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Circulating Levels of Testosterone, Sex Hormone Binding Globulin and Colorectal Cancer Risk: Observational and Mendelian Randomization Analyses

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ABSTRACT

Background: Epidemiologic studies evaluating associations between sex steroid hormones and colorectal cancer risk have yielded inconsistent results. To elucidate the role of circulating levels of testosterone, and sex hormone-binding globulin (SHBG) in colorectal cancer risk, we conducted observational and Mendelian randomization (MR) analyses.

Methods: The observational analyses included 333,530 participants enrolled in the UK Biobank with testosterone and SHBG measured. HRs and 95% confidence intervals (CI) were estimated using multivariable Cox proportional hazards models. For MR analyses, genetic variants robustly associated with hormone levels were identified and their association with colorectal cancer (42,866 cases/42,752 controls) was examined using two-sample MR.

Results: In the observational analysis, there was little evidence that circulating levels of total testosterone were associated with colorectal cancer risk; the MR analyses showed a greater risk for women (OR per 1-SD = 1.09; 95% CI, 1.01–1.17), although pleiotropy may have biased this result. Higher SHBG concentrations were associated with greater colorectal cancer risk for women (HR per 1-SD = 1.16; 95% CI, 1.05–1.29), but was unsupported by the MR analysis. There was little evidence of associations between free testosterone and colorectal cancer in observational and MR analyses.

Conclusions: Circulating concentrations of sex hormones are unlikely to be causally associated with colorectal cancer. Additional experimental studies are required to better understand the possible role of androgens in colorectal cancer development.

Impact: Our results from large-scale analyses provide little evidence for sex hormone pathways playing a causal role in colorectal cancer development.

Introduction

Colorectal cancer is one of the most common cancers worldwide with lower incidence rates in women compared with men (1). It has been proposed that differing exposures to endogenous and exogenous sex steroid hormones may contribute to this sex disparity (2). Higher concentrations of endogenous or exogenous estrogens in women may confer a protective role against colorectal cancer development (2, 3), whereas longer-term use of androgen deprivation therapy has been associated with elevated colorectal cancer risk in men (4).

Inconsistent results have been found in the few relatively small epidemiologic studies that examined the association between circulating testosterone concentrations and colorectal cancer risk. In a pooled analysis of four US based studies, an inverse association was found between testosterone levels and colorectal cancer among men, but not women (5), whereas a recent Japanese prospective study of postmenopausal women, reported a positive association between testosterone and colorectal cancer risk (6).

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Sex hormone-binding globulin (SHBG) is a hepatically-derived glycoprotein and principal transport protein of estrogens and testosterone, and is therefore an important regulator of their bioactivity. In an analysis nested within the Women's Health Initiative Clinical Trial (WHI-CT), we reported a more than twofold higher colorectal cancer risk when the highest and lowest SHBG concentrations exposure groups were compared (7). However, inconsistent results have been found in other smaller studies that have examined the relationship between circulating SHBG concentrations and colorectal cancer (5, 6, 8).

To further examine associations of circulating testosterone and SHBG concentrations with colorectal cancer risk, we conducted complementary observational and Mendelian randomization (MR) analyses. First, we investigated associations of prediagnostic circulating concentrations of total testosterone, free testosterone, and SHBG with colorectal cancer risk in the UK Biobank study, a large prospective cohort of more than 500,000 participants. We then employed MR to help strengthen causal inference by using genetic variants robustly related to circulating sex steroid hormone concentrations from a recent genome-wide association study (GWAS) in UK Biobank (9), and then assessed the relation of these variants with colorectal cancer from large genetic consortia including 42,886 colorectal cancer cases and 42,752 controls (10).

Materials and Methods

UK biobank: observational analysis

Study participants

The UK Biobank is a prospective cohort study of 502,656 adults ages 40 to 69 years who were recruited between 2006 and 2010 (11). The UK Biobank is approved by the North West Multi-centre Research Ethics Committee, the National Information Governance Board for Health and Social Care in England and Wales, and the Community Health Index Advisory Group in Scotland. Since 2004, an independent Ethics and Governance Council additionally oversees UK Biobank's continuous adherence to the Ethics and Governance Framework (http://www.ukbiobank.ac.uk/ethics/). This research has been conducted using the UK Biobank Resource under application number 25897.

At baseline, participants completed a self-administered touchscreen questionnaire, with questions on sociodemographics (such as age, sex, educational level, and postcode, which were used to calculate the Townsend deprivation score; ref. 12), health/medical history, and lifestyle exposures (including smoking related phenotypes, physical activity, dietary intakes, and alcohol consumption). Several anthropometric measurements were also collected, such as body weight, height, and waist circumference. At baseline, blood samples were collected from all participants, and from a subset of ~20,000 participants repeat blood samples were also collected during a follow-up visit between 2012 and 2013. Blood samples were centrifuged, and serum stored at −80°C (13).

We excluded the following participants: those who reported having had a diagnosis of cancer at recruitment to help reduce reverse causality as an explanation for any observed associations (n = 27,264 prevalent cases, self-reported and cancer registry identified); participants with missing data on body-size measurements (n = 3,032); self-reported prevalent type-2 diabetes (T2D) or unknown diabetes status at recruitment (as diabetes medications can affect the concentrations of sex steroid hormones (14–16); n = 26,698); women who reported oral contraceptive or menopausal hormone use (as our focus was on endogenous circulating hormone levels; n = 19,802); and participants without a total testosterone, SHBG, or albumin (required to estimate free testosterone concentration) measurement (n = 92,330). Our analysis therefore included 333,530 participants (160,650 women and 172,880 men; **Fig. 1**).

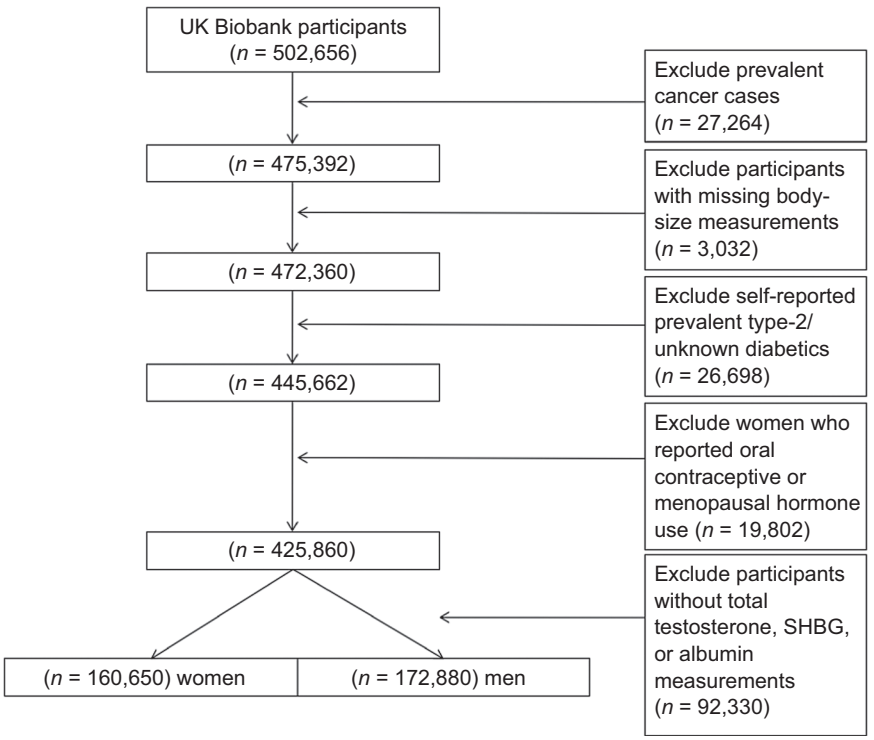

Figure 1.
Flowchart of the exclusion criteria of the study participants in UK Biobank.

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Laboratory methods

As part of the UK Biobank Biomarker Project, serum concentrations of testosterone, SHBG, and insulin-like growth factor-1 (IGF-1) were determined by a chemiluminescent immunoassay. Serum high sensitivity C-reactive protein (CRP) concentrations were assayed by the immuno-turbidimetric method. For glycated hemoglobin (HbA1c), the HPLC Variant II Turbo 2.0 system was used. A detailed description of assay performance can be found elsewhere (17). The average within-laboratory (total) coefficient of variation (CV) for low, medium, and high internal quality control level samples for each biomarker ranged from 3.7% to 8.3% for total testosterone and 5.2% to 5.7% for SHBG (17). Free testosterone concentrations were calculated with the Vermeulen equation using measured albumin concentration available for each participant (18, 19). A total of 10,573 and 11,519 participants had SHBG and testosterone concentrations measured, respectively, in blood samples collected both at recruitment and at the repeat assessment visit (median of 4 years apart).

Assessment of outcome

The UK Biobank cohort is linked to national cancer and death registries used to determine incident colorectal cancer cases and cancer cases recorded first in death certificates. Complete follow-up was available through March 31, 2016, for England and Wales and October 31, 2015, for Scotland. The 10th Revision of the International Classification of Diseases (ICD10) was used to code incidence cancer data. We classified as proximal colon cancers those found within the caecum, appendix, ascending colon, hepatic flexure, transverse colon, and splenic flexure (C18.0–18.5). Distal colon cancers were considered those found within the descending (C18.6) and sigmoid (C18.7) colon. Overlapping (C18.8) and unspecified (C18.9) lesions of the colon were included in colon cancers only. Rectal cancers were classified those at the recto-sigmoid junction (C19) and rectum (C20).

Statistical analysis

Intraclass correlation coefficients (ICC) were used to estimate the reproducibility between the two measurements of SHBG and testosterone available in a subsample of participants. These were obtained dividing the between-person variance by the sum of the between-person and within-person variances.

Cox proportional hazards models were used to estimate HRs and 95% confidence intervals (CI). We used age was the primary time variable. In particular, time at entry was age at recruitment and exit time was age at whichever of the following came first: colorectal cancer diagnosis, death, or the last date at which follow-up was considered complete. Stratification by age at recruitment in 5-year categories, Townsend deprivation index (quintiles), and region of the recruitment assessment center was used in all models. Analyses were conducted separately for men and women, and also according to anatomical subsite (colon, proximal colon, distal colon, and rectal cancer). Total testosterone, free testosterone, and SHBG were modelled with participants grouped into sex-specific quintiles of circulating concentrations and on the continuous scale. To allow us to compare the continuous model results with the MR estimates we used the following transformations: for total testosterone concentration, an inverse normal transformation of the rank was used for women and men; for free testosterone, a natural logarithmic transformation was used for women and an inverse normal transformation of the rank for men; and for SHBG, an inverse normal transformation of the rank was used for women and a natural logarithmic transformation for men (Supplementary Fig. S1).

Statistical tests for trend were calculated using the ordinal quintiles of sex steroid hormones entered into the model as a continuous variable. Continuous scale HRs were additionally corrected for regression dilution using regression dilution ratios obtained from the subsample of participants with repeated testosterone and SHBG measurements (20, 21). Regression dilution ratios are calculated as the ratio of the difference between the means of the follow-up measurements of sex steroid hormones of participants in the highest and lowest and quintiles divided by the respective estimates at baseline. To obtain the corrected continuous HRs, the log HRs and their standard errors were divided by the regression dilution ratio for total testosterone (i.e., 0.65 in women and 0.68 in men), free testosterone (i.e., 0.71 in women and 0.57 in men), and SHBG (i.e., 0.82 in women and 0.83 in men), and then exponentiated (22). All models met the proportional hazards assumption, assessed through analyses of Schoenfeld residuals (23).

Our primary multivariable model 1 was adjusted for a set of *a priori*-determined colorectal cancer risk factors. In particular we adjusted for waist circumference, total physical activity, height, alcohol consumption frequency, smoking status and intensity, frequency of red and processed meat consumption, family history of colorectal cancer, educational level, regular aspirin/ibuprofen use, and ever use of hormone replacement therapy. We also considered models additionally adjusted for inflammation markers and glycemic pathways that correlate with sex steroid hormone concentrations, and are associated with colorectal cancer risk, namely CRP, IGF-1, and HbA1c (6, 7, 24, 25). The testosterone and SHBG multivariable model were mutually adjusted.

Sensitivity analyses excluding colorectal cancer cases occurring in the first 2 years of follow-up were performed. We also performed sensitivity analyses excluding women who were ever menopausal hormone users ($N = 50{,}948$) or those with polycystic ovary syndrome (PCOS; $N = 329$). Analyses for sex steroid hormones on the continuous scale were repeated excluding possible outliers (defined as sex hormone concentrations more than 1.5 times the interquartile range above the third quartile or below the first quartile). We further assessed associations of circulating total testosterone, free testosterone and SHBG with colorectal cancer across subgroups of body mass index (BMI; <25, ≥25 kg/m^2), waist-to-hip ratio (WHR; <median, ≥above median), age at recruitment (<60, ≥60 years), follow-up time (<5, ≥5 years), and menopausal status (pre-, post-). The likelihood ratio test was used to evaluate interactions between these variables and circulating sex steroid hormones concentrations.

MR analysis

Data on total testosterone, free testosterone, and SHBG

We selected genetic variants associated with circulating total testosterone, free testosterone, and SHBG 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 (9). We used data from 230,454 women and 194,453 men of European ancestry for total testosterone, 188,507 women and 178,782 men for free testosterone, and 189,473 for women and 180,726 for men for SHBG. Genotyping chip, age at baseline and 10 genetically derived principal components to account for population stratification were included as covariates in the analysis. For SHBG, BMI was also included as a covariate. However, in the MR analysis for SHBG, genetic loci from the BMI-adjusted analyses were used with corresponding effect estimates from the BMI-unadjusted analyses to mitigate possible collider bias (26).

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Data on colorectal cancer

Summary data for associations of the hormone-related variants with colorectal cancer were obtained from a meta-analysis of GWAS involving 85,638 participants (42,886 colorectal cancer cases and 42,752 controls) within the ColoRectal Transdisciplinary Study (CORECT), the Colon Cancer Family Registry (CCFR), and the Genetics and Epidemiology of Colorectal Cancer (GECCO) consortia (10). The GWAS was adjusted for age, sex, genotyping platform, and genomic principal components.

Statistical analysis

We conducted two-sample MR analyses to appraise the potential causal nature of the associations between total testosterone, free testosterone, and SHBG with colorectal cancer risk. Where a variant used as an instrument for one of the hormones of interest was not present in the colorectal cancer GWAS, we identified a 1,000 Genomes proxy with $r^2 > 0.8$. For our main analysis, we used a random-effects inverse-variance weighted (IVW) method (27, 28).

Sensitivity analyses

We conducted sensitivity analyses to mitigate against any pleiotropic effects. We undertook MR-Egger regression (29) and computed the estimator from the weighted median approach (30) to assess the possible influence of horizontal pleiotropy on the effect estimates. We calculated the Cochran *Q* statistic that quantifies the heterogeneity in effect sizes attributed to the selected genetic variants (31). We also estimated the intercept term from the MR-Egger regression, with a deviation from zero being indicative of directional (nonbalanced horizontal) pleiotropy (29). We excluded genetic variants having larger effects (based on standardized beta) on any one of 11 metabolic traits available in the UK Biobank (fasting glucose, T2D, coronary artery disease, high density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, total-cholesterol, and diastolic and systolic blood pressure, BMI and waist-to-hip ratio adjusted for BMI), an approach known as Steiger filtering (32). A list of pleiotropic variants for total testosterone, free testosterone, and SHBG can be found in the published GWAS (9). Finally, we used only *cis* variants at the *SHBG* gene locus (rs1799941, rs6258). Variant rs1799941 is common, whereas rs6258 is rare and alters SHBG's binding affinity for testosterone (33, 34).

All the observational analyses were implemented in Stata 13.1, whereas for the MR analyses, we used the Mendelian randomization R package (35).

Results

UK biobank: observational analysis

After a median follow-up time of 7.1 years (interquartile range = 6.4–7.7), 2,258 colorectal cancer cases were recorded (833 in women and 1,425 in men). In both women and men, compared with noncases, individuals with colorectal cancer were older, had higher BMI, were more likely to have a family history of colorectal cancer and eat red and processed meat more frequently, and were less likely to be current smokers (**Table 1**). Participant characteristics according to quintiles of total testosterone, free testosterone, and SHBG are presented in Supplementary Table S1.

The reproducibility (ICC) of testosterone ($n = 11,519$ participants; 4,669 women and 6,850 men; median of 4 years apart) was 0.59 (95% CI, 0.58–0.61) for women and 0.66 (95% CI, 0.64–0.67) for men. The ICC of SHBG concentrations measured at both the recruitment and repeat assessment visit ($n = 10,573$ participants; 4,459 women and 6,114 men) was 0.77 (95% CI, 0.76–0.79) for women and 0.82 (95% CI, 0.75–0.87) for men.

Association of circulating total testosterone and free testosterone concentrations with colorectal cancer risk

In the multivariable model 2 additionally adjusted for circulating concentrations of CRP, HbA1c, SHBG, and IGF-1, there was little evidence that a 1-SD increment of total testosterone concentration was associated with colorectal cancer risk for women (HR = 1.00; 95% CI, 0.90–1.11) and men (HR = 0.97; 95% CI, 0.88–1.07; **Table 2**; Supplementary Table S2). When stratifying by anatomical subsite, no association between circulating total testosterone concentration and colon cancer was found for women (HR per 1-SD increment = 1.04; 95% CI, 0.92–1.18) and men (HR per 1-SD increment = 0.94; 95% CI, 0.83–1.07); a similar pattern of associations were found for proximal and distal colon cancers (**Table 2**; Supplementary Table S2).

There was little evidence that circulating concentrations of free testosterone were associated with colorectal cancer risk for women (HR per 1 unit increment in log concentration = 0.90; 95% CI, 0.75–1.08) and men (HR per 1-SD increment = 0.98; 95% CI, 0.89–1.08; **Table 2**; Supplementary Table S2). There was little evidence for an association between circulating levels of free testosterone and colorectal cancer across anatomical subsites for both men and women. Heterogeneity for the circulating free testosterone concentrations and colorectal cancer association was found for men by follow-up time ($P_{interaction} = 0.01$; **Table 3**).

Association between circulating SHBG concentrations and colorectal cancer risk

In the multivariable model 2 additionally adjusted for circulating concentrations of CRP, HbA1c, testosterone, and IGF-1, a 1-SD increment of SHBG concentrations was associated with a higher colorectal cancer risk amongst women (HR = 1.16; 95% CI, 1.05–1.29; **Table 2**; Supplementary Table S2). No association between SHBG concentrations and colorectal cancer risk was found for men (HR per 1 unit increment in log concentration = 1.04; 95% CI, 0.84–1.28). Associations of similar magnitude between SHBG concentrations and colorectal cancer risk were found in the quintile models, by anatomical subsite, and according to subgroups of BMI, WHR, age at recruitment, follow-up time, and menopausal status (**Table 3**; all $P_{interactions} \geq 0.05$).

Sensitivity analyses

Similar results for total testosterone, free testosterone, and SHBG with colorectal cancer were found when: participants with outlier concentrations were excluded (Supplementary Table S3); cases occurring in the first 2 years of follow-up were excluded ($n = 564$ colorectal cancer cases excluded; Supplementary Table S4); and ever users of menopausal hormones or women with PCOS were excluded (Supplementary Table S5).

Mendelian randomization analyses

Effect estimates for the association between circulating total testosterone and free testosterone concentrations and colorectal cancer risk

In the random-effects IVW models, higher genetically predicted circulating total testosterone concentration was associated with greater risk of colorectal cancer for women (OR per 1 SD increment in testosterone concentrations = 1.09; 95% CI, 1.01–1.17), but not for

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Table 1. Characteristics of UK Biobank study participants (n = 333,530 participants).

	Women (n = 160,650)		Men (n = 172,880)	
	Colorectal cancer cases (N = 833)	Noncases (N = 159,817)	Colorectal cancer cases (N = 1,425)	Noncases (N = 171,455)
Age at recruitment (years)[a]	59.5 (7.1)	55.8 (8.1)	61.1 (6.3)	56.3 (8.2)
Body mass index (kg/m^2)[a]	27.2 (4.9)	27.0 (5.1)	28.1 (4.0)	27.6 (4.0)
Waist circumference (cm)[a]	85.5 (12.3)	84.4 (12.2)	98.6 (10.6)	96.2 (10.9)
Height (cm)[a]	162.2 (6.2)	162.6 (6.3)	175.4 (6.6)	175.8 (6.8)
Total physical activity (MET hours per week)				
<10	25.0%	23.0%	19.6%	20.5%
≥60	19.4%	20.0%	26.0%	24.8%
Smoking status				
Never	57.6%	60.4%	39.8%	49.9%
Current	8.3%	9.1%	11.8%	12.6%
Alcohol consumption				
Never	9.2%	8.7%	4.4%	5.8%
daily/almost daily	19.3%	16.3%	31.7%	25.8%
Socio-economic status (Townsend deprivation index)				
Highest quintile	20.9%	19.6%	18.9%	20.3%
Family history (first degree relative) of colorectal cancer				
Yes	12.2%	10.4%	15.0%	11.0%
Regular aspirin/ibuprofen use				
Yes	23.5%	24.4%	27.6%	26.3%
Red and processed meat				
<2 occasions per week	17.2%	18.9%	6.0%	9.0%
≥4 occasions per week	32.3%	30.8%	57.1%	51.7%
Ever menopausal hormone use[b]				
Yes	41.9%	31.7%		
Menopausal status[b]				
Postmenopausal	82.0%	66.3%		
C-reactive protein (CRP; mg/L)[a]	2.9 (4.5)	2.5 (4.0)	3.0 (4.6)	2.4 (4.2)
Total testosterone (nmol/L)[a]	1.1 (0.9)	1.1 (0.6)	11.7 (3.5)	12.1 (3.7)
Free testosterone (pmol/L)[a]	14.7 (13.8)	14.6 (10.5)	199.0 (53.8)	210.7 (60.6)
Sex hormone binding globulin (SHBG; nmol/L)[a]	61.5 (28.4)	60.8 (27.9)	41.5 (17.7)	39.8 (16.6)
IGF-1 (nmol/L)[a]	20.9 (5.5)	21.3 (5.6)	21.5 (5.8)	22.0 (5.4)
Glycated hemoglobin (HbA1c; mmol/mol)[a]	35.8 (4.3)	35.1 (4.3)	35.7 (4.5)	35.2 (5.0)

Abbreviation: MET, metabolic equivalents.
[a]Mean and SD.
[b]Among women only.

men (OR = 0.99; 95% CI, 0.91–1.07); although heterogeneity was observed (P-value for heterogeneity was 0.01 for women and <0.01 men). Positive associations were also found for distal colon cancer and rectal cancer for women (distal colon cancer, OR = 1.15; 95% CI, 1.03–1.28; rectal cancer, OR = 1.13; 95% CI, 1.00–1.28), but not for men (distal colon cancer, OR = 1.06; 95% CI, 0.93–1.20; rectal cancer, OR = 1.02; 95% CI, 0.91–1.15; **Table 4**; Supplementary Table S6). However, these positive associations were slightly attenuated for the weighted median and Steiger filtered analyses, and were null in the lower powered MR-Egger models (Supplementary Table S6).

No association was estimated between genetically predicted circulating free testosterone concentrations and risk of colorectal cancer for both women (OR per 1 unit increment in log-concentrations = 1.05; 95% CI, 0.93–1.18) and men (OR per 1-SD increment = 1.00; 95% CI, 0.89–1.13; **Table 4**; Supplementary Table S6). Associations of similar magnitude were estimated for all anatomic subsites in both men and women. The MR-Egger test showed evidence of directional pleiotropy for rectal cancer in women (MR-Egger intercept P value = 0.03). The weighted median approach showed effect estimates of similar magnitude with wider CIs in all models. Steiger filtered analysis showed nearly identical null associations with risk of colorectal cancer in both women and men (**Table 4**; Supplementary Table S6).

Effect estimates for the association between circulating SHBG concentrations and colorectal cancer risk

In the random-effects IVW models, we found no association between genetically predicted circulating SHBG concentrations and risk of colorectal cancer for women (OR per 1 SD increment = 1.07; 95% CI, 0.94–1.23) and men (OR per 1 unit increment in log-concentrations = 1.06; 95% CI, 0.92–1.21), with evidence for heterogeneity in all analyses (Cochran Q P values <0.001; **Table 4**; Supplementary Table S6). Similar magnitude effect estimates were found for all anatomic subsites in both men and women. The MR-Egger test showed evidence of directional pleiotropy for rectal cancer in women (MR-Egger intercept P value = 0.03). The weighted median approach showed effect estimates of similar magnitude in all models. Almost identical null associations were estimated for circulating SHBG concentrations and colorectal cancer in both women and men excluding pleiotropic variants indicated by Steiger filtering. No associations were observed using *cis* variants in the *SHBG* gene as the genetic instrument (Supplementary Table S7).

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Table 2. Risk (HRs) of colorectal cancer associated with circulating total testosterone, free testosterone, and sex hormone binding globulin (SHBG) levels in the UK Biobank.

	Colorectal cancer	Colon cancer	Proximal colon cancer	Distal colon cancer	Rectal cancer
Total testosterone[a]					
Women					
Q1	1	1	1	1	1
Q2	0.95 (0.77–1.17)	1.01 (0.79–1.29)	1.05 (0.77–1.42)	0.97 (0.64–1.48)	0.79 (0.52–1.19)
Q3	1.01 (0.82–1.25)	1.03 (0.81–1.32)	0.70 (0.49–1.00)	1.48 (1.01–2.16)	0.95 (0.64–1.42)
Q4	0.95 (0.77–1.18)	1.00 (0.78–1.29)	0.90 (0.64–1.26)	1.17 (0.78–1.75)	0.82 (0.54–1.25)
Q5	0.98 (0.79–1.22)	1.03 (0.80–1.33)	0.99 (0.71–1.38)	1.03 (0.68–1.57)	0.86 (0.56–1.30)
P-trend	0.89	0.86	0.59	0.59	0.56
HR per 1-SD increment	1.00 (0.93–1.07)	1.03 (0.95–1.11)	0.99 (0.89–1.11)	1.04 (0.92–1.18)	0.92 (0.80–1.05)
HR per 1-SD increment (adjusted)[b]	1.00 (0.90–1.11)	1.04 (0.92–1.18)	0.99 (0.84–1.17)	1.07 (0.88–1.30)	0.88 (0.71–1.08)
Men					
Q1	1	1	1	1	1
Q2	1.12 (0.95–1.31)	1.13 (0.92–1.38)	0.95 (0.71–1.27)	1.30 (0.97–1.78)	1.11 (0.86–1.44)
Q3	1.01 (0.85–1.21)	1.08 (0.87–1.34)	0.82 (0.60–1.12)	1.36 (0.99–1.88)	0.92 (0.69–1.23)
Q4	0.96 (0.80–1.16)	0.94 (0.74–1.19)	0.85 (0.61–1.19)	1.01 (0.70–1.45)	1.00 (0.75–1.35)
Q5	0.91 (0.74–1.13)	0.87 (0.66–1.13)	0.69 (0.47–1.01)	1.14 (0.78–1.69)	0.99 (0.72–1.37)
P-trend	0.19	0.16	0.06	0.97	0.75
HR per 1-SD increment	0.98 (0.92–1.05)	0.96 (0.88–1.05)	0.92 (0.82–1.04)	1.01 (0.89–1.14)	1.01 (0.91–1.12)
HR per 1-SD increment (adjusted)[b]	0.97 (0.88–1.07)	0.94 (0.83–1.07)	0.89 (0.74–1.06)	1.01 (0.84–1.22)	1.01 (0.87–1.18)
Free testosterone					
Women					
Q1	1	1	1	1	1
Q2	0.80 (0.65–0.99)	0.90 (0.70–1.16)	0.90 (0.65–1.24)	0.86 (0.57–1.29)	0.58 (0.38–0.88)
Q3	0.82 (0.66–1.01)	0.90 (0.70–1.16)	0.76 (0.54–1.07)	1.02 (0.69–1.51)	0.64 (0.43–0.97)
Q4	0.87 (0.70–1.08)	0.94 (0.73–1.21)	0.92 (0.66–1.28)	0.88 (0.58–1.33)	0.73 (0.49–1.10)
Q5	0.83 (0.66–1.04)	0.91 (0.69–1.18)	0.77 (0.53–1.10)	0.94 (0.62–1.43)	0.66 (0.43–1.02)
P-trend	0.23	0.59	0.21	0.85	0.16
HR per 1-unit increment (log scale)	0.93 (0.81–1.06)	0.98 (0.84–1.14)	0.89 (0.72–1.09)	1.01 (0.80–1.29)	0.79 (0.61–1.03)
HR per 1-unit increment (log scale-adjusted)[c]	0.90 (0.75–1.08)	0.97 (0.78–1.20)	0.85 (0.63–1.13)	1.02 (0.73–1.43)	0.72 (0.51–1.04)
Men					
Q1	1	1	1	1	1
Q2	0.95 (0.82–1.11)	0.96 (0.80–1.17)	0.75 (0.57–0.99)	1.23 (0.93–1.61)	0.94 (0.73–1.20)
Q3	0.95 (0.81–1.11)	0.83 (0.68–1.02)	0.74 (0.56–0.99)	0.91 (0.67–1.24)	1.15 (0.90–1.48)
Q4	1.01 (0.86–1.19)	0.98 (0.80–1.21)	0.87 (0.65–1.16)	1.15 (0.85–1.56)	1.08 (0.83–1.40)
Q5	0.91 (0.76–1.09)	0.87 (0.69–1.10)	0.77 (0.56–1.07)	1.02 (0.72–1.44)	0.99 (0.74–1.32)
P-trend	0.59	0.29	0.20	0.98	0.62
HR per 1-SD increment	0.99 (0.93–1.05)	0.98 (0.91–1.05)	0.94 (0.85–1.04)	1.03 (0.92–1.14)	1.01 (0.92–1.10)
HR per 1-SD increment (adjusted)[c]	0.98 (0.89–1.08)	0.96 (0.85–1.09)	0.90 (0.75–1.08)	1.04 (0.87–1.26)	1.01 (0.86–1.18)
SHBG[d]					
Women					
Q1	1	1	1	1	1
Q2	1.12 (0.90–1.40)	1.07 (0.83–1.37)	1.17 (0.84–1.64)	1.00 (0.68–1.49)	1.31 (0.83–2.06)
Q3	1.02 (0.81–1.29)	0.93 (0.71–1.22)	0.91 (0.62–1.32)	0.94 (0.61–1.43)	1.33 (0.83–2.14)
Q4	1.39 (1.10–1.76)	1.26 (0.96–1.65)	1.37 (0.95–1.98)	1.25 (0.82–1.91)	1.86 (1.16–2.98)
Q5	1.40 (1.09–1.81)	1.30 (0.97–1.74)	1.48 (1.00–2.19)	1.28 (0.81–2.02)	1.76 (1.05–2.94)
P-trend	0.002	0.045	0.044	0.18	0.012
HR per 1-SD increment	1.13 (1.04–1.23)	1.13 (1.02–1.24)	1.19 (1.04–1.36)	1.11 (0.96–1.30)	1.15 (0.97–1.35)
HR per 1-SD increment (adjusted)[e]	1.16 (1.05–1.29)	1.15 (1.03–1.30)	1.24 (1.05–1.45)	1.14 (0.95–1.37)	1.18 (0.97–1.44)
Men					
Q1	1	1	1	1	1
Q2	0.92 (0.77–1.10)	0.94 (0.75–1.18)	0.84 (0.61–1.17)	0.92 (0.66–1.30)	0.89 (0.68–1.18)
Q3	0.81 (0.67–0.97)	0.91 (0.72–1.15)	0.91 (0.65–1.27)	0.90 (0.64–1.28)	0.66 (0.49–0.90)
Q4	0.91 (0.75–1.11)	0.97 (0.76–1.24)	0.87 (0.61–1.23)	0.96 (0.67–1.37)	0.83 (0.61–1.12)
Q5	1.01 (0.82–1.25)	1.09 (0.83–1.42)	1.12 (0.77–1.64)	0.99 (0.66–1.47)	0.90 (0.65–1.26)
P-trend	0.86	0.51	0.60	0.93	0.56
HR per 1-unit increment (log scale)	1.03 (0.86–1.23)	1.08 (0.86–1.36)	1.13 (0.82–1.57)	0.95 (0.68–1.32)	0.95 (0.72–1.26)
HR per 1-unit increment (log scale-adjusted)[e]	1.04 (0.84–1.28)	1.10 (0.84–1.45)	1.16 (0.79–1.72)	0.94 (0.63–1.40)	0.94 (0.67–1.32)

Note: Multivariable Cox regression model using age as the underlying time variable and stratified by sex, Townsend deprivation index (quintiles), region of the recruitment assessment center, and age at recruitment. Models adjusted for waist circumference (per 5 cm), total physical activity (<10, 10–<20, 20–<40, 40–<60, ≥60 MET hours per week, unknown), height (per 10 cm), alcohol consumption frequency (never, special occasions only, 1 to 3 times per month, 1 to 2 times per week, 3 to 4 times per week, daily/almost daily, unknown), smoking status and intensity (never, former, current- <15 per day, current- ≥15 per day, current- intensity unknown,

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Discussion

In our MR analysis, we found a positive effect estimate for circulating total testosterone levels with colorectal cancer risk among women; however, we cannot rule out the possibility of pleiotropy biasing this finding (i.e., the effect is explained by an independent biological pathway). There was little evidence that circulating testosterone levels were associated with risk of colorectal cancer for men in the observational and MR analyses. In observational analyses of UK Biobank data, we found that higher prediagnostic concentrations of circulating SHBG were associated with a greater risk of colorectal cancer, with this relationship limited to women only. These findings were not, however, corroborated by our MR analyses, which showed little evidence of an association between genetically predicted SHBG concentrations and colorectal cancer risk in women.

In our observational analyses in UK Biobank, there was little evidence that circulating testosterone levels were associated with colorectal cancer risk for women. Our findings for total testosterone and free testosterone concentrations are generally similar to those published in other recent UK Biobank studies (36, 37). In the MR analyses, we found positive effect estimates between total testosterone concentrations and colorectal, distal colon, and rectal cancer risk for women. However, the effect estimates were null in the MR-Egger models, indicating that there may be alternative pathways explaining these associations (pleiotropy). Possible biological pathways linking testosterone with colorectal cancer development for women are unclear. In women, testosterone is mainly produced by the ovaries, suprarenal glands, and adipose tissue, with its secretion regulated by aromatase activity. After menopause, testosterone becomes the main source of estradiol when ovarian production of estrogens ceases. Thus, the positive association found between total testosterone and colorectal cancer for women may be an indicator of estrogenic pathways. However, epidemiological studies examining the associations between prediagnostic levels of estrogens and colorectal cancer have reported mixed results (5–8), and stronger genetic instruments for circulating estrogen concentrations are required to undertake suitably powered MR analyses with colorectal cancer. Overall, further studies are needed to better understand the biological pathways through which testosterone may influence colorectal cancer risk for women.

The positive association we found between SHBG concentrations and colorectal cancer for women in our UK Biobank observational analysis was consistent with a prior analysis in the WHI-CT study (7). However, other previous observational studies have reported no association between circulating SHBG levels and colorectal cancer risk (5, 6, 8). For men, the null association we found in our observational analysis was inconsistent with a prior Health Professional Follow-up Study/Physicians' Health Study II analysis (5) and a recently published study in UK Biobank (37) that reported an inverse association. This prior UK Biobank study, however, did not statistically adjust for markers of inflammation and glycemic pathways that are known to be correlated with sex steroid hormone concentrations, and have been linked to colorectal cancer risk, namely CRP, IGF-1, and HbA1c (6, 7, 24, 25). After we adjusted our multivariable models for these serologic factors the inverse SHBG risk estimate attenuated to the null. For our MR analysis, we found little evidence of an association between SHBG concentrations and colorectal cancer risk for both men and women. It is possible that this inconsistency in results between observational and MR evidence is a consequence of measurement error, residual confounding, and/or reverse causality, characteristic of observational epidemiology. MR is an increasingly used method that uses genetic variants robustly associated with the exposure of interest in an instrumental variable analysis to appraise the causal nature of the effects of the exposure on an outcome (38). The random and fixed allocation of alleles at conception makes confounding and reverse causation less likely explanations for associations identified in MR studies (39).

This study is the most comprehensive investigation of the associations between circulating sex steroid hormone concentrations and colorectal cancer incorporating complementary observational and MR analyses. Our observational study, using data from the UK Biobank, was the largest to date (including >2,000 incident cases) which meant we were able to examine circulating sex steroid hormones levels and colorectal cancer association by anatomical subsite and by subgroups of colorectal cancer risk factors. We were also able to control statistically for other factors that are related to the sex hormone pathway, and have been linked to colorectal cancer incidence in some studies, namely CRP, IGF-1, and HbA1c (6, 7, 24, 25). A limitation of our analysis was that single hormones measures were available for most participants and it is possible that these measurements may not reflect longer term exposures. However, in our reproducibility analysis, we estimated a within-person ICC of ∼0.6 and ∼0.8 for SHBG for testosterone over a four-year period, indicating that a single measurement provided moderate to good estimates of longer-term exposures of testosterone and SHBG. Uniquely, the availability of second SHBG measurements in a subset of cohort participants also allowed us to correct for regression dilution bias, resulting in HRs of greater magnitude in all models. A further limitation was that we were unable to estimate the association of circulating concentrations of estrogens with colorectal cancer risk as the assay used in the UK Biobank to assess estradiol levels was not sufficiently sensitive to measure low concentrations commonly found in postmenopausal women and men. For our MR analyses, the summary level data that we used meant we were unable to conduct subgroup analyses by other colorectal cancer risk factors (e.g., age, BMI, smoking, menopausal status). In addition, our two-sample MR analyses using summary-level data assumed a linear relationship between sex

unknown), frequency of red and processed meat consumption (<2, 2–<3, 3–<4, ≥4 occasions per week, unknown), family history of colorectal cancer (no, yes, unknown), educational level (CSEs/O-levels/GCSEs or equivalent, NVQ/HND/HNC/A-levels/AS-levels or equivalent, other professional qualifications, college/university degree, none of the above, unknown), regular aspirin/ibuprofen use (no, yes, unknown), ever use of hormone replacement therapy (no, yes, unknown), circulating levels (sex-specific quintiles, missing/unknown) of C-reactive protein (CRP; mg/L), glycated hemoglobin (HbA1c; mmol/mol), and IGF-1 (nmol/L).

[a]Plus additional adjustment for SHBG (nmol/L).

[b]HRs were additionally corrected for regression dilution using a regression dilution ratio (0.65 in women and 0.68 in men) obtained from the subsample of participants with repeat total testosterone measurements.

[c]HRs were additionally corrected for regression dilution using a regression dilution ratio (0.71 in women and 0.57 in men) obtained from the subsample of participants with repeat free testosterone measurements.

[d]Plus additional adjustment for total testosterone (nmol/L).

[e]HRs were additionally corrected for regression dilution using a regression dilution ratio (0.82 in women and 0.83 in men) obtained from the subsample of participants with repeat SHBG measurements.

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Table 3. Subgroup analyses of the association between circulating total testosterone, free testosterone, and sex hormone binding globulin (SHBG) levels and colorectal cancer risk in the UK Biobank.

	Total testosterone			Free testosterone			SHBG		
	HR[a]	HR (adjusted)[b]	$P_{interaction}$	HR[c]	HR (adjusted)[d]	$P_{interaction}$	HR[e]	HR (adjusted)[f]	$P_{interaction}$
Women									
Body mass index (kg/m^2)									
<25	0.95 (0.85–1.07)	0.93 (0.78–1.11)	0.33	0.87 (0.70–1.08)	0.82 (0.61–1.11)	0.46	1.12 (0.98–1.29)	1.15 (0.98–1.36)	0.90
≥25	1.02 (0.94–1.11)	1.03 (0.91–1.18)		0.96 (0.82–1.13)	0.94 (0.75–1.18)		1.14 (1.03–1.26)	1.17 (1.03–1.32)	
Waist to hip ratio									
below median (<0.81)	1.05 (0.95–1.17)	1.08 (0.93–1.27)	0.16	0.99 (0.82–1.20)	0.99 (0.76–1.29)	0.31	1.15 (1.02–1.30)	1.19 (1.03–1.37)	0.75
above median (≥0.81)	0.96 (0.87–1.05)	0.93 (0.81–1.07)		0.87 (0.74–1.04)	0.83 (0.65–1.05)		1.12 (1.01–1.25)	1.15 (1.01–1.31)	
Age at recruitment (years)									
<60	0.99 (0.89–1.11)	0.99 (0.84–1.17)	0.94	0.94 (0.77–1.14)	0.91 (0.69–1.20)	0.84	1.10 (0.99–1.24)	1.13 (0.98–1.30)	0.47
≥60	1.00 (0.91–1.09)	1.00 (0.87–1.14)		0.91 (0.77–1.08)	0.88 (0.70–1.11)		1.16 (1.05–1.29)	1.20 (1.06–1.37)	
Follow-up time (years)									
<5	0.94 (0.86–1.03)	0.92 (0.80–1.05)	0.28	0.84 (0.71–1.00)	0.79 (0.62–1.00)	0.16	1.13 (1.02–1.25)	1.16 (1.02–1.31)	0.33
≥5	1.02 (0.91–1.15)	1.04 (0.87–1.24)		1.02 (0.82–1.25)	1.02 (0.76–1.38)		1.05 (0.92–1.19)	1.06 (0.90–1.24)	
Menopausal status									
Premenopausal	1.08 (0.89–1.31)	1.12 (0.84–1.51)	0.32	0.92 (0.66–1.27)	0.88 (0.56–1.40)	1.00	1.25 (1.04–1.50)	1.31 (1.04–1.64)	0.20
Postmenopausal	0.97 (0.90–1.05)	0.96 (0.85–1.07)		0.92 (0.79–1.06)	0.88 (0.72–1.08)		1.10 (1.00–1.20)	1.12 (1.00–1.25)	
Men									
Body mass index (kg/m^2)									
<25	0.96 (0.85–1.09)	0.94 (0.79–1.13)	0.73	0.91 (0.81–1.02)	0.85 (0.69–1.04)	0.11	1.37 (0.98–1.91)	1.46 (0.98–2.18)	0.05
≥25	0.98 (0.91–1.06)	0.98 (0.88–1.09)		1.01 (0.95–1.08)	1.02 (0.91–1.14)		0.96 (0.80–1.16)	0.96 (0.76–1.20)	
Waist-to-hip ratio									
below median (<0.93)	0.91 (0.83–1.00)	0.87 (0.76–1.00)	0.05	0.94 (0.86–1.02)	0.89 (0.76–1.04)	0.16	1.00 (0.78–1.28)	1.00 (0.74–1.35)	0.61
above median (≥0.93)	1.02 (0.94–1.10)	1.02 (0.91–1.15)		1.02 (0.95–1.09)	1.03 (0.91–1.16)		1.07 (0.87–1.32)	1.09 (0.85–1.39)	
Age at recruitment (years)									
<60	1.04 (0.94–1.14)	1.05 (0.91–1.22)	0.13	1.03 (0.93–1.13)	1.05 (0.89–1.24)	0.29	1.07 (0.83–1.38)	1.09 (0.80–1.47)	0.80
≥60	0.95 (0.88–1.02)	0.93 (0.83–1.04)		0.97 (0.90–1.03)	0.94 (0.83–1.06)		1.03 (0.84–1.27)	1.04 (0.81–1.33)	
Follow-up time (years)									
<5	1.00 (0.93–1.08)	1.00 (0.90–1.12)	0.46	1.05 (0.98–1.13)	1.10 (0.97–1.24)	0.01	0.85 (0.69–1.04)	0.82 (0.64–1.05)	0.27
≥5	0.96 (0.87–1.06)	0.94 (0.81–1.09)		0.91 (0.82–1.00)	0.84 (0.71–1.00)		1.00 (0.77–1.30)	1.00 (0.73–1.38)	

Note: Multivariable Cox regression model using age as the underlying time variable and stratified by sex, Townsend deprivation index (quintiles), region of the recruitment assessment center, and age at recruitment. Models adjusted for waist circumference (per 5 cm), total physical activity (<10, 10–<20, 20–<40, 40–<60, ≥60 MET hours per week, unknown), height (per 10 cm), alcohol consumption frequency (never, special occasions only, 1 to 3 times per month, 1 to 2 times per week, 3 to 4 times per week, daily/almost daily, unknown), smoking status and intensity (never, former, current- <15 per day, current- ≥15 per day, current- intensity unknown, unknown), frequency of red and processed meat consumption (<2, 2–<3, 3–<4, ≥4 occasions per week, unknown), family history of colorectal cancer (no, yes, unknown), educational level (CSEs/O-levels/GCSEs or equivalent, NVQ/HND/HNC/A-levels/AS-levels or equivalent, other professional qualifications, college/university degree, none of the above, unknown), regular aspirin/ibuprofen use (no, yes, unknown), ever use of hormone replacement therapy (no, yes, unknown), circulating levels (sex-specific quintiles, missing/unknown) of C-reactive protein (CRP; mg/L), glycated hemoglobin (HbA1c; mmol/mol), and IGF-1 (nmol/L).

[a]HRs per 1-SD increment in both women and men additional adjusted for sex hormone binding globulin (SHBG; nmol/L);.

[b]HRs were additionally corrected for regression dilution using a regression dilution ratio (0.65 in women and 0.68 in men) obtained from the subsample of participants with repeat total testosterone measurements;.

[c]HRs per 1-unit increment (log scale) in women and per 1-SD increment in men;.

[d]HRs were additionally corrected for regression dilution using a regression dilution ratio (0.71 in women and 0.57 in men) obtained from the subsample of participants with repeat free testosterone measurements;.

[e]HRs per 1-SD increment in women and per 1-unit increment (log scale) in men additionally adjusted for total testosterone (nmol/L);.

[f]HRs were additionally corrected for regression dilution using a regression dilution ratio (0.82 in women and 0.83 in men) obtained from the subsample of participants with repeat SHBG measurements.

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Table 4. MR estimates for the effect of total testosterone, free testosterone, and sex hormone binding globulin (SHBG) on colorectal cancer risk.

	Women				Men			
Methods	**OR[a]**	**95% CI**	***P* value**	***P* value for pleiotropy or heterogeneity**	**OR[a]**	**95% CI**	***P* value**	***P* value for pleiotropy or heterogeneity**
Total testosterone								
Colorectal cancer								
IVW (random effects)	1.09	(1.01–1.17)	0.04	0.01	0.99	(0.91–1.07)	0.76	<0.01
MR-Egger (slope)	1.01	(0.88–1.17)	0.85	0.28	0.95	(0.84–1.09)	0.49	0.52
Weighted median	1.08	(0.94–1.25)	0.27	NA	1.02	(0.91–1.14)	0.75	NA
Colon cancer								
IVW (random effects)	1.06	(0.97–1.16)	0.17	0.14	1.03	(0.94–1.12)	0.58	<0.01
MR-Egger (slope)	0.94	(0.80–1.11)	0.47	0.09	1.00	(0.86–1.16)	0.99	0.65
Weighted median	1.05	(0.90–1.24)	0.53	NA	1.08	(0.94–1.25)	0.27	NA
Distal colon cancer								
IVW (random effects)	1.15	(1.03–1.28)	0.01	0.52	1.06	(0.93–1.20)	0.37	<0.01
MR-Egger (slope)	1.02	(0.83–1.25)	0.87	0.18	1.14	(0.92–1.40)	0.23	0.40
Weighted median	1.13	(0.91–1.40)	0.26	NA	1.27	(1.06–1.53)	0.01	NA
Proximal colon cancer								
IVW (random effects)	1.02	(0.91–1.14)	0.75	0.02	1.00	(0.90–1.12)	0.99	0.07
MR-Egger (slope)	0.91	(0.74–1.13)	0.40	0.24	0.90	(0.75–1.08)	0.25	0.14
Weighted median	1.05	(0.85–1.28)	0.67	NA	0.94	(0.79–1.13)	0.53	NA
Rectal cancer								
IVW (random effects)	1.13	(1.00–1.28)	0.05	0.06	1.02	(0.91–1.15)	0.68	<0.01
MR-Egger (slope)	1.16	(0.91–1.46)	0.23	0.82	0.97	(0.81–1.17)	0.77	0.48
Weighted median	1.25	(1.00–1.57)	0.05	NA	1.05	(0.88–1.24)	0.61	NA
Free testosterone								
Colorectal cancer								
IVW (random effects)	1.05	(0.93–1.18)	0.42	<0.01	1.00	(0.89–1.13)	0.98	<0.01
MR-Egger (slope)	1.14	(0.93–1.40)	0.19	0.31	1.00	(0.79–1.28)	0.98	0.98
Weighted median	1.06	(0.89–1.26)	0.49	NA	1.06	(0.90–1.24)	0.49	NA
Colon cancer								
IVW (random effects)	1.01	(0.89–1.16)	0.85	0.01	1.02	(0.89–1.17)	0.78	0.06
MR-Egger (slope)	1.07	(0.85–1.35)	0.55	0.55	0.90	(0.68–1.19)	0.45	0.31
Weighted median	1.07	(0.87–1.32)	0.50	NA	1.00	(0.83–1.22)	0.97	NA
Distal colon cancer								
IVW (random effects)	1.08	(0.91–1.27)	0.37	0.17	0.92	(0.77–1.08)	0.30	0.10
MR-Egger (slope)	0.99	(0.74–1.33)	0.96	0.50	0.80	(0.57–1.12)	0.19	0.36
Weighted median	1.04	(0.80–1.35)	0.78	NA	0.88	(0.69–1.12)	0.30	NA
Proximal colon cancer								
IVW (random effects)	0.98	(0.83–1.14)	0.76	0.01	1.09	(0.92–1.29)	0.31	0.12
MR-Egger (slope)	1.18	(0.89–1.55)	0.24	0.10	1.05	(0.74–1.49)	0.78	0.81
Weighted median	1.11	(0.85–1.45)	0.43	NA	1.09	(0.85–1.39)	0.51	NA
Rectal cancer								
IVW (random effects)	1.13	(0.94–1.36)	0.19	0.02	1.00	(0.83–1.20)	0.99	<0.01
MR-Egger (slope)	1.51	(1.10–2.07)	0.01	0.03	1.09	(0.75–1.58)	0.65	0.60
Weighted median	1.10	(0.83–1.45)	0.51	NA	1.01	(0.78–1.30)	0.95	NA
SHBG								
Colorectal cancer								
IVW (random effects)	1.07	(0.94–1.23)	0.32	<0.01	1.06	(0.92–1.21)	0.42	<0.01
MR-Egger (slope)	1.02	(0.84–1.23)	0.88	0.45	1.03	(0.86–1.24)	0.71	0.74
Weighted median	1.05	(0.84–1.31)	0.67	NA	0.98	(0.82–1.16)	0.80	NA
Colon cancer								
IVW (random effects)	1.04	(0.89–1.21)	0.64	<0.01	1.06	(0.91–1.24)	0.44	<0.01
MR-Egger (slope)	1.00	(0.80–1.25)	0.99	0.64	1.07	(0.87–1.32)	0.52	0.91
Weighted median	0.93	(0.73–1.18)	0.53	NA	1.09	(0.87–1.37)	0.43	NA
Distal colon cancer								
IVW (random effects)	1.01	(0.83–1.23)	0.93	<0.01	1.16	(0.96–1.40)	0.12	<0.01
MR-Egger (slope)	0.95	(0.71–1.27)	0.71	0.55	1.15	(0.89–1.49)	0.27	0.97
Weighted median	0.98	(0.74–1.29)	0.88	NA	1.41	(1.01–1.96)	0.04	NA
Proximal colon cancer								
IVW (random effects)	1.07	(0.89–1.29)	0.45	<0.01	0.99	(0.82–1.20)	0.91	<0.01
MR-Egger (slope)	1.05	(0.81–1.36)	0.72	0.81	1.00	(0.77–1.29)	0.98	0.93

(Continued on the following page)

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Table 4. MR estimates for the effect of total testosterone, free testosterone, and sex hormone binding globulin (SHBG) on colorectal cancer risk. (Cont'd)

	Women				Men			
Methods	**OR[a]**	**95% CI**	***P* value**	***P* value for pleiotropy or heterogeneity**	**OR[a]**	**95% CI**	***P* value**	***P* value for pleiotropy or heterogeneity**
Weighted median	0.93	(0.68–1.28)	0.65	NA	0.90	(0.68–1.19)	0.47	NA
Rectal cancer								
IVW (random effects)	1.06	(0.86–1.30)	0.58	<0.01	1.09	(0.92–1.31)	0.32	<0.01
MR-Egger (slope)	0.84	(0.63–1.13)	0.25	0.03	1.06	(0.84–1.36)	0.61	0.74
Weighted median	0.89	(0.63–1.27)	0.53	NA	0.92	(0.70–1.21)	0.55	NA

Note: *P* value for pleiotropy in MR-Egger regression; *P* value for heterogeneity in IVW analysis.
[a]ORs per 1-SD increment in total testosterone concentrations in both women and men, per 1-unit increment (log scale) in free testosterone concentrations in women and per 1-SD increment in men, and per 1-SD increment in SHBG concentrations in women and per 1-unit increment (log scale) in men.

steroid hormones and risk of colorectal cancer; consequently, potential nonlinear effects could not be examined.

In conclusion, our complementary observational and MR analyses did not support causal associations of circulating SHBG and free testosterone concentrations with colorectal cancer risk. For total testosterone, our MR analyses found positive associations with colorectal cancer among women only; however, we identified some evidence of pleiotropy that may have biased this result indicating the influence of independent biological pathways. Additional experimental studies are required to better understand the possible role of androgens in colorectal cancer development.

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Authors' Contributions

N. Dimou: Formal analysis, validation, methodology, writing–original draft. **N. Mori:** Writing–review and editing. **S. Harlid:** Writing–review and editing. **J. Harbs:** Writing–review and editing. **R.M. Martin:** Writing-review and editing. **K. Smith-Byrne:** Writing–review and editing. **N. Papadimitriou:** Writing–review and editing. **D.T. Bishop:** Writing–review and editing. **G. Casey:** Writing–review and editing. **S.M. Colorado-Yohar:** Writing–review and editing. **M. Cotterchio:** Writing–review and editing. **A.J. Cross:** Writing–review and editing. **L. Le Marchand:** Writing–review and editing. **Y. Lin:** Writing–review and editing. **K. Offit:** Writing–review and editing. **N.C. Onland-Moret:** Writing–review and editing. **U. Peters:** Writing–review and editing. **J.D. Potter:** Writing–review and editing. **T.E. Rohan:** Writing–review and editing. **E. Weiderpass:** Writing–review and editing. **M.J. Gunter:** Conceptualization, resources, supervision, funding acquisition, validation, methodology, writing–original draft. **N. Murphy:** Conceptualization, resources, formal analysis, supervision, funding acquisition, validation, methodology, writing–original draft.

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