

Prospective analyses of testosterone and sex hormone-binding globulin with the risk of 19 types of cancer in men and postmenopausal women in UK Biobank

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Abstract

We investigated the associations of estimated free and total circulating testosterone and sex hormone-binding globulin (SHBG) with cancer risk in men and postmenopausal women, using a pan-cancer approach, including 19 cancers in UK Biobank. Risk was estimated using multivariable-adjusted Cox regression in up to 182 608 men and 122 112 postmenopausal women who were cancer-free at baseline. Participants diagnosed with cancer within 2 years of baseline were excluded. Hazard ratios (HRs) and confidence intervals (CIs) were corrected for regression dilution bias using repeat measurements. We accounted for multiple testing using the false discovery rate. In men, higher free testosterone was associated with higher risks of melanoma and prostate cancer (HR per 50 pmol/L increase = 1.35, 95% CI 1.14-1.61 and 1.10, 1.04-1.18, respectively). Higher total testosterone was associated with an elevated risk of liver cancer (HR per 5 nmol/L = 2.45, 1.56-3.84), and higher SHBG was associated with a higher risk of liver cancer (HR per 10 nmol/L = 1.56, 1.31-1.87) and a lower risk of prostate cancer (0.93, 0.91-0.96); the associations with liver cancer were partially attenuated after excluding men diagnosed within 4.7 years from baseline. In postmenopausal women, free and total testosterone and SHBG were associated with risks of endometrial (HR per 10 pmol/L = 1.59, 1.32-1.90; HR per 0.5 nmol/L = 1.34, 1.18-1.52 and HR per 25 nmol/L = 0.78, 0.67-0.91, respectively) and breast cancer (1.32, 1.22-1.43; 1.24, 1.17-1.31 and 0.88, 0.83-0.94, respectively). We report a novel association of free testosterone with malignant melanoma in men, and confirm known associations between testosterone and risks for prostate, breast and endometrial cancers. The association with liver cancer in men may be attributable to reverse causation.

KEYWORDS

cancer, prospective analysis, SHBG, testosterone, UK Biobank

Abbreviations: BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; HbA1c, glycated hemoglobin; HPG, hypothalamic-pituitary-gonadal; HR, hazard ratio; HRT, hormone replacement therapy; ICD-10, International Classification of Diseases Tenth revision; IGF-I, insulin-like growth factor-I; NHL, non-Hodgkin lymphoma; NHS, National Health Service; SHBG, sex hormone-binding globulin.

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1 | INTRODUCTION

It is well-established that men have a higher risk of most nonsex-specific cancers than women.^{1,2} While some of these differences are due to life-style factors, there is also evidence that sex hormones are involved in the development of several cancers.^{3–6} However, aside from prostate,^{7–9} breast^{9–11} and endometrial^{9,12,13} cancers, previous population-based observational studies with hormone measurements have had limited power to assess associations with other cancer sites.^{4,5,14} Current evidence of associations with other cancers is largely based on animal models and tumor cell lines,^{15–17} or investigated indirectly via associations with self-reported menstrual and reproductive factors in women.^{18,19}

There are large sex differences in the regulation and quantities of serum androgens in men and postmenopausal women, therefore associations with disease risk should be assessed separately.²⁰ In men, testosterone is primarily synthesized by the testes and serum concentrations of free testosterone are regulated by the hypothalamic-pituitary-gonadal (HPG) axis.²¹ In postmenopausal women, testosterone is primarily synthesized peripherally from androstenedione and other androgen precursors as well as by the ovaries, and the HPG axis is thought to have little direct effect in the regulation of testosterone concentrations.^{22,23}

In both sexes, sex hormone-binding globulin (SHBG) is primarily synthesized by the liver and is secreted into the bloodstream, where it tightly binds to sex hormones and modulates their bioavailability.²⁴

In this study, we aimed to examine the associations of serum concentrations of free testosterone, total testosterone and SHBG with the diagnosis of 19 types of cancer in a cohort of 182 600 men and 122 100 postmenopausal women in the UK Biobank, who were cancer-free >2 years from study baseline. This comprehensive pan-cancer approach, using standardized hormone and cancer endpoint analyses, enables the comparison of effect estimates and the exploration of the specificity of our findings.

2 | MATERIALS AND METHODS

2.1 | Study design

UK Biobank is a prospective cohort for public health research. Details of the study protocol and data collection are available online (<http://www.ukbiobank.ac.uk/wp-content/uploads/2011/11/UK-Biobank-Protocol.pdf>) and elsewhere.^{25,26}

In brief, all participants were registered with the UK National Health Service (NHS) and lived within 40 km of one of the UK Biobank assessment centers. Approximately 9.2 million people were initially invited to participate. Overall, 503 317 participants aged 40–69 years consented to join the cohort and attended one of 22 assessment centers throughout England, Wales and Scotland between 2006–2010, a participation rate of 5.5%.²⁶

The UK Biobank study was approved by the North West Multi-Centre Research Ethics Committee (reference number 06/MRE08/65), and at recruitment, all participants gave written informed

What's new?

Significant differences exist in serum androgen regulation and quantity between men and postmenopausal women, implying key differences in risk of hormone-associated cancers. Previous studies, however, have been limited in their ability to assess relationships between androgens and many site-specific cancers. Here, analysis of circulating free and total testosterone and sex hormone-binding globulin in a cohort of men and postmenopausal women reveals positive associations between free testosterone and risk of prostate cancer and melanoma in men and breast and endometrial cancer in postmenopausal women. Sex- and site-specific associations with testosterone do not support a general role for testosterone in cancer risk.

consent to participate and for their health to be followed-up through linkage to electronic medical records.

2.2 | Baseline assessment

At the baseline assessment visit, participants provided information on a range of sociodemographic, anthropometric, lifestyle and health-related factors via a self-completed touch-screen questionnaire and a computer-assisted personal interview.²⁶

2.3 | Blood sampling and biomarker assays

At recruitment, blood sampling was performed successfully in 99.7% of the cohort. Blood was collected in a serum separator tube and shipped to the central processing laboratory in temperature-controlled boxes at 4°C,²⁷ then aliquoted and stored in a central working archive at –80°C.²⁸ Measurement of serum concentrations of SHBG, testosterone and albumin was attempted in all participants. SHBG and testosterone were measured by chemiluminescent immunoassays (Beckman Coulter AU5800), and albumin was measured by a colorimetric assay (Beckman Coulter AU5800). Average within-laboratory (total) coefficients of variation for low, medium and high internal quality control level samples for each biomarker ranged from 2.1% to 8.3%.

Participants with total testosterone below the limit of detection were assigned a serum concentration of 0.26 nmol/L (3/4 of the lower limit of detection) (n = 16 men and 25 253 postmenopausal women).

Serum insulin-like growth factor-I (IGF-I), glycated hemoglobin (HbA1c) and C-reactive protein (CRP) concentrations were also assayed in serum from blood collected at study baseline. Full details of the assay methods and quality assurance protocols are available online (https://biobank.ndph.ox.ac.uk/showcase/showcase/docs/serum_biochemistry.pdf).

2.4 | Free testosterone calculation

Testosterone in the circulation is bound to SHBG and albumin. Approximately 2% of total testosterone circulates unbound or “free” and is postulated to be biologically active.²⁴ Free testosterone concentrations were estimated using a formula based on the law of mass action and measured total testosterone, SHBG and albumin concentrations.^{7,29}

2.5 | Repeat measurements

Participants who lived within a 35 km radius were invited via email to attend a repeat assessment clinic at the UK Biobank Co-ordinating Centre in Stockport between August 2012 and June 2013, with a response rate of 21%.³⁰ Repeat assessments were completed in approximately 20 000 individuals.

2.6 | Participant follow-up

Cancer registration and death data were provided from national registries via record linkage to NHS Digital (England and Wales) and the NHS Central Register (Scotland). Linkage was achieved using participants' NHS numbers in England and Wales, and the Community Health Index in Scotland. Data were available until the censoring date (March 31, 2016, in England and Wales and October 31, 2015, in Scotland) or until participants died, withdrew consent for future linkage or were reported to have left the United Kingdom. Further information on data linkage is available from <https://biobank.ctsu.ox.ac.uk/crystal/crystal/docs/DataLinkageProcess.pdf>.

Endpoints were defined as the first incident cancer diagnosis, or cancer recorded on death certificate if not previously diagnosed with cancer. Cancers were coded using the International Classification of Diseases Tenth revision code (ICD-10)³¹ and we restricted analyses to cancers with a minimum of 100 recorded cases in men, and 100 cases for female only cancers (for consistency across sexes): lip, oral and pharynx (C00-14), esophagus (C15), adenocarcinoma of esophagus (C15, morphology codes ICD-O-3 8140-8573), stomach (C16), colorectum (C18-20) including colon (C18) and rectum (including rectosigmoid junction; C19-20), liver (C22), pancreas (C25), lung (C34), malignant melanoma (C43), mesothelioma (C45), female breast (C50), endometrial (C54.1), ovarian (C56), prostate (C61), kidney (C64-65), bladder (C67), brain (C71), non-Hodgkin lymphoma (NHL) (C82-85), multiple myeloma (C90) and leukemia (C91-95), and the NHL subtype diffuse NHL (C83). Person-years were calculated from the date of recruitment to the date of the first cancer registration, death, loss to follow-up, or censoring date, whichever occurred first.

2.7 | Exclusion criteria

Our analytical dataset included up to 182 608 men and 122 112 postmenopausal women (Figure S1). We excluded 27 170 participants with

prevalent cancer (except C44: nonmelanoma skin cancer), 32 563 with no blood measurement data available or who had biomarker measurements that did not pass quality control procedures,³² 1533 who were missing BMI measurement data and 22 697 who were diabetic at baseline (self-reported). We also excluded 6070 participants who were diagnosed with cancer <2 years from baseline, 2629 who were taking hormone-related medication at baseline, and 3843 participants for whom it was not possible to determine genetic sex or whose genetic sex was identified as being different from their self-reported sex.

After applying the exclusion criteria listed above, 156 045 women were identified as postmenopausal (defined as stopped menstrual periods or aged >55 years; all other women were excluded, $n = 66\,526$). We also excluded 12 745 women who were current users of hormone replacement therapy (HRT) or the contraceptive pill, 930 who had prevalent in situ breast cancer at study baseline and 19 786 who reported having had a hysterectomy or bilateral oophorectomy at study baseline (Figure S1).

2.8 | Statistical analysis

Cox proportional hazards models were used to estimate the associations with cancer diagnosis, with age as the underlying time variable. Primary analyses were stratified by geographic area (10 UK regions) and age at recruitment (<45, 45-49, 50-54, 55-59, 60-64, ≥65 years), and adjusted for Townsend deprivation score (fifths, unknown [0.1%]), education level (college or university degree/vocational qualification, national examination at ages 17/18, national examination at age 16, other qualification or unknown [19.0%]), ethnic group (white, Asian, black, mixed background and other, and unknown [1.2%]), height (<170, ≥170-175, ≥175-180, ≥180 cm and unknown [0.1%], for men; and <160, ≥160-165, ≥165-170, ≥170 cm and unknown [0.02%], for women), BMI (<25, ≥25-30, ≥30-35, ≥35 kg/m²), cigarette smoking (never, former, current light smoker [1-15 cigarettes per day], current heavy smoker [≥15 cigarettes per day], current [number of cigarettes per day unknown] and smoking status unknown [0.5%]), alcohol consumption (nondrinkers, <1-10, ≥10-20, ≥20 g ethanol/day, unknown [0.6%]), and total physical activity (<10, 10-19, 20-39, 40-59, ≥60 metabolic equivalent of task hours per week and unknown [0.6%]). For postmenopausal women, we additionally adjusted for past HRT use (never, ever), past oral contraceptive pill use (never, ever), parity and age at first birth (nulliparous; 1-2, <25; 1-2, 25-29; 1-2, ≥30; 1-2, unknown; ≥3, <25; ≥3, 25-29; ≥3, ≥30 years, unknown [16.0%]), age at menarche (<12, 12-13, ≥14 years, unknown [2.9%]) and age at menopause (<45, 45-49, 50-54, ≥55 years, unknown [14.9%]). Adjustment covariates were defined a priori based on previous analyses of UK Biobank data.³³

Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated per increment in each hormone, using the trend across the medians of the quartiles to estimate the linear trends. The increments were per 50 pmol/L, per 5 nmol/L and 10 nmol/L for free testosterone, total testosterone and SHBG, respectively for men and per 10 pmol/L, per 0.5 nmol/L and 25 nmol/L, respectively for postmenopausal women, and were chosen based on the sex-specific hormone SDs).

TABLE 1 Baseline characteristics and blood data in men and postmenopausal women (in the analytic sample and incident cancers cases) in UK Biobank

	Men		Postmenopausal women	
	All (n = 182 608)	Incident cancer ^a (n = 9519)	All (n = 122 112)	Incident cancer ^a (n = 5632)
Sociodemographic				
Age at recruitment (years), mean (SD)	56.13 (8.22)	61.07 (6.18)	60.15 (5.29)	61.29 (4.97)
Most deprived quintile, % (N)	19.58 (35716)	19.02 (1808)	17.24 (21037)	19.04 (1072)
Black ethnicity, % (N)	1.38 (2485)	0.94 (88)	1.02 (1238)	0.59 (33)
Not in paid/self-employment, % (N)	36.52 (66693)	53.50 (5093)	54.93 (67082)	60.58 (3412)
Living with partner, % (N)	76.91 (140452)	77.08 (7337)	69.48 (84849)	67.86 (3822)
Anthropometric, mean (SD)				
Height (cm)	175.76 (6.82)	175.32 (6.70)	161.92 (6.19)	162.09 (6.32)
BMI (kg/m ²)	27.61 (4.05)	27.69 (4.02)	26.96 (4.90)	27.53 (5.15)
Lifestyle, % (N)				
Current cigarette smokers	12.53 (22744)	15.14 (1430)	7.90 (9610)	10.77 (604)
Drinking alcohol ≥ 20 g per day	44.37 (80618)	46.38 (4390)	13.50 (16378)	14.61 (818)
Low physical activity (0-10 METs per week)	27.32 (48357)	27.64 (2545)	30.83 (36136)	32.82 (1770)
Health history, % (N)				
Hypertension	51.82 (94587)	59.27 (5637)	46.25 (56422)	50.11 (2817)
Poor self-rated health	3.95 (7164)	4.49 (425)	2.76 (3355)	3.39 (190)
Reproductive factors, % (N)				
Previous use of HRT	—	—	42.56 (51965)	46.64 (2627)
Previous use of oral contraceptive pill	—	—	78.26 (95570)	75.30 (4241)
Nulliparous	—	—	15.99 (19514)	16.67 (938)
<25 years at first child	—	—	55.06 (47996)	57.96 (2301)
≥ 14 years at menarche	—	—	36.80 (43619)	36.34 (1984)
<50 years at menopause	—	—	32.19 (33439)	29.64 (1401)
Baseline blood measures, mean (SD)				
Free testosterone (pmol/L)	210.49 (59.54)	199.55 (54.92)	14.41 (10.13)	15.25 (10.59)
Total testosterone (nmol/L)	12.11 (3.63)	11.97 (3.62)	1.09 (0.63)	1.14 (0.73)
SHBG (nmol/L)	39.65 (16.51)	42.34 (17.21)	58.91 (26.51)	57.24 (26.00)
Albumin (g/L)	45.59 (2.58)	45.06 (2.60)	45.11 (2.53)	44.85 (2.56)

Abbreviations: BMI, body mass index; HRT, hormone replacement therapy; METs, metabolic equivalent of tasks; N, number; SHBG, sex hormone binding globulin.

^aDiagnosed >2 years from baseline.

Measurement error and within-person variability using single measures at baseline leads to underestimation of risk (ie, regression dilution bias).³⁴ Therefore, estimates for trend were corrected for regression dilution bias by assigning the median values from the fourths of blood biomarker concentrations measured in the repeat blood samples for the subcohort who attended the repeat assessment (up to 7669 men and 4568 postmenopausal women, median of 4.3 years after first blood collection³⁰) to participants categorized by fourths of hormone concentrations measured at study baseline,³⁵ excluding those who were diagnosed with cancer between baseline and repeat assessment (up to 132 men and 48 women).

In the categorical analyses, biomarker measurements were categorized into fourths and HRs were calculated relative to the lowest fourth of each blood parameter. The variance of the log risk in

each group was calculated and used to obtain group-specific 95% CIs.^{36,37}

2.9 | Sensitivity analyses

2.9.1 | Subgroup analyses

Subgroup analyses for incident cancer were examined with participants categorized according to: (a) time from blood collection to diagnosis (>2 - ≤ 4.7 , >4.7 years); (b) age at diagnosis (≤ 65 , >65 years) and (c) age at blood collection (≤ 60 , >60 years), with these categories chosen based on the median values. Heterogeneity in the associations for case-specific variables (ie, time to diagnosis and age at

diagnosis) was examined using stratified Cox models based on competing risks, comparing the risk coefficients and standard errors in the two subgroups, and testing with a χ^2 for heterogeneity. For age at blood collection, heterogeneity was assessed using a χ^2 interaction term.

2.9.2 | Adjustment for other factors

To assess the role of other biomarkers, we further adjusted the primary analysis for serum IGF-I, HbA1c and CRP concentrations (fourths, unknown). The association of testosterone concentration

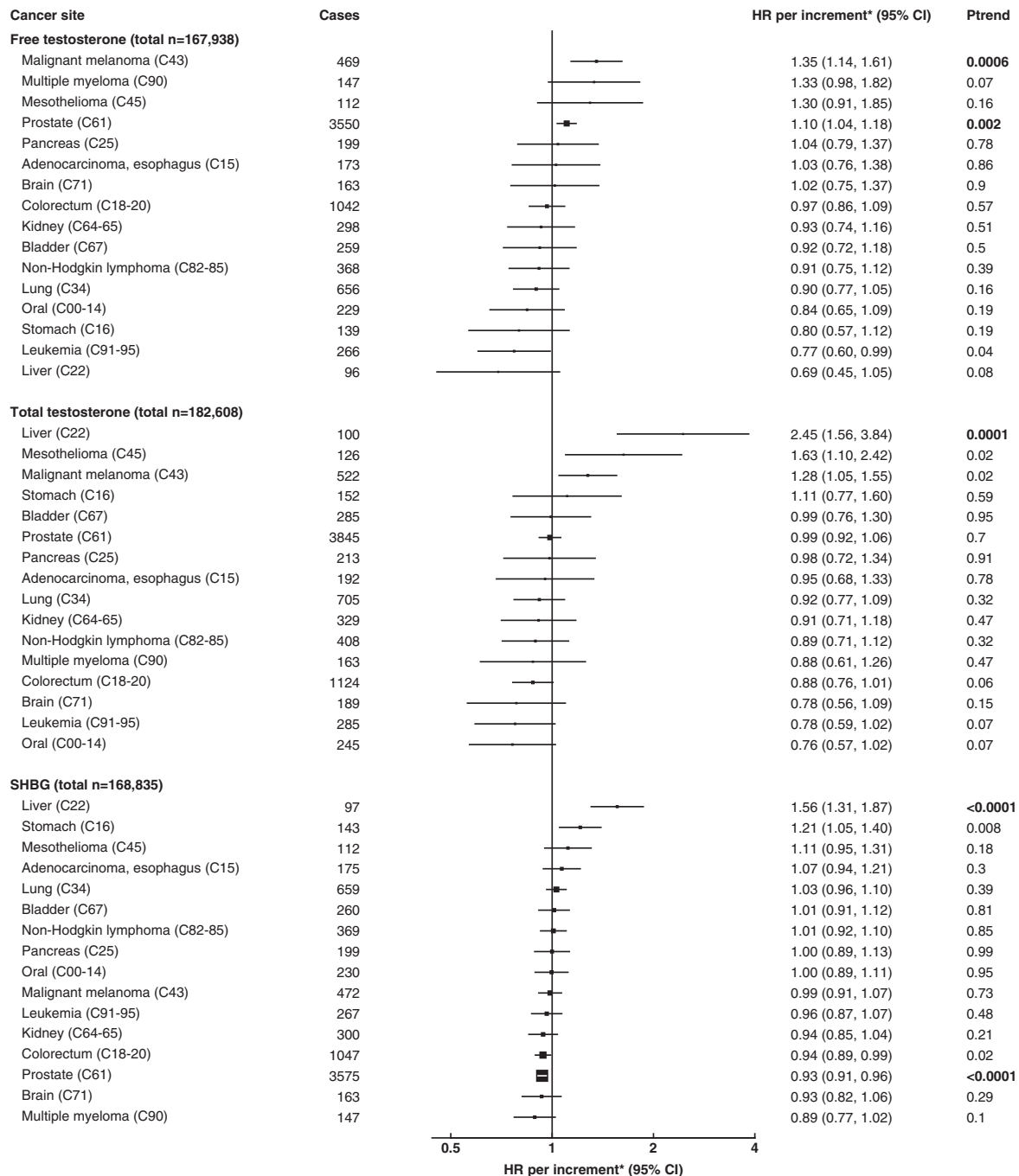


FIGURE 1 Hazard ratios and 95% confidence intervals for cancer diagnosis per increment in free testosterone, total testosterone and SHBG concentrations by cancer site in men. Associations stratified for age group, geographical region and adjusted for Townsend deprivation score, ethnic group, height, body mass index, cigarette smoking, alcohol consumption, and total physical activity and corrected for regression dilution bias. HRs are presented by squares with their 95% CIs as horizontal lines, the size of the squares is inversely proportional to the variance of the log HR. P_{trends} are bold if associations are statistically significant after accounting for false discovery rates. *Increments are free testosterone, per 50 pmol/L; total testosterone, per 5 nmol/L; SHBG, per 10 nmol/L. CI, confidence interval; HR, hazard ratio; SHBG, sex hormone binding globulin

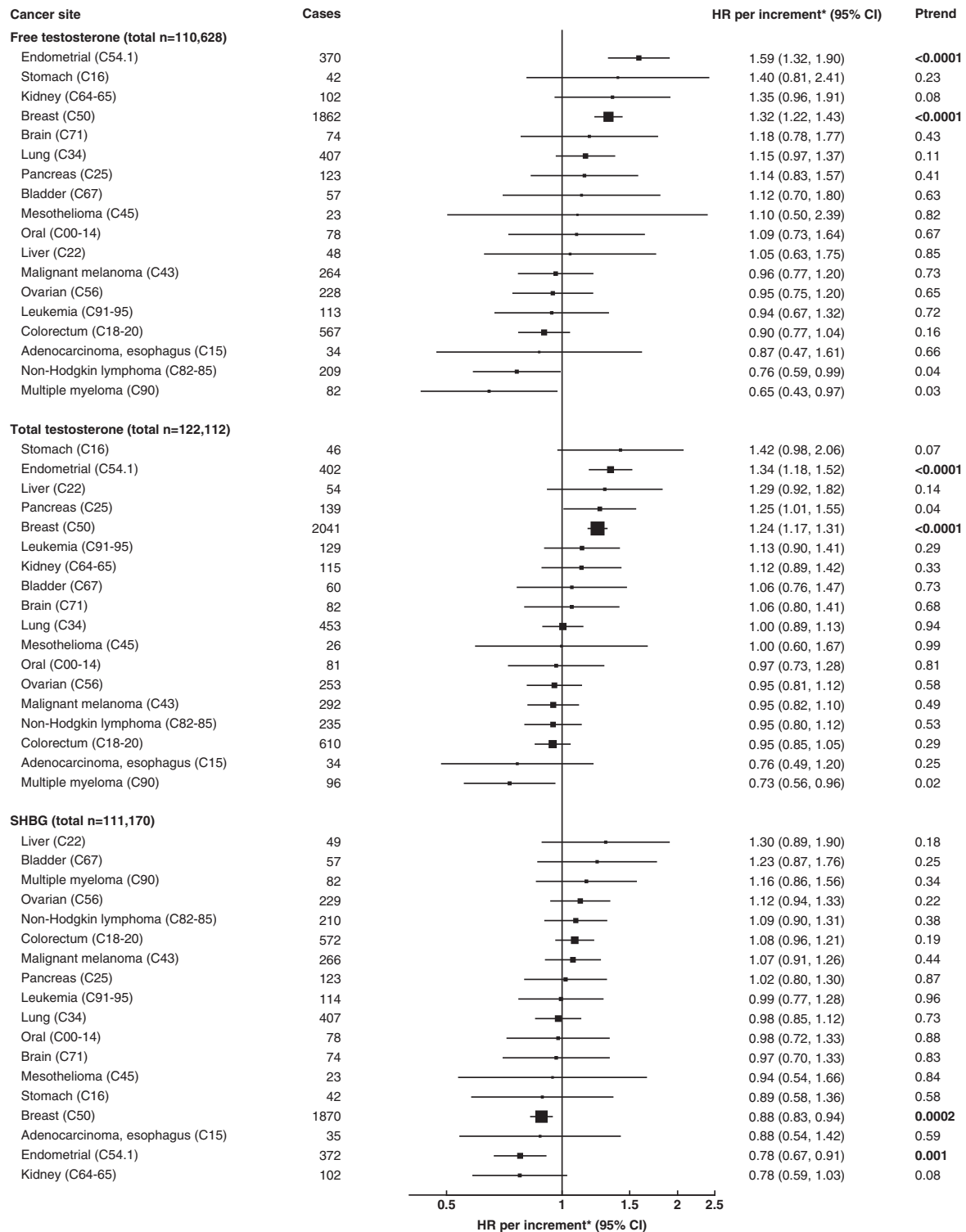


FIGURE 2 Hazard ratios and 95% confidence intervals for cancer diagnosis per increment in free testosterone, total testosterone and SHBG concentrations by cancer site in postmenopausal women. Associations stratified for age group, geographical region and adjusted for Townsend deprivation score, ethnic group, height, body mass index, cigarette smoking, alcohol consumption, total physical activity, hormone replacement therapy use, oral contraceptive use, and parity and age at first birth, and corrected for regression dilution bias. HRs are presented by squares with their 95% CIs as horizontal lines, the size of the squares is inversely proportional to the variance of the log HR. P_{trends} are bold if associations are statistically significant after accounting for false discovery rates. *Increments are free testosterone, per 10 pmol/L; total testosterone, per 0.5 nmol/L; SHBG, per 25 nmol/L. CI, confidence interval; HR, hazard ratio; SHBG, sex hormone binding globulin

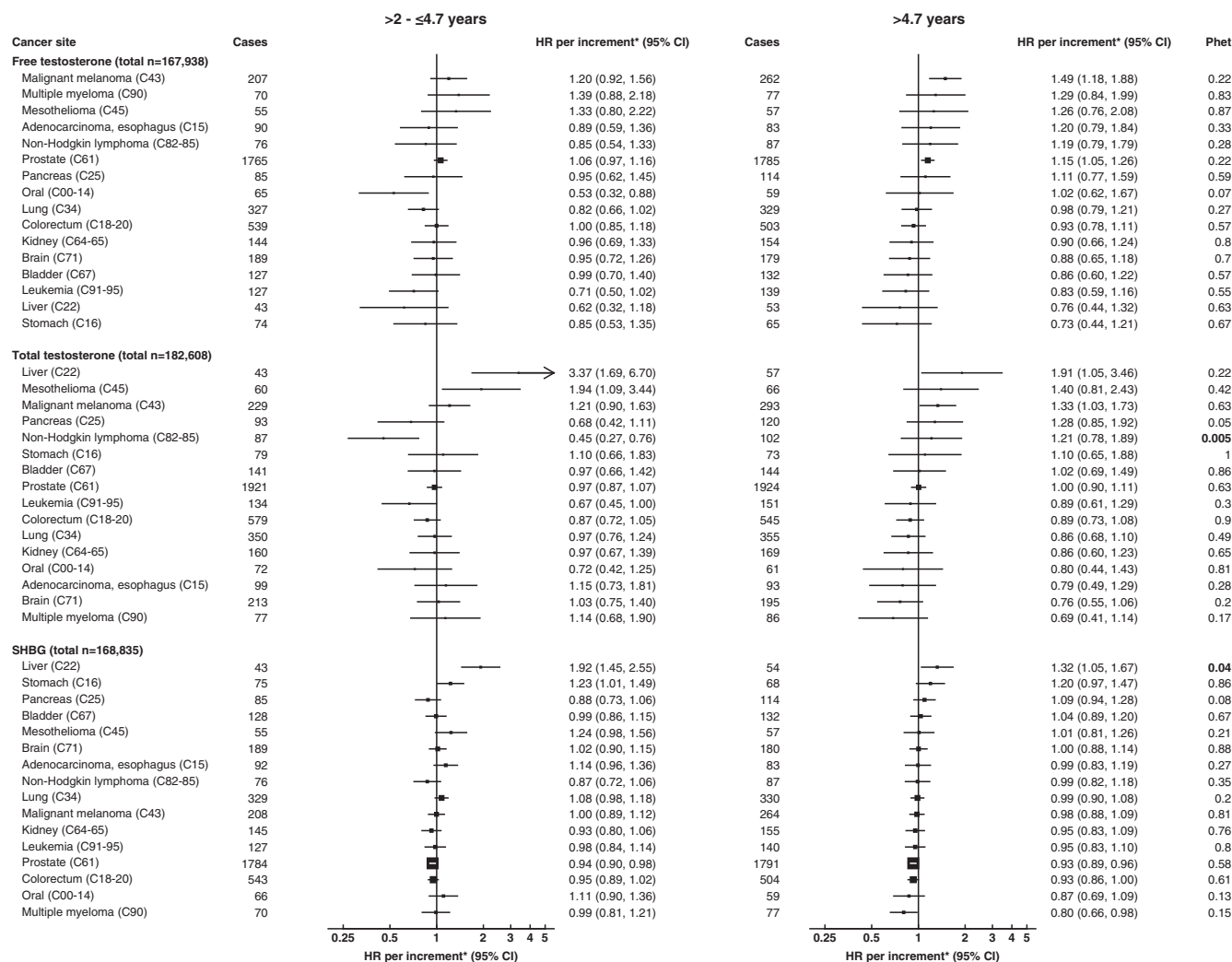


FIGURE 3 Hazard ratios and 95% confidence intervals per increment in free testosterone, total testosterone and SHBG for cancer diagnosis by cancer site and time to diagnosis in men. Associations stratified for age group, geographical region and adjusted for Townsend deprivation score, ethnic group, height, body mass index, cigarette smoking, alcohol consumption and total physical activity. Follow-up time was based on the median time to diagnosis. HRs are presented by squares with their 95% CIs as horizontal lines, the size of the squares is inversely proportional to the variance of the log HR. HRs are presented by squares with their 95% CIs as horizontal lines, the size of the squares is inversely proportional to the variance of the log HR. $P_{\text{heterogeneity}}$ (P_{het}) comparing associations below and above time to diagnosis obtained using competing risks, P -values are bold if $P < .05$. *Increments are free testosterone, per 50 pmol/L; total testosterone, per 5 nmol/L; SHBG, per 10 nmol/L. CI, confidence interval; HR, hazard ratio; SHBG, sex hormone binding globulin

and melanoma may have been confounded by factors relating to sun exposure and sensitivity, therefore we additionally adjusted for: skin color (very fair, fair, light olive, dark olive, brown/black, missing); hair color (blonde, red, light brown, dark brown, black, missing); skin reaction to sun exposure (get very tanned, moderately tanning, mildly/occasionally tanning, never tanning only burning, missing); and sunburn before age 15 (never, ever, missing).

All analyses were performed using Stata version 14.1 (Stata Corporation, College Station, TX), and figures were plotted in R version 3.6.3. All tests of significance were two-sided, and P -values $< .05$ were considered statistically significant. In the primary analysis, we additionally accounted for multiple testing using false discovery rates (FDR; 48 statistical tests in men and 54 statistical tests in women).³⁸

3 | RESULTS

After a mean follow-up of 7.0 years ($SD = 1$), 9519 men (5.2%) and 5632 postmenopausal women (4.6%) were diagnosed with any type of malignant cancer (excluding nonmelanoma skin cancer, C44).

Table 1 summarizes the baseline characteristics of study participants. Mean age at recruitment was 56.1 ($SD = 8.2$) in men and 60.2 years (5.3) in postmenopausal women. Participants who subsequently developed cancer were older, were more likely to smoke and had worse self-rated health at recruitment. Men who were subsequently diagnosed with cancer on average had a slightly lower socioeconomic status, while women who were diagnosed with cancer had a slightly higher socioeconomic status and were more likely to have previously used HRT compared to those without a cancer diagnosis

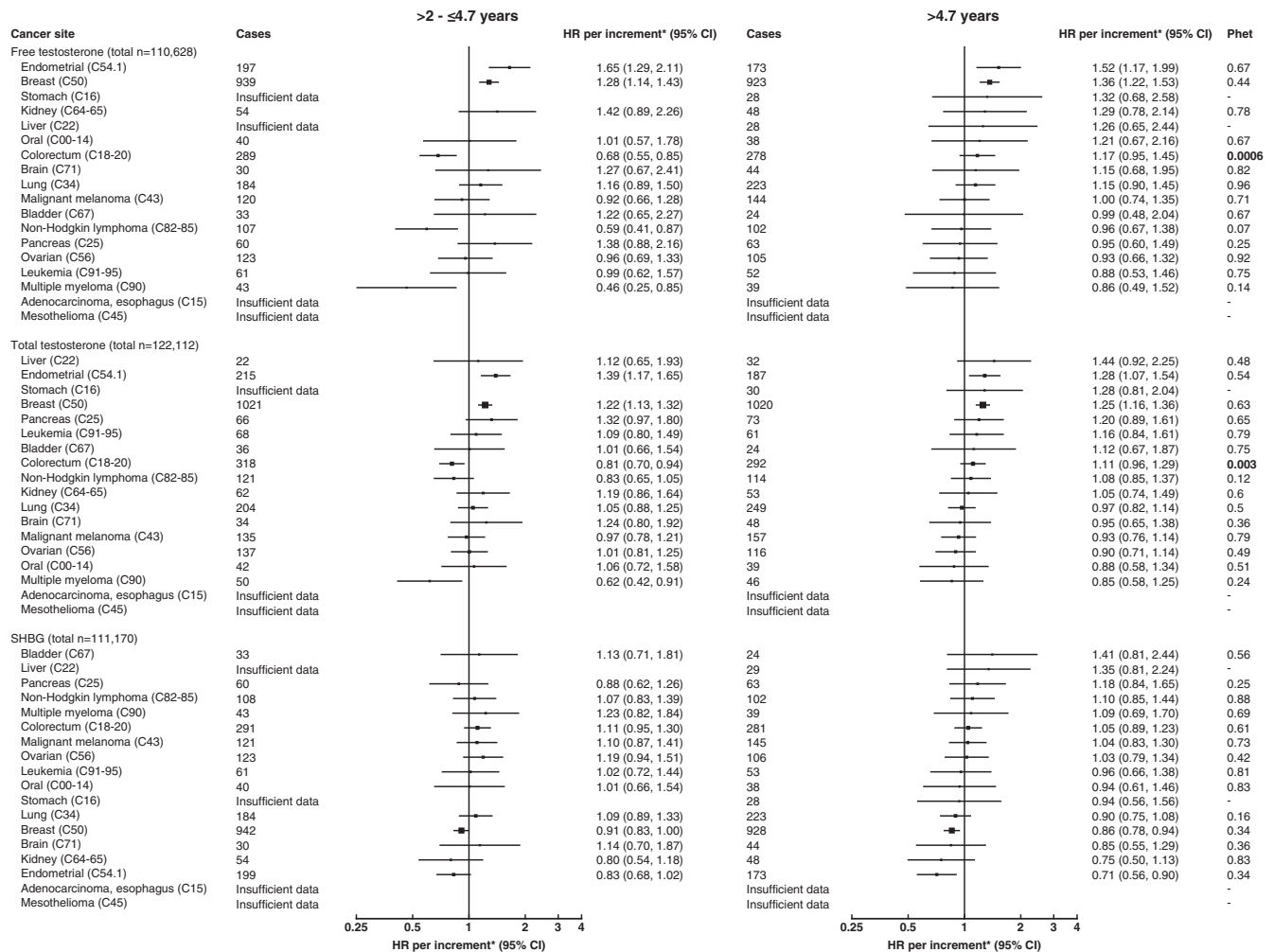


FIGURE 4 Hazard ratios and 95% confidence intervals per free testosterone, total testosterone and SHBG increment for cancer diagnosis by cancer site and time to diagnosis in postmenopausal women. Associations stratified for age group, geographical region and adjusted for Townsend deprivation score, ethnic group, height, body mass index, cigarette smoking, alcohol consumption, total physical activity, hormone replacement therapy use, oral contraceptive use, and parity and age at first birth. Follow-up time was based on the median time to diagnosis. HRs are presented by squares with their 95% CIs as horizontal lines, the size of the squares is inversely proportional to the variance of the log HR. HRs are presented by squares with their 95% CIs as horizontal lines, the size of the squares is inversely proportional to the variance of the log HR. $P_{\text{heterogeneity}}$ (P_{het}) comparing associations below and above time to diagnosis obtained using competing risks, P -values are bold if $P < 0.05$. *Increments are free testosterone, per 10 pmol/L; total testosterone, per 0.5 nmol/L; SHBG, per 25 nmol/L. CI, confidence interval; HR, hazard ratio; SHBG, sex hormone binding globulin

(Table 1). Mean and SD values for baseline biomarker measurements are also displayed in Table 1.

3.1 | Associations between testosterone and SHBG concentrations and cancer diagnosis

3.1.1 | Men

Serum-free testosterone concentration was positively associated with risks of malignant melanoma and prostate cancer (HR per 50 pmol/L increment = 1.35, 95% CI 1.14-1.61; $P_{\text{trend}} = 0.0006$, and 1.10, 1.04-1.18; $P_{\text{trend}} = 0.002$, respectively) and inversely associated with the risk of leukemia (0.77, 0.60-0.99; $P_{\text{trend}} = 0.04$) (Figure 1).

Serum total testosterone concentration was positively associated with risks of liver cancer (HR per 5 nmol/L increment = 2.45, 1.56-3.84; $P_{\text{trend}} = 0.0001$), mesothelioma (1.63, 1.10-2.42; $P_{\text{trend}} = 0.02$) and malignant melanoma (1.28, 1.05-1.55; $P_{\text{trend}} = 0.02$) (Figure 1).

Serum SHBG concentrations were positively associated with risks of liver (HR per 10 nmol/L increment = 1.56, 1.31-1.87; $P_{\text{trend}} = <0.0001$) and stomach cancer (1.21, 1.05-1.40; $P_{\text{trend}} = 0.008$) and inversely with risks of prostate (0.93, 0.91-0.96; $P_{\text{trend}} = <0.0001$) and colorectal cancer (0.94, 0.89-0.99; $P_{\text{trend}} = 0.02$) (Figure 1).

After accounting for multiple testing, the associations of free testosterone with prostate cancer and melanoma, total testosterone with liver cancer, and SHBG with liver and prostate cancer remained statistically significant.

3.1.2 | Postmenopausal women

Serum-free testosterone concentration was positively associated with risks of endometrial and breast cancer (HR per 10 pmol/L increment = 1.59, 1.32-1.90; $P_{\text{trend}} < 0.0001$ and 1.32, 1.22-1.43; $P_{\text{trend}} < 0.0001$, respectively), and inversely with risks of multiple myeloma (0.65, 0.43-0.97; $P_{\text{trend}} = 0.03$) and NHL (0.76, 0.59-0.99; $P_{\text{trend}} = 0.04$) (Figure 2).

Serum total testosterone concentration was positively associated with risks of endometrial (HR per 0.5 nmol/L increment = 1.34, 1.18-1.52; $P_{\text{trend}} < 0.0001$), breast (1.24, 1.17-1.31; $P_{\text{trend}} < 0.0001$) and pancreatic cancer (1.25, 1.01-1.55; $P_{\text{trend}} = 0.04$) and inversely with risk of multiple myeloma (0.73, 0.56-0.96; $P_{\text{trend}} = 0.02$) (Figure 2).

Serum SHBG concentrations were inversely associated with risks of endometrial (HR per 25 nmol/L increment = 0.78, 0.67-0.91; $P_{\text{trend}} = 0.001$) and breast cancer (0.88, 0.83-0.94; $P_{\text{trend}} = 0.0002$) (Figure 2).

After accounting for multiple testing, the associations of free and total testosterone and SHBG with endometrial and breast cancer remained statistically significant.

Further results including relationships for cancer subtypes, in fourths of the hormones distributions, different adjustment models and associations with and without adjustment for regression dilution bias are displayed in Tables S1-S6.

3.2 | Sensitivity analyses

3.2.1 | Subgroup analyses

In men, the significant associations between testosterone and cancer diagnosis showed no significant heterogeneity by length of follow-up, age at diagnosis, or age at blood collection (Figure 3, Figures S2 and S3). There was evidence of heterogeneity in the association of SHBG with liver cancer by length of follow-up; men diagnosed with liver cancer 2-4.7 years from baseline had a higher risk of liver cancer diagnosis in relation to SHBG (HR per 10 nmol/L increment = 1.92, 95% CI 1.45-2.55), while the magnitude of this association was smaller in men diagnosed >4.7 years from baseline (1.32, 1.05-1.67; $P_{\text{het}} = 0.04$).

In postmenopausal women, associations of testosterone and SHBG with cancer risk showed no significant heterogeneity by length of follow-up, age at diagnosis or age at blood collection (Figure 4, Figures S4 and S5), with the exception of total testosterone concentration and endometrial cancer by age at diagnosis and age at blood collection; postmenopausal women diagnosed with endometrial cancer aged ≤ 65 years had a larger magnitude of association of total testosterone with endometrial cancer risk (HR per 0.5 nmol/L increment = 1.58, 95% CI 1.30-1.91), than women diagnosed aged >65 years (1.17, 0.98-1.39; $P_{\text{het}} = 0.02$) (Figure S4). Similarly, women aged ≤ 60 years at blood collection had a larger magnitude of association (1.67, 1.32-2.12), than women >60 years at baseline (1.22, 1.05-1.42; $P_{\text{het}} = 0.03$) (Figure S5).

3.2.2 | Adjustment for other factors

In men, after further adjustment for IGF-I, HbA1c and CRP, the association of SHBG concentration with colorectal cancer slightly attenuated (HR per 10 nmol/L increment = 0.95, 95% CI 0.90-1.00; $P_{\text{trend}} = 0.07$) (Table S3).

In postmenopausal women, further adjustment for these biomarkers slightly attenuated the association of serum-free testosterone with NHL (HR per 10 pmol/L increment = 0.78, 95% CI = 0.60-1.01; $P_{\text{trend}} = 0.06$) (Table S4).

The associations of free and total testosterone with melanoma in men were not materially different after further adjustment for sun exposure and sensitivity factors (Table S7).

4 | DISCUSSION

This comprehensive analysis of serum-free and total testosterone and SHBG concentrations with cancer risk in 182 600 men and 122 100 postmenopausal women shows a novel association of free testosterone with melanoma risk in men. We also confirm previously observed associations with prostate,⁷⁻⁹ breast⁹⁻¹¹ and endometrial^{9,12,13} cancer. We additionally report several possible associations with other cancer sites, such as SHBG with stomach cancer, but these associations were not statistically significant after accounting for multiple testing.

4.1 | Associations with incident cancer in men

Higher total and free testosterone concentrations were associated with an increased risk of malignant melanoma. Until now, associations between serum sex hormone concentrations and melanoma have not been robustly assessed in the context of other large prospective cohort studies.¹⁴ However, it has been observed that men diagnosed with melanoma have a higher risk of developing prostate cancer and vice versa, suggesting a common biological or behavioral cause.³⁹ There is also biological evidence of plausibility; androgens are involved in several skin processes including melanogenesis^{15,40}; and in vitro models implicate a role of androgen signaling in promoting melanoma.¹⁵

We have previously observed associations between free testosterone and prostate cancer risk and an inverse association with SHBG in UK Biobank and in an international consortium of 20 cohorts (6933 cases and 12 088 controls).^{7,8} These associations are also consistent with evidence from two large randomized controlled trials (which aimed to reduce intraprostatic androgen signaling using 5 α -reductase inhibitors to reduce the conversion of testosterone to dihydrotestosterone)^{41,42} and a Mendelian randomization study.⁹

We note the large magnitude of the associations between total testosterone and SHBG and liver cancer, which has been observed previously.³ The liver is integral in sex hormone signaling and metabolism, and synthesis of SHBG. Liver damage often leads to abnormal concentrations of blood biomarkers. For instance, liver fibrosis, which can

develop into liver cancer, is associated with higher SHBG concentrations in men.⁴³ Therefore, higher SHBG and total testosterone concentrations may be markers of preclinical disease, and this interpretation is supported by the marked attenuation of risk estimates we observed when restricting cases to those diagnosed after the median follow-up.

We also observed nominally significant inverse associations between free testosterone and leukemia, total testosterone and mesothelioma, and SHBG and colorectal cancer, and a positive association between SHBG and stomach cancer risk. However, these associations did not withstand correction for multiple testing. While current evidence concerning the possible inverse associations between testosterone and/or SHBG and these cancers is limited, associations between sex hormones and stomach cancer risk have been observed previously.⁴ Speculatively, this could perhaps be due to the role of SHBG in limiting estradiol bioactivity, as estradiol has been shown to reduce the viability and carcinogenic potential of *Helicobacter pylori* in animal models.^{44,45} Nevertheless, we are unable to rule out the possibility of reverse causation⁴⁶ or chance.

4.2 | Associations with incident cancer in postmenopausal women

In postmenopausal women, we observed positive associations of free and total testosterone with risks for endometrial and breast cancer, while higher SHBG was associated with a lower risk of cancer at these sites. These associations have been observed previously in prospective nested case-control studies,^{10,12,13} in Mendelian randomization analyses,⁹ and associations with breast cancer have been reported in an international consortium of 15 cohorts with 3112 cases and 6117 controls.¹¹ Higher androgen concentrations might increase the risk of these cancers indirectly via their conversion to estrogens which stimulate cell proliferation, increasing the probability of mutations and uncontrolled cell growth.⁴⁷⁻⁴⁹ There is also some evidence that testosterone might affect endometrial and breast cancer risk more directly via androgen receptor signaling.^{50,51} In previous epidemiological analyses, when estimates of testosterone and breast cancer risk additionally adjusted for circulating estradiol concentrations, the association with testosterone somewhat attenuated but remained statistically significant,¹⁰ while the association of testosterone with endometrial cancer attenuated to the null.^{12,13}

We also observed possible inverse associations of testosterone with multiple myeloma, NHL and pancreatic cancer in postmenopausal women. However, these associations did not withstand correction for multiple testing.

4.3 | Differences in cancer risk in men vs postmenopausal women

Although men have a greater risk of most cancers in comparison with women,^{1,2} this analysis suggests that differences in testosterone and SHBG are not likely to make a large contribution to this difference. Men were more likely to smoke and drink alcohol than postmenopausal women, which may indicate a greater importance of sex-

differences in lifestyle factors in risk disparities, or that differences in risk may be related to other sexual dimorphisms which are independent of sex hormones,² including greater body size.⁵² We did, however, observe a sex-specific association of free testosterone with melanoma. It is possible that this difference might be due to the more than 10-fold greater free testosterone concentrations in men.

4.4 | Strengths and limitations

UK Biobank is currently the largest prospective study with hormone data available for nearly the whole cohort, providing a unique opportunity to robustly assess associations of serum testosterone and SHBG concentrations with cancer risk using a comprehensive pan-cancer approach. The statistical power improved the precision of our risk estimates and enabled us to investigate associations with less common cancers, and the availability of repeat measurements enabled us to adjust for regression dilution bias.³⁴ UK Biobank is also a well-characterized study population, and therefore we were able to adjust our risk estimates for a wide range of possible confounders and to examine the role of other related biomarkers.

Limitations of the analysis are that tumor characterization information is not currently available, which may be relevant for the etiology of some cancer sites. We were also underpowered to investigate heterogeneity in the associations, particularly for the less common cancers. The UK Biobank participants are predominantly of white European ancestry and are healthier than the sampling population, therefore risk estimates may not be generalizable,²⁶ though this is unlikely to affect the direction of the associations.⁵³ Although all our analyses excluded participants diagnosed with cancer in the first 2 years of follow-up, the follow-up period is not very long and we are not able to rule out the possibility of reverse causation. Although we employed FDR to address the increased risk of chance findings due to the large number of statistical tests, we cannot rule out the possibility of chance findings, particularly in the sensitivity analyses.

SHBG also modulates the bioavailability of other sex hormones including estradiol, some of which is synthesized from testosterone and may independently affect cancer risk via estrogen receptor signaling. However, estradiol concentrations are too low in men and in postmenopausal women to obtain accurate data with the assays used in the UK Biobank. Furthermore, 20.7% of postmenopausal women included in this analysis had testosterone concentrations below the limit of detection, and this would be expected to reduce statistical power and to attenuate risk estimates. Free testosterone concentrations were calculated using a commonly-employed formula derived from mass action equations, however, the extent to which this estimated free testosterone represents the biologically active component of total testosterone remains under debate.⁵⁴

In conclusion, our study reports a novel positive association of free testosterone with melanoma in men. Our findings also support established associations of serum testosterone concentrations with prostate cancer risk in men, and with breast and endometrial cancer risk in postmenopausal women. Associations with

testosterone were sex and site-specific and therefore do not support a general major role of testosterone in cancer risk. Studies with longer follow-up time and tumor subtype data, as well as pooled datasets including genetic information, are needed to further assess these associations.

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CONFLICT OF INTEREST

The authors declare no potential conflicts of interest.

DATA AVAILABILITY STATEMENT

This work has been conducted using the UK Biobank Resource under application numbers 3282 and 24 494. The UK Biobank is an open-access resource and bona fide researchers can apply to use the UK Biobank dataset by registering and applying at <http://ukbiobank.ac.uk/register-apply/>. Further information is available from the corresponding author upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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