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Epidemiology

Testosterone, sex hormone-binding globulin, insulin-like growth factor-1 and endometrial cancer risk: observational and Mendelian randomization analyses

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BACKGROUND: Dysregulation of endocrine pathways related to steroid and growth hormones may modify endometrial cancer risk; however, prospective data on testosterone, sex hormone-binding globulin (SHBG) and insulin-like growth factor (IGF)−1 are limited. To elucidate the role of these hormones in endometrial cancer risk we conducted complementary observational and Mendelian randomization (MR) analyses.

METHODS: The observational analyses included 159,702 women (80% postmenopausal) enrolled in the UK Biobank. Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. For MR analyses, genetic variants associated with hormone levels were identified and their association with endometrial cancer (12,906 cases/108,979 controls) was examined using two-sample MR.

RESULTS: In the observational analysis, higher circulating concentrations of total (HR per unit inverse normal scale = 1.38, 95% CI = 1.22–1.57) and free testosterone (HR per unit log scale = 2.07, 95% CI = 1.66–2.58) were associated with higher endometrial cancer risk. An inverse association was found for SHBG (HR per unit inverse normal scale = 0.76, 95% CI = 0.67–0.86). Results for testosterone and SHBG were supported by the MR analyses. No association was found between genetically predicted IGF-1 concentration and endometrial cancer risk.

CONCLUSIONS: Our results support probable causal associations between circulating concentrations of testosterone and SHBG with endometrial cancer risk.

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INTRODUCTION

Exposure to unopposed estradiol is an established risk factor for endometrial cancer [1–5]; however, the role of androgens is less clear. Testosterone is a biologically potent androgen and is the main source of estradiol after the menopause when ovarian estrogen synthesis ceases. Relatively few prospective studies have examined the association between testosterone concentrations and endometrial cancer risk. Prior studies have been of comparatively small size and most have reported statistically non-significant positive associations between circulating total or free testosterone concentrations and endometrial cancer risk after menopause [1, 4, 6, 7]. However, the extent to which the relationship between testosterone and endometrial cancer risk differs by population subgroups (e.g. by body mass index [BMI]) and menopausal status) is uncertain. Furthermore, since free testosterone levels are regulated by sex hormone-binding globulin (SHBG), separation of the apparent effects of testosterone from those of SHBG on disease remains a major challenge. SHBG is a hepatically derived glycoprotein and the principal transport protein of testosterone and estradiol and is therefore an important regulator of their bioactivity [8]. SHBG has not been robustly associated with endometrial cancer risk in previous epidemiological studies, with statistically non-significant inverse associations reported in two smaller size nested case-control studies [4, 5].

Insulin-like growth factor-1 (IGF-1) is a polypeptide hormone that has potent mitogenic effects [9, 10]. IGF-1 shares downstream signalling pathways with insulin and is considered as a risk factor for several cancers as it exerts stronger mitotic and antiapoptotic activity than insulin [11, 12]. Prior epidemiological studies investigating the role of IGF-1 in endometrial cancer development have reported inconsistent results [3, 13–18]. However, there is biological crosstalk between sex hormone and IGF-related pathways [19]; for example, IGF-1 activity is regulated by estrogens in the uterus [20]. Consequently, joint evaluations of the associations between circulating levels of sex hormones and IGF-1 with endometrial cancer are warranted.

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To further examine the association between circulating levels of testosterone, SHBG, and IGF-1 with endometrial cancer risk, we conducted complementary observational and Mendelian randomization (MR) analyses. First, we investigated the association between pre-diagnostic circulating concentrations of total testosterone, free testosterone (i.e. biologically active), SHBG, IGF-1 and endometrial cancer risk in the UK Biobank, a large prospective cohort that includes more than 270,000 women. We then used MR to examine potential causality using genetic variants associated with testosterone, SHBG and IGF-1 from recent genome-wide association studies (GWAS) [21, 22], and assessed the relation of these variants with endometrial cancer using data from the Endometrial Cancer Association Consortium involving 12,906 endometrial cancer cases and 108,979 controls [23].

METHODS

UK Biobank—observational analysis

Study participants. The UK Biobank is a prospective cohort of ~503,000 adults aged between 40 and 69 years (229,182 men and 273,474 women) who were recruited between 2006 and 2010 [24]. The UK Biobank invited ~9.2 million people to participate through postal invitation, with a response rate of 5.5% [25]. All participants were registered with the UK National Health Service and lived within ~25 miles (40 km) of one of the 22 study assessment centres in England, Wales and Scotland. The UK Biobank study was approved by the North-West Multi-Centre Research Ethics Committee (06/MRE08/65), and at recruitment, all participants gave written informed consent to participate and for their health to be followed-up through linkage to electronic medical records. This research has been conducted using the UK Biobank Resource under application numbers 8294.

At the baseline recruitment visit, participants completed a self-administered touchscreen questionnaire and a computer-assisted personal interview, which included questions on medical history and lifestyle factors (including smoking habits, dietary intake and alcohol consumption). Anthropometric measurements (standing height, weight, waist and hip circumferences) were taken by trained research clinic staff at the assessment centre, and BMI was assessed through bioimpedance measures.

For the current analysis, we excluded the following: men ($n = 229,182$); women with prevalent cancer (excluding non-malignant skin cancer) at recruitment (identified by linkage to cancer registry data; $n = 18,621$); type-2 diabetics or those with unknown diabetes status at recruitment based on self-report, diabetes medication use and age of diagnosis over 36 years (because diabetes medications can affect circulating concentrations of sex steroid hormones and IGF-1 [19]; $n = 10,551$); women who reported oral contraceptive or hormone-replacement therapy (HRT) use at recruitment (as our focus was on endogenous circulating hormone levels; $n = 19,802$); and participants with missing measurements of total testosterone, SHBG, IGF-1 or albumin (required to estimate free testosterone concentration) ($n = 64,678$). After these exclusion criteria, our analysis included 159,702 women.

Blood collection and laboratory methods. Blood samples were also collected from 99.7% of the cohort at recruitment. Blood was collected in a serum separator tube and shipped to the central processing laboratory at 4 °C prior to serum preparation, aliquoting and cryopreservation in a central working archive [26]. Measurement of serum concentrations of IGF-1 (Liaison XL, DiaSorin S.p.A., Italy), total testosterone and sex hormone-binding globulin (SHBG) were determined by a chemiluminescent immunoassay (DXI 800, Beckman Coulter, London, UK). The immunoturbidimetric method (DXI 800) was used to assay serum high sensitivity C-reactive protein (CRP) concentrations. Glycated haemoglobin (HbA1c) concentrations were determined using the high-performance liquid chromatography (HPLC) Variant II Turbo 2.0 system (Bio-Rad, Hercules, CA). Average within-laboratory (total) coefficients of variation for low, medium and high internal quality control level samples for each biomarker ranged from 1.7 to 15.3% (for total testosterone, SHBG and IGF-1, the coefficients of variation ranged from 3.7 to 8.7%) [27]. Blood samples were also collected from a subset of cohort participants who re-attended an assessment centre, between 2012 and 2013. From the analytical dataset of 159,702 women, 4633, 4426 and 5144 women had circulating concentrations of total testosterone, SHBG and IGF-1 measured, respectively, in blood samples collected at both the recruitment and repeat assessment visit (median of 4 years apart).

Estimation of free testosterone. Free testosterone concentrations were estimated using a formula based on the law of mass action from measured total testosterone, SHBG and albumin concentrations [28, 29].

Assessment of outcome. The endpoint was first diagnosis of incident endometrial cancer cases as identified through linkage to national cancer registries and death records. Complete follow-up was available until 31 March 2016 for England and Wales and 31 October 2015 for Scotland. Endometrial cancer was defined using the 10th Revision of the International Classification of Diseases (ICD-10: C54).

Statistical analysis. To assess reproducibility between the two measurements of testosterone, free testosterone, SHBG and IGF-1 available in a subsample of participants, we calculated intraclass correlation coefficients (ICC) by dividing the between-person variance by the sum of the between-person and within-person variances.

Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards models. Age was the primary time variable in all models. Time at entry was the age at recruitment. Exit time was age at censoring (i.e. whichever came first from: first diagnosis of incident cancer, loss to follow-up or death or the last date at which follow-up was considered complete). Models were stratified by age at recruitment (5-year categories), Townsend deprivation index (quintiles) [30], and region of the recruitment assessment centre (East Midlands, London, North-East England, North-West England, Scotland, South-East England, South-West England, Wales, West Midlands and Yorkshire). The linearity of the associations for each biomarker with endometrial cancer was investigated in restricted cubic spline models. Overall, no strong evidence for departure from linearity for the associations between circulating levels of testosterone (P-non-linearity = 0.14), free testosterone (P-non-linearity = 0.08), SHBG (P-non-linearity = 0.94) and IGF-1 (P-non-linearity = 0.68) with endometrial cancer was detected. Primary analyses were conducted on the continuous scale (per unit inverse normal scale of total testosterone, per unit natural log scale of free testosterone, per unit inverse normal scale of SHBG, and per 1 standard deviation [SD; 5.6 nmol/l] of IGF-1). HRs were additionally corrected for regression dilution using regression dilution ratios obtained from the subsample of women with repeated biomarker measurement [24, 31]; to obtain corrected HRs, the log HRs and their standard errors were divided by the regression dilution ratio of each circulating concentration for total testosterone (0.69), free testosterone (0.72), SHBG (0.81) and for IGF-1 (0.74), and then exponentiated [32]. False discovery rate correction was computed (qvalue) for the continuous model using the Benjamini–Hochberg method [33]. Total testosterone, free testosterone, SHBG and IGF-1 were also modelled with participants of the full cohort grouped into quintiles of circulating concentrations based on the distributions of the full cohort. The multivariable model (model 2) was adjusted for a set of endometrial cancer risk factors determined a priori, namely ever use of HRT, parity, age of menopause, age of menarche and BMI. We also mutually adjusted the sex steroid hormone and IGF-1 models, and additionally adjusted for markers of inflammatory and glycaemic pathways that are known to interrelate/crosstalk with concentrations of these hormones [19], CRP and HbA1c (multivariable model 3). Deviations from proportionality were assessed using an analysis of Schoenfeld residuals [34], with no evidence of non-proportionality being detected. Statistical tests for trend were calculated using the ordinal quintiles of total testosterone, free testosterone, SHBG and IGF-1 entered into the model as a continuous variable. The circulating total testosterone, free testosterone, SHBG and IGF-1 and endometrial cancer associations were further assessed across subgroups of BMI, menopausal status at recruitment, age of menarche, parity, follow-up time and age at diagnosis. Interaction terms (multiplicative scale) between these variables and circulating total testosterone, free testosterone, SHBG and IGF-1 were included in separate models, and the statistical significance of the cross-product terms were evaluated using likelihood ratio tests. In sensitivity analyses, we excluded endometrial cancer cases that occurred within the first 2 years of follow-up; additionally adjusted the multivariable models for smoking status, total physical activity and education level; and excluded ever users of HRT (self-reported at enrolment).

Statistical tests were all two-sided and a P-value < 0.05 was considered statistically significant. Analyses were conducted using Stata version 14 (StataCorp, College Station, TX).

MR analysis

Data on total testosterone, free testosterone, SHBG and IGF-1. We selected genetic variants associated with sex steroid hormones (total testosterone, free testosterone and SHBG) and IGF-1 concentrations at the genome-wide significant level (i.e. *P* value threshold for inclusion at $<5 \times 10^{-8}$) from the largest GWAS conducted to date [22, 35]. For total testosterone ($N = 230{,}454$), free testosterone ($N = 188{,}507$) and SHBG ($N = 189{,}473$), we used data for women of European ancestry in the UK Biobank (Supplementary Tables S1, 2) [22]. For IGF-1, the GWAS included 194,174 women of European ancestry from the UK Biobank (Supplementary Table S3) [21] and have been presented elsewhere [35]. Specific covariates used for each of the hormones assessed can be found in Supplementary Tables 1–3 For SHBG, although BMI was used as an additional covariate for signal identification, genetic loci from the BMI-adjusted analyses were used with corresponding effect estimates from the BMI-unadjusted analyses to mitigate possible collider bias in the MR analyses [36]. Genetic variants were pruned based on a linkage disequilibrium (LD) threshold $R^2 < 0.01$ (Supplementary Tables 1–3).

Data on endometrial cancer. Summary data for the associations of the IGF-1 and sex steroid hormones-related variants with endometrial cancer were obtained from a GWAS involving 121,885 participants (12,906 endometrial cancer cases and 108,979 controls) within the Endometrial Cancer Association Consortium [23]. All women were of European ancestry. GWAS analyses were adjusted for principal components.

Statistical analysis. We conducted two-sample MR analyses to examine a potential causal relationship between sex steroid hormones, and IGF-1 with endometrial cancer risk. Where a signal was not present in the endometrial cancer GWAS, we identified a 1000 Genomes proxy with $r^2 > 0.8$. Causal effects were estimated using a random-effects inverse-variance weighted (IVW) method [37–39]. MR requires that genetic variants are strongly associated with the exposure of interest and this assumption was very likely to be satisfied by using variants only associated with hormones at a genome-wide significance level. We calculated the Cochran's *Q* statistic that quantifies the heterogeneity in effect sizes attributed to the selected genetic variants [40]. In addition, we performed MR-Egger regression [41] and the estimator from the weighted median approach [42] to account for any pleiotropic effects. The MR pleiotropy residual sum and outlier test (MR-PRESSO) was performed to identify, and exclude, any outlying variants (*p* value threshold set at <0.05) [43]. We also created scatter plots of the genetic associations of the hormones and endometrial cancer and labelled outlying variants identified by MR-PRESSO. Leave-one-variant out and single-variant analyses were conducted to assess the influence of individual variants on the observed associations.

If causal effects were significant for any of the exposures tested, we excluded genetic variants having larger effects (based on standardised beta) on any one of 11 metabolic traits available in the UK Biobank (fasting glucose, type-2 diabetes, coronary artery disease, HDL-C, LDL-C, triglycerides, total-cholesterol and diastolic and systolic blood pressure, BMI and waist-to-hip ratio adjusted for BMI) [44], to ensure that these significant effects were not driven by pleiotropy. In particular, if the selected genetic variants were associated with other traits (for instance with adiposity and insulin resistance given their strong correlation with hormones) and these were known to be associated with endometrial cancer without involving sex hormones and IGF-1 concentrations first, then that would represent a violation of the MR assumptions. A list of pleiotropic variants for sex steroid hormones can be found in the respective GWAS [22] and are also presented in Supplementary Tables S1, 2.

Sensitivity analyses for sex steroid hormones excluding variants related to IGF-1 were also conducted. We additionally conducted multivariable MR analyses to account for possible pleiotropic effects of variants related to both total testosterone and SHBG concentrations [39]. Variants related to these exposures were further pruned based on an LD threshold $R^2 < 0.01$, and a total of 505 variants was used in the multivariable model (Supplementary Table S1).

Comparing the R^2 of the selected genetic variants on the hormones versus the R^2 of the variants on endometrial cancer we assessed that the hypothesised direction of causality (i.e. hormones being associated with endometrial cancer and not vice versa) is likely for all exposures [45]. False discovery rate correction was computed (qvalue) for the IVW model using the Benjamini–Hochberg method [33].

The MR analyses were conducted using the MR R package [46] and MR-PRESSO version 1.0 in the R environment [43].

RESULTS

UK Biobank—observational analysis

After a median follow-up of 7.1 years, 549 cases of incident endometrial cancer were recorded. Compared with those in the lowest quintile, women in the highest quintile of total testosterone were younger, had higher BMI, were less likely to be ever HRT users and have experienced menopause at a younger age. Women in the highest quintile of SHBG concentrations had similar characteristics with those in the highest quintile of total testosterone but had a lower BMI (Table 1). Women in the highest quintile of circulating IGF-1 concentrations were younger, had lower BMI and were more likely to be never smokers, and less likely to be ever users of oral contraceptives and HRT (Table 1).

The reproducibility (ICC) of concentrations of testosterone, free testosterone, SHBG and IGF-1 measured at both the recruitment and repeat assessment visit (median of 4 years apart) was 0.63 (95% CI: 0.61–0.65), 0.70 (95% CI: 0.68–0.71), 0.81 (95% CI: 0.80–0.82) and 0.77 (95% CI: 0.76–0.78), respectively.

Circulating total testosterone, free testosterone, SHBG concentrations and endometrial cancer risk. Circulating concentrations of total testosterone and free testosterone were positively associated with endometrial cancer risk in the maximally adjusted multivariable model (model 3) that controlled for established risk factors and circulating concentrations of CRP, HbA1c, IGF-1 and SHBG (total testosterone model only) (HR per unit inverse normal scale = 1.25, 95% CI = 1.15–1.36 for total testosterone; HR per unit natural log scale = 1.69, 95% CI = 1.44–1.98 for free testosterone) (Table 2, Fig. 1). These positive associations were strengthened after correction for regression dilution bias (total testosterone, HR per unit inverse normal scale increment = 1.38, 95% CI = 1.22–1.57; free testosterone, HR per unit natural log scale = 2.07, 95% CI = 1.66–2.58) (Fig. 1, Table 2) and remained statistically significant after correction for multiple comparisons (Fig. 1). In the quintile models, positive associations were also observed between circulating concentration of testosterone and free testosterone with endometrial cancer risk (Table 2). Similar associations for total testosterone and free testosterone and endometrial cancer risk were found according to subgroups of follow-up time, age at diagnosis, menopausal status at recruitment and other endometrial cancer risk factors (Pinteractions > 0.13) (Fig. 2, Supplementary Table S4). Notably, positive associations were found between circulating testosterone concentration and incident endometrial cancer risk for women who were premenopausal (HR per unit inverse normal scale = 1.49, 95% CI = 1.05–2.09) and postmenopausal (HR per unit inverse normal scale = 1.35, 95% CI = 1.18–1.55) at enrolment into the study (Pinteraction = 0.42) (Fig. 2).

Higher circulating concentration of SHBG was associated with lower endometrial cancer risk (multivariable model 3, HR per unit inverse normal scale = 0.76, 95% CI = 0.67–0.86; Table 2); this association remained statistically significant after we corrected for multiple comparisons (Fig. 1). An inverse association between SHBG concentration and endometrial cancer risk was also found in the quintile models (Table 2). Similar associations for SHBG concentrations and endometrial cancer risk were found according to subgroups of follow-up time, age at diagnosis, menopausal status at recruitment and other endometrial cancer risk factors (Pinteractions > 0.15; Fig. 3).

Circulating IGF-1 concentration and endometrial cancer risk. An inverse association not reaching the threshold of statistical significance was found between circulating IGF-1 concentration and endometrial cancer risk in the maximally adjusted multivariable (model 3 (HR per 1 SD increment = 0.89, 95% CI = 0.78–1.01) (Fig. 1, Table 2). Heterogeneity for the circulating IGF-1 concentration and endometrial cancer risk was found according to BMI, with an inverse

Table 1. Characteristics of UK Biobank study participants by lowest and highest quintile of circulating concentrations of total testosterone, sex hormone-binding globulin (SHBG) and insulin-like growth factor-1 (IGF-1) ($n = 159{,}702$ women).

Characteristic	Total testosterone concentration		SHBG concentration		IGF-1 concentration	
	Quintile 1	Quintile 5	Quintile 1	Quintile 5	Quintile 1	Quintile 5
Women, *n*	31,902	31,758	31,765	31,874	31,719	31,858
Endometrial cancer cases, *n*	59	147	192	50	148	79
Age at recruitment[a], years	57.6 (7.5)	54.2 (8.4)	56.2 (7.6)	54.6 (8.4)	59.0 (7.0)	52.2 (8.1)
BMI[a], kg/m^2	26.5 (4.8)	27.9 (5.5)	30.6 (5.5)	24.1 (3.6)	28.6 (6.1)	26.1 (4.2)
Waist circumference[a], cm	83.6 (11.7)	86.0 (12.9)	93.4 (12.2)	76.8 (9.17)	88.1 (14.0)	81.97 (10.6)
Smoking status, *n* (%)						
Never	19,040 (59.6)	19,171 (60.1)	19,081 (59.8)	19,674 (61.6)	18,450 (57.8)	20,126 (63.0)
Current	2389 (7.5)	3572 (11.2)	2530 (7.92)	3216 (10.1)	2892 (9.1)	2997 (9.4)
Physical activity, *n* (%)						
<10 MET h/week	6948 (21.7)	7939 (24.9)	9153 (28.6)	6139 (19.2)	8136 (25.5)	6864 (21.5)
60+ MET h/week	6758 (21.1)	5859 (18.4)	5325 (16.7)	7376 (23.1)	6332 (19.8)	6206 (19.4)
Alcohol consumption, *n* (%)						
Never	2943 (9.2)	2723 (8.5)	3268 (10.2)	3001 (9.4)	3781 (11.8)	2390 (7.8)
Daily or almost daily	5065 (15.9)	5429 (17.0)	4785 (15.0)	4753 (14.9)	5383 (16.9)	4349 (13.6)
Townsend deprivation index, *n* (%)						
Quintile 1	6691 (20.9)	6282 (19.7)	5962 (18.7)	6552 (20.5)	5892 (18.4)	6702 (21.0)
Quintile 5	5798 (18.4)	6436 (20.2)	6750 (21.1)	5988 (18.8)	6733 (21.1)	5806 (18.2)
Qualification, *n* (%)						
College/University degree	3336 (10.4)	3420 (10.7)	2943 (9.21)	3967 (12.4)	2981 (9.3)	3879 (12.2)
Oral contraceptive pill, *n* (%)						
Ever users	28,811 (80.8)	25,917 (80.5)	20,061 (81.6)	25,426 (79.7)	7418 (23.2)	4943 (15.5)
Hormone-replacement therapy (HRT), *n* (%)						
Ever users	12,812 (40.1)	7858 (24.6)	10,914 (34.2)	8544 (26.8)	13,071 (40.9)	7317 (22.9)
Age of menarche, *n* (%)						
≤13 years old	19,437 (60.8)	20,031 (62.8)	20,750 (64.9)	18,796 (58.9)	18,952 (59.3)	20,472 (64.1)
>13 years old	11,568 (36.2)	10,943 (34.4)	10,275 (32.2)	12,125 (38.0)	12,055 (37.7)	10,506 (32.9)
Age of Menopause, *n* (%)						
<50 years	7253 (22.7)	5638 (17.7)	6955 (21.8)	5476 (17.2)	7901 (24.7)	4739 (14.8)
≥50 years	12,722 (39.8)	10,429 (32.7)	12,011 (37.6)	10,180 (31.9)	13,220 (41.4)	9058 (28.4)
Parity, *n* (%)						
None	5497 (17.2)	6467 (20.3)	5441 (17.0)	6942 (21.8)	5248 (16.4)	6830 (21.4)
1–2	18,387 (57.5)	18,065 (56.6)	18,392 (57.6)	17,798 (55.8)	17,633 (55.2)	18,528 (58.0)
>2	8041 (25.2)	7331 (23.0)	8088 (25.3)	7154 (22.4)	9026 (28.3)	6552 (20.5)
CRP[a], mg/l	2.5 (4.2)	2.6 (3.89)	4.0 (5.0)	1.5 (3.0)	3.9 (5.4)	1.6 (2.9)
HbA1c[a], mmol/mol	35.4 (4.2)	34.9 (4.3)	37.0 (5.6)	33.8 (3.5)	35.9 (5.0)	34.5 (3.9)
Total testosterone[a], nmol/l	0.5 (0.1)	2.0 (0.9)	1.1 (0.7)	1.1 (0.6)	1.1 (0.6)	1.2 (0.7)
Free testosterone[a], pmol/l	6.7 (2.4)	26.3 (16.2)	21.8 (13.6)	8.9 (5.3)	13.7 (10.0)	15.9 (11.8)
SHBG[a], nmol/l	61.2 (28.3)	59.8 (28.2)	28.7 (6.2)	103.9 (22.3)	64.0 (31.7)	57.3 (25.0)
IGF-1[a], nmol/l	20.6 (5.5)	22.0 (5.7)	21.5 (5.7)	20.4 (5.3)	14.0 (2.0)	29.4 (3.8)

BMI body mass index, *CRP* C-reactive protein, *HbA1c* glycated haemoglobin, *IGF* insulin-like growth factor, *METh* metabolic equivalent hours, *SHBG* sex hormone-binding globulin.
[a]Mean and standard deviation unless specified.

association found among obese women (BMI ≥ 30 kg/m^2; HR per 1 SD increment = 0.78, 95% CI 0.64–0.95), and no association among non-obese women (BMI < 30 kg/m^2; HR per 1 SD increment = 0.98, 95% CI = 0.82–1.16; Pinteraction = 0.02) (Supplementary Fig. S1, Supplementary Table S5). No heterogeneity was found according to other endometrial cancer risk factors.

Sensitivity analyses

Similar associations between concentrations of total testosterone, free testosterone, SHBG and IGF-1 with endometrial cancer were found when cases occurring in the first 2 years of follow-up were excluded (Supplementary Table S6); when we additionally adjusted the multivariable models for smoking status, total physical activity and education level (Supplementary Table S7);

Table 2. Risk (hazard ratios) of endometrial cancer associated with circulating concentrations of total testosterone, free testosterone, sex hormone-binding globulin (SHBG) and insulin growth factor-1 (IGF-1) in the UK Biobank.

Biomarker	Quintile cutpoints	*n* cases/participants	HR (95% CI) Model 1	HR (95% CI) Model 2	HR (95% CI) Model 3
Total testosterone (nmol/l)					
per unit inverse normal scale		549/159,702	1.36 (1.25–1.47)	1.24 (1.14–1.35)	1.25 (1.15–1.36)
per unit inverse normal scale (corrected)[a]		549/159,702	1.55 (1.38–1.75)	1.36 (1.21–1.54)	1.38 (1.22–1.57)
Quintile 1	0.35–0.67	59/31,961	1 (reference)	1 (reference)	1 (reference)
Quintile 2	0.68–0.91	108/31,929	1.94 (1.41–2.66)	1.79 (1.30–2.46)	1.81 (1.32–2.49)
Quintile 3	0.92–1.15	116/31,968	2.15 (1.57–2.95)	1.88 (1.37–2.58)	1.93 (1.41–2.64)
Quintile 4	1.16–1.48	119/31,939	2.34 (1.71–3.21)	1.94 (1.42–2.66)	2.00 (1.46–2.74)
Quintile 5	>1.48	147/31,905	3.07 (2.27–4.16)	2.33 (1.72–3.17)	2.41 (1.77–3.28)
ptrend			<0.001	<0.001	<0.001
Free testosterone (pmol/l)					
per unit natural log scale		549/159,702	2.24 (1.95–2.58)	1.70 (1.46–1.98)	1.69 (1.44–1.98)
per unit natural log scale (corrected)[a]		549/159,702	3.07 (2.53–3.73)	2.09 (1.69–2.59)	2.07 (1.66–2.58)
Quintile 1	1.41–7.63	55/31,941	1 (reference)	1 (reference)	1 (reference)
Quintile 2	7.64–10.73	63/31,940	1.16 (0.81–1.67)	1.02 (0.71–1.46)	1.02 (0.71–1.47)
Quintile 3	10.74–14.34	104/31,941	1.98 (1.42–2.74)	1.60 (1.15–2.23)	1.62 (1.16–2.25)
Quintile 4	14.35–19.91	137/31,940	2.63 (1.93–3.61)	1.89 (1.37–2.60)	1.91 (1.38–2.63)
Quintile 5	>19.91	190/31,940	3.80 (2.81–5.13)	2.32 (1.70–3.17)	2.32 (1.68–3.19)
Ptrend			<0.001	<0.001	<0.001
SHBG (nmol/l)					
per unit inverse normal scale		549/159,702	0.66 (0.60–0.71)	0.80 (0.73–0.88)	0.80 (0.73–0.89)
per unit inverse normal scale (corrected)[a]		549/159,702	0.59 (0.53–0.66)	0.76 (0.68–0.86)	0.76 (0.67–0.86)
Quintile 1	0.58–37.29	192/31,957	1 (reference)	1 (reference)	1 (reference)
Quintile 2	37.3–50.01	131/31,957	0.68 (0.54–0.85)	0.82 (0.65–1.03)	0.83 (0.66–1.04)
Quintile 3	50.02–63.16	100/31,927	0.53 (0.41–0.67)	0.74 (0.57–0.95)	0.74 (0.58–0.96)
Quintile 4	63.17–81.30	76/31,949	0.43 (0.32–0.55)	0.65 (0.49–0.87)	0.66 (0.49–0.88)
Quintile 5	>81.30	50/31,924	0.29 (0.21–0.40)	0.50 (0.36–0.70)	0.49 (0.35–0.70)
Ptrend			<0.001	<0.001	<0.001
IGF-1 (nmol/l)					
per SD		549/159,702	0.88 (0.81–0.97)	0.96 (0.88–1.05)	0.92 (0.83–1.01)
per SD (corrected)[a]		549/159,702	0.84 (0.75–0.96)	0.95 (0.84–1.07)	0.89 (0.78–1.01)
Quintile 1	1.45–16.55	148/31,946	1 (reference)	1 (reference)	1 (reference)
Quintile 2	16.56–19.72	136/31,946	0.99 (0.79–1.26)	1.12 (0.88–1.41)	1.07 (0.84–1.35)
Quintile 3	19.73–22.45	101/31,937	0.79 (0.61–1.02)	0.93 (0.72–1.20)	0.87 (0.67–1.13)
Quintile 4	22.46–25.64	85/31,936	0.72 (0.55–0.95)	0.86 (0.66–1.13)	0.79 (0.60–1.05)
Quintile 5	>25.64	79/31,937	0.77 (0.58–1.01)	0.95 (0.71–1.26)	0.83 (0.62–1.11)
ptrend			0.005	0.24	0.042

Model 1: minimally adjusted model using age as the underlying time variable stratified by Townsend deprivation index (quintiles), region of recruitment assessment centre and age category (5-year categories).

Model 2: multivariable Cox regression model using age as the underlying time variable stratified by Townsend deprivation index (quintiles), region of recruitment assessment centre and age category (5-year categories). Models adjusted for ever use of hormone-replacement therapy (yes, no, unknown); age of menopause (<50 years, 50 years, >50 years, not applicable, unknown); parity (0, 1–2, ≥3, unknown); body mass index (kg/m^2); age of menarche (≤13 years old, >13 years old, unknown).

Model 3: Model 1 plus additional adjustment for other circulating concentrations (quintiles, missing/unknown) of insulin-like growth factor (IGF-1; nmol/l), C-reactive protein (CRP; mg/l), glycated haemoglobin (HbA1c; mmol/mol), total testosterone (nmol/l) and sex hormone-binding globulin (SHBG; nmol/l; not included in free testosterone analysis).

SD (IGF-1 = 5.6 nmol/l).

HR hazard ratio, *CI* confidence interval, *SD* standard deviation.

[a]HRs were additionally corrected for regression dilution using a regression dilution ratio (IGF-1 = 0.74; total testosterone = 0.69; free testosterone = 0.72; SHBG = 0.81) obtained from the subsample of women with repeat biomarker measurements.

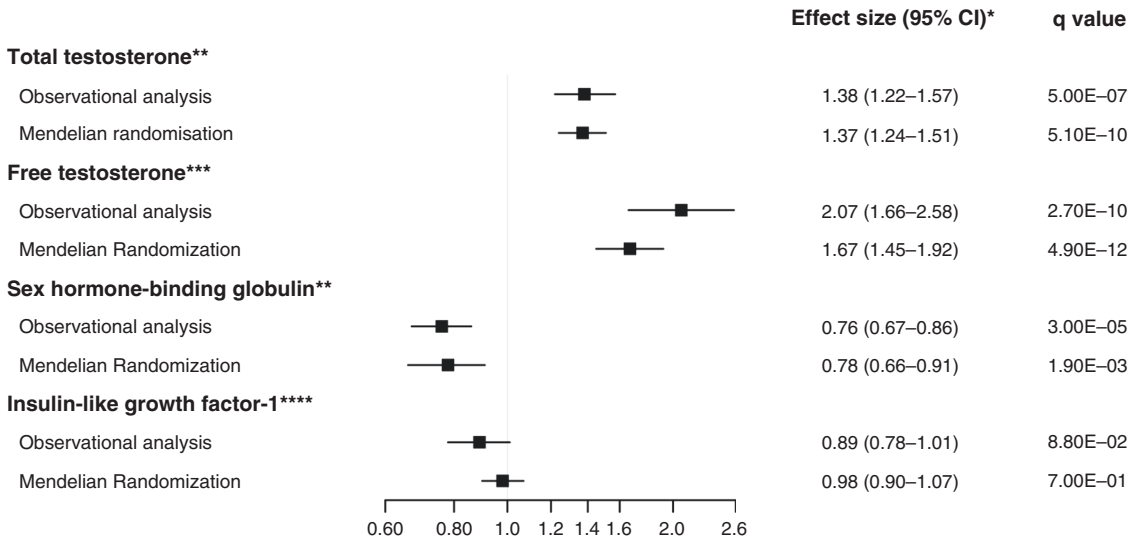

Fig. 1 Observational and Mendelian randomization estimates for total testosterone, free testosterone, sex hormone-binding globulin (SHBG) and insulin-like growth factor-1 (IGF-1) and endometrial cancer risk. UK Biobank—observational analysis: Multivariable Cox regression model using age as the underlying time variable stratified by Townsend deprivation index (quintiles), region of recruitment assessment centre and age category (5-year categories). Models adjusted for ever use of hormone-replacement therapy (yes, no, unknown); age of menopause (<50 years, 50 years, >50 years, not applicable, unknown); parity (0, 1–2, ≥3, unknown); body mass index (kg/m^2); age of menarche (≤13 years old, >13 years old, unknown), plus additional adjustment for other circulating concentrations (quintiles, missing/unknown) of insulin-like growth factor (IGF-1; nmol/l), C-reactive protein (CRP; mg/l), glycated haemoglobin (HbA1c; mmol/mol), total testosterone (nmol/l), and sex hormone-binding globulin (SHBG; nmol/l). *HRs corrected for regression dilution using a regression dilution ratio (IGF-1 = 0.74; total testosterone = 0.69; free testosterone = 0.72; SHBG = 0.81) obtained from the subsample of women with repeat biomarker measurements are reported in the observational analyses, whereas ORs from the random-effects inverse-variance weighted models are reported in the Mendelian randomization analyses. False discovery rate (FDR) = qvalue SD (IGF-1 = 5.6 nmol/l) **(per per unit inverse normal scale ***(per unit per unit natural log scale) ****(per 1 SD). HR hazard ratio, OR odds ratio, CI confidence interval, SD standard deviation, CRP C-reactive protein, HbA1c glycated haemoglobin, IGF insulin-like growth factor, SHBG sex hormone-binding globulin.

and when ever users of HRT were excluded from the analyses (Supplementary Table S8).

MR analyses

Genetically predicted circulating total testosterone, free testosterone, SHBG concentrations and endometrial cancer risk. In the IVW random-effects models, we found a positive association between genetically predicted circulating total testosterone (OR per unit inverse normal scale increment = 1.37, 95% CI = 1.24–1.51, $p < 0.01$) and free testosterone concentrations (OR per unit natural log scale increment = 1.67, 95% CI = 1.45–1.92, $p < 0.01$) and risk of endometrial cancer (Fig. 1, Supplementary Table S9). Conversely, genetically predicted SHBG was inversely associated with endometrial cancer risk (OR per unit inverse normal scale increment = 0.78, 95% CI = 0.66–0.91, $p < 0.01$). All of these associations remained statistically significant after we corrected for multiple comparisons (Fig. 1). There was evidence for heterogeneity in all analyses (Cochran's Q p-values < 0.001). The MR-Egger test showed evidence of pleiotropy for total testosterone (MR-Egger intercept p-values < 0.01); however, a positive effect estimate was found for the MR-Egger model accounting for pleiotropy (OR per unit inverse normal scale increment = 1.76, 95% CI = 1.47–2.11, $p < 0.01$). The MR-PRESSO method identified few outlying variants for total testosterone, free testosterone and SHBG, with similar results obtained after these variants were excluded (Supplementary Table S9; Supplementary Fig. 2). The leave-one-variant out analyses did not identify any influential variants (Supplementary Table S10), while the single-variant analyses showed low precision of the MR effect estimates (wide confidence intervals) for some variants (Supplementary Table S11) for total testosterone, free testosterone and SHBG. Additionally, removing pleiotropic variants related to metabolic traits yielded similar results for total testosterone (OR = 1.41, 95% CI = 1.27–1.56, $p < 0.01$) (Supplementary Table S12). The weighted median approach showed effect estimates of similar magnitude for all models (Supplementary Tables S9, S12). Removing variants related to IGF-1 had little impact on the effect of total testosterone, free testosterone and SHBG concentrations on endometrial cancer risk. The multivariable MR analyses for total testosterone and SHBG concentrations resulted in similar effect estimates (Supplementary Table S9). Consistent effect estimates across all methods and sensitivity analyses suggest that the MR assumptions (i.e. that the genetic variants are not associated with any confounder of the hormones-endometrial cancer association, and that they do not influence endometrial cancer via any pathway other than through the hormones of interest) were likely to have been satisfied.

Genetically predicted circulating IGF-1 concentration and endometrial cancer risk. No association was found between genetically predicted circulating IGF-1 concentrations and endometrial cancer risk (OR per 1 SD increment = 0.98, 95% CI = 0.90–1.07, $P = 0.69$) (Fig. 1, Supplementary Table S9). A similar null result was found for the MR-Egger and weighted median approaches.

DISCUSSION

In this complementary analysis using data from the UK Biobank and genome-wide association data from a large endometrial cancer consortium, we found that higher circulating concentrations of total testosterone and free testosterone were positively associated with endometrial cancer risk, and that SHBG levels were inversely associated with risk. These observational relationships were consistent across subgroups of BMI, menopausal status, follow-up time and other endometrial cancer risk factors. Consistent with these findings, in our MR analyses, we found positive effect estimates between genetically predicted concentrations of total testosterone, free testosterone and an inverse effect estimate between genetically predicted concentrations of

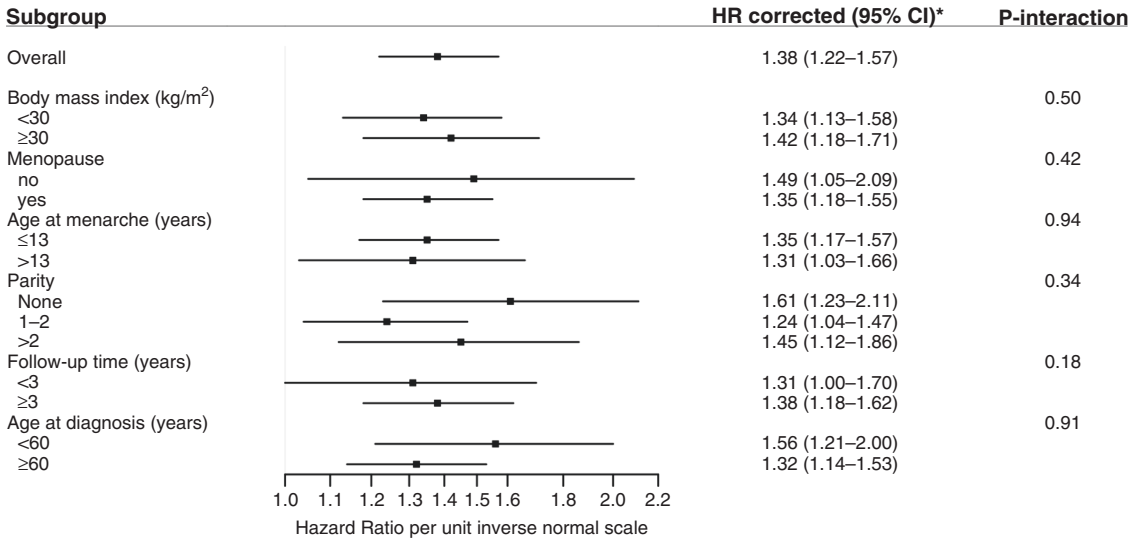

Fig. 2 Subgroup analyses of association between total testosterone concentration and endometrial cancer risk in the UK Biobank (per unit inverse normal scale). Multivariable Cox regression model using age as the underlying time variable stratified by Townsend deprivation index (quintiles), region of recruitment assessment centre, and age category (5-year categories). Models adjusted for ever use of hormone-replacement therapy (yes, no, unknown); age of menopause (<50 years, 50 years, >50 years, not applicable, unknown); parity (0, 1–2, ≥3, unknown); body mass index (kg/m^2); age of menarche (≤13 years old, >13 years old, unknown), plus additional adjustment for other circulating concentrations (quintiles, missing/unknown) of insulin-like growth factor-1 (IGF-1), C-reactive protein (CRP; mg/l), glycated haemoglobin (HbA1c; mmol/mol), sex hormone-binding globulin (SHBG; nmol/l). *HRs were corrected for regression dilution using a regression dilution ratio of 0.69 obtained from the subsample of women with repeat total testosterone measurements. HR hazard ratio, CI confidence interval, SD standard deviation.

SHBG, with endometrial cancer risk. Collectively, these results support probable causal roles of testosterone and SHBG in endometrial cancer development.

Relatively few prospective studies have examined the associations between circulating concentrations of testosterone and endometrial cancer risk. Prior studies have been of comparatively small size (all including fewer than 320 endometrial cancer cases), and although positive associations were usually reported between circulating testosterone concentration and endometrial cancer risk, the risk estimates often did not reach the threshold for statistical significance [1, 4, 6, 7]. Our observational analysis, including 549 incident cases, was the largest to date to examine the association between testosterone and endometrial cancer risk. We found positive relationships for both total and free (bioavailable) testosterone, with consistent associations observed across subgroups of other endometrial cancer risk factors and by follow-up time. Interestingly, we found no heterogeneity for the testosterone and endometrial cancer positive association by menopausal status. To our knowledge, this is the first observational study to observe a positive association between testosterone concentration and endometrial cancer risk for premenopausal women. However, this finding should be interpreted cautiously as the number of endometrial cancer cases recorded in premenopausal women in our study was relatively small ($n = 80$ cases). In addition, we only had data on menopausal status at study enrolment and the menopausal status of women was unknown when endometrial cancer was diagnosed. Further studies are needed to examine the role of androgens in the development of endometrial cancer prior to menopause.

Higher circulating testosterone concentrations have been more consistently associated with greater endometrial cancer risk in postmenopausal women. Most recently, a case-control nested in the Women's Health Initiative Observational Study (WHI-OS) (313 endometrial cancer cases), reported a near twofold higher risk when the highest and lowest exposure groups of circulating testosterone concentrations were compared, with similar magnitude associations also found for dehydroepiandrosterone (DHEA), androstenedione [7] and free testosterone [1]. Interestingly, these positive associations attenuated after adjustment for circulating estradiol concentration and remained significant only for androstenedione, supporting the hypothesis that these androgens influence endometrial cancer risk via estrogenic pathways. Further support for this hypothesis comes from experimental studies that have found estrogens, but not testosterone, to have direct proliferative effect in endometrial cancer cell models [47]. Higher circulating concentrations of androgens lead to raised tissue levels of estrogens via aromatisation in peripheral or local tissues.

The association between pre-diagnostic circulating SHBG concentration and endometrial cancer risk has rarely been studied. Two previous nested case-control studies of postmenopausal women found statistically non-significant inverse associations in their maximally adjusted multivariable models [1, 4, 5]. In our analysis, we found that higher circulating SHBG concentration was associated with a statistically significant lower endometrial cancer risk; this inverse association was consistent by follow-up time and across subgroups of other endometrial cancer risk factors, including by BMI and age at diagnosis. Similar strength associations were also found by menopausal status, although the inverse relationship was only statistically significant for the postmenopausal women and not premenopausal women. This suggestive inverse association found between SHBG concentrations and premenopausal endometrial cancer is contrary to a previous null association reported from a smaller pooled analysis of three prospective studies [6]. Further examination of the role of pre-diagnostic SHBG levels in endometrial cancer development is now warranted, especially for premenopausal women.

Despite the robustness of these associations found in our UK Biobank observational analyses, to enable causal inference, we conducted MR analyses to examine the associations between testosterone and SHBG with endometrial cancer risk. Compared to observational analyses, MR is less prone conventional confounding and reverse causality due to the random assortment of alleles during meiosis, and germline genetic variants being fixed at conception and therefore unaffected by the disease process. Our MR estimates likely represent lifetime exposures to hormones as opposed to measured levels at enrolment into the study as were

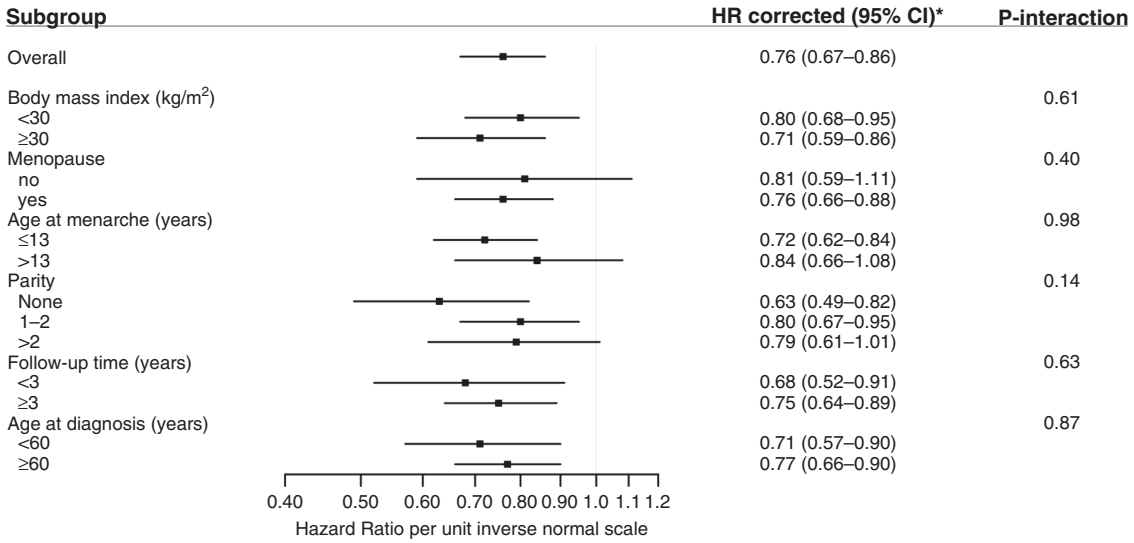

Fig. 3 Subgroup analyses of association between sex-hormone-binding globulin (SHBG) concentration and endometrial cancer risk in the UK Biobank (per unit inverse normal scale). Multivariable Cox regression model using age as the underlying time variable stratified by Townsend deprivation index (quintiles), region of recruitment assessment centre and age category (5-year categories). Models adjusted for ever use of hormone-replacement therapy (yes, no, unknown); age of menopause (<50 years, 50 years, >50 years, not applicable, unknown); parity (0, 1–2, ≥3, unknown); body mass index (kg/m^2); age of menarche (≤13 years old, >13 years old, unknown), plus additional adjustment for other circulating concentrations (quintiles, missing/unknown) of insulin-like growth factor-1 (IGF-1), C-reactive protein (CRP; mg/l), glycated haemoglobin (HbA1c; mmol/mol) and total testosterone (nmol/l). *HRs were corrected for regression dilution using a regression dilution ratio of 0.81 obtained from the subsample of women with repeat SHBG measurements. HR hazard ratio, CI confidence interval, SD standard deviation.

used in our UK Biobank observational analyses. Despite these differences, findings from our MR analyses were coherent with our observational results, with positive effect estimates found for total testosterone and free testosterone, and an inverse effect estimate found for SHBG, with endometrial cancer risk. These results, similar to those from a prior MR study [22], were robust according to the various sensitivity analyses (i.e. MR-PRESSO method, and leave-one-variant out analyses) and in analyses we conducted to examine the possible influence of pleiotropy. These included novel multivariable MR analyses to estimate the effects of total testosterone and SHBG concentrations on endometrial cancer risk accounting for possible pleiotropic effects, with robust results obtained for both traits. Our MR results for total testosterone, free testosterone and SHBG with endometrial cancer risk were also robust in sensitivity analyses excluding variants related to IGF-1.

Our observational finding of an inverse trend between circulating IGF-1 concentration and endometrial cancer risk is contrary to two previous prospective studies that reported null associations [3, 15]. It is of note that the risk estimates for our current study did not reach the threshold of statistical significance. Furthermore, the null effect estimates we found in our MR analysis, similar to an earlier smaller study [48], suggest that the inverse trend we found in our observational analyses may be a consequence of confounding and/or reverse causality. Overall, current evidence is unsupportive to circulating IGF-1 levels being a risk factor for endometrial cancer development. However, unlike other tissues in which IGF-1 synthesis is predominantly stimulated by growth hormone, the major determinant of IGF-1 concentration in the uterus is estradiol [20, 49]. Consequently, it is possible that measuring (or genetically-predicting) IGF-1 levels in the circulation may not be a good marker for local expression and any biological effects of IGF-1 in the endometrium.

The current study is the largest and most comprehensive investigation on the role of total testosterone, free testosterone, SHBG and IGF-1 in endometrial cancer development. The availability of biomarker measurements in most UK Biobank participants meant we were able to conduct full cohort analyses rather than follow nested study designs as previous investigations have undertaken. A further strength of our analysis was our correction for regression dilution bias using the repeat biomarker measures available in a subset of women, therefore diminishing the effects of measurement error and within-person variability [50]. Importantly, this correction resulted in a strengthening of the relationships between total testosterone, free testosterone and SHBG and endometrial cancer risk, supporting the likelihood that previous epidemiological studies that used single measurements of these hormones may have underestimated the strength of the association with endometrial cancer risk. A limitation of our observational analysis was that androgens were measured by chemiluminescent immunoassay and not by the current gold standard mass spectrometry method [51]; however, the positive association we found for testosterone was consistent with the WHI-OS study that measured androgens using liquid chromatography with tandem mass spectrometry (LC-MS/MS) [7]. Additionally, we were unable to adjust our multivariable models for circulating concentrations of estrogens as the assay used in the UK Biobank to assess estradiol levels was not sufficiently sensitive to measure the typically lower concentrations found in postmenopausal women. Consequently, we were unable to assess the influence of estrogens in mediating the relationship between testosterone and endometrial cancer association. For our MR analyses, our use of summary level data meant that we were unable to examine whether the associations between circulating concentrations of sex hormones and IGF-1 differed according to subgroups of other endometrial cancer risk factors. However, our observational analyses generally did not find any heterogeneity of associations across subgroups of these other risk factors. A further potential limitation of our MR analyses is that UK Biobank participants were included in both the exposures and endometrial cancer GWAS datasets, which might have introduced some bias in the MR estimates. However, due to the strong genetic instruments we used for testosterone, free testosterone, SHBG and IGF-1 (all F statistics > 10), simulation studies suggest that any bias from sample overlap is likely to be small [52].

In conclusion, our complementary observational and MR results support probable causal associations between circulating total testosterone, free testosterone and SHBG with endometrial cancer. These results suggest that interventions targeting androgenic pathways may aid the prevention of endometrial carcinogenesis.

DISCLAIMER

Where authors are identified as personnel of the International Agency for Research on Cancer/World Health Organization, the authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer/World Health Organization.

DATA AVAILABILITY

Data used in the MR analyses can be found in supplementary material. Researchers can apply to use the UK Biobank dataset by registering and applying at http://ukbiobank.ac.uk/register-apply/.

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AUTHOR CONTRIBUTIONS

AM, ND, MJG and NM: Designed the study and developed the methodology, analysed the data, interpreted the results, drafted the paper and approved the final version. NA and TO'M: Interpreted the results, edited the manuscript and approved the final version.

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COMPETING INTERESTS

The authors declare no competing interests.

ETHICAL APPROVAL AND CONSENT TO PARTICIPATE

UK Biobank has approval from the North-West Multi-Centre Research Ethics Committee, the National Information Governance Board for Health & Social Care in England and Wales and the Community Health Index Advisory Group in Scotland. Written informed consent was provided by all participants.

CONSENT TO PUBLISH

Not applicable.

ADDITIONAL INFORMATION

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