*The Journal of Clinical Endocrinology & Metabolism*, 2021, Vol. 106, No. 2, e625–e637
doi:10.1210/clinem/dgaa743
Clinical Research Article

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# Serum Testosterone is Inversely and Sex Hormone-binding Globulin is Directly Associated with All-cause Mortality in Men

**Bu B. Yeap,[1,2] Ross J. Marriott,[3] Leen Antonio,[4] Yi X. Chan,[1,2] Suchitra Raj,[2] Girish Dwivedi,[1,5] Christopher M. Reid,[6] Bradley D. Anawalt,[7] Shalender Bhasin,[8] Adrian S. Dobs,[9] Graeme J. Hankey,[1] Alvin M. Matsumoto,[7,10] Paul E. Norman,[1] Terence W. O'Neill,[11] Claes Ohlsson,[12,13] Eric S. Orwoll,[14] Dirk Vanderschueren,[4] Gary A. Wittert,[15] Frederick C.W. Wu,[16] and Kevin Murray[3]**

[1]Medical School, University of Western Australia, Perth 6009, Australia; [2]Department of Endocrinology and Diabetes, Fiona Stanley Hospital, Perth 6150, Australia; [3]School of Population and Global Health, University of Western Australia, Perth 6009, Australia; [4]Laboratory of Clinical and Experimental Endocrinology, KU Leuven, Leuven 3000, Belgium; [5]Harry Perkins Institute of Medical Research and Fiona Stanley Hospital, Perth 6150, Australia; [6]School of Public Health, Curtin University, Perth 6102, Australia; [7]Department of Medicine, University of Washington School of Medicine, Seattle 98195, Washington; [8]Brigham and Women's Hospital, Harvard Medical School, Boston 02115, Massachusetts; [9]Division of Endocrinology, Johns Hopkins University School of Medicine, Baltimore 21287, Maryland; [10]Geriatric Research, Education and Clinical Center, VA Puget Sound Health Care System, Seattle 98108, Washington; [11]Manchester Institute for Collaborative Research on Ageing, University of Manchester, Manchester M13 9PL, UK; [12]Centre for Bone and Arthritis Research, Department of Internal Medicine and Clinical Nutrition, Sahlgrenska Academy, University of Gothenburg, Gothenburg 405 30, Sweden; [13]Region Vastra Gotaland, Sahlgrenska University Hospital, Gothenburg 405 30, Sweden; [14]Oregon Health and Science University, Portland, Oregon 97239; [15]Freemasons Centre for Men's Health and Wellbeing, School of Medicine, University of Adelaide, Adelaide 5005, Australia; and [16]Division of Endocrinology, Diabetes and Gastroenterology, School of Medical Sciences, University of Manchester, Manchester M13 9PL, UK

**ORCiD numbers:** 0000-0002-7612-5892 (B. B. Yeap); 0000-0002-3982-6200 (S. Bhasin); 0000-0002-9633-2805 (C. Ohlsson); 0000-0002-8856-6046 (K. Murray).

Received: 27 August 2020; Editorial Decision: 8 October 2020; First Published Online: 16 October 2020; Corrected and Typeset: 30 November 2020.

## Abstract

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

**Objective:** To analyze 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 aged 40–69 years old, followed for 11 years.

ISSN Print 0021-972X ISSN Online 1945-7197
Printed in USA

**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 a complete case analysis of 149 436 men with 10 053 deaths (1925 CVD and 4927 cancer-related), men with lower testosterone had a higher mortality rate 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 a lower mortality rate 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, 3128 CVD-related and 7468 cancer-related) and analysis excluding deaths within the first 2 years (9261, 1734, and 4534 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.

**Freeform/Key Words:** testosterone, sex hormone-binding globulin, mortality, cardiovascular disease, cancer

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, is associated with lower SHBG concentrations, and liver or thyroid disease are associated 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), while others reported inverse associations (11, 12, 14, 15, 19). Cancer is another major cause of death. Testosterone concentrations have been inversely associated with cancer mortality in some studies (11, 16), positively associated in one study (20), and not associated in other studies (12, 19). Several cohort studies have reported no association of SHBG concentrations with mortality, nor with deaths from CVD (6, 8, 17–19, 21). Other studies in middle-aged and older men (20, 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 (20). 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, CVD, and those that are cancer-related could be performed. Associations of serum testosterone and SHBG with mortality outcomes could also be compared with associations of cFT. The 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, 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 (1) lower testosterone and lower SHBG are independently associated with a higher mortality in men, after adjusting for potential confounders, and (2) cFT provides additional information beyond that of testosterone and SHBG as a predictor of mortality risk in men.

## Participants and Methods

### The UK Biobank

Over 500 000 participants aged 40–69 years were recruited across 22 assessment centers in the United Kingdom from 2006 to 2010 (28). Detailed characterization was undertaken using self-completed questionnaires, brief interviews, physical and functional measures, and blood collection. The 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 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 (10–1599 ng/dL, DXI 800; Beckman Coulter, High Wycombe, 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 844–1424 ng/dL), respectively. Serum SHBG was assayed using a 2-step sandwich chemiluminescent immunoassay, analytical range 0.33–242 nmol/L (DXI 800; Beckman Coulter). 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), respectively. 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 to October 2010) until April 30, 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 CVD were categorized as such if the primary cause of death was due to angina pectoris, myocardial infarction, other acute ischemic heart disease, chronic ischemic heart disease, dilated cardiomyopathy, cardiac arrest, heart failure, hemorrhagic or ischemic stroke, atherosclerosis, or aortic aneurysm and dissection (Table S1 from the supplementary material located in (33). Men who died of cancer were categorized as such if the primary cause of death was due to cancer (Table S1 (33). For analyses of CVD deaths and cancer-related deaths, individuals were censored at their earliest date of death (for analyses of cause-specific deaths, if not attributed to that cause) or at the 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 in Table S1 (33).

**Covariates.** Participants' age, ethnicity, living status (ie, with a partner), qualifications, alcohol consumption, dietary patterns, physical activity, and smoking status were obtained from self-reporting. (South) Asian refers to men of India, Pakistan, Bangladesh, and Sri Lanka extraction. Qualifications were categorized 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 categorized (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-reporting, physical examination, blood chemistry, and linked general practice records and medical datasets (eg, surgical codes, hospital admission diagnosis codes, and cancer registries). Medications were determined from self-reporting. Prevalent CVD (defined as prior myocardial infarction, stroke, or heart failure), angina, atrial fibrillation, chronic obstructive pulmonary disease (COPD), human immunodeficiency virus (HIV), and liver disease were determined from self-reporting or previous hospital admission diagnoses. Diabetes, dementia, renal impairment, and hypertension were defined from combinations of self-reporting, medications, physical measures, and biochemical data (33). Thyroid disease was determined by the use of antithyroid, thyroxine, or liothyronine medications, prior hospital admission diagnosis, or self-reported conditions. Hyperlipidemia was categorized by the use of lipid-lowering medications. Other medication variables included baseline use of anticonvulsant, glucocorticoids, and opioid medications, and the number of medications taken (33). The full set of hospital admission and diagnosis codes used is provided in 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 calculated follow-up times. Cox proportional hazards models were fitted, with separate analyses done for each exposure (testosterone, SHBG, and cFT) and outcome. The site was modeled 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 2 years of follow-up, to infer the potential effects of missing data and to reverse causation on results (33).

Each analysis involved fitting an unadjusted model, as well as 2 multivariable models, which included additional covariates, to adjust for known risk factors and possible confounders. Multivariable Model 1 included lifestyle and demographic variables (ie, age, alcohol consumption, BMI, cholesterol, diet, ethnicity, living with partner, physical activity, qualifications, smoking status, and waist circumference), prevalent conditions (eg, angina, atrial fibrillation, COPD, dementia, diabetes, HIV, hypertension, liver disease, renal impairment, thyroid disease), and the medication used at baseline (eg, anticonvulsants, lipid, glucocorticoids, opioids, and 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 nonlinearity, adjusted analyses were performed and modeled 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 5$^{th}$ quintile were plotted against the exposure variable, with 95% confidence intervals (CIs). Hazard ratios 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 3 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

The UK Biobank recruited 229 122 men aged 40–69 years from 2006 to 2010. Excluding men who were infertile or with prior pituitary disease, orchidectomy, or were hospitalized for adrenogenital/testicular disorders, or taking androgen, antiandrogen, estrogen, antiestrogen, progesterone, or 5α-reductase medications left 224 266 men for analyses. 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 men for analyses (Fig. 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-related deaths.

### Participant characteristics

Men were predominantly white (95.3%), living with a partner (78.3%), on a "low red meat" diet (80.5%), with a median age of 58.0 years, a BMI of 27.1 kg/m$^2$, and testosterone of 11.7 nmol/L (Table 1). A total of 7800 (5.2%) had prevalent CVD and 9070 (6.1%) had a history of cancer. During follow-up, 10 053 (6.7%) died, of which 1925 had the cause of death attributed to atherosclerotic CVD and 4927 had the cause of death attributed to cancer. Men with prevalent CVD were older, had a 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 with more on lipid-lowering medication, had more prevalent health conditions, took more medications, and had lower testosterone and higher SHBG compared with men without prevalent CVD. 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 with men without a history of cancer (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 (Fig. S2 (33).

In univariable analysis, there was a U-shaped association of serum testosterone with all-cause mortality, while

**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[b])

| Characteristic[a] | All[b] (n = 149 436) | CVD (n = 7800) | No CVD (n = 141 636) | Cancer (n = 9070) | No Cancer (n = 140 366) |
|---|---|---|---|---|---|
| **Sociodemographic and 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 (2815) | 2.4 (191) | 1.9 (2624) | 0.7 (66) | 2.0 (2749) |
| Black | 1.3 (2016) | 0.7 (58) | 1.4 (1958) | 0.9 (78) | 1.4 (1938) |
| 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 (1145) | 0.6 (45) | 0.8 (1100) | 0.4 (37) | 0.8 (1108) |
| White | 95.3 (142 389) | 95.8 (7470) | 95.3 (134 919) | 97.6 (8852) | 95.1 (133 537) |
| Living with partner (Yes) | 78.3 (117 030) | 74.3 (5795) | 78.5 (111 235) | 80.4 (7296) | 78.2 (109 734) |
| Quals | | | | | |
| Below A levels | 42.3 (63 196) | 55.4 (4321) | 41.6 (58 875) | 42.9 (3895) | 42.2 (59 301) |
| A level (high school) | 7.0 (10 530) | 6.2 (482) | 7.1 (10 048) | 6.5 (586) | 7.1 (9944) |
| College/University | 36.4 (54 401) | 24.0 (1873) | 37.1 (52 528) | 35.8 (3247) | 36.4 (51 154) |
| Professional/other | 14.3 (21 309) | 14.4 (1124) | 14.3 (20 185) | 14.8 (1342) | 14.2 (19 967) |
| Alcohol | | | | | |
| Abstainers | 25.7 (38 403) | 32.5 (2537) | 25.3 (35 866) | 26.0 (2355) | 25.7 (36 048) |
| Low | 14.1 (21 137) | 12.8 (997) | 14.2 (20 140) | 14.7 (1334) | 14.1 (19 803) |
| Moderate | 15.0 (22 437) | 14.0 (1089) | 15.1 (21 348) | 15.1 (1369) | 15.0 (21 068) |
| Medium | 14.9 (22 337) | 14.6 (1140) | 15.0 (21 197) | 15.0 (1362) | 14.9 (20 975) |
| High | 30.2 (45 122) | 26.1 (2037) | 30.4 (43 085) | 29.2 (2650) | 30.3 (42 472) |
| Diet | | | | | |
| High red meat | 16.0 (23 914) | 17.7 (1378) | 15.9 (22 536) | 16.6 (1503) | 16.0 (22 411) |
| Low red meat | 80.5 (120 256) | 80.3 (6267) | 80.5 (113 989) | 80.5 (7304) | 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 (2304) | 0.7 (57) | 1.6 (2247) | 1.3 (119) | 1.6 (2185) |
| Vegetarian | 1.3 (1915) | 0.5 (38) | 1.3 (1877) | 0.8 (70) | 1.3 (1845) |
| Vegan | 0.1 (128) | 0.1 (4) | 0.1 (124) | 0.1 (6) | 0.1 (122) |
| Physical activity, PA | | | | | |
| Insufficient | 30.4 (45 407) | 32.4 (2525) | 30.3 (42 882) | 31.3 (2840) | 30.3 (42 567) |
| Sufficient | 18.4 (27 469) | 18.5 (1442) | 18.4 (26 027) | 18.7 (1695) | 18.4 (25774) |
| Additional | 51.2 (76 560) | 49.1 (3833) | 51.3 (72 727) | 50.0 (4535) | 51.3 (72 025) |
| Smoking | | | | | |
| Never | 50.2 (75 016) | 33.2 (2588) | 51.1 (72 428) | 44.6 (4046) | 50.6 (70 970) |
| Previous | 38.5 (57 529) | 54.0 (4212) | 37.6 (53 317) | 46.1 (4177) | 38.0 (53 352) |
| Current | 11.3 (16 891) | 12.8 (1000) | 11.2 (15 891) | 9.3 (847) | 11.4 (16 044) |

**Table 1.** Continued

| Characteristic[a] | All[b] (n = 149 436) | CVD (n = 7800) | No CVD (n = 141 636) | Cancer (n = 9070) | No Cancer (n = 140 366) |
|---|---|---|---|---|---|
| **Prevalent health conditions and medication usage** | | | | | |
| CVD | 5.2 (7800) | 100.0 (7800) | 0.0 (0) | 7.3 (661) | 5.1 (7139) |
| Cancer | 6.1 (9070) | 8.5 (661) | 5.9 (8409) | 100.0 (9070) | 0.0 (0) |
| Diabetes | 6.9 (10 343) | 18.6 (1452) | 6.3 (8891) | 7.8 (706) | 6.9 (9637) |
| Dementia | 0.1 (89) | 0.2 (18) | 0.1 (71) | 0.1 (8) | 0.1 (81) |
| Angina | 5.0 (7435) | 40.5 (3159) | 3.0 (4276) | 7.0 (637) | 4.8 (6798) |
| Atrial fibrillation | 2.1 (3186) | 11.4 (888) | 1.6 (2298) | 3.6 (326) | 2.0 (2860) |
| Renal impairment | 0.6 (961) | 2.6 (202) | 0.5 (759) | 1.4 (129) | 0.6 (832) |
| Hypertension | 62.1 (92 787) | 86.1 (6716) | 60.8 (86 071) | 69.5 (6302) | 61.6 (86 485) |
| COPD | 0.7 (1060) | 3.0 (231) | 0.6 (829) | 1.4 (126) | 0.7 (934) |
| Liver disease | 1.3 (1919) | 2.1 (165) | 1.2 (1754) | 1.9 (172) | 1.2 (1747) |
| Thyroid disease | 2.1 (3143) | 4.1 (316) | 2.0 (2827) | 3.5 (318) | 2.0 (2825) |
| 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 (6169) | 19.4 (27 526) | 28.6 (2596) | 22.2 (31 099) |
| Glucocorticoid use | 6.9 (10 329) | 8.4 (659) | 6.8 (9670) | 8.0 (730) | 6.8 (9599) |
| Opioid use | 3.6 (5354) | 8.4 (658) | 3.3 (4696) | 5.6 (511) | 3.5 (4843) |
| Anticonvulsant use | 1.3 (1886) | 3.1 (242) | 1.2 (1644) | 1.8 (167) | 1.2 (1719) |
| Number of Medications | | | | | |
| 0 | 33.4 (49 932) | 2.0 (155) | 35.1 (49 777) | 23.1 (2091) | 34.1 (47 841) |
| 1–2 | 33.3 (49 759) | 8.9 (691) | 34.6 (49 068) | 32.8 (2973) | 33.3 (46 786) |
| 3–4 | 18.2 (27 151) | 26.5 (2065) | 17.7 (25 086) | 21.4 (1945) | 18.0 (25 206) |
| 5+ | 15.1 (22 594) | 62.7 (4889) | 12.5 (17 705) | 22.7 (2061) | 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) |

Abbreviations: Alcohol, level of alcohol consumption (standard units of alcohol consumed/week; see "Methods" section); BMI, body mass index (kg/m$^2$); cFT, calculated free testosterone; COPD, chronic obstructive pulmonary disease; CVD, Cardiovascular Disease; HIV, Human Immunodeficiency Virus; PA, level of physical activity categories (min/week; see "Methods" section); Quals, qualifications; SHBG, sex hormone-binding globulin; Smoking, smoking status.

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

[b]Summary data presented for complete cases data, after excluding men with prior orchidectomy or taking androgens, antiandrogen, 5α-reductase, estrogen, antiestrogen, progesterone medications, infertile men, men with pituitary disease, adrenogenital or testicular disorders, or with missing testosterone values.

men with lower serum testosterone had higher CVD and cancer-related mortality (Fig. 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 (Fig. 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 (Fig. 1).

Associations of testosterone in quintiles with all-cause mortality are tabulated (Table 2). In univariable analysis,

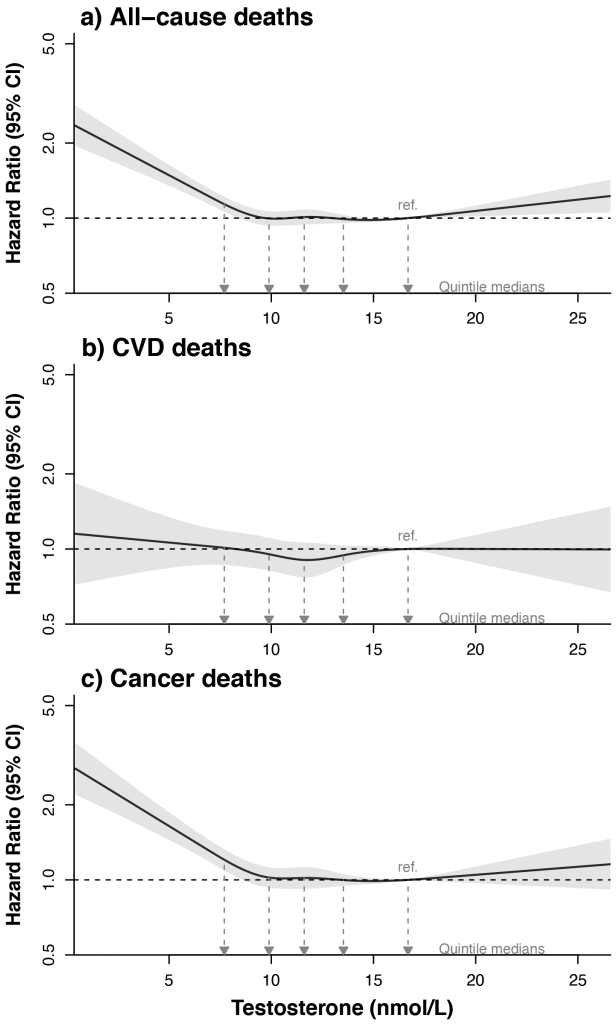


**Figure 1.** Multivariable model showing the effect of baseline serum testosterone on risk of death from any cause (**A**), CVD death (**B**), cancer death (**C**), adjusted for risk factors and potential confounders, and for SHBG. The horizontal dashed line is at the reference hazard (median of the 5$^{th}$ quintile). Shaded areas are the 95% confidence intervals. Horizontal plot axes are truncated to exclude values greater than 4 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. Abbreviations: CVD, cardiovascular disease; SHBG, sex hormone-binding globulin.

**Table 2.** Hazard ratios of all-cause mortality, deaths due to CVD, and deaths due tocancer by quintiles of testosterone

| Model | | Q5 (Highest T) (n = 30 467)[a] | 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 | | 2047 events | 1913 events | 1832 events | 1995 events | 2266 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[c] |
| | Multivariable 1[b] | 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[c] |
| | 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[c] |
| CVD deaths: 1925 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[c] |
| | 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: 4927 events | | 938 events | 908 events | 915 events | 1006 events | 1160 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[c] |
| | 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[c] |
| | 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[c] |

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; HIV, Human Immunodeficiency Virus; ref., reference quintile; SHBG, sex hormone-binding globulin; T, testosterone.
[a]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). 2.5$^{th}$ percentile = 5.9 nmol/L (170 ng/dL), 97.5$^{th}$ = 20.1 nmol/L (579 ng/dL). Presented numbers are for complete cases, after exclusions.
[b]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 center 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 all the covariates as in Multivariable model 1 and SHBG as an additional covariate.
[c]Result interpreted as significant.

HR for the lowest versus the 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 to Q4 were all significantly <1.00, with Q2 being 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 a 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 to Q4 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, 3128 CVD-related and 7468 cancer-related) showed similar results (Table S2 (33). Analyses excluding deaths within the first 2 years (N = 149 185, 9261 deaths, 1734 CVD-related and 4534 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 (Fig. 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 (Fig. 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 (Fig. S7 (33). In Multivariable Model 2 (including testosterone), linear associations of serum SHBG with all-cause, CVD, and cancer mortality were present (Fig. 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 $P < 0.001$).

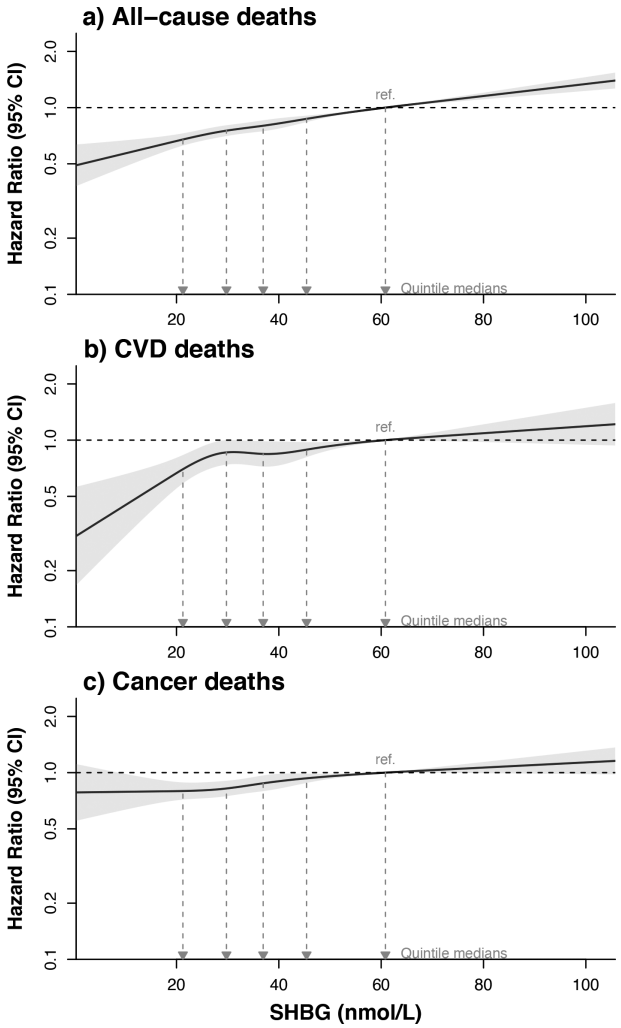


**Figure 2.** Multivariable model showing the effect of baseline serum SHBG on risk of death from any cause (**A**), CVD death (**B**), cancer death (**C**), adjusted for risk factors and potential confounders, and for testosterone. The horizontal dashed line is at the reference hazard (median of the 5th 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. Abbreviations: CVD, cardiovascular disease; SHBG, sex hormone-binding globulin.

A multiply imputed dataset (Table S4 (33) and analyses excluding deaths within the first 2 years (Table S5 (33) showed similar results.

## Associations of cFT values with mortality

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

In univariable analyses, stepwise increases in the risk of all-cause, CVD, and cancer mortality were seen decreasing quintiles of cFT (Fig. S9 (33). In multivariable analyses

**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)[a] | 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 | | 2973 events | 2175 events | 1883 events | 1692 events | 1330 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[c] |
| | Multivariable 1[b] | 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[c] |
| | 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[c] |
| CVD deaths: 1925 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[c] |
| | 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[c] |
| | 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[c] |
| Cancer deaths: 4927 events | | 1326 events | 1082 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[c] |
| | 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[c] |
| | 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[c] |

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; HIV, Human Immunodeficiency Virus; ref., reference quintile; SHBG, sex hormone-binding globulin.
[a]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. 2.5$^{th}$ percentile = 15.2 nmol/L, 97.5$^{th}$ = 79.1 nmol/L. Presented numbers are for complete cases, after exclusions.
[b]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 center 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.
[c]Result interpreted as significant.

(Multivariable Model 1), lower cFT was associated with higher all-cause and cancer-related mortality, but not with CVD deaths (Fig. 3)—results resembling those seen with testosterone (Fig. 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 versus 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 mortality, and CVD-related and cancer-related mortality.

Previous studies of testosterone and mortality have been smaller, generally analyzing between 1 to 5 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 deaths, 304 cancer-related deaths) and 1489 controls (11). The largest previous cohort analysis involved 5350 men, with 1533 deaths (428 CVD-related, 480 cancer-related) (19). Our analysis of 149 436 men from the UK Biobank, with 10 053 deaths (1925 CVD-related, 4927 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 were typically adjusted for between 5 to 10 covariates (7, 9, 10, 12–15, 19, 20), 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 adjustments 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 a higher risk 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

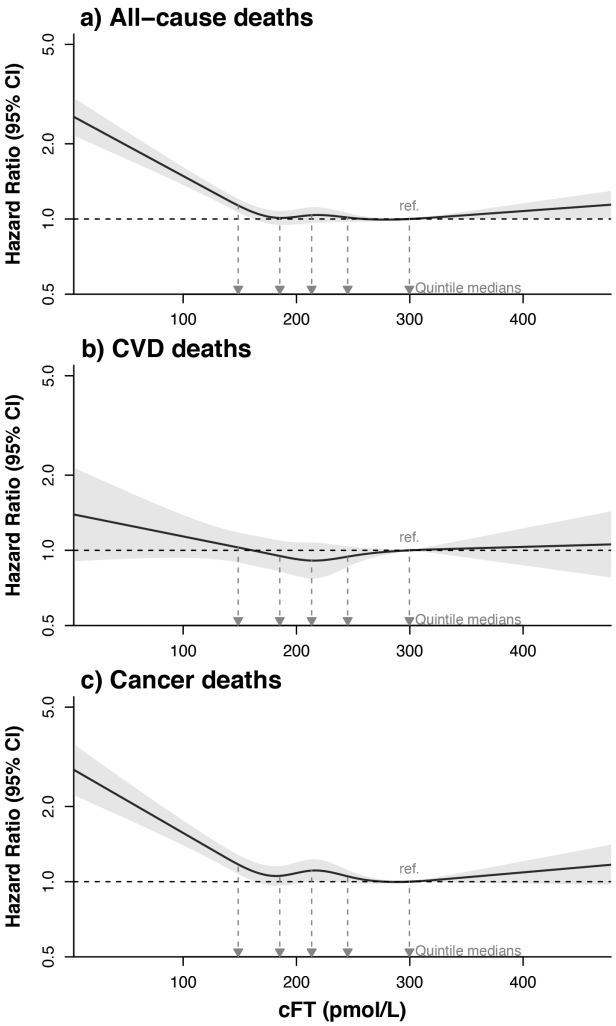


**Figure 3.** Multivariable model showing the effect of baseline calculated free testosterone (cFT) value on risk of death from any cause (**A**), CVD death (**B**), cancer death (**C**), adjusted for risk factors and potential confounders. The horizontal dashed line is at the reference hazard (median of the 5th 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. Abbreviations: cFT, calculated free testosterone; SHBG, sex hormone-binding globulin.

mortality. Other studies adjusting for risk factors and comorbidities, not including SHBG, reported inverse (12, 13, 15, 16) or neutral (6, 7, 19, 20) 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

**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)[a] | 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 | | 1210 events | 1605 events | 1927 events | 2275 events | 3036 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[c] |
| | Multivariable[b] | 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[c] |
| CVD deaths: 1925 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[c] |
| | 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: 4927 events | | 588 events | 803 events | 979 events | 1153 events | 1404 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[c] |
| | 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[c] |

Abbreviations: BMI, body mass index; cFT, calculated free testosterone; COPD, chronic obstructive pulmonary disease; CVD, cardiovascular disease; HIV, Human Immunodeficiency Virus; ref., reference quintile.
[a]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.
[b]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 center as a stratification factor. Prevalent CVD and cancer status were included as additional terms in analyses of CVD deaths and cancer deaths, respectively.
[c]Result interpreted as significant.

testosterone was associated with risk of CVD death. The HR was 1.01, with a narrow CI (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 (20), 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 with the highest quintile, with a narrow 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, 21) or including testosterone in the model (17). Two studies associated higher SHBG with all-cause mortality, without testosterone in the model (20), and with testosterone in the model (22). 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 (21). 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 with highest quintile with a 30% lower risk, with a narrow CI (0.59–0.83). Furthermore, we found that lower SHBG was associated with lower cancer mortality, in a linear fashion, independent of other covariates, including testosterone. This contrasts with previous studies, which reported no association of SHBG with cancer mortality (6, 9, 16, 19, 22).

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 aging men. Sex hormone-binding globulin is the major binding protein for testosterone in the circulation, with the liver its main site of production (35). Sex hormone-binding globulin 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-reporting and was 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 and were 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 underestimates testosterone concentrations slightly compared with 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 widely used Vermeulen formula (26, 27). Participants in the 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.

## Acknowledgments

This research has been conducted using the UK Biobank Resource (project 54680). 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.

***Financial Support:*** 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.

***Author Contributions:*** B.B.Y., R.M., and K.M. led the project; all authors approved the analysis plan; R.M. and K.M. performed the statistical analyses; and B.B.Y. drafted the manuscript. All authors contributed to interpretation of the results and the revision of the manuscript for important intellectual content and approved its submission.

## Additional Information

***Correspondence and Reprint Requests:*** Professor Bu B. Yeap, Medical School, M582, University of Western Australia, 35 Stirling Highway, Crawley 6009, Western Australia, Australia. E-mail: bu.yeap@uwa.edu.au.

***Disclosure Summary:*** The authors have no conflicts of interest to declare in relation to this work.

***Data Availability:*** Datasets analyzed during the current study are not publicly available but are available from the UK Biobank via application to the UK Biobank.

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