

The Relationship Between Bilateral Oophorectomy and Plasma Hormone Levels in Postmenopausal Women

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Abstract Oophorectomy prior to natural menopause reduces breast cancer risk. We evaluated whether timing of oophorectomy (during premenopause vs. postmenopause) or hysterectomy was associated with hormone levels, specifically estradiol, estrone, estrone sulfate, testosterone, sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), and prolactin, using data from the Nurses' Health Study. We included 2,251 postmenopausal women not using hormones who provided blood samples in 1989–1990 and/or 2000–2002, and who were controls in various nested case–control studies. We used multivariate linear mixed-effects models to assess geometric mean hormone levels by surgery

status. Bilateral oophorectomy was associated with 25 % lower testosterone levels versus women with natural menopause (20.8 vs. 15.5 ng/dL) ($P < 0.0001$) with no effect of timing of surgery ($P = 0.80$). SHBG levels were lower among women with a premenopausal oophorectomy (52.2 nmol/L) versus those with natural menopause (58.1 nmol/L) or a postmenopausal oophorectomy (62.0 nmol/L) ($P = 0.02$). There was no significant association of oophorectomy with estradiol, estrone, estrone sulfate, DHEAS, or prolactin levels ($P \geq 0.23$). A simple hysterectomy was associated with a significant 8 % lower testosterone ($P = 0.03$) and 14 % lower DHEAS ($P = 0.02$) levels compared with women with natural menopause but not with other hormone levels. Although limited by small numbers, our findings suggest no differential influence of timing of surgery on sex hormone levels. The reduction of testosterone levels in women with oophorectomy or hysterectomy suggests a possible role of this hormone in postmenopausal breast cancer development.

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Introduction

A woman's reproductive history is an important determinant of her subsequent risk of breast cancer. Specifically, an earlier age at menopause has been associated with a reduction in breast cancer risk likely due to a reduction in the duration and dose of ovarian hormonal exposure [1]. The hormonal state associated with menopause may vary depending upon whether menopause was natural or surgical (including bilateral oophorectomy) and whether postmenopausal hormonal therapy or antiestrogens (e.g., tamoxifen or aromatase inhibitors) are used.

Among women in the general population, studies consistently report a stronger protective effect of surgical versus natural menopause on breast cancer risk, especially if performed at an early age (i.e., prior to age at which natural

menopause would be expected to occur) and with increasing time since surgery [2–11]. Most studies have reported that an oophorectomy performed after natural menopause does not protect against breast cancer risk [10, 5, 9, 2]. Premenopausal oophorectomy may affect breast cancer risk by reducing lifetime exposure to circulating ovarian hormones. In the large analysis of 13 prospective cohort studies of postmenopausal women (which included a subset of the women included in the current analysis), the authors reported that bilateral oophorectomy was associated with lower testosterone levels and no difference in estradiol or estrone levels, but no distinction was made regarding the timing of oophorectomy (e.g., before or after natural menopause) [12]. More recently, Gaudet et al. reported a significant reduction in breast cancer risk with oophorectomy, irrespective of the timing of surgery [11]. Similarly, a study conducted among women at high risk due to an inherited *BRCA* mutation reported a significant protective effect of oophorectomy on breast cancer risk even when performed after natural menopause [13]. To our knowledge, the effect of timing of oophorectomy on sex hormone levels has never been evaluated.

Thus, the goal of the current study was to evaluate the relationship between timing of oophorectomy in relation to menopause (i.e., natural menopause vs. a bilateral oophorectomy during premenopause or following natural menopause) and plasma concentrations of estrogens, androgens, prolactin, and sex hormone binding globulin (SHBG). We studied 2,251 postmenopausal women (who were not using hormone therapy [HT]) from the Nurses' Health Study (NHS). In addition, we evaluated the relationship between a simple hysterectomy and circulating sex hormone levels in postmenopausal women by timing of surgery (during premenopause vs. postmenopause).

Materials and Methods

Study Population

The NHS was established in 1976 among 121,700 US female registered nurses, ages 30 to 55 years. All women completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses. Data have been collected on numerous reproductive, hormonal, and other factors including parity, HT use, tubal ligation, and family history of cancer.

From 1989 to 1990, 32,826 NHS participants (ages 43–70 years) provided blood samples and completed a short questionnaire [14]. Women arranged to have their blood drawn and shipped on ice, via overnight courier, to our laboratory, where it was separated into plasma, red blood cell, and white blood cell components. From 2000 to 2002, we collected a second blood sample from a subset of these women ($n=18,743$ women, ages 53–80 years, and >98 % postmenopausal) using the same

protocol as in the original collection [15]. Since collection, samples have been stored in monitored liquid nitrogen freezers. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital (Boston, MA).

Participants in the current study were postmenopausal controls who had not used HT for at least 5 months prior to blood draw from previous nested case–control studies of breast, colon, and ovarian cancer, as well as stroke and rheumatoid arthritis [15–20]. Through 2010, 2,251 women had their blood assayed for at least one of the hormones of interest. Since 312 women were included in both the 1989–1990 and 2000–2002 blood collections, this analysis includes 2,563 blood samples with 2,067 from the 1989 to 1990 blood collection and 496 from the 2000 to 2002 blood collection. The number of blood samples assayed for each hormone varied and ranged from a minimum of 1,645 (estrone sulfate) to a maximum of 2,309 (testosterone). We included women from both blood draws to increase the sample size of women with surgery, particularly after menopause.

Exposures

We obtained information on menopausal status and oophorectomy/hysterectomy history from the blood collection questionnaires completed at the time of each collection. Data on timing of oophorectomy and hysterectomy were obtained from the 2002 main questionnaire for those giving a second blood. We did not collect information on the indication for a gynecologic surgery. Women were classified into three categories for our primary exposure: (1) those who underwent natural menopause (i.e., no menstrual cycles during previous 12 months) and had both ovaries and uterus intact, (2) those who had surgical menopause defined as a bilateral oophorectomy with or without a hysterectomy during premenopause, and (3) those who had a bilateral oophorectomy with or without a hysterectomy following natural menopause. We created similar categories for simple hysterectomy. Women with an unknown oophorectomy status or a unilateral oophorectomy were excluded from the both the oophorectomy and hysterectomy analyses.

Laboratory Assays

The methods used to assay postmenopausal hormones in the NHS have been published previously [15–23]. The following postmenopausal hormone levels have been quantified: estrogens including total estradiol, estrone, estrone sulfate; androgens including total testosterone, dehydroepiandrosterone sulfate (DHEAS); prolactin; and sex hormone-binding globulin (SHBG). The inter-assay coefficients of variation based on blinded replicates were <10 % for 59 % of batches and between 10 % and 16 % otherwise.

When hormone values were less than the detection limit, we set the value to one half the limit. The detection limits of the assays and the number of samples below the limit were 2 pg/ml estradiol ($n=56$), 10 pg/ml estrone ($n=55$), 40 pg/ml estrone sulfate ($n=6$), 2 ng/dl testosterone ($n=9$), 5 $\mu\text{g/dl}$ DHEAS ($n=53$), 0.6 ng/ml prolactin ($n=0$), and 2 nmol/L SHBG ($n=1$). The stability of these hormones in whole blood not processed for 24–48 h has been shown previously [24].

Statistical Analysis

For each analyte, we excluded women with missing values related to assay difficulties or who had low plasma volume. Women with estradiol levels above 30 pg/ml were excluded from all analyses as estradiol levels higher than 30 pg/ml likely indicate that a woman had currently or very recently used HT. We used the generalized extreme studentized deviate many-outlier detection approach to identify statistical outliers [25]. The following statistical outliers were excluded from analyses— ≤ 0.2 pg/ml estradiol ($n=2$), ≤ 2.2 pg/ml estrone ($n=2$), ≤ 1.3 ng/dl testosterone ($n=11$), >66 ng/ml prolactin ($n=8$), and 1 nmol/L SHBG ($n=1$) [25]. After these exclusions, levels of estradiol, estrone, estrone sulfate, testosterone, DHEAS, prolactin, and SHBG were recalibrated to have a comparable distribution to an average batch according to the methods described by Rosner and colleagues [26]. Each hormone was log-transformed. Women with missing hormone information were excluded for the specific analysis with missing data.

We used linear mixed-effects models, with a random effect for ID to account for correlation among participants providing two blood samples, to calculate adjusted geometric means for each log-transformed hormone by our exposures. We adjusted for key characteristics at the time of blood draw that might be associated with biomarker levels as well as factors that have been associated with these biomarkers in prior studies. For all hormones, we adjusted for age at blood draw (55, 55–60, 60–65, >65 years), time of day of blood draw (1–8 a.m., 9 AM–noon, 1 PM–midnight), fasting status (>8 h since last meal, <8 h since last meal, or unknown), past HT use assessed at blood draw (ever, never, missing), date of blood draw (first blood collection: on or before June 1989, July 1989–Jan 1990, Feb 1990–June 1990, after June 1990; second blood collection: on or before June 2000, July 2000–Jan 2001, Feb 2001–June 2001, after June 2001), body mass index (BMI) at blood draw (continuous), age at first birth/parity (nulliparous, age at first birth <25 years/1–4 children, age at first birth 25–29 years/1–4 children, age at first birth ≥ 30 years/1–4 children, age at first birth <25 years/5 children, age at first birth >25 years/5 children), daily alcohol consumption (0 g/day, >0 to ≤ 10 g/day, >10 to ≤ 20 g/day, >20 to ≤ 30 g/day, >30 g/day), family history of breast cancer (yes/no), personal history of benign breast disease (yes/no), and age at menopause

(continuous). Waist-to-hip ratio (WHR; continuous) was adjusted for in secondary analyses as we only had information on WHR among a proportion of the participants. Values for women missing information on WHR were set to the median, and a separate missing indicator was created. We used the global F -test to evaluate the overall association for the geometric means comparing natural menopause, premenopausal oophorectomy, and postmenopausal oophorectomy (or for hysterectomy) and used pairwise t tests to assess the association between each combination.

For timing of oophorectomy and hysterectomy, we additionally assessed whether the associations differed by BMI, a major source of hormone production in postmenopausal women, and age at blood draw using multiplicative interaction terms.

All analyses were conducted using SAS, version 9.3 (SAS Institute Inc, Cary, North Carolina). All P values were two-sided and considered statistically significant if less than 0.05.

Results

There was a total of 2,251 women included in the current study. Table 1 displays the distribution of various characteristics of the women included in the study by timing of bilateral oophorectomy and year of blood collection. Women were, on average, overweight in all groups (i.e., BMI >25). As expected, age at blood draw was higher among women in the 2000 blood collection, and age at menopause was lower in the premenopausal oophorectomy group. Mean daily alcohol consumption was higher among women who had a postmenopausal oophorectomy. In addition, past HT use was higher among women with a natural menopause in the 2000 blood collection (43 %) versus those in the 1990 blood collection (25 %). Sex hormone levels were in the expected ranges [27].

Among postmenopausal women, plasma testosterone levels were significantly lower in women without ovaries compared with those women who had both ovaries intact (geometric mean, 15.4 vs. 20.8 ng/dL; $P<0.0001$) (Table 2). Geometric mean testosterone levels did not differ by timing of oophorectomy (15.4 vs. 15.6 ng/dL for premenopausal and postmenopausal oophorectomy, respectively, $P=0.80$ for pairwise comparison). There was a modest significant difference in geometric mean SHBG levels among women who had an oophorectomy versus those who had natural menopause ($P=0.04$). In particular, SHBG levels were significantly lower in women with a premenopausal oophorectomy (52.2 nmol/L) compared with women who had a postmenopausal oophorectomy (62.0 nmol/L) or natural menopause (58.1 nmol/L) (global F test $P=0.02$). Adjustment for WHR did not materially alter the results (57.9, 53.2, and 63.4 nmol/L for natural menopause, premenopausal oophorectomy, and postmenopausal oophorectomy, respectively; global F test $P=0.04$).

Table 1 Characteristics of postmenopausal women not taking hormone therapy in the Nurses' Health Study by timing of bilateral oophorectomy and year of blood collection

Characteristic	1990 Blood collection			2000 Blood collection		
	Natural menopause (<i>n</i> =1,335)	Premenopausal oophorectomy (<i>n</i> =190)	Postmenopausal oophorectomy (<i>n</i> =13)	Natural menopause (<i>n</i> =318)	Premenopausal oophorectomy (<i>n</i> =28)	Postmenopausal oophorectomy (<i>n</i> =17)
Age at blood draw, years (mean, SD)	61.3 (4.8)	61.5 (5.3)	64.3 (3.2)	69.2 (6.7)	71.6 (5.2)	69.9 (5.4)
Age at menarche, years (mean, SD)	12.5 (1.8)	12.5 (1.5)	12.7 (1.7)	12.6 (1.5)	12.3 (1.4)	12.5 (1.2)
Parity (mean, SD) ^a	3.5 (1.7)	3.4 (1.7)	3.6 (0.7)	3.5 (1.7)	3.0 (1.1)	3.4 (2.0)
Past HT use (<i>n</i> , %) ^b	332 (25 %)	132 (70 %)	8 (62 %)	138 (43 %)	26 (93 %)	11 (65 %)
Age at menopause, years (mean, SD)	50.3 (3.4)	44.5 (6.4)	50.3 (3.5)	50.9 (3.3)	45.6 (4.6)	50.7 (3.5)
Alcohol consumption, g/day (mean, SD)	5.5 (10.4)	5.3 (9.2)	7.1 (9.6)	5.5 (8.8)	3.5 (4.2)	6.2 (8.3)
BMI at blood draw, kg/m ² (mean, SD)	25.8 (4.8)	26.7 (4.9)	25.0 (3.5)	26.8 (5.7)	26.5 (4.2)	27.7 (7.4)
WHR, (mean, SD)	0.8 (0.1)	0.8 (0.1)	0.8 (0.1)	0.9 (0.1)	0.9 (0.1)	0.9 (0.1)
Median plasma hormone levels (10th–90th percentile)						
Estradiol (pg/mL)	5.3 (2.7–11.0)	4.9 (2.7–11.1)	4.7 (2.8–9.0)	5.2 (2.8–17.6)	5.7 (1.8–8.9)	6.0 (2.2–6.8)
Estrone (pg/mL)	24.8 (13.2–44.4)	23.4 (13.7–44.5)	29.9 (13.8–45.4)	25.8 (13.8–50.6)	24.7 (10.7–46.6)	25.6 (17.5–31.4)
Estrone sulfate (pg/mL)	223.1 (98.2–510.8)	219.2 (100.6–629.4)	221.9 (65.2–472.3)	218.2 (79.4–600.8)	216.7 (81.1–545.3)	198.2 (59.7–609.1)
Testosterone (ng/dL)	20.8 (10.9–39.3)	15.6 (8.5–27.7)	14.5 (8.3–26.9)	22.3 (10.9–42.5)	12.8 (4.0–31.1)	16.8 (7.6–30.6)
DHEAS (μg/dL)	64.6 (24.9–141.7)	63.7 (25.3–125.7)	44.5 (14.0–100.0)	65.5 (12.5–166.4)	35.2 (3.8–103.8)	68.1 (4.1–164.6)
Prolactin (ng/mL)	8.9 (5.2–16.1)	9.4 (5.2–16.8)	9.7 (4.9–20.7)	8.9 (5.8–17.4)	8.5 (5.2–16.6)	7.3 (5.8–13.3)
SHBG (nmol/L)	60.9 (29.4–109.1)	54.6 (27.4–91.1)	56.8 (28.0–93.2)	55.7 (29.6–109.2)	62.3 (23.6–105.8)	57.3 (22.5–115.0)

^a Among parous women only

^b The percent total does not add up to 100 % due to missing data

Table 2 Adjusted geometric means of sex hormone levels by timing of bilateral oophorectomy

Hormone	Natural menopause		Any oophorectomy		P^b	Premenopausal oophorectomy		Postmenopausal oophorectomy		P^c
	<i>n</i>	Mean (95 %CI) ^a	<i>n</i>	Mean (95 %CI)		<i>n</i>	Mean (95 %CI)	<i>n</i>	Mean (95 %CI)	
Estradiol (pg/ml)	1,388	5.4 (5.3, 5.6)	225	5.3 (4.9, 5.7)	0.52	199	5.3 (4.9, 5.8)	26	5.3 (4.4, 6.5)	0.81
Estrone (pg/ml)	1,332	24.2 (23.5, 24.8)	211	23.8 (22.2, 25.8)	0.79	187	23.6 (21.8, 25.6)	24	25.2 (21.0, 30.3)	0.78
Estrone sulfate (pg/ml)	1,066	222.4 (213.1, 231.9)	160	240.6 (213.9, 270.7)	0.23	142	244.5 (215.2, 277.7)	18	219.7 (162.6, 296.8)	0.40
Testosterone (ng/dL)	1,494	20.8 (20.2, 21.3)	225	15.5 (14.4, 16.7)	<0.0001	199	15.4 (14.2, 16.7)	26	15.6 (13.2, 18.9)	<0.0001 ^d
DHEAS (μg/dL)	1,090	59.8 (57.0, 62.9)	163	60.0 (51.6, 68.3)	0.95	146	60.1 (51.9, 69.5)	17	56.5 (39.1, 81.7)	0.93
Prolactin (ng/mL)	1,150	9.2 (8.9, 9.4)	176	9.1 (8.4, 9.8)	0.88	155	9.1 (8.4, 10.0)	21	8.8 (7.2, 10.8)	0.93
SHBG (nmol/L)	1,460	58.1 (56.7, 59.5)	237	53.8 (50.4, 57.5)	0.04	208	52.2 (48.6, 56.2)	29	62.0 (53.0, 72.4)	0.02 ^e

Oophorectomy defined as both ovaries removed with or without a hysterectomy (one woman had a premenopausal bilateral oophorectomy without a hysterectomy, and four women who had a postmenopausal bilateral oophorectomy without a hysterectomy)

^a Adjusted for age at blood draw, fasting status, date of blood draw, time of blood draw, age at first birth/parity, BMI at blood draw, alcohol consumption, family history of breast cancer, personal history of benign breast disease, past HT use, and age at menopause

^b P value based on a t test from a fixed-effects model

^c P value based on global F test from a fixed-effects model

^d P value for pairwise comparisons: premenopause oophorectomy versus natural menopause $p<0.0001$; postmenopause oophorectomy versus natural menopause $p=0.003$; premenopause oophorectomy versus postmenopause oophorectomy $p=0.80$

^e P value for pairwise comparisons: premenopause oophorectomy versus natural menopause $p=0.008$; postmenopause oophorectomy versus natural menopause $p=0.42$; premenopause oophorectomy versus postmenopause oophorectomy $p=0.05$

Plasma estradiol, estrone, estrone sulfate, DHEAS, or prolactin levels were similar in women who underwent a natural menopause compared with women who had a bilateral oophorectomy during premenopause or postmenopause ($P \geq 0.23$). Patterns for free testosterone were similar with significantly lower levels among women who had an oophorectomy compared with those with natural menopause ($P < 0.0001$) (data not shown). There was no significant difference in free estradiol levels ($P = 0.36$) (data not shown).

Comparing women who had a simple hysterectomy to those who had natural menopause, we observed significantly lower plasma testosterone levels (19.1 vs. 20.7 ng/dL; $P = 0.03$), which was likely attributed to the lower levels in women who had surgery during premenopause (18.9 ng/dL) (global F -test $P = 0.05$) (Table 3). DHEAS levels were significantly lower in women who had a hysterectomy at any time compared to women who had natural menopause (51.1 vs. 59.6 $\mu\text{g/dL}$; $P = 0.02$). Further, DHEAS level varied significantly by timing of surgery, with significantly lower levels among women who had a hysterectomy during premenopause (48.2 $\mu\text{g/dL}$; global F -test $P = 0.004$). There was no significant difference in circulating levels of estradiol, estrone, estrone sulfate, prolactin or SHBG among women who had natural menopause compared to those who had an hysterectomy during premenopause or postmenopause ($P \geq 0.14$).

There were no significant interactions between BMI and oophorectomy or hysterectomy for any of the hormones (all P interactions ≥ 0.14) (data not shown). Additionally, no significant interactions were observed for age at blood draw (all P interactions ≥ 0.09), with the exception of prolactin

(P interaction = 0.004). Among women younger than 65 years at blood draw, those who had a natural menopause had higher levels of prolactin (9.4 ng/ml) compared with women with any oophorectomy (8.7 ng/ml). However, among women 65 years or older at blood draw, women who had a natural menopause had lower levels of prolactin compared with women with any oophorectomy (8.7 vs. 9.8 ng/ml, respectively).

We also categorized women based on the median number of years that lapsed between surgery and blood draw (i.e., ≤ 15 vs. > 15 years for oophorectomy and ≤ 20 vs. > 20 years for hysterectomy) and then evaluated the effect of timing of surgery with respect to blood draw on circulating hormone levels. We found no significant difference in hormone levels when comparing women above and below the median time lapse between blood draw and surgery ($P \geq 0.09$ and 0.32, for oophorectomy and hysterectomy, respectively).

Discussion

In this large cross-sectional study of postmenopausal women, we observed that a bilateral oophorectomy was associated with a significant 25 % lower level of circulating testosterone, irrespective of the timing of surgery. Interestingly, a simple hysterectomy was associated with significantly lower testosterone levels, although the magnitude of the association was smaller than for bilateral oophorectomy (8 %). We also observed significantly lower circulating SHBG levels with a premenopausal oophorectomy compared with those with natural menopause or a postmenopausal oophorectomy. A

Table 3 Adjusted geometric means of sex hormone levels by timing of simple hysterectomy

Hormone	Natural menopause		Any hysterectomy		P^b	Premenopausal hysterectomy		Postmenopausal hysterectomy		P^c
	<i>n</i>	Mean (95 %CI) ^a	<i>n</i>	Mean (95 %CI)		<i>n</i>	Mean (95 %CI)	<i>n</i>	Mean (95 %CI)	
Estradiol (pg/ml)	1,388	5.5 (5.3, 5.6)	242	5.8 (5.4, 6.2)	0.14	205	5.7 (5.3, 6.2)	37	5.8 (5.0, 6.9))	0.35
Estrone (pg/ml)	1,332	24.2 (23.5, 24.9)	229	24.9 (23.4, 26.6)	0.38	194	24.6 (22.9, 26.4)	35	26.4 (22.8, 30.6)	0.49
Estrone sulfate (pg/ml)	1,066	223.9 (214.9, 233.4)	177	216.6 (195.9, 239.5)	0.55	149	209.0 (187.2, 233.3)	28	257.5 (204.3, 324.5)	0.23
Testosterone (ng/dL)	1,494	20.7 (20.2, 21.3)	256	19.1 (17.9, 20.4)	0.03	219	18.9 (17.6, 20.2)	37	20.1 (17.3, 23.4)	0.05 ^d
DHEAS ($\mu\text{g/dL}$)	1,090	59.6 (56.8, 62.5)	191	51.1 (45.6, 57.3)	0.02	163	48.2 (42.6, 54.5)	28	69.5 (52.3, 92.5)	0.004 ^e
Prolactin (ng/mL)	1,150	9.1 (8.9, 9.4)	181	9.6 (8.9, 10.3)	0.23	155	9.6 (8.9, 10.4)	36	9.3 (7.7, 11.1)	0.45
SHBG (nmol/L)	1,460	58.1 (56.7, 59.5)	249	55.4 (52.3, 58.7)	0.15	213	55.5 (52.1, 59.2)	26	54.8 (47.9, 62.7)	0.34

Simple hysterectomy defined as uterus removed and both ovaries intact

^a Adjusted for age at blood draw, fasting status, date of blood draw, time of blood draw, age at first birth/parity, BMI at blood draw, alcohol consumption, family history of breast cancer, personal history of benign breast disease, past HT use, and age at menopause

^b P value based on a t test from a fixed-effects model

^c P value based on a global F test from a fixed-effects model

^d P value for pairwise comparisons: premenopausal hysterectomy versus natural menopause $p = 0.01$; postmenopausal hysterectomy versus natural menopause $p = 0.70$; premenopausal hysterectomy versus postmenopausal hysterectomy $p = 0.44$

^e P value for pairwise comparisons: premenopausal hysterectomy versus natural menopause $p = 0.002$; postmenopausal hysterectomy versus natural menopause $p = 0.29$; premenopausal hysterectomy versus postmenopausal hysterectomy $p = 0.02$

premenopausal hysterectomy was associated with significantly lower DHEAS levels. Given that adipose tissue is an important source of sex hormones in postmenopausal women, we also evaluated whether these associations varied by BMI [28, 29]. There was no evidence for effect modification by adiposity. Collectively, our findings support a role of testosterone in the etiology of breast cancer, irrespective of menopausal status.

In the general population, a bilateral oophorectomy when performed before menopause is associated with a significant reduction in breast cancer risk [2–10]. Although attributed to a reduction in ovarian hormones, whether this protective effect is mediated by a reduction in estrogen, testosterone, or both has not been elucidated. In an analysis of 13 studies which included a subset participants from the current analysis ($n=6,291$), Key et al. compared circulating sex hormone levels in postmenopausal women by type of menopause (i.e., natural vs. hysterectomy vs. bilateral oophorectomy) [12]. Compared with women with a natural menopause, androstenedione, DHEAS, testosterone, and free testosterone levels were 13 %, 11 %, and 30 % lower for women with a bilateral oophorectomy, respectively, with intermediate circulating androgen levels among women who had a simple hysterectomy. They reported no significant difference in SHBG levels, and similar to our findings, they observed no differences in estradiol or estrone levels across groups. The intermediate androgen levels reported with a hysterectomy have been attributed to the misclassification of women with a hysterectomy status (i.e., due to an unknown oophorectomy status) or damage to the ovarian artery during surgery leading to impaired ovarian hormonal secretion [30–32]. Notably, in our study, androgen levels were similar by timing of bilateral oophorectomy, suggesting that removal of the ovaries has a similar influence on testosterone production regardless of when the surgery occurs.

It has been well-documented that hormonal changes at menopause include a large, sustained drop in circulating estrogen levels, but only a small, gradual decline in androgen synthesis by the ovaries and adrenal glands [27]. In turn, the testosterone and androstenedione that continues to be secreted by the ovaries are used as substrates for subsequent conversion to estradiol and estrone by aromatization in the breast and other tissues [33]. In the general population, there is epidemiologic evidence to support an etiologic role of androgens for both premenopausal and postmenopausal breast cancer [34–37, 22]. Androgens may influence breast cancer risk by two mechanisms: (1) directly by increasing cellular growth and proliferation or (2) indirectly through their aromatization to estrogens [38]. The findings of the current study suggest that either a premenopausal and postmenopausal oophorectomy are associated with substantially lower testosterone levels. We reported similarly, but less dramatic, lower levels with a simple hysterectomy. Of particular importance is that we observed no significant effect of oophorectomy or hysterectomy on circulating

estrogen levels, despite the relatively high correlation of circulating estrogens with testosterone [39]. Our findings corroborate the underlying production of testosterone by the ovaries even in postmenopausal women and that the removal of the ovaries specifically affects levels of this hormone. Collectively, these observations suggest that the lowering of circulating androgens (i.e., testosterone) represents one mechanism by which a bilateral oophorectomy protects against breast cancer development. A possible protective effect of a shorter duration of exposure to high levels of premenopausal estrogens associated with an oophorectomy performed prior to menopause cannot be excluded.

Although the epidemiologic evidence to date generally supports a protective role of only premenopausal oophorectomy for breast cancer, studies with substantially longer follow-up of women with a postmenopausal oophorectomy may confirm similar reductions in breast cancer risk given that we reported comparable lowering of testosterone irrespective of timing of surgery. In contrast, a recent publication using data from the large Cancer Prevention Study–II Nutrition Cohort ($n=66,802$; median follow-up of 13.9 years), Gaudet et al. reported a significant inverse relationship between hysterectomy plus oophorectomy performed at any age and breast cancer risk relative to no surgery (overall HR=0.80; 95 %CI 0.73–0.88) [11]. The HR based on oophorectomy performed at ages <45, 45–54, and ≥ 55 were 0.73 (95 %CI 0.62–0.86), 0.81 (95 %CI 0.72–0.92), and 0.85 (95 %CI 0.73–0.98), respectively. Interestingly, Kotsopoulos and colleagues have previously reported a significant reduction in breast cancer risk among *BRCA* mutation carriers who had an oophorectomy after natural menopause [13]. The substantially different findings in the latter two studies compared with the earlier publications may be due to substantially longer follow-up as well as differences in the distribution of the age at oophorectomy. It is clear that well-powered prospective studies with long follow-up are needed to evaluate the role of timing of oophorectomy versus natural menopause on breast cancer risk.

SHBG levels were different only for women with premenopausal oophorectomy. It is possible that women who undergo a premenopausal oophorectomy, and thus have an early menopause, may be more susceptible to weight gain than women with a postmenopausal oophorectomy, leading to lower SHBG levels [40, 41]. Although this hypothesis is supported by the fact that the association in our study was slightly attenuated with additional adjustment for WHR, a metric of central adiposity, it does not completely explain the differences by oophorectomy status.

Why a simple hysterectomy would lead to lower DHEAS levels if performed prior to menopause is unclear, given that DHEAS is produced exclusively by the adrenal gland [42]. This finding may be due to chance or uncontrolled confounding but requires further investigation in a larger study population. A hysterectomy alone does not appear to be associated with the

risk of breast cancer in the general population [43, 44]. In the aforementioned study by Danforth et al., there was no significant association of hysterectomy and/or oophorectomy with DHEA or DHEAS levels, although the sample size in that study was smaller than in the current analysis [45]. We observed no relationship between oophorectomy or hysterectomy and circulating prolactin levels, and this represents, to our knowledge, the first evaluation of surgical menopause and prolactin levels.

The distinguishing feature of our study was the ability to classify women into those who underwent a premenopausal versus postmenopausal oophorectomy. We were able to limit our analysis to postmenopausal women not using HT and utilized strict criteria to create accurate categories for timing of oophorectomy and hysterectomy. The main limitation of our study is that plasma hormone levels may not necessarily be reflective of tissue hormone levels. Much of the evidence for hormonal breast carcinogenesis comes from clinical and epidemiologic studies of circulating estrogen and other hormones [37]; however, it is believed that local breast tissue levels of hormones are much more relevant than blood levels [46]. Studies that quantify breast hormone levels prior to and following oophorectomy are necessary to accurately evaluate the impact of oophorectomy on tissue levels of these hormones. In addition, we were not sufficiently powered to decipher an effect of timing of surgery on hormone levels given the small number of women who had undergone postmenopausal surgery. Although we did not have information on indications for surgery, Nichols et al. have shown no confounding by nonmalignant conditions when evaluating the relationship between surgery and breast cancer risk [47].

In summary, our findings confirm that oophorectomy prior to and after menopause modestly lowers plasma concentrations of androgens, specifically testosterone. For women in the general population, surgical menopause, specifically a bilateral oophorectomy, may influence the risk of premenopausal and postmenopausal breast cancer by affecting circulating levels of testosterone. Whether testosterone has a direct effect on breast cancer risk or through conversion to estrogen remains unclear. Future studies that quantify breast hormone levels, and possibly aromatase activity, before and after oophorectomy, are necessary to confirm how androgens influence risk (i.e., directly or indirectly) and will help delineate the most appropriate hormone inhibitor (i.e., androgen blockade vs. aromatase inhibitor) to prevent postmenopausal breast cancer.

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

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