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Endogenous Levels of Circulating Androgens and Risk of Crohn's Disease and Ulcerative Colitis Among Women: A Nested Case-Control Study From the Nurses' Health Study Cohorts

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Data sharing: Requests for access to data, statistical code, questionnaires, and technical processes may be made by contacting the corresponding author at hkhalili@mgh.harvard.edu.

Authors Contributions

HK - study concept and design; acquisition of data; statistical analysis; interpretation of data; drafting of the manuscript;

ANA- acquisition of data; critical revision of the manuscript.

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Abstract

Background—Androgens, which are known to be altered by exogenous hormone use have recently been linked to alterations of the gut microbiome and mucosal immune function. No study has evaluated the association between circulating levels of androgens and risk of Crohn’s disease (CD) and ulcerative colitis (UC).

Methods—We conducted a nested case-control study of women enrolled in the Nurses’ Health Study (NHS) and NHSII who provided a blood specimen. Cases of CD and UC were each matched to two controls. Pre-diagnosis plasma levels of dehydroepiandrosterone sulfate (DHEAS), testosterone, and sex hormone binding globulin (SHBG) were measured. We examined the association of each analyte with risk of CD or UC using conditional logistic regression models.

Results—Compared to women in the lowest quintile of testosterone, the multivariable-adjusted odds ratios (ORs) for CD were 0.86 (95% CI, 0.39–1.90) for women in the second quintile; 0.49 (95% CI, 0.21–1.15) for the third quartile; 0.22 (0.08–0.65) for the fourth quintile and 0.39 (95% CI, 0.16–0.99) for the highest quintile ($P_{\text{linear trend}} = 0.004$). In contrast, we did not observe a consistent association between prediagnostic testosterone and risk of UC ($P_{\text{linear trend}} = 0.84$). We also did not observe any association between plasma levels of SHBG or DHEAS and risk of UC or CD (all $P_{\text{linear trends}} > 0.10$).

Conclusion—Among women, pre-diagnostic circulating testosterone is associated with a lower risk of CD but not UC. Further studies to understand the biological mechanisms by which endogenous androgens may mediate the etiopathogenesis of CD are warranted.

Keywords

Inflammatory Bowel Disease; Crohn’s Disease; Ulcerative Colitis; Androgens; Testosterone; Nurses’ Health Study

INTRODUCTION

Crohn’s disease (CD) and ulcerative colitis (UC), collectively known as inflammatory bowel disease (IBD), are chronic inflammatory disorders of the gastrointestinal tract affecting nearly 1.4 million Americans. Although the exact pathophysiology of the disease remains largely unknown, it is thought that IBD occurs as a result of inappropriate immune response to the intestinal microbiome in genetically susceptible individuals. (1) In turn the composition of human gut microbiome appears to be closely linked to environmental factors such as diet, stress, and medications. (2–4)

Genetic studies have identified a number of common variants associated with risk of CD and UC. (5–7) Collectively, the contribution of these variants to risk of IBD is modest. Therefore identification of novel exogenous and endogenous factors with potential biologic links to human immune function and/or the microbiome will likely better shape our overall understanding of the complex interplay of the environment and genetics on risk of CD and

UC. An association between oral contraceptives (OC) and risk of CD has been consistently demonstrated. (8) Recently, we confirmed this association in two prospective cohorts of women. (9) In addition, we have also shown an association between use of menopausal hormone therapy (MHT) and risk of UC. (10) Although the exact biologic mechanism underlying these associations is unknown, these medications are known to influence endogenous circulating levels of sex hormones. (11–13) In turn, compelling data have linked these hormones to immune function and/or gut microbiome composition and function. (14, 15) To date, no studies have evaluated the association between endogenous sex hormones and risk of CD or UC.

We therefore sought to examine the association between prediagnostic levels of circulating androgens and risk of CD and UC in two large ongoing prospective cohort studies of U.S. women, the Nurses' Health Study (NHS) and NHSII. With more than 20 years of biennially updated and validated data on use of OC, menopausal hormones, diet, and medical diagnoses as well as archived blood specimens, these cohorts offered us the unique opportunity to examine the association between prediagnostic measures of plasma androgens and subsequent risk of CD and UC.

METHODS

Study Population

The NHS is a prospective cohort that began in 1976 when 121,700 U.S. female registered nurses, ages 30 to 55 years, completed a mailed questionnaire. Follow-up questionnaires are mailed every two years to update health information. In 1989, a parallel cohort, the NHS II, enrolled 116,430 U.S. female nurses between the ages of 25–42 years. These women have been followed with similar biennial questionnaires. Follow-up for these participants has consistently exceeded 95%.

In 1989–1990, 32,826 NHS participants (aged 43–69 years) returned a blood sample on ice packs by overnight courier and completed a short questionnaire. (16) Between 1996 and 1999, 29,611 NHSII participants (aged 32–54 years) provided blood samples and completed a short questionnaire in a similar protocol. (17) Blood samples were processed upon receipt and subsequently stored in the vapor phase of liquid nitrogen freezers. Our study is a nested case-control study of women with available blood samples in the two large prospective cohorts of NHS and NHSII. Institutional review board at the Brigham and Women's Hospital approved this study.

Ascertainment of Cases and Controls

We have previously detailed our methods for confirming self-reported cases of CD and UC. (18–20) In brief, since 1976, participants have reported diagnoses of UC or CD through an open-ended response on biennial surveys. In addition, participants were asked about diagnoses of UC since 1982 and CD since 1992. In NHS II, we have specifically queried participants about diagnoses of both CD and UC since 1993. When a diagnosis was reported on any biennial questionnaire, a supplementary questionnaire and related medical records were requested and reviewed by two gastroenterologists blinded to exposure information.

We excluded participants who subsequently denied either the diagnosis on the supplementary questionnaire or permission to review their records. Data were extracted on diagnostic tests, histopathology, anatomic location of disease, and disease behavior. Using standard criteria,(21–24) UC diagnosis was based on a typical clinical presentation for 4 weeks and endoscopic or surgical pathological specimen consistent with UC (e.g. evidence of colitis). CD diagnosis was based on a typical clinical history for 4 weeks and endoscopy or radiologic evaluation demonstrating small bowel findings, or surgical findings consistent with CD combined with pathology suggesting transmural inflammation or granuloma. Disagreements were resolved through consensus. Among those women whom we received adequate medical records, the case confirmation rate for IBD was 78%.(19)

From among participants who provided a blood specimen, we matched 83 CD cases (NHS = 59, NHSII = 24) and 91 UC cases (NHS = 59, NHSII = 32) that were diagnosed after blood collection to two controls on age, menopausal status at the time of blood collection, month of blood collection, fasting status, and use of menopausal hormone therapy at the time of blood collection. In NHSII, premenopausal blood samples that were timed to the luteal phase of the menstrual cycle were also matched on the day of the luteal phase (date of next period minus date of blood draw). Consistent with prior studies, we identified and excluded two outliers: one control with a DHEAS < 15 ug/dL and one control with a testosterone = 4.5 ng/dL. (25) Thus, our final analysis included 83 CD cases matched to 165 controls and 91 UC cases matched to 181 controls.

Ascertainment of Exposures

Our primary exposures of interest were androgens, which included testosterone, dehydroepiandrosterone sulfate (DHEAS), and sex hormone binding globulin (SHBG). All laboratory assays were conducted by personnel blinded to case-control status at the Mayo Clinic (Rochester MN, USA). Samples were assayed for testosterone in three batches by liquid chromatography-tandem mass spectrometry; DHEAS and SHBG were measured by a solid-phase, chemiluminescent enzyme immunoassay (Siemens Healthcare Diagnostics, Deerfield, IL, USA). Masked replicate quality control samples (10% of the samples) were included in each batch to assess coefficients of variation (CVs). Overall CVs within batches were 6.4% to 9.6% for DHEAS, 4.0% to 5.1% for testosterone, and 4.3% to 6.3% for SHBG. Free testosterone was calculated using the formula described by Södergard *et al.* (26)

Assessment of Other Covariates

On each biennial questionnaire, women were asked about pertinent lifestyle factors, including body weight, smoking status, parity, and use of OCs and menopausal hormone therapy. Participants' self-report of body weight, height, and use of oral contraceptives have been previously validated. (27, 28) At baseline, we collected data on age at menarche in both cohorts. On each questionnaire, menopausal status was determined by asking whether the participants' menstrual periods had ceased permanently and, if so, at what age and for what reason (occurring naturally or after radiation therapy or surgery). If menopause was due to surgery, the participant was asked to report the number of ovaries removed. Self-

reported type of menopause and age at time of menopause was highly accurate compared to medical records. (29)

Information on physical activity was also collected every 2–4 years. Participants' self-report of physical activity and use of OC have been previously validated. (27, 28) Intake of dietary vitamin D was assessed using validated, self-administered, semi- quantitative food frequency questionnaires (FFQs) administered in 1991, 1995, 1999 in NHSII and 1984, 1986, 1990 in NHS prior to blood collections. For these analyses, data on weight, and menopause status were taken directly from the short questionnaires administered at the time of blood collection; data from other covariates were obtained from the general questionnaires that were completed closest to the time of blood draw.

Statistical Analysis

We examined the possibility that there is a non-linear association between androgens and risk of CD and UC using a previously reported non-parametric cubic spline method. (30) This method is unique in allowing for controlling for covariates. It also allows stepwise selection among spline variables. The output is the set of p-values from the likelihood ratio tests for non-linearity, a linear relation, and any relation, as well as a graph of the predicted incidence rate, with or without its confidence band. Using this method the likelihood ratio test comparing models with linear terms with those with spline terms were not statistically significant (All $P_{\text{comparisons}} > 0.40$) indicating that the relationship between androgens and risk of CD and UC is linear. As these analyses are particularly sensitive to outliers we performed further sensitivity analyses removing observations beyond 3 interquartile ranges. In these analyses, the likelihood ratio test comparing models with linear terms with those with spline terms continue to not be statistically significant (All $P_{\text{comparisons}} > 0.30$). Based on these findings, we used continuous and quintile categories of androgens in our main analyses.

We calculated cut offs for quintiles based upon the distribution of each analyte within controls separately for each cohort (NHS and NHSII) to account for differences in the distribution of menopausal status between studies. We used conditional logistic regression to estimate the odds ratio (OR) and 95% confidence interval (CI). Multivariable analyses were adjusted for physical activity, body mass index (BMI), total dietary vitamin D intake, OC use, parity, menopause status (include type of menopause), cohort, and smoking as these variables have previously been associated with CD or UC or may influence the levels of androgens. We did not include use of non-steroidal inflammatory drugs, appendectomy, age at menarche, and age at menopause as these variables did not alter our effect estimates. We also evaluated if the association between androgens and risk of CD and UC varied by menopause status (premenopausal, postmenopausal), current menopausal hormone therapy (yes, no), BMI (< 25 , > 25 kg/m²), and OC use (never, ever).

We used SAS version 9.3 (Cary, NC) for these analyses. All *P*-values were 2-sided and < 0.05 was considered statistically significant.

RESULTS

Within both cohorts, our analysis included 83 cases of CD matched to 165 controls and 91 cases of UC matched to 181 controls with prediagnostic blood specimens (Table 1). The mean time between blood collection and diagnosis of either CD or UC or index date for controls was 5.0 years (SD = 5.1). At the time of blood collection, the age range was 36 to 68 years for the CD case-control set and 36 to 69 years for the UC case-control set. There were no significant differences in baseline characteristics of CD and UC cases with their matched controls (Table 1). The median concentration of testosterone was significantly lower in CD cases (16.0 ng/dl) compared to controls (19.0 ng/dl, $P_{\text{difference}} = 0.01$) (Table 1). There were no significant differences in plasma concentration of SHBG and DHEAS comparing cases of CD to controls (all $P_{\text{difference}} > 0.30$). Comparing UC cases to their matched controls, there were no significant differences in plasma concentrations of any androgens (all $P_{\text{difference}} > 0.40$).

Compared to matched controls, the risk of CD decreased with higher plasma levels of testosterone ($P_{\text{trend}} = 0.004$) (Table 2). Specifically, compared to the lowest quintile of plasma levels of testosterone the multivariable-adjusted ORs of CD were 0.86 (95% CI, 0.39–1.90) for the second quintile, 0.49 (95% CI, 0.21–1.15) for the third quintile, 0.22 (95% CI, 0.08–0.65) for the fourth quintile, and 0.39 (95% CI, 0.16–0.99) for the highest quintile. (Table 2). Although the risk of CD appeared to decrease with higher plasma levels of SHBG, the trend did not reach statistical significance ($P_{\text{trend}} = 0.11$). We also evaluated the effect of total plasma testosterone on risk of CD using plasma testosterone as a continuous variable and found that for every 10 ng/ml increase in plasma levels of testosterone the risk of CD decreases by 15% (OR 0.85, 95% CI 0.74–0.97).

As the level of free testosterone is strongly influenced by SHBG, the inverse association between testosterone and risk of CD was significantly attenuated in analysis of free testosterone, calculated from SHBG and total testosterone, and risk of CD ($P_{\text{trend}} = 0.24$). In addition, we did not find an association between plasma levels of DHEAS ($P_{\text{trend}} = 0.46$) and risk of CD.

We explored the possibility that changes in endogenous hormones due to subclinical disease may explain our observed association. Thus, we repeated our analyses after excluding cases of CD in whom blood samples were collected less than 2 years prior to diagnosis. Compared to women in the lowest quintile of plasma testosterone level, the multivariable-adjusted ORs of CD were 0.68 (95% CI, 0.24–1.89) among women in the second quintile, 0.24 (95% CI, 0.07–0.84) among women in the third quintile, 0.10 (95% CI, 0.02–0.49) among women in the fourth quintile, and 0.42 (95% CI, 0.14–1.27) among women in the highest quintile ($P_{\text{trend}} = 0.02$).

In contrast, plasma levels of testosterone or free testosterone were not associated with risk of UC ($P_{\text{trend}} = 0.84$ and 0.25, respectively) (Table 3). Although, the risk of UC appeared to increase with increasing plasma levels of SHBG, the trend did not reach statistical significance ($P_{\text{trend}} = 0.09$). Compared to the women in the lowest quintile of plasma levels of SHBG, the multivariable-adjusted ORs of UC were 1.00 (95% CI, 0.44–2.28) among

women in the second quintile, 1.43 (95% CI, 0.55–3.69) among women in the third quintile, 1.01 (95% CI, 0.39–2.58) among women in the fourth quintile, and 2.80 (95% CI, 1.05–7.49) among women in the highest quintile. We did not find an association between plasma levels of DHEAS ($P_{\text{trend}} = 0.41$) and risk of UC.

We explored the possibility that the association of testosterone with risk of CD may be modified by other putative risk factors (Table 4). The association of plasma testosterone and risk of CD did not appear to be modified by OC use, menopausal status, or BMI (all $P_{\text{interactions}} > 0.30$). Although the effect of circulating testosterone on risk of CD appeared to be modified by use of MHT, a formal statistical test for interaction did not reach statistical significance ($P_{\text{interaction}} = 0.06$). Specifically, among postmenopausal women who were not using MHT at the time of blood draw, the multivariable-adjusted OR of CD for every 10 ng/dL increase in testosterone was 0.66 (95% CI, 0.45–0.95). In contrast, among postmenopausal women who were using MHT at the time of blood collection, the multivariable-adjusted OR of CD for every 10 ng/dL increase in testosterone was 1.17 (95% CI, 0.88–1.56). As levels of endogenous testosterone changes significantly with age, we also assessed whether the effect of testosterone on risk of CD varies according to age as a continuous variable and observed no effect modification ($P_{\text{interaction}} = 0.62$). We could not evaluate the possibility that smoking may modify the effect of androgens on risk of CD due to low number of CD cases among smokers ($n = 13$). However, among never smokers the multivariable-adjusted OR of CD for every 10 ng/dL increase in testosterone was 0.63 (95% CI, 0.48–0.83). The association of testosterone on risk of UC did not vary by menopausal status, BMI, or use of MHT or OCs (Table 4).

DISCUSSION

In this large prospective, nested case-control study, we observed that higher levels of prediagnostic plasma testosterone are associated with lower risk of CD but not UC. In addition, there was a suggestion that the association was more evident among individuals who did not concurrently use MHT. There was also a trend for a direct association between plasma levels of SHBG and risk and UC as well as an inverse association between plasma levels of SHBG and risk of CD. To our knowledge no prior study has investigated the link between endogenous levels of plasma androgens and risk of CD and UC. A prior study from our cohort has shown an association between OC use and risk of CD, which has been supported by many other studies. (8, 9) OC use has been shown to reduce endogenous levels of testosterone. (11, 13) Thus, the consistent association of OC and risk of incident CD may be mediated by the biological consequences of lower circulating testosterone. Moreover, the apparent benefit of higher circulating testosterone on risk of CD may be attenuated by the use of high doses of exogenous estrogen in the form of MHT.

Although our understanding of the pathogenesis of CD and UC remains incomplete, the discovery of distinct genetic susceptibility loci for both diseases points to potential diverging biological pathways that may be differentially influenced by endogenous levels of testosterone. (5) In addition, CD and UC have immunologically distinct gastrointestinal mucosal cytokine profiles, with mucosal inflammation in CD primarily mediated by Th1-related cytokines and UC mediated by Th2-related cytokines. The potential differential

effect of sex hormones including estrogen and testosterone on Th1- and Th2-mediated disease processes may partially explain the specific link between endogenous testosterone and risk of CD. In particular, testosterone has been shown to modulate immune function, including cytokine production. In animal models, endogenous levels of testosterone are linked to reduction in expression of Toll-like receptor 4 (TLR4) on macrophages, which play a fundamental role in pathogen recognition and innate immunity. (15) In addition, recent animal data suggest that gut commensal microbes may modulate levels of endogenous testosterone, leading to development of autoimmune diseases. (14) Thus, our observed association between endogenous testosterone and development of CD may be biologically mediated by a complex interaction between endogenous hormones, the gut microbiome, and immune function.

Previously, we have shown an association between MHT and increased risk of UC. (10) Although the exact biologic mechanism of this is unknown, estrogen compounds modify colonic barrier function, (31, 32) which is a biologic pathway that may be uniquely related to pathogenesis of UC. (33) Thus, modification of distinct biologic processes by OC use compared with MHT may have potentially divergent effects on the pathogenesis of CD and UC. Of note, the apparent lack of association between free testosterone and risk of CD in our study is likely explained by the significant influence of plasma levels of SHBG on free testosterone levels with higher SHBG levels being associated with lower free testosterone.

Our study has several notable strengths. First, the availability of detailed and validated information on BMI, use of OC or MHT, physical activity, smoking, and other reproductive factors allowed us to control for a number of potential confounders that may affect our observed associations. Second, we have confirmed all of the cases of CD and UC through medical record review, an advantage over studies that rely on self-reported or diagnoses codes that may not be accurate. Third, blood samples were collected prior to diagnosis of CD and UC allowing us to assess pre-diagnosis levels of androgens in relation to risk of disease.

We acknowledge several limitations. First, plasma levels of androgens were based on a single measurement that may not be an accurate reflection of long-term endogenous androgen levels. However, the intraclass correlation over 3 years for these androgens in luteal phase ranged from 0.73 (for testosterone) to 0.89 (for SHBG and DHEAS) in premenopausal women (34) and from 0.88 (for testosterone and DHEAS) to 0.92 (for SHBG) in postmenopausal women (35), suggesting that a single measurement reasonably reflects longer-term levels. Second, unlike NHSII, blood samples collected from premenopausal women in NHS were not timed according to menstrual phase (luteal vs. follicular). However, androgen levels do not vary substantially by menstrual phase (36, 37). In addition, our findings were consistent across the two cohorts of NHS and NHSII and premenopausal cases accounted for only 30% of all cases and controls. Third, we acknowledge that because of our small number of cases, we had limited power to detect more modest associations (e.g. SHBG). In addition, because of our sample size, the results from our stratified analyses that endogenous levels of androgens may modulate the effect of exogenous hormones on risk of CD should be interpreted with caution. Finally, only 10 participants (controls and cases) were taking oral contraceptives at the time of blood

collection and therefore we were unable to fully evaluate the hypothesis that oral contraceptives through modulating the endogenous level of androgens alter risk of developing CD. We estimated that at least 40% of our participants would have had to be on oral contraceptives at the time of blood collection in order for us to have nearly 80% power to detect the potential mediation effect of testosterone on the associations between oral contraceptives and risk of CD.

In conclusion, we show that pre-diagnosis levels of circulating total testosterone are associated with a lower risk of CD but not UC among women. This finding may, at least in part, explain the consistent association observed between use of OC and subsequent risk of CD. Although the exact mechanism underlying the association between exogenous and endogenous sex hormones and risk of CD is largely unknown, we believe that these initial studies justify the need for further translational studies carried out at the intersection of epidemiology, sex hormones, the gut microbiome, and immune function. In particular, further investigation into evaluating the complex interaction between exogenous and endogenous sex hormones and community structure of human gut microbiome on risk and progression of CD are warranted. Finally, whether exogenous and endogenous sex hormones also play a role in IBD progression is yet to be determined and is the topic of future research.

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Table 1

Baseline Characteristics of Cases and Controls at the Time of Blood Collection*

	Crohn's disease		P _{difference} [†]	Ulcerative colitis		P _{difference} [†]
	Cases N = 83	Controls N = 165		Cases N = 91	Controls N = 181	
Cohort, %**						
NHS	71	71	0.98	65	65	0.97
NHSII	29	29		35	35	
Age (yrs), mean (std) **	53 (8)	53 (8)	0.99	52 (8)	52 (8)	0.92
Body mass index (kg/m ²)	24.4 (4.9)	25.1 (5.3)	0.20	25.5 (5.1)	24.5 (5.3)	0.11
Physical activity (Met-hr/wk)	11.1 (17.4)	10.6 (15.6)	0.58	10.3 (17.2)	11.0 (17.2)	0.51
Smoking, %			0.34			0.58
Never	48	56		37	47	
Past	35	33		48	44	
Current	17	11		15	9	
Oral contraceptive use, %	59	65	0.33	71	65	0.34
Premenopause, %**	31	32	1.00	37	38	1.00
Menopausal hormone therapy [‡] , %						
Never	47	46		40	41	
Past	35	36		40	40	
Current	18	18		20	19	
Parity	2.0 (1.6)	3.0 (1.8)	0.07	3.0 (1.7)	3.0 (1.6)	0.43
Vitamin D intake (IU/daily)	300 (231)	324 (267)	0.30	293 (238)	298 (226)	0.87
Testosterone (ng/dL)	16.0 (10.8)	19.0 (13.4)	0.01	20.0 (10.2)	19.0 (9.0)	0.85
SHBG (nmol/L)	57.7 (52.7)	65.7 (46.4)	0.36	66.7 (53.7)	64.6 (40.9)	0.42
DHEAS (ug/dL)	57.0 (38.2)	63.0 (52.3)	0.99	62.0 (49.6)	62.0 (56.6)	0.53

* Unless otherwise noted variables are presented as median. Abbreviations: Nurses' Health Study (NHS), standard deviation (std), sex hormone binding globulin (SHBG), and Dehydroepiandrosterone (DHEAS).

** Matching factor.

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Represents percentages among post-menopausal women.

† Difference was estimated by chi-square test for categorical variables and Wilcoxon-sum rank test for contiguous variables.

Table 2
Risk of Crohn's Disease According to Quintiles of Plasma Androgen Concentrations*

	Q1	Q2	Q3	Q4	Q5	P _{trend} [†]
Testosterone, ng/dL						
Median (range), NHS	10 (7–11)	13 (12–15)	17 (16–19)	23 (20–25)	33 (26–64)	
Median (range), NHSII	12 (10–15)	19 (17–20)	22 (21–25)	31 (26–34)	50 (35–99)	
Cases/controls	23/31	24/36	16/34	7/30	13/33	
Unadjusted OR	1.00	0.88 (0.41–1.87)	0.61 (0.27–1.36)	0.32 (0.12–0.85)	0.52 (0.22–1.24)	0.02
Adjusted OR [‡]	1.00	0.86 (0.39–1.90)	0.49 (0.21–1.15)	0.22 (0.08–0.65)	0.39 (0.16–0.99)	0.004
DHEAS, µg/dL						
Median (range), NHS	16 (15–23)	37 (24–45)	53 (46–62)	72 (63–87)	102 (88–210)	
Median (range), NHSII	53 (19–56)	66 (61–75)	90 (76–106)	133 (117–163)	197 (167–329)	
Cases/controls	21/31	18/36	17/32	11/33	16/32	
Unadjusted OR	1.00	0.72 (0.30–1.72)	0.78 (0.35–1.75)	0.51 (0.21–1.20)	0.74 (0.30–1.83)	0.30
Adjusted OR [‡]	1.00	0.55 (0.22–1.38)	0.68 (0.29–1.60)	0.41 (0.16–1.09)	0.83 (0.32–2.20)	0.46
SHBG, mmol/L						
Median (range), NHS	29.1 (17.3–38.1)	46.8 (39.8–53.5)	64.3 (55.9–77.3)	89.5 (77.4–120.0)	143.0 (124.0–278.0)	
Median (range), NHSII	24.4 (4.8–34.6)	44.7 (35.0–56.6)	66.1 (57.8–71.1)	80.4 (72.1–89.8)	131.0 (91.0–197.0)	
Cases/controls	22/33	15/33	18/34	11/33	17/32	
Unadjusted OR	1.00	0.68 (0.30–1.51)	0.81 (0.37–1.77)	0.50 (0.21–1.17)	0.75 (0.32–1.80)	0.33
Adjusted OR [‡]	1.00	0.48 (0.20–1.15)	0.51 (0.21–1.27)	0.25 (0.08–0.73)	0.53 (0.20–1.41)	0.11

* Abbreviations: Odds ratio (OR), Sex hormone binding globulin (SHBG), and Dehydroepiandrosterone (DHEAS).

[‡] Adjusted for body mass index (kg/m²), cohort (NHS, NHSII), smoking (never, past, current), cumulative physical activity (MET-hr/wk), total vitamin D intake (IU/daily), parity, menopause status (premenopause, natural menopause, and menopause from surgery), menopausal hormone therapy (never, past, and current), and oral contraceptive use (never, ever).

[†] P_{trend} was estimated by entering the median value of testosterone for each quintile category.

Table 3
Risk of Ulcerative Colitis According to Quintiles of Plasma Androgen Concentrations*

	Q1	Q2	Q3	Q4	Q5	P _{trend} [†]
Testosterone, ng/dL						
Median (range), NHS	10 (7–11)	13 (12–15)	17 (16–19)	23 (20–25)	33 (26–64)	
Median (range), NHSII	12 (10–15)	19 (17–20)	22 (21–25)	31 (26–34)	50 (35–99)	
Cases/controls	22/32	17/43	17/33	12/36	23/37	
Unadjusted OR	1.00	0.53 (0.23–1.24)	0.70 (0.31–1.61)	0.48 (0.20–1.14)	0.84 (0.37–1.89)	0.75
Adjusted OR [‡]	1.00	0.63 (0.26–1.52)	0.81 (0.34–1.92)	0.55 (0.22–1.34)	0.91 (0.39–2.13)	0.84
DHEAS, µg/dL						
Median (range), NHS	16 (15–23)	37 (24–45)	53 (46–62)	72 (63–87)	102 (88–210)	
Median (range), NHSII	53 (19–56)	66 (61–75)	90 (76–106)	133 (117–163)	197 (167–329)	
Cases/controls	22/36	12/37	23/36	17/37	17/35	
Unadjusted OR	1.00	0.55 (0.24–1.24)	1.05 (0.51–2.16)	0.75 (0.34–1.67)	0.79 (0.34–1.82)	0.75
Adjusted OR [‡]	1.00	0.50 (0.21–1.15)	0.95 (0.45–2.01)	0.62 (0.27–1.45)	0.62 (0.26–1.52)	0.41
SHBG, mmol/L						
Median (range), NHS	29.1 (17.3–38.1)	46.8 (39.8–53.5)	64.3 (55.9–77.3)	89.5 (77.4–120.0)	143.0 (124.0–278.0)	
Median (range), NHSII	24.4 (4.8–34.6)	44.7 (35.0–56.6)	66.1 (57.8–71.1)	80.4 (72.1–89.8)	131.0 (91.0–197.0)	
Cases/controls	16/35	18/37	18/36	13/37	26/36	
Unadjusted OR	1.00	1.05 (0.47–2.31)	1.17 (0.49–2.82)	0.79 (0.33–1.89)	1.93 (0.79–4.70)	0.35
Adjusted OR [‡]	1.00	1.00 (0.44–2.28)	1.43 (0.55–3.69)	1.01 (0.39–2.58)	2.80 (1.05–7.49)	0.09

* Abbreviations: Odds ratio (OR), Sex hormone binding globulin (SHBG), and Dehydroepiandrosterone (DHEAS).

[‡] Adjusted for body mass index (kg/m²), cohort (NHS, NHSII), smoking (never, past, current), cumulative physical activity (MET-hr/wk), total vitamin D intake (IU/daily), parity, menopause status (premenopause, natural menopause, and menopause from surgery), menopausal hormone therapy (never, past, and current), and oral contraceptive use (never, ever).

[†] P_{trend} was estimated by entering the median value of testosterone for each quintile category.

Table 4
Risk of Crohn's Disease and Ulcerative Colitis According to Plasma Testosterone Concentrations in Selected Strata*

	Crohn's Disease			Ulcerative Colitis		
	Cases/Controls	MV-adjusted [†]	P _{interaction}	Cases/Controls	MV-adjusted [†]	P _{interaction}
Cohorts						
NHS + NHSII	83/164	0.85 (0.74-0.97)	0.64	91/181	1.03 (0.89-1.19)	0.33
NHS	59/116	0.86 (0.72-1.02)		59/117	1.08 (0.89-1.31)	
NHSII	24/48	0.77 (0.57-1.02)		32/64	0.90 (0.69-1.17)	
Menopausal status at blood collection			0.56			0.64
Pre-menopausal	26/52	0.74 (0.54-1.02)		34/68	1.05 (0.83-1.31)	
Post-menopausal	47/92	0.87 (0.74-1.03)		46/91	1.01 (0.82-1.25)	
Menopausal hormone therapy at blood collection			0.06			0.86
No	27/52	0.66 (0.45-0.95)		23/46	0.94 (0.61-1.43)	
Yes	20/40	1.17 (0.88-1.56)		23/45	0.94 (0.65-1.37)	
Oral contraceptive use			0.76			0.86
Never	34/56	0.83 (0.61-1.13)		26/62	1.07 (0.73-1.58)	
Ever	49/108	0.78 (0.62-0.97)		65/119	0.89 (0.73-1.08)	
Body mass index			0.39			0.07
< 25 kg/m ²	47/80	0.86 (0.66-1.11)		43/98	1.28 (0.93-1.76)	
≥ 25 kg/m ²	36/84	0.76 (0.55-1.06)		48/83	0.81 (0.59-1.13)	

* Odds ratios are calculated for every 10 ng/dl increase in plasma levels of testosterone.

[†] Adjusted for age (years), body mass index (kg/m²), cohort (NHS, NHSII), smoking (never, past, current), cumulative physical activity (MET-hr/wk), total vitamin D intake (IU/daily), parity, fasting status, month of blood draw, menopausal status (pre-menopausal, non-user of menopausal hormone therapy, current user of menopausal hormone therapy, missing/unknown) and oral contraceptive use (never, ever).