

Premenopausal Circulating Androgens and Risk of Endometrial Cancer: results of a Prospective Study

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Abstract Endometrial cancer risk is increased by estrogens unopposed by progesterone. In premenopausal women, androgen excess is often associated with progesterone insufficiency, suggesting that premenopausal androgen concentrations may be associated with risk. In a case–control study nested within three cohorts, we assessed the relationship between premenopausal androgens and risk of endometrial cancer (161 cases and 303 controls matched on age and date of blood donation). Testosterone, DHEAS, androstenedione, and SHBG were measured in serum or plasma. Free testosterone was calculated from testosterone and SHBG. We observed trends of increasing risk across tertiles of testosterone ($OR_{T3-T1} = 1.59$, 95 % CI=0.96, 2.64, $p=0.08$) and free testosterone ($OR_{T3-T1} = 1.76$, 95 % CI=1.01, 3.07, $p=0.047$), which were not statistically significant after adjustment for body mass index (BMI). There was no association for DHEAS, androstenedione, or SHBG. There were significant interactions by age at diagnosis (<55 years, $n=51$ cases; ≥ 55 years, $n=110$ cases). Among

women who were ≥ 55 years of age (predominantly postmenopausal) at diagnosis, the BMI-adjusted OR was 2.08 (95 % CI=1.25, 3.44, $p=0.005$) for a doubling in testosterone and 1.55 (95 % CI=1.04, 2.31, $p=0.049$) for a doubling in free testosterone. There was no association among women aged <55 years at diagnosis, consistent with the only other prospective study to date. If pre- and post-menopausal concentrations of androgens are correlated, our observation of an association of premenopausal androgens with risk among women aged ≥ 55 years at diagnosis could be due to the effect on the endometrium of postmenopausal androgen-derived estrogens in the absence of progesterone, which is no longer secreted.

Introduction

The different effects of the various types of hormonal contraceptives (sequential/combined/progestin-only) and hormone

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replacement therapy (estrogen-only/estrogen + progestin) on risk of endometrial cancer have long provided support to the hypothesis that exposure to estrogen unopposed by progesterone is an important contributor to endometrial cancer risk [1, 2]. The proposed mechanism involves proliferation of epithelial cells in the endometrium, which results in increased potential for accumulation of genetic errors: estrogens stimulate endometrial cell proliferation but this action is counteracted by progesterone/progestins. As would be expected under this mechanism, risk of endometrial cancer has been shown to be positively associated with circulating estrogen concentrations in postmenopausal women, who have very low or undetectable progesterone levels [3–6]. Circulating androgens, which are the main source of estrogens in postmenopausal women (through aromatization in adipose tissue), have also been shown to be associated with risk [4, 6].

In premenopausal women, the mitotic rate of endometrial cells increases in parallel with the increase in estrogen concentrations observed during the early follicular phase, but then levels off despite further increase in estrogen concentrations. After ovulation at mid-cycle, the concentration of progesterone starts rising and the mitotic rate drops to very low levels, despite estrogen concentrations as high or higher than in the early follicular phase. Based on these and other observations, Key and Pike proposed that, in premenopausal women, progesterone insufficiency rather than estrogen excess contributes to endometrial risk [1]. It is very difficult to study the circulating estrogens and progesterone in premenopausal women because of the large variations in the concentrations of these hormones during the menstrual cycle. Though testosterone may increase slightly during the ovulatory phase [7], this increase appears limited to younger women [8] and other androgens vary little during the menstrual cycle, suggesting that their association with disease risk can be evaluated using a single measurement. The observation that androgen excess is associated with chronic anovulation and consequently with progesterone insufficiency, as observed in polycystic ovary syndrome (PCOS), suggests that premenopausal concentrations of androgens may be positively associated with risk of endometrial cancer [2].

Data on premenopausal androgen concentrations and risk of endometrial cancer are limited and not consistent [6, 9, 10]. One case–control study reported lower concentrations of testosterone among premenopausal cases vs. controls [10], while a second found that circulating androstenedione was higher among cases [9]. The only prospective study to date that assessed prediagnostic concentrations of DHEAS, androstenedione, testosterone, and free testosterone in premenopausal women (55 cases and 107 controls) did not report differences in the concentrations of these hormones between cases and controls (odds ratios not reported) [6]. The purpose of this study was to assess the association of circulating premenopausal concentrations of androgens (total testosterone,

free testosterone, dehydroepiandrosterone sulfate (DHEAS), and androstenedione) and sex hormone binding globulin (SHBG) with risk of subsequent endometrial cancer.

Methods

Study Design and Parent Cohorts

We conducted a nested case–control study in women premenopausal at blood donation in three prospective cohorts: the New York University Women’s Health Study (NYUWHS), the Northern Sweden Health and Disease Study (NSHDS), and the ORDET study in Italy. We reported previously the results of a similar nested case–control study in women postmenopausal at blood donation, and details about the parent cohorts can be found in [4]. Briefly, the NYUWHS includes 14,274 women enrolled in 1985–1991 (ages 35–65 years) at a mammography screening clinic in New York City. Information on lifestyle and reproductive and medical history was obtained through a self-administered questionnaire. The NSHDS continuously enrolls women ages 30–65 years from health screening programs in Northern Sweden (the Västerbotten Intervention Program, VIP and the Mammary Screening Cohort, MSC). Participants complete a self-administered questionnaire on lifestyle at blood donation; cases and controls selected for this study were also asked to complete a retrospective questionnaire about reproductive history. The ORDET cohort enrolled 10,788 healthy women, ages 35–70 years, from the Varese Province in Northern Italy who participated in a breast cancer screening program between 1987 and 1992. Reproductive and health history information was collected through questionnaire and height and weight measured by nurses at enrollment.

Women in all three cohorts donated blood at the time of enrollment, and serum (NYUWHS) or plasma (NSHDS and ORDET) was stored at -80°C . Women who were pregnant, lactating, or using oral contraceptives at the time of blood donation were not eligible to enter the ORDET and NYUWHS cohorts and were excluded from this study for the NSHDS cohort.

The Institutional Review Board of New York University School of Medicine, the Ethical Review Board of the National Cancer Institute of Milan (Italy) and the Regional Ethical Committee of the University of Umeå, Sweden, and the Swedish Data Inspection Board reviewed and approved this study. Participants provided written informed consent.

Definition of Menopausal Status at Blood Donation

Only women premenopausal at blood collection were considered for this study. In the NYUWHS, women were classified as premenopausal if they reported having one menstrual cycle

in the 6 months prior to blood donation. In the NSHDS, women were considered premenopausal if they were ≤ 45 years of age at blood donation and had not had a bilateral oophorectomy or if they reported on a follow-up questionnaire that menopause had occurred after blood donation. For NSHDS participants who did not complete a questionnaire on reproductive history, FSH measurements were performed and women with values < 20 IU/L were considered premenopausal. In ORDET, women who reported > 6 menstrual cycles in the 12 months prior to blood donation were considered premenopausal (women with bilateral oophorectomy were not eligible for entry in the cohort).

Case Ascertainment

In the NYUWHS, endometrial cancer cases were identified through self-report on mailed questionnaires or telephone interviews, followed by review of medical records. This active follow-up was supplemented by linkages with state tumor registries in New York, New Jersey, and Florida and to the National Death Index. For NSHDS and ORDET, cases were identified through linkages with the regional and/or national cancer registries in Sweden and Italy, respectively.

Case–Control Selection

Cases were all women diagnosed with endometrial cancer (International Classification of Disease [ICD] codes: 182.0 to 182.9) during follow-up (irrespective of their menopausal status at diagnosis), excluding women with a history of prior cancer (other than non-melanoma skin cancer). For each case, two controls were selected at random among women who matched the case on cohort (NYUWHS, NSHDS-VIP, NSHDS-MS, and ORDET) and age and date at blood donation (± 6 months). The NYUWHS cases and controls were also matched on day of menstrual cycle. All women in the ORDET cohort donated blood during the luteal phase (days 20–24) of the menstrual cycle. Case–control sets from the NSHDS were not matched on day of menstrual cycle. To be eligible for selection as a control, women had to be alive, without prior hysterectomy or diagnosis of cancer (except non-melanoma skin cancer) at the time of diagnosis of the case. For NYUWHS, we included all cases ascertained up to 2010, while for NSHDS and ORDET cases diagnosed up to December 2006 were included. In total, the study included 161 cases and 303 controls (NYUWHS; 108 cases and 209 controls, NSHDS; 30 cases and 50 controls, and ORDET; 23 cases and 44 controls).

Laboratory Analyses

All assays for NSHDS and ORDET were conducted at the International Agency for Research on Cancer (IARC) in

Lyon, France. Assays for NYUWHS were conducted in two different laboratories: IARC in 2007 (101 cases and 181 controls) and DKFZ in Germany in 2014 (60 cases and 122 controls). At IARC, total testosterone and DHEAS were measured using radioimmunoassays (RIA, Beckman-Coulter, previously Immunotech), androstenedione using a double antibody RIA, and SHBG using an immunoradiometric assay (IRMA, Cis-Bio) [11, 12]. Total testosterone, DHEAS, and SHBG were run in duplicate whereas androstenedione was run singly. At DKFZ, testosterone, DHEAS, and androstenedione were measured with RIAs (Beckman-Coulter) and SHBG with an IRMA (Cis-Bio) using the same kits (IM1119, IM0729, DSL-3800, and SHBG-RIACT) as the IARC laboratory [13]. All measurements were performed in duplicate when sample volume allowed, except for DHEAS, which was measured once. SHBG measurements were not available from 79 women measured at DKFZ due to a laboratory assay error pertaining to the standards (remaining volume was insufficient to re-run this batch). Free testosterone was calculated using mass action equations incorporating the absolute concentrations of testosterone and SHBG [14].

In both laboratories, each case–control set was analyzed together in the same assay batch. Laboratory personnel were blinded to the case–control status of the samples. Blinded quality control samples consisting of duplicate samples or samples from a large pool were interspersed randomly in each batch from NYUWHS and NSHDS to assess laboratory variability. The intra-batch CVs were less than 10 % for all analytes except for total testosterone measured at IARC (intra-batch CV 12 %), and inter-batch CVs were less than 20 %, except for total testosterone IARC measurements (inter-batch CV 22 %).

Statistical Analysis

To reduce departures from the normal distribution, androgens were \log_2 -transformed and SHBG was square-root transformed [15, 16]. Geometric means of hormone concentrations in cases and controls were compared using mixed-effects models, which accounted for the matched design. Conditional logistic regression, appropriate for the matched design, was used to estimate odds ratios (ORs) and 95 % confidence intervals for risk of endometrial cancer. Hormonal and SHBG concentrations were examined both as tertiles and as continuous variables. Tertile cut points were based on the frequency distribution of cases and controls combined and were determined separately within each cohort and laboratory (NYUWHS only). Heterogeneity between cohorts was assessed by comparing models including interaction terms for androgens (or SHBG) and cohort to those without the interaction term. The likelihood ratio test was used to assess statistical significance.

Potential confounders considered for inclusion were BMI at blood donation (continuous, \log_2 scale), age at menarche (continuous), number of full term pregnancies (0, 1–2, 3+), OC use (ever/never), and HRT use prior to index date (ever/never). BMI was the only covariate found to affect the associations of androgens and SHBG with risk by more than 10 %. Models unadjusted and adjusted for BMI are shown. Because insulin resistance is a risk factor for endometrial cancer and is also associated with higher concentrations of androgens, we also examined the effect of adjusting for SHBG, a marker of insulin resistance independent of BMI, on the total testosterone-endometrial cancer risk association (we did not examine free testosterone because of its high correlation with SHBG). Missing data for BMI (0.6 % for NYUWHS, 25 % for NSHDS, and 1.5 % for ORDET) were imputed for each cohort separately using multiple imputation method [17] by using fully conditional specification and including age at sampling (continuous) and age at menarche (categorical, as shown in Table 1) in the model. Analyses including all women (using imputed data for BMI) gave similar results as analyses including only subjects with no missing data for BMI (complete case method), thus we only show the analyses including all women and imputed BMI data. Additional analyses were conducted restricted to women with Type I endometrial cancer ($n=109$ cases) and their matched controls. All endometrial tumors histologically identified as endometrioid or mucinous adenocarcinoma were classified as type I, except those with a tumor grade of 3 or higher. We assessed the effect of androgens on endometrial cancer risk by age at blood donation (<45 vs. ≥ 45 years) and by age at diagnosis (<55 vs. ≥ 55 years) as a surrogate for menopausal status at diagnosis. Analyses restricted to never-users of HRT, and analyses stratified by BMI at enrollment (<25 vs. ≥ 25 kg/m²), were performed using an unconditional logistic regression model adjusted for the matching factors and cohort. Prior to breaking the matching, we verified that odds ratios were not appreciably different for conditional logistic regression models and unconditional logistic regression models adjusted for the matching factors. All p values are two-sided ($p < 0.05$ was used to identify statistical significance). SAS version 9.3 was used for statistical analyses.

Results

Table 1 shows characteristics of the cases and controls. As expected, cases tended to have a younger age at menarche ($p=0.02$) and higher BMI ($p < 0.0001$) than controls. The proportion of women who were nulliparous was higher ($p=0.01$) while ever use of oral contraceptives was less frequent ($p=0.03$) among cases.

The geometric means for biomarkers by case–control status are presented in Table 2. The geometric means of testosterone (1.26 vs. 1.16 nmol/L, $p=0.04$) and free testosterone (0.016

Table 1 Characteristics of cases and controls

	Cases ($N=161$)	Controls ($N=303$)	p value ^a
Age at blood donation			
≤45 years	71 (44.1)	143 (47.2)	
45–50 years	62 (38.5)	103 (34.0)	
≥50 years	28 (17.4)	57 (18.8)	Matched
Age at diagnosis			
<55 years	51 (31.7)		
≥55 years	110 (68.3)		
Lagtime			
≤10 years	54 (33.5)		
>10 years	107 (66.5)		
Age at menarche			
≤12 years	85 (52.8)	127 (42.1)	
12–14 years	43 (26.7)	95 (31.5)	
≥14 years	33 (20.5)	80 (26.5)	0.02
Missing		1	
Height			
≤160 cm	70 (44.3)	145 (48.0)	
>160 cm	88 (55.7)	157 (52.0)	0.57
Missing	3	1	
Weight			
≤65 kg	76 (49.4)	186 (64.6)	
>65 kg	78 (50.6)	102 (35.4)	0.003
Missing	7	15	
BMI			
<25 kg/m ²	82 (53.6)	199 (69.1)	
25–30 kg/m ²	38 (24.8)	69 (24.0)	
≥30 kg/m ²	33 (21.6)	20 (6.9)	<0.0001
Missing	8	15	
Number of full term pregnancies			
0	53 (34.6)	76 (26.3)	
1–2	81 (52.9)	152 (52.6)	
3+	19 (12.4)	61 (21.1)	0.01
Missing	8	14	
Oral contraceptive use			
Never	78 (52.7)	120 (42.3)	
Ever	70 (47.3)	164 (57.7)	0.03
Missing	13	19	
HRT use ^b			
Never	83 (63.8)	149 (62.1)	
Ever	47 (36.2)	91 (37.9)	
Missing	31	63	0.77

^a p values from conditional logistic regression models for ordered categorical variables

^bUse of estrogen alone and estrogen together with progesterone formulations prior to index date

vs. 0.014 nmol/L, $p=0.01$) were higher among cases than controls. Geometric means for DHEAS and androstenedione were similar for cases and controls. The mean of SHBG (on

Table 2 Descriptive statistics of androgen and SHBG concentrations by case control status

Hormone	Number of cases/controls	Cases, geometric mean ^a (95 % CI)	Controls, geometric mean ^a (95 % CI)	<i>p</i> value ^b
Total testosterone (nmol/L)	156/292	1.26 (1.18, 1.35)	1.16 (1.10, 1.23)	0.04
Free testosterone (nmol/L)	131/238	0.016 (0.015, 0.018)	0.014 (0.013, 0.015)	0.01
DHEAS (μmol/L)	160/302	3.16 (2.89, 3.45)	3.00 (2.81, 3.20)	0.34
Androstenedione (nmol/L)	159/302	5.20 (4.88, 5.53)	5.09 (4.84, 5.34)	0.54
SHBG (nmol/L)	136/249	52.7 (47.9, 57.7)	58.9 (55.0, 62.9)	0.05

Hormone measurements were converted to the International Systems of Units (testosterone, 1 ng/mL = 3.47 nmol/L, DHEAS, 1 ng/mL = 2.71 nmol/L, androstenedione, 1 ng/mL = 3.45 nmol/L)

^a Except for SHBG for which back-transformed mean of square-root (SHBG) is reported

^b Mixed effects model, accounting for matching status

the square-root scale) was lower in cases than in controls (52.7 vs. 58.9 nmol/L, $p=0.05$). We observed the expected inverse correlations between androgen concentrations and age and positive correlations between androgens and BMI as well as between the different androgens (Supplementary Table 1).

Table 3 shows the odds ratios and 95 % confidence intervals for risk of endometrial cancer by androgen or SHBG tertiles. We observed marginally significant trends of increasing risk with increasing tertiles of testosterone ($OR_{T3-T1}=1.59$, 95 % CI=0.96, 2.64, $p=0.08$) and free testosterone

($OR_{T3-T1}=1.76$, 95 % CI=1.01, 3.07, $p=0.047$), which were reduced after adjustment for BMI (testosterone, $OR_{T3-T1}=1.37$, 95 % CI=0.81, 2.32, $p=0.26$, and free testosterone, $OR_{T3-T1}=1.33$, 95 % CI=0.73, 2.42, $p=0.36$). Endometrial cancer risk was not significantly associated with DHEAS, androstenedione, or SHBG. Tests for heterogeneity by cohort were not statistically significant except for DHEAS ($p=0.03$) which was not associated with risk overall.

Table 4 shows analyses on the continuous scale overall and for some subgroups. Results for type I tumors were similar to

Table 3 Odds ratios for endometrial cancer associated with tertiles of androgen and SHBG concentrations, with and without adjustment for BMI

Hormone	Number of cases/controls	Unadjusted model ^a		Model adjusted for BMI ^b	
		OR (95 % CI)	<i>p</i> -trend	OR (95 % CI)	<i>p</i> -trend
Total testosterone					
Tertile 1	45/103	1.0 (ref)		1.0 (ref)	
Tertile 2	52/94	1.31 (0.79, 2.15)		1.30 (0.78, 2.19)	
Tertile 3	58/87	1.59 (0.96, 2.64)	0.08	1.37 (0.81, 2.32)	0.26
Free testosterone					
Tertile 1	37/79	1.0 (ref)		1.0 (ref)	
Tertile 2	41/81	1.08 (0.64, 1.94)		0.98 (0.57, 1.70)	
Tertile 3	52/68	1.76 (1.01, 3.07)	0.047	1.33 (0.73, 2.42)	0.36
DHEAS					
Tertile 1	53/98	1.0 (ref)		1.0 (ref)	
Tertile 2	50/104	0.85 (0.53, 1.36)		0.80 (0.50, 1.30)	
Tertile 3	57/98	1.11 (0.69, 1.78)	0.70	0.89 (0.54, 1.46)	0.60
Androstenedione					
Tertile 1	49/102	1.0 (ref)		1.0 (ref)	
Tertile 2	59/95	1.30 (0.81, 2.09)		1.26 (0.78, 2.03)	
Tertile 3	51/101	1.07 (0.65, 1.78)	0.80	0.94 (0.56, 1.59)	0.82
SHBG					
Tertile 1	59/68	1.0 (ref)		1.0 (ref)	
Tertile 2	29/99	0.37 (0.22, 0.63)		0.43 (0.25, 0.76)	
Tertile 3	48/80	0.71 (0.43, 1.18)	0.16	0.99 (0.57, 1.73)	0.92

^a Conditional logistic regression (matching factors: cohort, age, and date of blood donation)

^b Conditional logistic regression (matching factors: cohort, age, and date of blood donation) adjusting for BMI

Table 4 Odds ratios for endometrial cancer associated with androgen and SHBG concentrations on the continuous scale

	All women	Type I endometrial cancer	Age <55 years at diagnosis	Age ≥55 years at diagnosis	Age at diagnosis p-interaction ^c
Total testosterone					
N cases/controls	155/284	103/189	49/86	106/198	
OR (95 % CI) ^a	1.48 (1.04, 2.12)	1.46 (0.95, 2.23)	0.93 (0.55, 1.58)	2.09 (1.29, 3.39)	
<i>p</i> value ^a	0.03	0.08	0.79	0.003	0.03
BMI-adjusted OR (95 % CI) ^b	1.34 (0.93, 1.94)	1.31 (0.84, 2.03)	0.83 (0.47, 1.45)	2.08 (1.25, 3.44)	
BMI-adjusted <i>p</i> value ^b	0.12	0.24	0.51	0.005	
Free testosterone					
N cases/controls	130/228	87/151	47/81	83/147	
OR (95 % CI) ^a	1.44 (1.10, 1.90)	1.44 (1.04, 1.99)	1.03 (0.68, 1.58)	1.81 (1.25, 2.63)	
<i>p</i> value ^a	0.009	0.03	0.88	0.002	0.049
BMI-adjusted OR (95 % CI) ^b	1.24 (0.92, 1.67)	1.23 (0.86, 1.75)	0.92 (0.57, 1.48)	1.55 (1.04, 2.31)	
BMI-adjusted <i>p</i> value ^b	0.16	0.25	0.73	0.03	
DHEAS					
N cases/controls	160/300	109/205	51/93	109/207	
OR (95 % CI) ^a	1.13 (0.89, 1.44)	1.12 (0.84, 1.50)	0.86 (0.59, 1.23)	1.38 (1.00, 1.90)	
<i>p</i> value ^a	0.33	0.44	0.40	0.05	0.05
BMI-adjusted OR (95 % CI) ^b	1.03 (0.80, 1.32)	1.01 (0.74, 1.36)	0.81 (0.55, 1.19)	1.27 (0.92, 1.76)	
BMI-adjusted <i>p</i> value ^b	0.81	0.97	0.29	0.15	
Androstenedione					
N cases/controls	159/298	108/203	50/92	109/206	
OR (95 % CI) ^a	1.15 (0.78, 1.69)	1.11 (0.70, 1.75)	0.66 (0.33, 1.32)	1.48 (0.93, 2.36)	
<i>p</i> value ^a	0.48	0.66	0.24	0.10	0.06
BMI-adjusted OR (95 % CI) ^b	1.06 (0.71, 1.58)	0.97 (0.60, 1.57)	0.62 (0.31, 1.27)	1.41 (0.86, 2.30)	
BMI-adjusted <i>p</i> value ^b	0.78	0.89	0.19	0.17	
SHBG					
N cases/controls	136/247	93/168	49/89	87/158	
OR (95 % CI) ^a	0.91 (0.81, 1.01)	0.92 (0.80, 1.04)	0.98 (0.80, 1.20)	0.88 (0.77, 1.00)	
<i>p</i> value ^a	0.08	0.18	0.84	0.05	
BMI-adjusted OR (95 % CI) ^b	0.98 (0.87, 1.11)	1.00 (0.86, 1.16)	1.01 (0.81, 1.25)	0.98 (0.84, 1.14)	
BMI-adjusted <i>p</i> value ^b	0.75	0.99	0.95	0.76	0.41

All analyses were performed on log₂ transformed measurements, except SHBG for which square-root transformation was used

^a Conditional logistic regression (matching factors: cohort, age, and date at blood donation)

^b Conditional logistic regression (matching factors: cohort, age, and date at blood donation) adjusting for BMI (missing values for BMI were imputed using multiple imputation)

^c *p*-interaction calculated with androgens modeled on the continuous (log₂) scale (or square root scale for SHBG)

the overall results. We present the results by age at diagnosis (<55 vs. ≥55 years) because we observed statistically significant interactions for this variable. We did not observe any associations between androgens and risk of endometrial cancer among women who were less than 55 years of age at diagnosis (and thus presumed premenopausal or recently menopausal). However, we observed a significant increase in endometrial cancer risk among women ≥55 years at diagnosis for testosterone (OR for a doubling in BMI-adjusted model = 2.08, 95 % CI = 1.25, 3.44) and free testosterone (OR = 1.55, 95 % CI = 1.04, 2.31). Odds ratios in analyses

limited to never users of hormone replacement therapy were similar to those observed in the subgroups by age at diagnosis (data not shown). Tests for interaction by age at blood donation (<45 vs. ≥45 years) or BMI (<25 vs. ≥25 kg/m²) were not statistically significant (data not shown).

Discussion

We observed trends of increasing risk of endometrial cancer with increasing premenopausal concentrations of total and free

testosterone, which were reduced and no longer significant after adjustment for BMI. We did not observe an association for DHEAS, androstenedione, or SHBG. Similar results were observed when analyses were limited to type I tumors. We observed statistically significant interactions with age at diagnosis (<55 vs. \geq 55 years), with significant associations of testosterone and free testosterone with risk among women who were ages 55 years and over at the time of diagnosis, but not among women younger than 55 years at diagnosis. These associations remained statistically significant after adjusting for BMI.

Because the mechanisms involving androgens in the development of endometrial cancer are thought to differ before and after menopause, we had decided a priori to conduct subgroup analyses in women aged <55 and \geq 55 years at diagnosis as a surrogate for menopausal status at diagnosis, since this variable was not available for two of the three participating cohorts. We first examined whether the differences we observed between the two age groups could be explained by other factors. The distribution of endometrial tumor types did not significantly differ by age at diagnosis (88 % type I in cases diagnosed at <55 years and 76 % in cases diagnosed at \geq 55 years), suggesting that this factor does not explain the interaction we observed. Other differences between the two case groups included age at blood donation and time interval (lagtime) between blood donation and diagnosis. Compared to cases diagnosed before age 55 years, cases diagnosed at age \geq 55 years were older at blood donation (median age, 48 vs. 42 years) and had a longer lagtime between blood donation and diagnosis (median, 11.2 years vs. 6.7 years). Due to the relationships between age at blood donation, age at diagnosis and lagtime, it is difficult to assess their individual effects; however, the test for interaction was significant only for age at diagnosis. Further, it is unclear why age at blood donation would affect the androgen-endometrial cancer risk association and, while the observation of an association with androgen concentrations in the more distant past only might suggest an effect only on the early stages of cancer development, this would be contradictory with what is known about the effect of hormones on carcinogenesis.

Key and Pike proposed that, in premenopausal women, progesterone insufficiency, rather than estrogen excess, contributes to endometrial risk [1]. It is difficult to directly examine this hypothesis in epidemiologic studies because of the large variations in estrogen and progesterone concentrations during the menstrual cycle. PCOS, which is present in 6 to 10 % of premenopausal women [18–21], is the most common cause (~70 %) of chronic anovulation and associated progesterone insufficiency [22, 23]; it is also characterized by ovarian hyperandrogenism [24, 25]. Further, it has been proposed that androgen excess is a key contributor to anovulation in PCOS patients [26, 27]. We therefore examined the hypothesis that premenopausal circulating levels of androgens are associated with risk of endometrial cancer. Only one other study examined this hypothesis. No differences in androgen levels

were found between cases and controls in this study, which included 55 cases, most of whom presumed to be either pre- or peri-menopausal at time of diagnosis because follow-up time was relatively short (mean ~3 years) [6]. The results from this and our study do not support the hypothesis of an association between circulating androgens and endometrial cancer risk before menopause. It is possible, though, that ovulatory dysfunction and progesterone insufficiency are associated only with androgen values higher than those observed in our study, which consisted of healthy volunteers. Though there are no established normative values in women [28], in the largest study published to date ($n=985$ aged 20–80 year), the upper reference limit for women in the 40–59 year age group was 2.00 nmol/L for testosterone and 0.0262 nmol/L for free testosterone using liquid chromatography-tandem mass spectrometry [29]. Despite the fact that we used RIA assays, which tend to overestimate testosterone concentrations [28, 30], only 19 case (12.2 %) and 27 control (9.2 %) women in our study had a testosterone concentration higher than 2.00 nmol/L and 28 case (21.4 %) and 32 control (14.3 %) women had a concentration of free testosterone higher than 0.0262 nmol/L. It is also possible that hyperandrogenemia is not a good indicator of progesterone insufficiency.

The only two prospective studies that examined androgens in postmenopausal women in relation to endometrial cancer risk reported positive associations. In a case-control study nested within the same three cohorts as this study and including 124 cases and 236 controls, we observed significant associations of postmenopausal concentrations of androstenedione and DHEAS, and a borderline association of testosterone, with endometrial cancer risk [3, 4]. In a pooled nested case-control analysis (192 cases and 374 controls) of postmenopausal women from the European EPIC cohorts, free testosterone and testosterone were associated with risk, though not androstenedione or DHEAS [6]. Studies in both older premenopausal and postmenopausal women have shown that circulating androgens have good temporal reliability, i.e., one measurement ranks women reasonably well in terms of mid- to long-term average concentration [31, 32]. Though we are not aware of any study that examined how well circulating testosterone and free testosterone concentrations track before and after menopause in the same women, if ranking is preserved and women who have higher levels before menopause also have higher levels after menopause, the association we observed between premenopausal levels with risk of postmenopausal endometrial cancer could be due to the increased peripheral production of estrogens from increased androgen concentrations during the postmenopausal years.

BMI is a strong risk factor for endometrial cancer and is also correlated with androgens through mechanisms that are not clearly understood. It could therefore be a confounder of the androgen-endometrial cancer associations. In postmenopausal women, BMI could also be an effect modifier

because aromatization of androgens into estrogens occurs in adipose tissue. We did not observe statistical evidence of effect modification, either overall or in subgroups by age at diagnosis. However, the power to test for interaction was limited, particularly in subgroups. Furthermore, too few women were obese in our study (12 %) for us to be able to examine the association of androgens with endometrial cancer risk in this subgroup. In overall analyses, adjusting for BMI resulted in a reduction in odds ratios for testosterone and free testosterone and loss of statistical significance. In the subgroup of women ≥ 55 years of age at diagnosis, odds ratios for testosterone and free testosterone were reduced but remained significant, suggesting that the association between testosterone and postmenopausal endometrial cancer in this subgroup is not completely explained by BMI.

Hyperandrogenism is associated with insulin resistance, which is a risk factor for endometrial cancer. Since SHBG has been suggested as a marker of insulin resistance we assessed whether adjusting for SHBG modified the testosterone-endometrial cancer association. We did not observe an appreciable effect on testosterone odds ratios of SHBG adjustment overall [unadjusted OR for a doubling in total testosterone = 1.48 (95 % CI = 1.04, 2.12) vs. SHBG-adjusted OR = 1.43 (95 % CI = 0.97, 2.11); BMI-adjusted OR = 1.34 (95 % CI = 0.93, 1.94) vs. BMI- and SHBG-adjusted OR = 1.34 (95 % CI = 0.90, 2.00)] or among women 55 and over at diagnosis (in BMI-adjusted and unadjusted models). These results suggest that insulin resistance does not explain the testosterone-endometrial cancer association we observed. However, we cannot completely discard this hypothesis since we did not directly assess insulin resistance.

Other potential mechanisms of action have been proposed for androgens. A direct inhibitory effect of androgens, via the androgen receptor, on cell growth and secretory activity of the endometrium has been observed in *in vitro* studies [33–36]. Some clinical studies have observed atrophic effects of exogenous testosterone on the endometrium [37–39], though others observed no effect (when testosterone treatment was given without estrogen) [40]. Our results for premenopausal androgens, and those of others, do not support a strong role for a direct effect of testosterone in endometrial cancer. The consistent positive association observed between postmenopausal androgen concentrations and risk suggests a prevailing estrogen effect (i.e., conversion of androgens to estrogen in adipose tissue), rather than a direct action of androgens.

There are some limitations to our study. A single blood sample was used to measure circulating androgens. We and others have shown, though, that among premenopausal women the reliability of measurements over a period of 2 years or more is high for testosterone, free testosterone, and DHEAS (intra-class correlations, ICCs > 0.73) and moderate for androstenedione (ICCs > 0.57) [32, 41]. Inter-batch CVs were as high as 15–22 % for some of the androgen measurements; however, intra-

batch CVs were < 12 % and all samples from one matched set were always assayed in the same batch. The power of our study was limited, particularly for analyses in subgroups. Also, we used age at diagnosis as a surrogate for menopausal status at diagnosis because this variable was not available in two of the participating cohorts. Misclassification of menopausal status is likely small in the group ≥ 55 (i.e., very few premenopausal women are expected in this group) so the association that we observed is likely to be representative of that of premenopausal hormonal concentration with risk of postmenopausal endometrial cancer. Further, the lack of association of premenopausal androgens with endometrial cancer risk in the < 55 -year-old group, despite the fact that this group includes some postmenopausal women (for whom risk is likely elevated as per previous analysis), supports our conclusion that premenopausal androgens are not associated with risk of premenopausal endometrial cancer. In addition, we did not observe an association in sensitivity analyses looking at the subgroup of women < 52 years at diagnosis (BMI-adjusted T OR = 1.01, 95 % CI = 0.51, 2.01, free T OR = 1.11, 95 % CI = 0.63, 1.95, 34 cases/62 controls). Finally, we were not able to adjust for the type of hormone therapy (estrogen-only/estrogen + progestin) because these data were missing for a large proportion of the women; however, an analysis limited to never users also showed that testosterone and free testosterone were associated with increased risk only among women ages 55 and over at diagnosis.

In this prospective study of healthy volunteers at enrollment, we did not observe an association between circulating androgens and premenopausal endometrial cancer. We observed, though, positive associations of testosterone and free testosterone with risk of endometrial cancer among women age 55 years or older and presumably postmenopausal, at the time of diagnosis.

Compliance with Ethical Standards All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Conflict of Interest The authors declare that they have no conflict of interest.

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