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The association of plasma androgen levels with breast, ovarian and endometrial cancer risk factors among postmenopausal women

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Although androgens may play an etiologic role in breast, ovarian and endometrial cancers, little is known about factors that influence circulating androgen levels. We conducted a cross-sectional analysis among 646 postmenopausal women in the Nurses' Health Study to examine associations between adult risk factors for cancer, including the Rosner/Colditz breast cancer risk score, and plasma levels of testosterone, free testosterone, androstenedione, dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). All analyses were adjusted for age, laboratory batch and other cancer risk factors. Free testosterone levels were 79% higher among women with a body mass index of ≥30 *vs.* <22 kg/m^2 (*p*-trend <0.01) and 25% higher among women with a waist circumference of >89 *vs.* ≤74 cm (*p*-trend = 0.02). Consuming >30 g of alcohol a day *vs.* none was associated with a 31% increase in DHEA and 59% increase in DHEAS levels (*p*-trend = 0.01 and <0.01, respectively). Smokers of ≥25 cigarettes per day had 35% higher androstenedione and 44% higher testosterone levels than never smokers (*p*-value, *F*-test = 0.03 and 0.01, respectively). No significant associations were observed for height or time since menopause with any androgen. Testosterone and free testosterone levels were ~30% lower among women with a hysterectomy *vs.* without (both *p*-values < 0.01). Overall breast cancer risk was not associated with any of the androgens. Thus, several risk factors, including body size, alcohol intake, smoking and hysterectomy, were related to androgen levels among postmenopausal women, while others, including height and time since menopause, were not. Future studies are needed to clarify further which lifestyle factors modulate androgen levels.

Sex steroid hormones play a key role in the development of breast, ovarian and endometrial cancers.[1–4] As associations with estrogens have been increasingly well-delineated, more attention has focused on androgens. Prospective studies have linked circulating androgen levels to the risk of postmenopausal breast cancer.[5–8] Excessive androgen levels also have been proposed as a causal mechanism in the pathogenesis of ovarian cancer, given the relatively high levels of circulating androgens compared with estrogens, presence of androgen receptors in normal ovarian epithelial cells, and animal data suggesting that testosterone may increase ovarian tumor growth.[9] Epidemiologic data have been conflicting but provide some support for an association between androgens and ovarian cancer risk.[10–13] Similarly, 2 prospective studies on endometrial cancer have produced discrepant results regarding associations with androgen levels.[14,15] Androgens have been hypothesized to increase endometrial cancer risk, likely through the aromatization of androgens into estrogens,[16] but alternatively might decrease risk by decreasing the proliferative effects of estrogens in the endometrium.[17,18]

Although androgens may be important in disease etiology, relatively little is known about nongenetic determinants of circulating levels among postmenopausal women. To assess whether factors associated with breast, ovarian and endometrial cancers might influence cancer risk by altering androgen levels, we examined associations between several adult risk factors for cancer and plasma levels of testosterone, free testosterone, androstenedione, dehydroepiandrosterone (DHEA)

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and DHEA sulfate (DHEAS) in a large cross-sectional study of over 600 participants of the Nurses' Health Study (NHS). We further examined associations between circulating androgen levels and overall risk for breast cancer using the Rosner/Colditz risk score for breast cancer.[19–21]

Material and Methods

Study population

The NHS began in 1976 when 121,701 female registered nurses age 30–55 years completed a self-administered, mailed questionnaire. Follow-up questionnaires have been mailed biennially to collect updated exposure and health information. In 1989 and 1990, a subset of NHS participants (n = 32,826) provided a blood sample. The nurses received instructions for taking the blood sample and returned it with an ice-pack *via* overnight courier; 97% of samples were received within 26 hr of being drawn. The nurses also completed a supplementary questionnaire at the time of blood sample collection that included questions on reason for menopause and use of postmenopausal hormones.

The study population consisted of controls from previous nested case-control studies in the NHS on hormone levels and breast cancer risk.[6,22] Over 600 postmenopausal women who had not used postmenopausal hormones in the 3 months before blood draw had data available for each androgen. The study was approved by the Institutional Review Board of Brigham and Women's Hospital, and all study participants provided informed consent.

Laboratory methods

Details on the collection, storage, and assays of the blood data have been published previously.[22] Upon receipt, blood samples were separated into plasma, white blood cells and red blood cells and stored in liquid nitrogen freezers. A validation study compared hormone levels, including testosterone and androstenedione, in blood processed immediately with those processed after mailing (24–48 hr delay). Although testosterone levels slightly increased while androstenedione levels slightly decreased, the between-person variability was 3 times as great as the within-person variability for both hormones.[23]

All laboratory assays were performed by the Nichols Institute (San Juan Capistrano, CA). Plasma samples were assayed by radioimmunoassay following extraction and celite column chromatography. No separation step was used before the radioimmunoassay of DHEAS. Masked replicate samples were included to assess within-batch (within-plate) variability, which was greatest for DHEA (13.6%).[22] Identical quality control samples were included across sets of samples assayed at different time periods (*i.e.*, meta-batch;1 replicate per 10 samples), and indicated some laboratory drift over time.[6,22] We therefore adjusted for laboratory meta-batch in all analyses. The assay detection limit was 5 ng/dl for androstenedione, 2 ng/dl for testosterone, 10 ng/dl for DHEA, and 5 μg/dl for DHEAS. Values below the detection limit were set to half the limit (androstenedione: n = 1; testosterone: n = 2; DHEA: n = 1, DHEAS: n = 5). Free testosterone was calculated using the Sodergard formula.[24,25]

Exposures

We examined associations with 4 anthropometric measures: body mass index (BMI), waist-to-hip ratio (WHR), waist circumference and height. Waist circumference and height were originally collected in inches but have been converted to centimeters and meters, respectively. Height was collected in 1976. BMI was calculated as kg/m^2 using weight from the questionnaire completed at blood collection or, if missing, from the regular 1990 NHS questionnaire (n = 11). Waist and hip measurements were obtained from the 1986 NHS questionnaire, which asked women to measure their waist (standing up) and hip (widest) circumference to the nearest quarter of an inch using a tape measure. Women who reported a "0" were set to missing, as was one woman who reported a hip circumference of <51 cm. We also assessed BMI and waist circumference together, comparing women with high BMI and high waist circumference (≥25 kg/m^2 and >81 cm, respectively) to women with low BMI and low waist circumference (<25 kg/m^2 and ≤81 cm, respectively).

For analyses of alcohol consumption, we used reported alcohol intake in the year prior to the 1990 questionnaire (or, where missing, from the 1986 questionnaire, n = 14) Information on cigarette smoking also was collected on the 1990 NHS questionnaire. Family history (mother or sister) of breast cancer was reported in 1976, 1982, 1988 and 1992; family history of ovarian cancer was reported in 1992. Information on hysterectomy and oophorectomy was obtained from the blood collection questionnaire. For women reporting natural menopause or a bilateral oophorectomy, time since menopause was calculated as the age at blood draw minus age at menopause. Tubal ligation was coded as any report of tubal ligation through 1982 or in 1994.

Risk scores for breast cancer using the Rosner/Colditz model were previously developed within the NHS and have been described in detail elsewhere.[19–21] Briefly, the Rosner/Colditz model incorporates data on BMI, alcohol intake, height, duration of premenopausal and postmenopausal time, type of menopause, postmenopausal hormone use, parity (including details on age at each birth), history of benign breast disease, and family history of breast cancer. Risk deciles were previously created within 5-year age groups in the larger NHS cohort, and those rankings were used for this analysis. Decile 1 represents the lowest risk group and decile 10 represents the highest risk group. Scores were calculated only for women with nonmissing and consistent data on a number of the risk factors (*e.g.*, parity and age at births from different questionnaire years) and with known age at menopause (hence women with simple hysterectomy were excluded).[21] Thus, the analysis on the breast cancer risk score included 469 women.

Data analysis

Hormone values were natural log-transformed to improve normality for analyses, and outliers were identified within batch by the extreme studentized deviate many-outlier procedure.[26] Using this method, 4 values were dropped for androstenedione, 3 for testosterone, 2 for free testosterone, 1 for DHEA, and 5 for DHEAS.

Linear regression models were used to compute geometric mean hormone levels across exposure categories. All analyses were run in SAS version 9.1.3 and were adjusted for age in years (continuous and centered at 60 years) and laboratory batch. In multivariable analyses, all exposures were mutually adjusted for one another, with the small amount of missing data coded to a missing category as necessary. Because results for analyses adjusting for age and batch only were similar to results from multivariable models, only results from multivariable models are presented. The exposures were defined as follows: BMI (<22, 22 to <25, 25 to <30, ≥ 30 kg/m^2), height (≤ 1.60, >1.60–1.63, >1.63–1.68, >1.68 m), alcohol intake (none, >0–5, >5–10, >10–15, >15–30, >30 g/day), cigarette smoking (never, past and current smokers of 1–14, 15–24, ≥ 25 cigarettes per day), time since natural menopause (≤ 5, >5 to ≤ 10, >10 to ≤ 15, >15 years), family history of breast cancer (yes/no), family history of ovarian cancer (yes/no), tubal ligation (yes/no) and hysterectomy (none, uterus only removed, uterus and both ovaries removed). Women who reported hysterectomy with one or unknown ovaries removed were included with the missing category. In addition, WHR (<0.75, 0.75 to <0.80, 0.80 to <0.84, ≥ 0.84) and waist circumference (≤ 74, >74 to ≤ 81, >81 to ≤ 89, >89 cm) were examined in multivariable models (but were not included in all models due to a larger amount of missing data).

Analyses of the Rosner/Colditz breast cancer risk score were adjusted for age and batch, as well as covariates from our multivariable models that were not already included in the risk scores (*i.e.*, we additionally adjusted for smoking, family history of ovarian cancer, and tubal ligation).

When appropriate, continuous values of an exposure were used to calculate tests for trend and partial Spearman correlations. For trend analyses of cigarettes per day, only current smokers were included and were assigned the midpoint of their smoking category (1–14, 15–24, ≥ 25 cigarettes per day). An *F*-test was used to assess the significance of smoking overall (including never, former and current smokers).

Results

There were 646 women in our study population: 627 had measures for androstenedione, 631 for testosterone, 613 for free testosterone, 606 for DHEA, and 635 for DHEAS. The average age was 62 years. Among women reporting natural menopause or bilateral oophorectomy, the median age at menopause was 51 years and median time since menopause was 12 years. The median BMI was 25 kg/m^2 and median WHR was 0.79 (Table 1). Additional characteristics of the study population are provided in Table 1.

Table 1. Study population characteristics among 646 postmenopausal women in the nurses' health study

Characteristic	Median (10th, 90th percentiles)
Age (years)	62 (56, 68)
Age at menopause[1] (years)	51 (46, 54)
Time since menopause[1] (years)	12 (4, 19)
Body mass index (kg/m^2)	25 (21, 32)
Waist-to-hip ratio	0.79 (0.73, 0.89)
Waist circumference (cm)	81 (69, 97)
Height (m)	1.6 (1.6, 1.7)
Alcohol consumption[2] (g/day)	4 (0.9, 28)
	N (%)
Smoking	
Never	300 (47)
Past	260 (40)
Current	83 (13)
Had a tubal ligation	59 (9)
Had a hysterectomy	
With no ovaries removed	85 (13)
With both ovaries removed	71 (11)
Family history[3] of cancer	
Breast cancer	100 (15)
Ovarian cancer	24 (4)
	Mean[4] (10th, 90th percentiles)
Androstenedione (ng/dl)	56 (29, 109)
Testosterone (ng/dl)	21 (11, 39)
Free testosterone (ng/dl)	0.21 (0.09, 0.46)
DHEA (ng/dl)	205 (88, 436)
DHEAS (μg/dl)	80 (33, 175)

DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate.
[1]For women reporting natural menopause or bilateral oophorectomy.
[2]Among women reporting any alcohol consumption ($n = 370$). [3]Mother or sister reported cancer. [4]Geometric mean.

Anthropometric measures

The anthropometric measures (Table 2) (BMI, WHR, waist circumference and height) were not associated with most circulating androgen levels. However, free testosterone levels were 79% higher among women with a BMI of ≥ 30 kg/m^2 *vs.* <22 kg/m^2 (p-trend < 0.01) and 25% higher among women with a waist circumference of >89 cm *vs.* ≤ 74 cm (p-trend $= 0.02$ adjusting for BMI and the other covariates), and a borderline significant association was observed with WHR (p-trend $= 0.08$). The correlation with free testosterone was strongest for BMI ($r = 0.33$). Results were similar when BMI and waist circumference were cross-classified (high BMI / high waist

Epidemiology

Table 2. Anthropometric measures and adjusted[1] geometric mean levels of circulating androgens among postmenopausal women

	N^2	Androstenedione (ng/dl)	Testosterone (ng/dl)	Free testosterone (ng/dl)	DHEA (ng/dl)	DHEAS (μg/dl)
BMI (kg/m^2) ($N = 606^2$)						
<22	102	49	17	0.14	156	62
22 to <25	184	53	17	0.16	180	73
25 to <30	203	50	16	0.18	175	73
≥30	117	57	18	0.25	170	73
p-trend		$p = 0.18$	$p = 0.22$	$p < 0.01$	$p = 0.93$	$p = 0.23$
Spearman (r_s, *p*-value)		0.05 (0.20)	0.05 (0.26)	0.33 (<0.01)	0.003 (0.94)	0.06 (0.15)
WHR ($N = 443^2$)						
<0.75	109	49	17	0.16	169	68
0.75 to <0.80	140	53	17	0.18	168	70
0.80 to <0.84	88	51	17	0.19	176	78
≥0.84	106	56	18	0.19	178	73
p-trend		$p = 0.40$	$p = 0.77$	$p = 0.08$	$p = 0.97$	$p = 0.65$
Spearman (r_s, *p*-value)		0.05 (0.32)	0.02 (0.73)	0.12 (0.02)	0.02 (0.72)	0.06 (0.19)
Waist (cm) ($N = 446^2$)						
≤74	119	48	16	0.16	158	64
>74 to ≤81	124	54	17	0.19	175	74
>81 to ≤89	101	53	18	0.19	178	74
>89	102	58	19	0.20	179	70
p-trend		$p = 0.20$	$p = 0.13$	$p = 0.02$	$p = 0.95$	$p = 0.87$
Spearman (r_s, *p*-value)		0.06 (0.21)	0.08 (0.11)	0.14 (<0.01)	0.01 (0.80)	0.04 (0.45)
Height (m) ($N = 606^2$)						
≤1.60	204	53	17	0.17	171	71
>1.60 to 1.63	102	55	18	0.20	177	72
>1.63 to 1.68	176	51	17	0.17	160	65
>1.68	124	51	17	0.17	172	71
p-trend		$p = 0.49$	$p = 0.66$	$p = 0.78$	$p = 0.68$	$p = 0.65$
Spearman (r_s, *p*-value)		−0.02 (0.58)	0.03 (0.50)	0.004 (0.92)	−0.02 (0.57)	−0.02 (0.69)

DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; BMI, body mass index; WHR, waist-to-hip ratio.
[1]All models include the following terms: age (in years), laboratory batch, BMI (<22, 22 to <25, 25 to <30, ≥30 kg/m^2), height (≤1.60, >1.60 to 1.63, >1.63 to 1.68, >1.68 m), alcohol intake (none, >0–5, >5–10, >10–15, >15–30, >30 g/day, missing), smoking (never, past, current smoking of 1–14, 15–24, ≥25 cigarettes/day, missing), time since menopause (≤5, >5 to ≤10, >10 to ≤15, >15 years, missing), family history of breast (yes/no) and ovarian (yes/no) cancer, tubal ligation (yes/no), hysterectomy (none, hysterectomy with no ovaries removed, hysterectomy with both ovaries removed, missing/hysterectomy with one or unknown ovaries removed). [2]Ns are presented for DHEA, the hormone available for the fewest number of women ($n = 606$); missing categories are not shown.

circumference *vs.* low BMI / low waist circumference). Combining the 2 measures did not result in larger differences in mean hormone values and remained significant for free testosterone only ($p < 0.01$).

Lifestyle factors

Alcohol intake (Table 3) was significantly associated with increasing levels of DHEA ($p = 0.01$) and DHEAS ($p < 0.01$) but was not associated with the other androgens ($p \geq 0.07$). When compared with nondrinkers, women who consumed 30 or more grams of alcohol per day (about 2 drinks) had 31% higher levels of DHEA and 59% higher levels of DHEAS; hormone levels among women with lower alcohol intakes were similar to or just modestly higher than those among nondrinkers. Cigarette smoking was significantly associated with increased levels of androstenedione ($p = 0.03$) and testosterone ($p = 0.01$). When compared with never smokers, current smokers of 25 or more cigarettes daily had 35% increased androstenedione levels and 44% increased testosterone levels. Never and past smokers had nearly identical levels of androstenedione, testosterone and free testosterone. For DHEA and DHEAS, the associations with smoking were somewhat U-shaped, with androgen levels highest among never smokers and current smokers of 15–24 or 25 or more

Table 3. Lifestyle factors and adjusted[1] geometric mean levels of circulating androgens among postmenopausal women

	N^2	Androstenedione (ng/dl)	Testosterone (ng/dl)	Free testosterone (ng/dl)	DHEA (ng/dl)	DHEAS (μg/dl)
Alcohol intake (g/day) ($N = 596^2$)						
None	245	52	18	0.18	162	61
>0–5	186	48	17	0.16	154	61
>5–10	53	50	17	0.18	155	62
>10–15	44	49	15	0.16	183	73
>15–30	39	54	18	0.18	167	78
>30	29	61	19	0.20	212	97
p-trend		$p = 0.07$	$p = 0.46$	$p = 0.23$	$p = 0.01$	$p < 0.01$
Spearman (r_s, *p*-value)		−0.01 (0.84)	−0.05 (0.20)	−0.01 (0.84)	0.05 (0.26)	0.11 (<0.01)
Cigarette smoking ($N = 601^2$)						
Never	284	52	16	0.18	195	75
Past	241	52	16	0.17	169	64
Current (cigarettes/day)						
1–14	30	60	19	0.21	173	66
15–24	30	55	19	0.17	183	75
≥25	16	70	23	0.22	195	91
p-trend[3]		$p = 0.25$	$p = 0.12$	$p = 0.89$	$p = 0.53$	$p = 0.25$
p-value (*F*-test[4])		$p = 0.03$	$p = 0.01$	$p = 0.19$	$p = 0.14$	$p = 0.05$

DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate.
[1]All models include the following terms: age (in years), laboratory batch, BMI (<22, 22 to <25, 25 to <30, ≥30 kg/m^2), height (≤1.60, >1.60 to 1.63, >1.63 to 1.68, >1.68 m), alcohol intake (none, >0–5, >5–10, >10–15, >15–30, >30 g/day, missing), smoking (never, past, current smoking of 1–14, 15–24, ≥25 cigarettes/day, missing), time since menopause (≤5, >5 to ≤10, >10 to ≤15, >15 years, missing), family history of breast (yes/no) and ovarian (yes/no) cancer, tubal ligation (yes/no), hysterectomy (none, hysterectomy with no ovaries removed, hysterectomy with both ovaries removed, missing/hysterectomy with one or unknown ovaries removed). [2]Ns are presented for DHEA, the hormone available for the fewest number of women ($n = 606$); missing categories are not shown. [3]Among current smokers, based on mid-point of category. [4]Including all smoking categories (never, past, current 1–14, 15–24, ≥25 cigarettes/day).

cigarettes per day. The trend among current smokers across cigarettes per day was not statistically significant for any androgen ($p \geq 0.12$).

Nonlifestyle factors

No associations were observed between androgen levels and time since natural menopause (Table 4). Analyses of time since menopause that included women with a bilateral oophorectomy were similarly null (data not shown). Hysterectomy was associated with ~30% lower levels of testosterone and free testosterone; the decreases were similar regardless of whether a bilateral oophorectomy also was performed. Borderline significant associations were observed between family history of ovarian cancer and DHEAS levels ($p = 0.05$), as well as between tubal ligation and DHEA ($p = 0.09$).

There were no significant associations between the breast cancer risk score and androgen levels. However, there was a weak positive association between overall risk for breast cancer and levels of free testosterone, which increased by 22% over risk groups (*p*-trend = 0.06; $r = 0.10$, $p = 0.04$).

Discussion

We examined the association between circulating levels of 5 androgens and anthropometric, lifestyle and nonlifestyle factors among postmenopausal women. Increasing BMI was associated with increasing levels of free testosterone but not with androstenedione, total testosterone, DHEA, or DHEAS. Two other large studies that examined associations between free testosterone and BMI among postmenopausal women also reported significant positive associations.[27,28] The observed association is likely due to the known inverse association between sex hormone-binding globulin levels and BMI.[27,28] In contrast to our findings, several,[27–31] but not all,[32,33] studies have observed positive associations with testosterone. Most studies did not find an association between BMI and androstenedione,[28,29,31–34] although significant associations have been reported.[27,30] Consistent with our findings, associations have not been observed between BMI and DHEA[29,30] or DHEAS[27–31,35] in previous studies.

Few studies have examined other anthropometric measures. Our WHR results were similar to our BMI results, with a borderline positive association for free testosterone but no

Table 4. Nonlifestyle factors and adjusted[1] geometric mean levels of circulating androgens among postmenopausal women

	N[2]	Androstenedione (ng/dl)	Testosterone (ng/dl)	Free testosterone (ng/dl)	DHEA (ng/dl)	DHEAS (μg/dl)
Time since natural menopause[3] (yrs) ($N = 436^2$)						
≤5	73	54	24	0.22	200	87
>5 to ≤10	121	51	22	0.21	174	87
>10 to ≤15	147	54	21	0.19	196	80
>15	95	57	23	0.21	209	89
p-trend		$p = 0.85$	$p = 0.51$	$p = 0.48$	$p = 0.51$	$p = 0.76$
Spearman (r_s, *p*-value)		0.01 (0.84)	−0.03 (0.47)	−0.04 (0.43)	0.04 (0.37)	−0.001 (0.98)
Tubal ligation ($N = 606^2$)						
No	551	55	18	0.18	184	71
Yes	55	50	16	0.17	157	69
p-value		$p = 0.18$	$p = 0.27$	$p = 0.34$	$p = 0.09$	$p = 0.74$
Hysterectomy ($N = 581^2$)						
None	438	54	23	0.22	173	76
Uterus only removed	79	48	14	0.16	156	64
Uterus and both ovaries removed	64	57	17	0.16	170	73
p-value (*F*-test)		$p = 0.78$	$p < 0.01$	$p < 0.01$	$p = 0.76$	$p = 0.95$
Family history of cancer						
Breast ($N = 606^2$)						
No	509	51	16	0.17	164	70
Yes	97	54	18	0.18	176	70
p-value		$p = 0.25$	$p = 0.17$	$p = 0.51$	$p = 0.34$	$p = 0.97$
Ovarian ($N = 606^2$)						
No	582	57	18	0.19	184	80
Yes	24	48	16	0.16	157	61
p-value		$p = 0.10$	$p = 0.30$	$p = 0.14$	$p = 0.24$	$p = 0.05$

DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate.
[1]All models include the following terms: age (in years), laboratory batch, BMI (<22, 22 to <25, 25 to <30, ≥30 kg/m^2), height (≤1.60, >1.60–1.63, >1.63–1.68, >1.68 m), alcohol intake (none, >0–5, >5–10, >10–15, >15–30, >30 g/day, missing), smoking (never, past, current smoking of 1–14, 15–24, ≥25 cigarettes/day, missing), time since menopause (≤5, >5 to ≤10, >10 to ≤15, >15 years, missing), family history of breast (yes/no) and ovarian (yes/no) cancer, tubal ligation (yes/no), hysterectomy (none, hysterectomy with no ovaries removed, hysterectomy with both ovaries removed, missing/hysterectomy with one or unknown ovaries removed). [2]Ns are presented for DHEA, the hormone available for the fewest number of women ($n = 606$); missing categories are not shown. [3]Results are presented for women with natural menopause only; findings were similar when women with bilateral oophorectomy were included.

associations with the other androgens. We also observed a weak positive correlation between free testosterone and waist circumference. In a prior study, a positive association was observed between free testosterone and waist circumference, as well as between free testosterone and WHR.[28] Another study found a positive association between waist circumference and testosterone and androstenedione, but not DHEAS,[31] while a small study of postmenopausal women ($n = 88$) did not observe relationships between WHR and androstenedione, testosterone, or free testosterone.[33] We did not observe any association between height and androgen levels, consistent with the few existing studies.[29,31,34,35] It is possible that height may primarily reflect hormone levels during youth that do not remain important in adulthood.

Significant, positive associations were observed between daily alcohol intake and levels of DHEA and DHEAS; compared with nondrinkers, women consuming about 2 or more drinks per day had 31% higher DHEA and 59% higher DHEAS levels. In a randomized controlled feeding study, DHEAS, but not DHEA, was significantly increased among postmenopausal women who drank 15–30 g of alcohol per day.[36] Other epidemiologic studies also support an increase in DHEAS levels with alcohol consumption, although the trend became nonsignificant after adjusting for covariates in one study.[35,37] In animal studies, alcohol stimulates the adrenal gland, suggesting that DHEAS, which is synthesized exclusively in the adrenal gland, might also increase.[37] Consistent with our findings, most prior studies did not find

associations between alcohol intake and levels of androstenedione and testosterone,[32,34,36–38] although it has been suggested that testosterone levels are lower among alcoholics than nonalcoholics.[39] Cumulatively, these data suggest that an increase in DHEAS levels (which have been associated with increases in breast cancer risk[5,6,8,40]) may be another mechanism through which alcohol influences cancer risk. The association with DHEA is less clear and warrants additional assessments.

Smoking was associated with higher levels of androstenedione and testosterone. Other studies also have observed elevated levels of testosterone among current smokers.[41,42] A meta-analysis[43] and summary of the literature[44] found significantly higher levels of androstenedione in smokers compared with nonsmokers, in concordance with our results, but also found higher levels of DHEAS. In our study, women smoking $\geq$25 cigarettes per day had higher mean DHEAS levels than nonsmokers and women smoking fewer cigarettes, although smoking overall did not significantly contribute to the model and the trend among current smokers across cigarettes per day was not significant. Smoking might impact androgen levels by increasing their production through stimulation of the adrenal gland; nicotine has been found to increase adrenocorticotropic hormone levels.[17,41,45] Alternatively, smoking might result in elevated androgen levels by decreasing their metabolic clearance rate; smoking has been found to inhibit both aromatase activity and enzymes that degrade androstenedione and testosterone.[17,41,44] The general lack of association between smoking and both breast[46] and ovarian[47,48] cancer may be due to the offsetting hormonal influences of this complex exposure.

Time since natural menopause was not associated with any of the androgens in our study. Previous studies also have reported null findings,[29,32,49] although associations have been observed with androstenedione,[34,50] testosterone,[50] and DHEAS[38] levels in some studies. The reasons for different findings are not clear, although the significant studies tended to control for more covariates than the nonsignificant studies. Further research is needed to better understand the changes in hormone levels after menopause. We did not observe any substantial associations between family history of breast or ovarian cancer and any of the androgens. When a variety of factors were combined into an overall breast cancer risk score, based on the Rosner/Colditz model, we did not observe any significant associations.

Tubal ligation was associated with marginally significantly lower DHEA levels. In the only prior study of this association, women with tubal ligation had nonsignificantly lower androgen levels.[51] Although further study is needed, these data suggest that tubal ligation might decrease risk of ovarian cancer[52,53] and possibly breast cancer[54,55] in part by altering androgen levels. We found significantly lower levels of testosterone and free testosterone among women with a hysterectomy compared with women with an intact uterus. Postmenopausally, the ovaries produce 40–50% of circulating testosterone[56,57] so we expected significant declines among women having a hysterectomy with bilateral oophorectomy. However, the lower levels of testosterone and free testosterone were similar for women who had a hysterectomy only and those who had a hysterectomy with bilateral oophorectomy. Other studies support a reduction in testosterone levels with a simple hysterectomy, but have observed greater decreases with a bilateral oophorectomy.[49–51] For androgens other than testosterone, both increasing and decreasing levels have been observed.[35,49,50] It is unclear why we observed similarly reduced testosterone levels among women with their uterus only removed and those who also had both ovaries removed, but it is unlikely to be due to measurement error. Menopausal status and reason for menopause were previously validated in this cohort.[58]

Our study has several strengths, including its relatively large size and the use of precise androgen assays. In addition, in a reproducibility study conducted in this population[59] we found intraclass correlations over a 3-year period of 0.64 for androstenedione, 0.84 for testosterone, 0.53 for DHEA, and 0.81 for DHEAS,[22] indicating that a single hormone measurement, as we have here, can reasonably characterize postmenopausal androgen levels.

Limitations of our study also need to be considered. Some exposures (*e.g.*, alcohol) may have acute effects on hormone levels that might be missed in our study, particularly for androgens with shorter half-lives. Information on cancer risk factors was self-reported, and for some factors, such as waist and hip measurements, we were missing data for a substantial number of women. Nondifferential misclassification of both cancer risk factor and androgen levels would most likely attenuate associations. Furthermore, in this cross-sectional study, it is not possible to identify the direction of the significant relationships that were observed. For instance, it is possible that cigarette smoking influences cancer risk through an effect on adrostenedione and testosterone, but it also is possible that higher levels of these hormones influence an individual's desire to smoke.

Additionally, our study population was primarily Caucasian and all women were nurses. Although this reduces the likelihood of confounding, results may not be generalizable to all women. However, while the distribution of health factors, such as cigarette smoking, may be different in our population compared with the general U.S. population, it seems likely that the associations observed for specific categories (*e.g.*, never or current smokers) will be relevant to older women in general. Studies in more diverse populations are needed to test this hypothesis and confirm findings. Finally, although our study was large overall, we had limited numbers for specific exposures (*e.g.*, current smokers, particularly heavy smokers).

In conclusion, we found that certain risk factors, such as BMI, alcohol intake, cigarette smoking and hysterectomy, were associated with circulating androgen levels among

postmenopausal women. However, we did not observe significant associations between many of the risk factors we examined and androgen levels. Given the relatively limited data, additional large studies are warranted to elucidate the relationship between androgen levels and risk factors for breast, endometrial and ovarian cancers.

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