8-29.7)	27.1 (24.8-29.6)	27.1 (24.9-29.8)
Cholesterol (mmol/L)	5.5 (4.8-6.2)	4.4 (3.8-5.0)	5.5 (4.8-6.3)	5.4 (4.7-6.2)	5.5 (4.8-6.2)
Waist circumference (cm)	95.0 (89.0-102.0)	99.0 (92.0-107.0)	95.0 (89.0-102.0)	96.0 (89.0-103.0)	95.0 (89.0-102.0)
Ethnicity: Asian	1.9 (2,815)	2.4 (191)	1.9 (2,624)	0.7 (66)	2.0 (2,749)
Black	1.3 (2,016)	0.7 (58)	1.4 (1,958)	0.9 (78)	1.4 (1,938)
Chinese	0.2 (356)	0.1 (6)	0.2 (350)	0.1 (8)	0.2 (348)
Mixed	0.5 (715)	0.4 (30)	0.5 (685)	0.3 (29)	0.5 (686)
Other	0.8 (1,145)	0.6 (45)	0.8 (1,100)	0.4 (37)	0.8 (1,108)
White	95.3 (142,389)	95.8 (7,470)	95.3 (134,919)	97.6 (8,852)	95.1 (133,537)
Living with partner (Yes)	78.3 (117,030)	74.3 (5,795)	78.5 (111,235)	80.4 (7,296)	78.2 (109,734)
Quals: Below A levels	42.3 (63,196)	55.4 (4,321)	41.6 (58,875)	42.9 (3,895)	42.2 (59,301)
A levels (high school)	7.0 (10,530)	6.2 (482)	7.1 (10,048)	6.5 (586)	7.1 (9,944)
College/University	36.4 (54,401)	24.0 (1,873)	37.1 (52,528)	35.8 (3,247)	36.4 (51,154)
Professional/Other	14.3 (21,309)	14.4 (1,124)	14.3 (20,185)	14.8 (1,342)	14.2 (19,967)
Alcohol: Abstainers	25.7 (38,403)	32.5 (2,537)	25.3 (35,866)	26.0 (2,355)	25.7 (36,048)
Low	14.1 (21,137)	12.8 (997)	14.2 (20,140)	14.7 (1,334)	14.1 (19,803)
Moderate	15.0 (22,437)	14.0 (1,089)	15.1 (21,348)	15.1 (1,369)	15.0 (21,068)
Medium	14.9 (22,337)	14.6 (1,140)	15.0 (21,197)	15.0 (1,362)	14.9 (20,975)
High	30.2 (45,122)	26.1 (2,037)	30.4 (43,085)	29.2 (2,650)	30.3 (42,472)

Diet: High Red Meat	16.0 (23,914)	17.7 (1,378)	15.9 (22,536)	16.6 (1,503)	16.0 (22,411)
Low Red Meat	80.5 (120,256)	80.3 (6,267)	80.5 (113,989)	80.5 (7,304)	80.5 (112,952)
Poultry eaters	0.6 (919)	0.7 (56)	0.6 (863)	0.7 (68)	0.6 (851)
Fish eaters	1.5 (2,304)	0.7 (57)	1.6 (2,247)	1.3 (119)	1.6 (2,185)
Vegetarian	1.3 (1,915)	0.5 (38)	1.3 (1,877)	0.8 (70)	1.3 (1,845)
Vegan	0.1 (128)	0.1 (4)	0.1 (124)	0.1 (6)	0.1 (122)
PA: Insufficient	30.4 (45,407)	32.4 (2,525)	30.3 (42,882)	31.3 (2,840)	30.3 (42,567)
Sufficient	18.4 (27,469)	18.5 (1,442)	18.4 (26,027)	18.7 (1,695)	18.4 (25,774)
Additional	51.2 (76,560)	49.1 (3,833)	51.3 (72,727)	50.0 (4,535)	51.3 (72,025)
Smoking: Never	50.2 (75,016)	33.2 (2,588)	51.1 (72,428)	44.6 (4,046)	50.6 (70,970)
Previous	38.5 (57,529)	54.0 (4,212)	37.6 (53,317)	46.1 (4,177)	38.0 (53,352)
Current	11.3 (16,891)	12.8 (1,000)	11.2 (15,891)	9.3 (847)	11.4 (16,044)
Prevalent health conditions and medication usage					
CVD	5.2 (7,800)	100.0 (7,800)	0.0 (0)	7.3 (661)	5.1 (7,139)
Cancer	6.1 (9,070)	8.5 (661)	5.9 (8,409)	100.0 (9,070)	0.0 (0)
Diabetes	6.9 (10,343)	18.6 (1,452)	6.3 (8,891)	7.8 (706)	6.9 (9,637)
Dementia	0.1 (89)	0.2 (18)	0.1 (71)	0.1 (8)	0.1 (81)
Angina	5.0 (7,435)	40.5 (3,159)	3.0 (4,276)	7.0 (637)	4.8 (6,798)
Atrial Fibrillation	2.1 (3,186)	11.4 (888)	1.6 (2,298)	3.6 (326)	2.0 (2,860)
Renal impairment	0.6 (961)	2.6 (202)	0.5 (759)	1.4 (129)	0.6 (832)
Hypertension	62.1 (92,787)	86.1 (6,716)	60.8 (86,071)	69.5 (6,302)	61.6 (86,485)
COPD	0.7 (1,060)	3.0 (231)	0.6 (829)	1.4 (126)	0.7 (934)
Liver disease	1.3 (1,919)	2.1 (165)	1.2 (1,754)	1.9 (172)	1.2 (1,747)
Thyroid disease	2.1 (3,143)	4.1 (316)	2.0 (2,827)	3.5 (318)	2.0 (2,825)
HIV	0.2 (245)	0.2 (13)	0.2 (232)	0.3 (26)	0.2 (219)
Lipid medication use	22.5 (33,695)	79.1 (6,169)	19.4 (27,526)	28.6 (2,596)	22.2 (31,099)
Glucocorticoid use	6.9 (10,329)	8.4 (659)	6.8 (9,670)	8.0 (730)	6.8 (9,599)
Opioid use	3.6 (5,354)	8.4 (658)	3.3 (4,696)	5.6 (511)	3.5 (4,843)
Anticonvulsant use	1.3 (1,886)	3.1 (242)	1.2 (1,644)	1.8 (167)	1.2 (1,719)
No. Meds: 0	33.4 (49,932)	2.0 (155)	35.1 (49,777)	23.1 (2,091)	34.1 (47,841)
1-2	33.3 (49,759)	8.9 (691)	34.6 (49,068)	32.8 (2,973)	33.3 (46,786)
3-4	18.2 (27,151)	26.5 (2,065)	17.7 (25,086)	21.4 (1,945)	18.0 (25,206)

5+	15.1 (22,594)	62.7 (4,889)	12.5 (17,705)	22.7 (2,061)	14.6 (20,533)
Hormone variables					
Testosterone (nmol/L)	11.7 (9.5-14.2)	11.0 (8.9-13.4)	11.7 (9.6-14.2)	11.4 (9.2-13.9)	11.7 (9.5-14.2)
Testosterone (ng/dL)	337 (274-409)	317 (256-386)	337 (277-409)	329 (265-401)	337 (274-409)
SHBG (nmol/L)	37.2 (28.2-48.3)	38.5 (29.3-49.3)	37.1 (28.2-48.3)	39.4 (30.1-51.2)	37.0 (28.1-48.1)
cFT (pmol/L)	214.4 (179.0-255.9)	197.4 (163.8-234.6)	215.4 (180.0-256.9)	201.6 (166.9-239.2)	215.2 (179.9-256.8)

* = Continuous variables (BMI, age, waist circumference, cholesterol, testosterone, SHBG) represented as median (interquartile range); other variables as percentages (numbers) per category.

** = Summary data presented for complete cases data, after excluding men with prior orchidectomy or taking androgens, anti-androgen, 5α-reductase, estrogen, anti-estrogen, progesterone medications, infertile men, men with pituitary disease, adrenogenital or testicular disorders, or with missing testosterone values.

§ = BMI, body mass index (kg/m^2); PA, level of physical activity categories (min/week; see Methods); Quals, qualifications (highest level of education / training attained); Alcohol, level of alcohol consumption (standard units of alcohol consumed/week; see Methods); Smoking, smoking status; CVD, Cardiovascular Disease.

Table 2. Hazard ratios of all-cause mortality, deaths due to CVD, and deaths due to cancer by quintiles of testosterone (T).

Model	Q5 (highest T) (n = 30,467) [§]	Q4 (n = 30,515)	Q3 (n = 30,224)	Q2 (n = 29,800)	Q1 (lowest T) (n = 28,430)	*P* Value
All-cause deaths: 10,053 events						
	2,047 events	***1,913 events***	***1,832 events***	***1,995 events***	***2,266 events***	
Univariable	ref.	0.96 (0.93-1.00)	0.98 (0.92-1.05)	0.96 (0.91-1.01)	1.21 (1.15-1.27)	< 0.001**
Multivariable 1*	ref.	0.93 (0.89-0.96)	0.89 (0.83-0.95)	0.82 (0.78-0.87)	0.90 (0.85-0.95)	< 0.001**
Multivariable 2	ref.	0.99 (0.95-1.03)	1.01 (0.94-1.08)	1.00 (0.93-1.06)	1.14 (1.06-1.22)	< 0.001**
CVD deaths: 1,925 events						
	365 events	***359 events***	***349 events***	***398 events***	***454 events***	
Univariable	ref.	0.98 (0.90-1.06)	1.00 (0.86-1.16)	1.09 (0.96-1.24)	1.33 (1.18-1.50)	< 0.001**
Multivariable 1	ref.	0.90 (0.83-0.98)	0.83 (0.71-0.96)	0.84 (0.73-0.95)	0.84 (0.74-0.96)	0.056
Multivariable 2	ref.	0.94 (0.86-1.03)	0.90 (0.77-1.06)	0.95 (0.82-1.11)	1.01 (0.86-1.18)	0.500
Cancer deaths: 4,927 events						
	938 events	***908 events***	***915 events***	***1,006 events***	***1,160 events***	
Univariable	ref.	1.02 (0.96-1.07)	1.05 (0.96-1.16)	1.01 (0.93-1.09)	1.32 (1.22-1.42)	< 0.001**
Multivariable 1	ref.	0.96 (0.92-1.02)	0.95 (0.86-1.04)	0.91 (0.84-0.99)	1.06 (0.97-1.14)	< 0.001**
Multivariable 2	ref.	1.00 (0.95-1.06)	1.02 (0.92-1.13)	1.02 (0.93-1.12)	1.20 (1.09-1.33)	< 0.001**

[§] = Quintile boundaries Q1/2 8.9 nmol/L (256 ng/dL), Q2/3 10.8 nmol/L (311 ng/dL), Q3/4 12.5 nmol/L (360 ng/dL) and Q4/5 14.8 nmol/L (427 ng/dL). 2.5th percentile = 5.9 nmol/L (170 ng/dL), 97.5th = 20.1 nmol/L (579 ng/dL). Presented numbers are for complete cases, after exclusions.

* = Multivariable 1 models included age, ethnicity, living with partner, qualifications, alcohol consumption, smoking status, diet, physical activity, BMI, waist circumference, cholesterol, prevalent diabetes, hypertension, angina, atrial fibrillation, COPD, dementia, renal impairment, liver disease, thyroid disease, HIV, and use of lipid medications (a proxy for hyperlipidemia), glucocorticoids, opioids, and anticonvulsants, with the number of medications included as a proxy for overall comorbidity status and assessment centre as a stratification factor. Prevalent CVD and Cancer status were included as additional terms in analyses of CVD deaths and Cancer deaths, respectively. Multivariable 2 models for testosterone included SHBG as an additional covariate.

** = Result interpreted as significant.

Table 3. Hazard ratios of all-cause mortality, deaths due to CVD, and deaths due to cancer by quintiles of SHBG.

Model	Q5 (highest) (n = 30,233) [§]	Q4 (n = 30,354)	Q3 (n = 30,143)	Q2 (n = 29,827)	Q1 (lowest) (n = 28,879)	*P* Value
All-cause deaths: 10,053 events						
	2,973 events	***2,175 events***	***1,883 events***	***1,692 events***	***1,330 events***	
Univariable	ref.	0.78 (0.75-0.81)	0.68 (0.64-0.72)	0.60 (0.57-0.63)	0.51 (0.48-0.54)	< 0.001**
Multivariable 1*	ref.	0.87 (0.84-0.91)	0.82 (0.77-0.87)	0.79 (0.74-0.83)	0.75 (0.71-0.80)	< 0.001**
Multivariable 2	ref.	0.87 (0.84-0.91)	0.80 (0.75-0.86)	0.75 (0.70-0.80)	0.68 (0.63-0.73)	< 0.001**
CVD deaths: 1,925 events						
	517 events	***406 events***	***358 events***	***382 events***	***262 events***	
Univariable	ref.	0.83 (0.76-0.90)	0.77 (0.67-0.88)	0.75 (0.66-0.85)	0.58 (0.51-0.67)	< 0.001**
Multivariable 1	ref.	0.88 (0.81-0.96)	0.84 (0.72-0.97)	0.86 (0.76-0.98)	0.72 (0.62-0.83)	< 0.001**
Multivariable 2	ref.	0.89 (0.81-0.97)	0.84 (0.72-0.99)	0.86 (0.74-1.00)	0.70 (0.59-0.83)	< 0.001**
Cancer deaths: 4,927 events						
	1,326 events	***1,082 events***	***976 events***	***841 events***	***702 events***	
Univariable	ref.	0.84 (0.80-0.89)	0.74 (0.68-0.81)	0.65 (0.60-0.70)	0.57 (0.53-0.62)	< 0.001**
Multivariable 1	ref.	0.94 (0.89-1.00)	0.91 (0.83-0.99)	0.88 (0.81-0.96)	0.93 (0.85-1.01)	0.002**
Multivariable 2	ref.	0.93 (0.88-0.99)	0.88 (0.80-0.97)	0.82 (0.75-0.90)	0.80 (0.72-0.89)	< 0.001**

[§] = Quintile boundaries Q1/2 26.0 nmol/L, Q2/3 33.3 nmol/L, Q3/4 40.9 nmol/L and Q4/5 51.4 nmol/L. 2.5th percentile = 15.2 nmol/L, 97.5th = 79.1 nmol/L; Presented numbers are for complete cases, after exclusions.

* = Multivariable 1 models included age, ethnicity, living with partner, qualifications, alcohol consumption, smoking status, diet, physical activity, BMI, waist circumference, cholesterol, prevalent diabetes, hypertension, angina, atrial fibrillation, COPD, dementia, renal impairment, liver disease, thyroid disease, HIV, and use of lipid medications (a proxy for hyperlipidemia), glucocorticoids, opioids, and anticonvulsants, with the total number of medications used included as a proxy for overall comorbidity status and assessment centre as a stratification factor. Prevalent CVD and Cancer status were included as additional terms in analyses of CVD deaths and Cancer deaths, respectively. Multivariable 2 models for SHBG included testosterone as an additional covariate.

** = Result interpreted as significant.

Table 4. Hazard ratios of all-cause mortality, deaths due to CVD, and deaths due to cancer by quintiles of cFT.

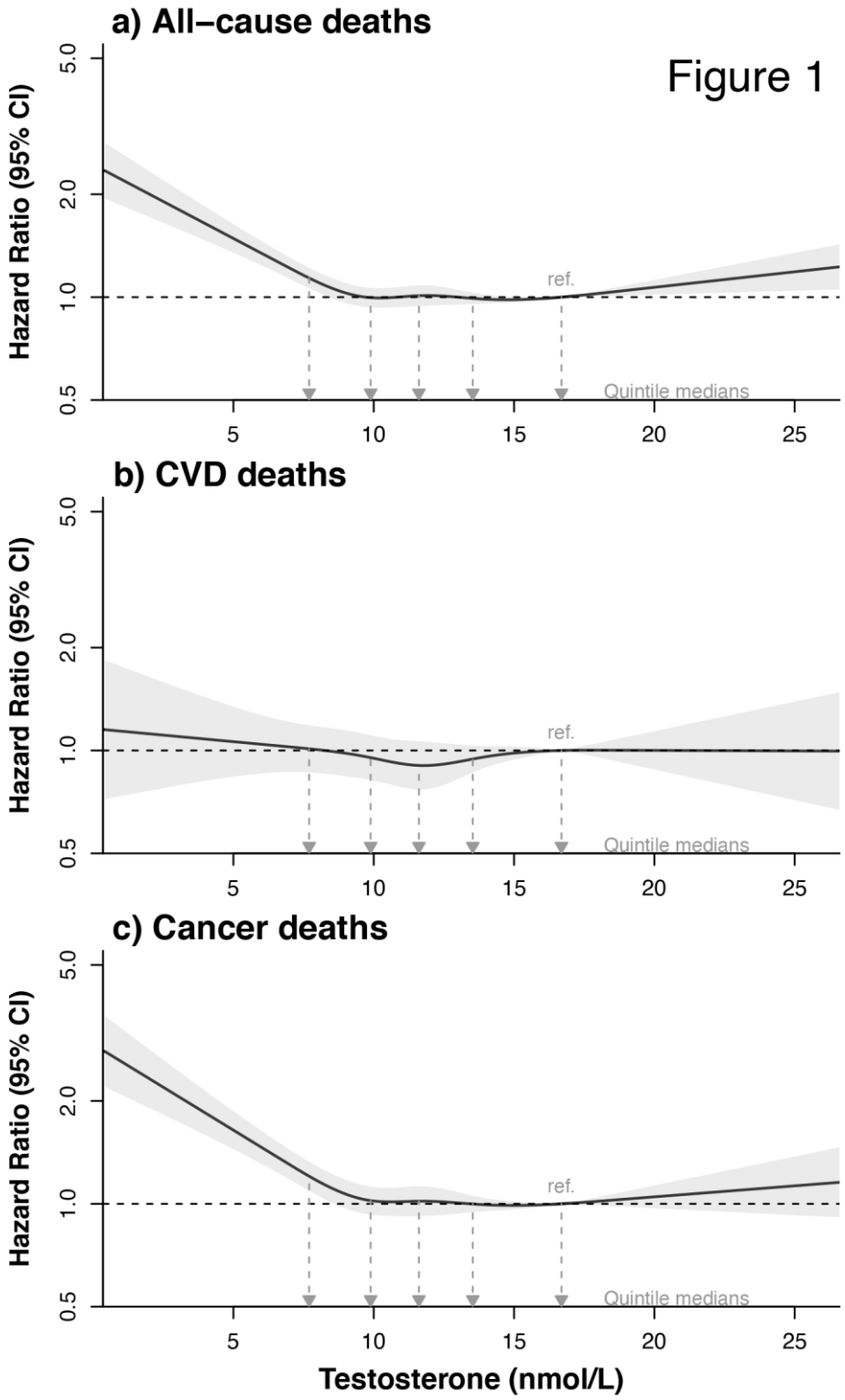
Model	Q5 (highest) (n = 30,401) [§]	Q4 (n = 30,241)	Q3 (n = 30,196)	Q2 (n = 29,916)	Q1 (lowest) (n = 28,682)	*P* Value
All-cause deaths: 10,053 events						
	1,210 events	***1,605 events***	***1,927 events***	***2,275 events***	***3,036 events***	
Univariable	ref.	1.25 (1.20-1.30)	1.62 (1.50-1.74)	1.88 (1.77-2.00)	2.52 (2.38-2.67)	< 0.001**
Multivariable*	ref.	1.01 (0.97-1.06)	1.04 (0.96-1.11)	1.01 (0.95-1.08)	1.13 (1.06-1.20)	< 0.001**
CVD deaths: 1,925 events						
	234 events	***320 events***	***368 events***	***412 events***	***591 events***	
Univariable	ref.	1.18 (1.08-1.29)	1.44 (1.22-1.69)	1.80 (1.57-2.07)	2.37 (2.08-2.70)	< 0.001**
Multivariable	ref.	0.94 (0.86-1.04)	0.91 (0.77-1.07)	0.95 (0.82-1.09)	1.03 (0.89-1.18)	0.143
Cancer deaths: 4,927 events						
	588 events	***803 events***	***979 events***	***1,153 events***	***1,404 events***	
Univariable	ref.	1.32 (1.25-1.40)	1.75 (1.58-1.94)	1.89 (1.74-2.07)	2.47 (2.27-2.68)	< 0.001**
Multivariable	ref.	1.05 (0.99-1.11)	1.11 (1.00-1.23)	1.05 (0.96-1.15)	1.17 (1.07-1.28)	< 0.001**

[§] = Quintile boundaries Q1/2 169 pmol/L, Q2/3 199 pmol/L, Q3/4 228 pmol/L and Q4/5 266 pmol/L. 2.5th percentile = 116 pmol/L, 97.5th = 360 pmol/L; Presented numbers are for complete cases, after exclusions.

* = Multivariable models included age, ethnicity, living with partner, qualifications, alcohol consumption, smoking status, diet, physical activity, BMI, waist circumference, cholesterol, prevalent diabetes, hypertension, angina, atrial fibrillation, COPD, dementia, renal impairment, liver disease, thyroid disease, HIV, and use of lipid medications (a proxy for hyperlipidemia), glucocorticoids, opioids, and anticonvulsants, with the total number of medications used included as a proxy for overall comorbidity status and assessment centre as a stratification factor. Prevalent CVD and Cancer status were included as additional terms in analyses of CVD deaths and Cancer deaths, respectively.

** = Result interpreted as significant; * = result considered suggestive evidence of an association, requiring confirmation.

Figure 1



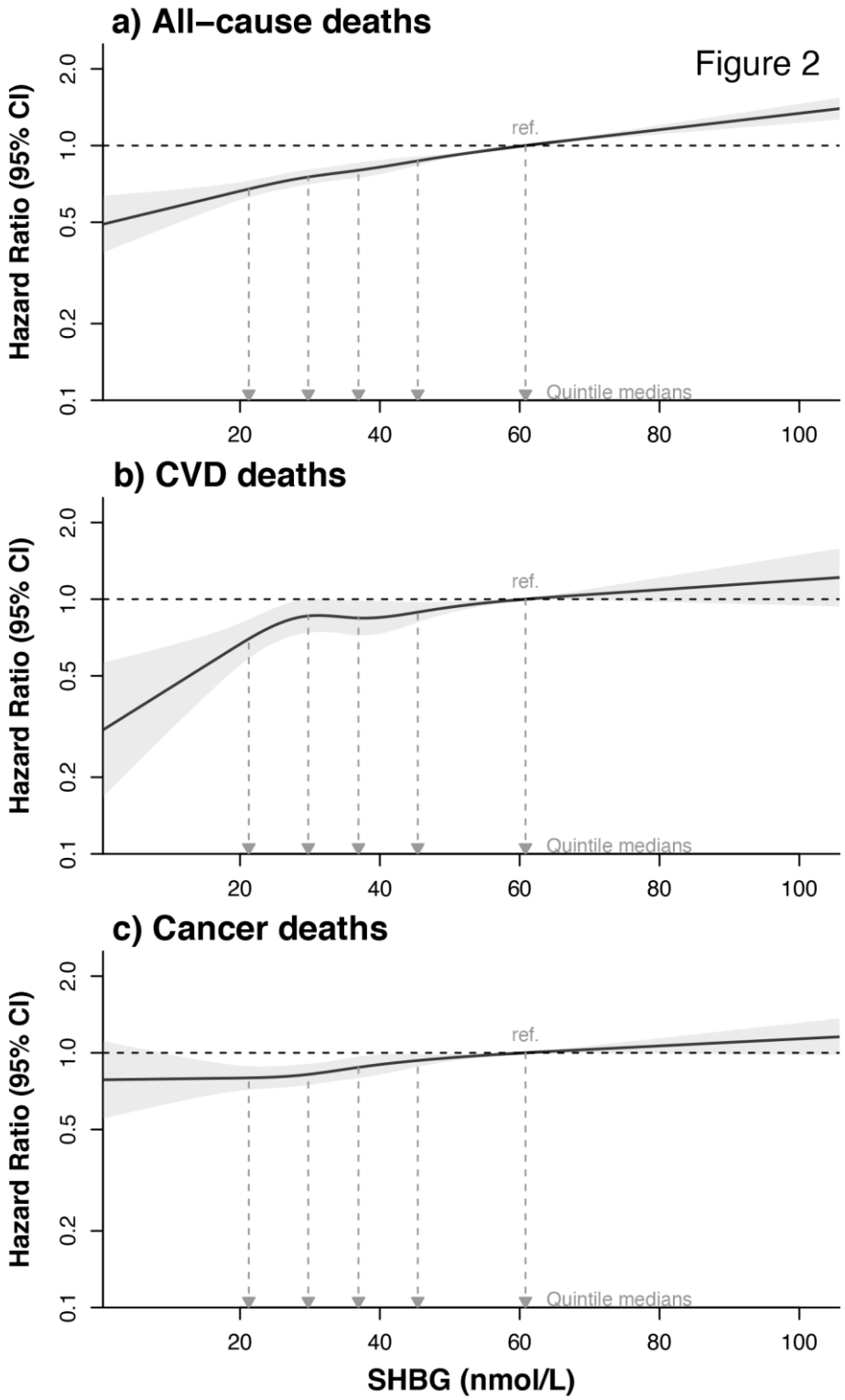


Figure 2

Figure 3