

Relation of Prediagnostic Serum Estrogen and Androgen Levels to Breast Cancer Risk

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Abstract

To evaluate the relation of serum sex hormones to breast cancer risk, we conducted a prospective nested case-control study using the Breast Cancer Serum Bank (Columbia, MO). This bank included serum from 3375 postmenopausal women free of cancer and not taking replacement estrogens when they donated blood between 1977 and 1987. Of these, 71 were diagnosed subsequently with breast cancer. For each case, two women alive and free of cancer at the age of the case's diagnosis and matched to the case on age and on date and time of day of blood collection were selected as controls. The median age of subjects at blood collection was 62 years, and the time from blood collection to diagnosis ranged from less than 1 to 9.5 years, with a median of 2.9 years. Postmenopausal women with elevated serum levels of total and non-sex hormone-binding globulin-bound E2 were at an increased risk of developing breast cancer. For non-sex hormone-binding globulin-bound E2, risks were elevated 4-5-fold for women in the upper three quartiles relative to those in the lowest quartile. Although breast cancer was not related to estrone or estrone sulfate concentration, the ratio of estrone sulfate to estrone was significantly inversely associated with risk, suggesting that women who develop breast cancer may be less able to metabolize estrone to its less active form. Serum testosterone was significantly positively associated with postmenopausal breast cancer; the relative risk for women in the highest *versus* the lowest quartile was 6.2 (95% confidence interval, 2.0-19.0). Our results support the hypothesis that prediagnostic serum estrogens and

androgens are related to the subsequent diagnosis of breast cancer in postmenopausal women.

Introduction

Numerous lines of evidence suggest that breast cancer is hormone related. Breast cancer is seen rarely in men; breast tumors are frequently estrogen dependent; and reproductive history, including ages at menarche and menopause, affects risk (1). Given this biological and epidemiological evidence, one could hypothesize a relation between serum sex hormones and postmenopausal breast cancer. However, results of five prospective studies that have evaluated the relation of serum estrogens to risk have been inconsistent (2-6). This may be explained partly by small sample sizes; four of the studies included fewer than 40 cases (3-6). Although the breast is metabolically active and can synthesize estrogens from androgen precursors (7-9), and some breast tumors are androgen dependent (10), the relation of prediagnostic serum androgens to postmenopausal breast cancer has been assessed in only three small studies (3-5).

In an effort to clarify the relation of serum sex hormones to breast cancer, we performed a prospective nested case-control study using the Breast Cancer Serum Bank. This serum bank included prediagnostic serum from 3375 postmenopausal women free of cancer and not taking replacement hormones when they donated blood to the bank between 1977 and 1987. Of these, 71 were diagnosed with breast cancer by the end of follow-up in 1989.

Materials and Methods

The Breast Cancer Serum Bank was established initially in 1977 as part of the National Cancer Institute's Biological Markers Project to identify serum markers for breast cancer. Columbia was one of three participating sites nationally and was charged specifically with collecting serum from women free of breast cancer. The other two sites, located in Michigan and Delaware, collected serum from women with breast cancer and benign breast disease.

Participants in Missouri were volunteers identified through three sources: the Breast Cancer Detection Demonstration Project; Women's Cancer Control Program at the Cancer Research Center, University of Missouri Hospital; and the Ellis Fischel Cancer Center. A total of 7224 women who were initially free of breast cancer donated blood to the bank on one or more occasions between 1977 and 1987. More than 90% of the women first gave blood in 1980 or earlier. Active follow-up by mail continued until 1989, but 70% of the cohort were last contacted in 1982-1983, at least partly because of funding changes. At the time of last contact, 91% of the total cohort were alive and free of breast cancer, 2% had been diagnosed with breast cancer, 5% were dead from a cause other than breast cancer, and 2% were considered lost to follow-up. Pathology reports were obtained for all women who reported a positive breast biopsy or mastectomy on follow-up.

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Women included in the current study were restricted to those who had at least 4 ml of blood remaining in the bank and who, at the time of blood collection, had no history of cancer other than nonmelanoma skin cancer, were not diagnosed with benign breast disease within the past 2 years, were postmenopausal, and did not report taking replacement estrogens. Women were initially classified as postmenopausal if they reported natural menopause, bilateral oophorectomy, or radiation to the ovaries prior to blood collection or were at least 51 years of age at blood collection with a history of hysterectomy without oophorectomy. Final determination of menopausal status was based on serum FSH² levels. Any woman with a FSH concentration less than 35 mIU/ml was considered potentially premenopausal, and her reported date of last menses, age, and hormonal profile were reviewed to determine eligibility.

Of the 3375 women who met these criteria, 72 were diagnosed subsequently with histologically confirmed breast cancer. For each of these cases, two controls were selected from among the eligible women using incidence density sampling. Controls were alive and free of cancer (except nonmelanoma skin cancer) at the age of the case's diagnosis and were matched to the case on exact age and on date (± 1 year) and time (± 2 h) of blood draw. Two controls who met the matching criteria could not be identified for 12 cases. For these cases, matching criteria were relaxed as follows: (a) age, ± 1 year ($n = 8$); (b) blood draw, ± 2 years ($n = 3$); (c) age, ± 4 years and blood draw ± 2 years and ± 4 h ($n = 1$). After review of FSH results, one case and four controls were dropped because they were premenopausal, and one control was dropped because her hormone profile was consistent with exogenous estrogen use. This left 66 case-control sets with two controls and 5 case-control sets with one control for analysis.

Serum specimens were collected, and clinical data, including age, height, weight, menstrual and reproductive histories, smoking, medication (including hormone) use, and family history of breast cancer, were obtained by self-report or medical record review after obtaining informed consent. Approximately 10 ml of serum were collected from each woman using standard procedures. Blood was chilled immediately, and serum was separated and aliquoted into glass vials within 2 h of collection. Serum was shipped on dry ice to the Mayo Foundation repository, where it was maintained at -70°C until analysis. Blood was stored for a median of 16 years prior to analysis for both cases and controls.

Hormones in serum were measured using commercially available RIA kits as follows: (a) E2 and testosterone (Diagnostic Products Co., Los Angeles, CA); (b) E1 and androstenedione (Diagnostic Systems Labs, Webster, TX); and (c) DHEAS. (ICN Biomedical, Costa Mesa, CA). E1S also was measured by RIA after solvolysis, extraction of hydrolyzed E1, and celite chromatography (11). SHBG was measured by an immunoradiometric assay kit (Farnos Group Ltd., Oulunsalo, Finland). Percentages of unbound and albumin-bound E2 were measured using centrifugal ultrafiltration (12), and SHBG-bound E2 was calculated.

Serum samples for each case-control set were grouped and analyzed in the same batch. Within-batch CVs for log_e-transformed hormone levels in blind replicate quality control samples included in these batches were 5.0% for E1, 23.7% for E2,

Table 1 Characteristics of cases and controls at blood collection

	Cases ($n = 71$)		Controls ($n = 133$)		<i>P</i> -value ^a
	Median	5-95 percentile	Median	5-95 percentile	
Age at blood collection (yr)	61	52-71	62	52-73	0.53
Age at menopause (yr)	50	43-56	50	42-55	0.66
Time since menopause at blood collection (yr)	11.1	2.3-23.5	12.4	2.6-25.7	0.49
Age at menarche (yr)	13	11-16	13	11-16	0.38
Parity	2	0-5	3	0-6	0.06
Age at first pregnancy (yr)	24	18-31	23	18-32	0.83
Height (cm)	163	152-170	160	152-169	0.02
Weight (kg)	68	56-94	65	52-99	0.24
Body mass index (kg/m ²)	26.0	21.3-32.9	25.2	20.1-37.3	0.44

^a *P* values from Wilcoxon rank sum test.

4.0% for E1S, 1.3% for androstenedione, 0.7% for DHEAS, 5.8% for testosterone, and 1.6% for SHBG. The high CV for E2 was likely due, in part, to the low level of this hormone in the quality control samples; the concentration in the 39 replicates averaged 5.3 pg/ml, which was close to the detection limit of the assay. Within-batch CVs for percentages of unbound and albumin-bound E2 were calculated on arc sine-transformed values and were 12.4 and 16.4%, respectively.

Geometric mean hormone levels for cases and controls were compared using Student's *t* tests. The relation of serum hormones to breast cancer risk for the matched sets was evaluated using conditional logistic regression (13). Women were stratified into quartiles based on their hormone levels relative to the distribution of hormone values in controls, and a set of categorical (dummy) variables was included in models. Models were also fit using quartile medians to test for trends. To adjust for known breast cancer risk factors, time since menopause, height, weight, parity, and family history of breast cancer were included in models. Interactions of hormone levels with age (a matching criterion) and breast cancer risk factors included in adjusted models were tested by including cross-product terms in models. Matching criteria were relaxed considerably for one case. When analyses were repeated excluding this case and her controls, findings were not changed and they, therefore, were included in the reported results. All analyses were performed using SAS Statistical Software (14).

Results

Characteristics of study subjects are summarized in Table 1. Their median age was 62 years, and except for one control, all subjects were white. Their weights and body mass indexes did not differ, but cases were significantly taller than controls. Cases tended to have fewer children, and a slightly larger proportion of cases (20%) than controls (14%) were nulliparous. Menopause occurred naturally in 54 cases (76%) compared to 96 controls (72%), and median times from menopause to blood collection did not differ significantly by case status. A positive family history of breast cancer among mothers, grandmothers, sisters, or blood-related aunts was reported by 34% of cases and 28% of controls ($P = 0.42$). The time from blood collection to diagnosis of breast cancer ranged from less than 1 to 9.5 years, with a median of 2.9 years.

Geometric mean serum hormone levels for cases and controls are shown in Table 2. Although cases had 17% higher total and 21% higher non-SHBG bound (bioavailable) E2 levels compared to controls, differences were not statistically signif-

² The abbreviations used are: FSH, follicle-stimulating hormone; DHEA, dehydroepiandrosterone; DHEAS, DHEA sulfate; SHBG, sex hormone-binding globulin; CV, coefficient of variation; RR, relative risk; CI, confidence interval; E1S, estrone sulfate; E1, estrone; E2, estradiol.

Table 2 Geometric mean serum hormone levels for cases and controls

	Cases (n = 71)		Controls (n = 133)		P value ^a
	Mean	95% CI	Mean	95% CI	
Estradiol (pM)	56.7	48.7–66.1	48.6	41.4–57.0	0.17
Non-SHBG-bound E2 (pM)	25.8	21.7–30.6	21.3	17.9–25.3	0.13
E1 (pM)	127.5	115.2–141.0	119.8	110.0–130.5	0.38
E1S (pM)	510.4	450.3–578.4	547.7	494.9–606.0	0.41
Testosterone (nM)	0.75	0.63–0.89	0.55	0.47–0.65	0.01
Androstenedione (nM) ^b	3.30	2.80–3.88	2.99	2.75–3.25	0.02
DHEAS (μM)	2.41	2.03–2.87	2.01	1.80–2.25	0.08
SHBG (nM)	46.4	39.8–54.2	49.1	44.2–54.6	0.55

^a P value (2-sided) from *t* test.

^b One matched set was deleted, because the case was an influential outlier.

icant. The differences between cases and controls in serum testosterone (36%) and androstenedione (10%), however, were significant, and the difference in DHEAS (20%) was marginally significant.

As shown for controls in Table 3, serum sex hormone levels, particularly the estrogens, are highly correlated. Correlations of total and non-SHBG bound E2 levels approached 1.0; otherwise, correlations among estrogens ranged between 0.54 and 0.68, and correlations among androgens ranged between 0.30 and 0.58. Correlations between the estrogens and androgens were slightly lower, but with the exception of that for E1S and testosterone, all were statistically significant. SHBG was inversely correlated with serum sex hormone levels and significantly so for the estrogens.

Table 4 presents RRs for breast cancer by quartile of serum hormone concentration. Unadjusted and adjusted RRs were similar. Relative to women in the lowest quartile, women in the upper three quartiles of total and non-SHBG bound E2 concentration were at an increased risk of breast cancer, but no gradient was observed. Although risk did not vary significantly by quartile of E1 or E1S concentration, the E1S:E1 ratio was inversely associated with risk (trend $P = 0.04$).

Serum testosterone level was associated significantly and positively with breast cancer. After adjustment for known breast cancer risk factors, women in the third and fourth (highest) quartiles had significant excess risks of 3- and 6-fold, respectively, compared to those in the lowest quartile, and the trend was significant ($P = 0.002$). Although the trend was not significant ($P = 0.13$), breast cancer risk also increased monotonically with serum androstenedione level. The relation of DHEAS with breast cancer was not consistent, and a significant association was observed only for women in the highest quartile.

No significant interactions were observed between serum hormone levels and age at blood collection, interval between blood collection and diagnosis, or covariates included in the final model. However, cases' serum testosterone levels increased significantly by 9% for each year closer to diagnosis that blood was collected compared to less than 1% per year over the same interval for controls. As shown in Table 5, when we restricted analysis to the 25 cases whose blood was collected within 2 years of diagnosis, the unadjusted RRs for women with testosterone levels in the middle and upper tertiles of the distribution were 2.5 and 4.1, respectively. For the 46 women whose blood was collected more than 2 years before diagnosis, the comparable RRs were 1.0 and 1.3. Similar analyses performed for other hormones did not reveal any other remarkable

discrepancies in results when women were stratified by time between blood collection and diagnosis.

Because of concerns about incomplete follow-up through the end of the study in 1989, we reanalyzed serum hormone-breast cancer associations after truncating the follow-up period to 1982–83 when follow-up was better than 90% complete. During this early phase of the study, 53 breast cancers were diagnosed. Risk of breast cancer in this subgroup in relation to serum estrogens and androgens did not differ materially from those reported for the entire cohort. Women in the three higher quartiles of non-SHBG-bound E2 were at an increased risk relative to women in the lowest; adjusted for years since menopause, height, weight, family history of breast cancer, and parity, RRs by increasing quartile were 1.0, 3.7 (95% CI, 1.1–13.2), 3.5 (95% CI, 1.0–12.2), and 4.0 (95% CI, 1.1–15.0). The risk of breast cancer in these women also increased monotonically with increasing serum testosterone level with RRs for the first through fourth quartiles of 1.0, 2.7 (95% CI, 0.5–13.9), 3.7 (95% CI, 0.9–16.3), and 7.1 (95% CI, 1.7–29.7). Furthermore, although it did not achieve statistical significance, the inverse association of the E1S:E1 ratio seen in the entire cohort was apparent in this subgroup; RRs by increasing quartile of this ratio were 1.0, 0.6 (95% CI, 0.2–1.9), 0.3 (95% CI, 0.1–1.1), and 0.4 (95% CI, 0.1–1.5).

Discussion

Findings of this prospective study provide strong support for an association between serum sex hormone levels and the subsequent development of breast cancer in postmenopausal women. Women with elevated levels of E2, particularly the bioavailable fraction, are at increased risk, whereas women who more readily inactivate E1 by converting it to E1S appear to be protected. Testosterone was strongly positively associated with postmenopausal breast cancer risk, but we could not discern from the available data whether elevated serum levels were a cause or effect of the malignancy. A larger prospective study is needed to clarify the relationship between serum testosterone and breast cancer.

Potential subjects for this study volunteered to donate blood on one or more occasions over a 10-year period. To obtain bloods furthest in time from diagnosis, for cases we selected the first postmenopausal collection, with a sufficient volume of serum remaining to perform the assays of interest. For 70 cases, this was the first postmenopausal collection, and for 1 case, it was the second. Only one potential case was lost because of insufficient volume remaining in the serum bank. Although we also selected control specimens from postmenopausal collections, we did not limit specimens to the first postmenopausal collection. For 109 (82%), it was the first; for 22 (17%), it was the second; and for 2 (1%), it was the third postmenopausal collection. When we refit all final adjusted models using only cases and controls whose serum was from their first postmenopausal collection, findings did not differ materially from those reported in Table 4.

The age-adjusted incidence of breast cancer among women 50 years of age and older in the cohort was 128/100,000 person years followed, which is less than the average incidence of 289/100,000/year reported for whites of the same age by the Surveillance, Epidemiology, and End Results Program during the period of case ascertainment for the study (15). The lower rate in our cohort may have been due to a lower incidence of breast cancer in the community, a lower incidence among women who volunteered to participate in the study, or incomplete follow-up. If the latter is correct, bias could have been

Table 3 Pearson correlations of log_e-transformed serum hormone levels in controls (n = 133)

	Estradiol	Non-SHBG-bound E2	E1	E1S	Testosterone	Androstenedione	DHEAS	SHBG
Estradiol								
Non-SHBG bound E2								
E1								
E1S								
Testosterone								
Androstenedione								
DHEAS								
SHBG								

introduced if serum hormones were related to follow-up, and this association differed for women who did and did not develop breast cancer. Because data on response rates were not tabulated during the conduct of the study, and these records were destroyed at completion of the contract, statistics on response rates cannot be reported. However, our findings of similar associations between serum hormones and breast cancer over the entire study period and during the early phase, when follow-up was more than 90% complete, suggest that the associations we observed were not biased by incomplete follow-up.

Women in the upper three quartiles of E2 were at an elevated risk of breast cancer. The relation was more consistent for non-SHBG-bound E2, the fraction that is biologically available, than for total E2. The increase in risk of breast cancer for women in the second quartile with no further increase in higher quartiles could reflect a threshold effect with a relatively low set-point, small numbers, or a noncausal association.

Toniolo *et al.* (2), in the largest prospective study to date, also observed a significant association of serum E2 and breast cancer risk in postmenopausal women. Similar to our findings, women in the two upper quartiles were at increased risk, and associations for the bioavailable fractions of E2 were stronger than for total E2. Helzlsouer *et al.* (3) also reported a 4-fold excess risk of breast cancer among postmenopausal women in the upper tertile of E2. Although the result was not statistically significant, the sample size was small, including only 30 cases. Moore *et al.* (6) reported cases having a significantly higher percentage of E2 in the free and albumin-bound fractions, and hence bioavailable. Although null associations also have been reported (4, 5), sample sizes generally have been small. Our findings, in combination with other recent reports, support a positive association between prediagnostic serum E2 and subsequent breast cancer in postmenopausal women.

Neither E1 nor E1S levels in serum were associated with the risk of developing breast cancer. The E1S:E1 ratio was, however, strongly inversely associated with risk. E1 is inactivated primarily in the liver by sulfurylation (16), and these data suggest that women who develop breast cancer may be less able to inactivate E1 compared to other women.

Toniolo *et al.* (2) reported a positive association between serum E1 and breast cancer, although Helzlsouer *et al.* (3) did not detect an association. Results for E1S were not reported by either of these investigators.

Breast tumors are metabolically active, and the observed differences in E2 levels and the E1S:E1 ratio in serum collected, on average, 3 years prior to diagnosis, could be consequences rather than causes of cancer. The lack of an association between serum estrogen levels and time from blood collection to diagnosis and the consistency of breast cancer associations when analyses were stratified by time interval from blood collection to diagnosis argue against this possibility.

Concentrations of E2 in normal breast tissue and tumor tissue are higher than and do not correlate with blood levels in postmenopausal women (7, 17–19). The breast could concentrate E2 from the serum or synthesize estrogens from androgens via aromatase, an enzyme that is present in both normal and cancerous breast tissue (8, 9, 20, 21). In our study, mean serum concentrations of testosterone and androstenedione in controls were, respectively, 11 and 61 times higher than E2. Serum androgens, therefore, could provide a large pool of available substrate for conversion to estrogens in the breast and, thereby, stimulate tumor growth. Alternatively, androgens could stimulate tumor growth directly (10).

Higher androstenedione and testosterone levels in postmenopausal women who developed breast cancer have been reported for the Washington County, Maryland cohort, although differences were not significant (3). Adami *et al.* (22) also reported significant elevations of serum androstenedione in postmenopausal women with breast cancer in a case-control study, and significant elevations of testosterone among cases have been reported in numerous case-control studies (22–26). In premenopausal women with breast cancer, an increased concentration of testosterone in breast fluid also has been observed (27).

Androstenedione and testosterone are secreted by ovarian interstitial cells, and ovarian interstitial hyperplasia is a common finding on autopsy of women with breast cancer (28). Grattarola (29) identified hyperplastic ovarian interstitial cells associated with elevated serum testosterone levels in premenopausal breast cancer patients and proposed that the ovaries may be the source of excess testosterone in these women. Six cases (8.4%) and 16 controls (12.0%) in our study had oophorectomies prior to blood collection ($P = 0.49$), which was too few to evaluate the association of serum testosterone and breast cancer risk in this group separately. However, exclusion of these women from analyses did not materially alter the relation of serum testosterone to breast cancer; RRs for successive quartiles of testosterone were 1.9 (95% CI, 0.5–6.9), 2.5 (95% CI, 0.8–7.7), and 5.5 (95% CI, 1.7–18.0).

In vitro, both normal and cancerous breast tissue can metabolize androstenedione to testosterone (30–32). Fan *et al.* (33) observed a significant decrease in plasma testosterone after breast tumor excision and proposed that the tumor may be secreting testosterone. Hamed *et al.* (34) also observed higher levels of free testosterone in lymph draining the tumor site in women with incomplete excision compared to women with complete excision. However, van Landeghem *et al.* (35) and Massobrio *et al.* (36) failed to observe elevated testosterone in venous drainage of cancerous breasts, which would be expected if the tumor were the source of elevated testosterone in peripheral blood.

Table 4 Relation of serum hormones to breast cancer risk

	Number of subjects		Unadjusted		Adjusted ^a		Trend P
	Cases	Controls	RR	95% CI	RR	95% CI	
E2 (pM)							
<29.3	6	32	1.0		1.0		0.12
29.3–51.4	24	31	3.9 ^b	1.4–10.7	4.9 ^b	1.6–14.9	
51.5–88.1	26	36	3.9 ^b	1.4–10.7	4.7 ^b	1.5–14.3	
88.2+	15	34	2.3	0.8–6.7	2.7	0.8–9.1	
Non-SHBG-bound E2 (pM)							
<11.4	5	33	1.0		1.0		0.12
11.4–22.1	24	33	4.8 ^b	1.7–14.1	5.9 ^b	1.8–19.3	
22.2–38.8	23	33	4.5 ^b	1.6–13.1	4.8 ^b	1.5–15.7	
38.9+	19	34	3.8 ^c	1.3–11.6	5.2 ^b	1.5–18.5	
E1 (pM)							
<85.1	14	31	1.0		1.0		
85.1–129.4	17	34	1.2	0.5–2.9	1.2	0.5–3.3	
129.5–162.7	16	34	1.1	0.4–2.8	1.0	0.4–2.9	
162.8+	24	34	1.7	0.7–4.2	1.8	0.6–5.1	
E1S (pM)							
<376.6	19	33	1.0		1.0		0.58
376.6–525.0	19	33	0.9	0.4–2.3	0.8	0.3–2.1	
525.1–759.0	17	33	0.8	0.3–2.0	0.8	0.3–2.2	
759.1+	16	34	0.8	0.3–2.0	0.8	0.3–2.2	
E1S:E1							
<3.30	28	33	1.0		1.0		0.04
3.30–4.69	26	33	0.4 ^c	0.2–1.0	0.5	0.2–1.2	
4.70–6.26	14	33	0.3 ^c	0.1–0.9	0.3 ^c	0.1–1.0	
6.27+	13	33	0.2 ^b	0.1–0.7	0.3 ^c	0.1–0.9	
Testosterone (nM)							
<0.34	9	32	1.0		1.0		
0.34–0.58	13	28	1.8	0.6–5.0	2.9	0.9–9.4	
0.59–0.90	20	39	2.1	0.8–5.6	2.9 ^c	1.0–8.6	
0.91+	29	34	3.7 ^b	1.4–10.0	6.2 ^b	2.0–19.0	
Androstenedione (nM)							
<2.23	10	30	1.0		1.0		0.02 ^d
2.23–3.10	20	34	1.9	0.7–4.7	1.7	0.6–4.7	
3.11–4.25	20	35	1.7	0.7–4.1	2.0	0.8–5.3	
4.26+	21	34	1.8	0.8–4.4	2.2	0.8–5.8	
DHEAS (μM)							
<1.32	13	33	1.0		1.0		0.05
1.32–2.19	19	31	1.4	0.6–3.4	1.6	0.6–4.1	
2.20–3.17	8	34	0.6	0.2–1.8	0.6	0.2–1.9	
3.18+	31	35	2.2	1.0–5.2	2.8 ^c	1.1–7.4	
SHBG (nM)							
<33.3	15	33	1.0		1.0		0.99
33.3–53.4	23	33	1.6	0.7–3.8	1.4	0.5–3.7	
53.5–73.9	17	33	1.2	0.5–3.0	1.4	0.5–4.0	
74.0+	16	34	1.1	0.5–2.6	1.3	0.5–3.9	

^a Adjusted for years since menopause, height, weight, family history of breast cancer, and parity.

^b $P \leq 0.01$.

^c $P \leq 0.05$.

^d One matched set was deleted because the case was an influential outlier.

Testosterone level was related inversely to time to diagnosis of breast cancer, and the association of testosterone to breast cancer risk was stronger in women who were diagnosed within 2 years of blood collection. However, CIs on RRs from stratified analyses were wide, and the interaction between testosterone level and time to diagnosis in the full model was not significant. Therefore, although serum testosterone was clearly related to breast cancer risk in our data, because of the relatively small sample size, we could not discern whether elevated levels were a cause or an effect of the malignancy.

DHEA and its sulfate ester, DHEAS, are intermediates in estrogen biosynthesis, and DHEAS may inhibit the metabolism of E2 to the less potent estrogen, E1 (37). Laboratory studies,

however, suggest a protective effect of DHEA and DHEAS for breast cancer (38).

Two previous prospective epidemiological studies reported no difference in postmenopausal breast cancer associated with serum DHEAS and an increased risk associated with elevated DHEA level (39, 40). In our study, women in the highest quartile of DHEAS were at a significantly increased risk of breast cancer, but women in the next lowest quartile were at a nonsignificant decreased risk. Although our findings do not provide strong evidence for a positive association between DHEAS and breast cancer, they are consistent with other epidemiological studies in not supporting a protective effect of DHEAS for breast cancer in humans.

Table 5 Unadjusted RRs of breast cancer for tertiles of serum hormone levels by time from blood collection to diagnosis in cases

	≤2 years (n = 25 cases)		>2 years (n = 46 cases)	
	RR	95% CI	RR	95% CI
E2 (pM)				
<33.0	1.0			
33.0–66.0	1.0	0.3–3.1	1.0 ^a	
66.1+	1.5	0.4–5.3	1.2	0.6–2.6
Non-SHBG-bound E2 (pM)				
<14.6	1.0		1.0	
14.6–33.3	0.8	0.3–2.3	5.6	1.7–18.0
33.4+	1.1	0.3–4.1	2.2	0.7–6.8
E1 (pM)				
<96.2	1.0		1.0	
96.2–147.9	0.8	0.2–2.6	3.6	1.1–11.2
148.0+	1.7	0.5–6.3	2.0	0.6–6.4
E1S (pM)				
<439.5	1.0		1.0	
439.5–647.7	0.7	0.2–2.2	0.6	0.2–1.6
647.8+	0.8	0.2–2.9	0.7	0.2–1.8
E1S:E1				
<3.76	1.0		1.0	
3.76–5.67	0.2	0.1–1.0	0.5	0.2–1.6
5.68+	0.3	0.1–1.6	0.1	0.0–0.5
Testosterone (nM)				
<0.45	1.0		1.0	
0.45–0.79	2.5	0.6–11.2	1.0	0.4–2.7
0.80+	4.1	0.9–18.0	1.3	0.5–3.4
Androstenedione (nM)				
<2.51	1.0		1.0	
2.51–3.80	1.8	0.5–5.7	1.3	0.6–3.2
3.81+	1.8	0.5–6.2	1.3	0.5–3.0
DHEAS (μM)				
<1.55	1.0		1.0	
1.55–2.87	1.4	0.4–4.9	1.2	0.5–3.2
2.88+	1.3	0.4–4.9	2.3	0.9–5.9
SHBG (nM)				
<37.9	1.0		1.0	
37.9–64.7	1.1	0.3–4.1	1.7	0.6–4.4
64.8+	0.8	0.2–3.0	1.2	0.5–3.3

^a The lower two tertiles were combined to create reference category, because only one case was in the lowest tertile for E2.

In conclusion, results of this prospective study support the hypothesized association of serum estrogens to breast cancer risk. Furthermore, they suggest a relation between serum testosterone and breast cancer, which needs to be explored in additional studies to elucidate whether it is causal.

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