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Serum levels of sex hormones and breast cancer risk in premenopausal women: a case–control study (USA)

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Abstract

High levels of serum estrogens and androgens have been convincingly linked with an increased risk of breast cancer among postmenopausal women. By contrast, the role of blood levels of these hormones in the etiology of premenopausal breast cancer is not well understood. In a case–control study, we sought to examine associations between levels of serum estradiol, sex-hormone binding globulin (SHBG), dehydroepiandrosterone (DHEA), testosterone, androstenedione and progesterone and risk of premenopausal breast cancer. Cases of breast cancer under age 45 were identified using rapid ascertainment systems in Seattle/Puget Sound, Washington and control subjects were identified from the same area through random digit dialing methods. A total of 169 eligible breast cancer cases and 195 control subjects donated blood (either before or six or more weeks after surgery) and were interviewed using a standardized questionnaire. The fully adjusted risk ratios and 95% confidence intervals for the highest *versus* lowest tertiles of estradiol, according to menstrual cycle phase, were 3.10 (0.8–12.7) for early follicular, 0.54 (0.2–1.7) for late follicular and 0.60 (0.3–1.4) for luteal. Risks for highest *versus* lowest quartiles of SHBG and androgens were 0.81 (0.4–1.6) for SHBG, 2.42 (1.1–5.2) for DHEA, 1.12 (0.6–2.5) for testosterone, and 1.33 (0.6–2.8) for androstenedione. For luteal progesterone, the RR for the highest *versus* lowest tertile was 0.55 (0.2–1.4). In summary, we did not find a convincing association between serum SHBG, estradiol, testosterone or androstenedione and premenopausal breast cancer risk. Observed differences between cases and controls subjects in serum levels of DHEA and luteal phase progesterone should be investigated further in large prospective studies.

Introduction

A recent combined analysis of nine prospective cohort studies found the risk of postmenopausal breast cancer to be substantially elevated among women with high blood levels of several estrogens, including total estradiol and free estradiol, as well as several androgens, including androstenedione, dehydroepiandrosterone (DHEA), DHEA sulfate, and testosterone [1]. Despite the strength of the sex hormone paradigm in breast cancer etiology, results from epidemiologic studies have been inconclusive about the association between serum sex hormones and premenopausal breast cancer risk [2, 3]. This lack of consistency may well reflect the limited numbers of early onset breast cancer cases that have been studied. For example, only four case–control studies nested within cohort studies have examined associations between serum estradiol levels and

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premenopausal breast cancer risk, with the number of breast cancer cases studied ranging from 20 to 79 [4–7]. Furthermore, of 13 published case–control studies focused on serum estradiol and premenopausal breast cancer risk, all but one included less than 40 breast cancer cases [2, 8–10]. Even fewer small studies have examined the association between serum androgen levels and premenopausal breast cancer risk [4, 5, 7, 10–16]. Taking special care to address potential methodologic concerns that may be associated with case–control studies of serum sex hormones and breast cancer risk (*e.g.*, extent of disease at diagnosis, adjuvant treatment), we therefore examined data from a population-based case–control study of 169 breast cancer cases and 195 control subjects.

Material and methods

Study population

Overview

Study subjects were part of a larger population-based interview case–control study of early onset breast cancer conducted in three geographic areas in the United States covered by cancer registries: Atlanta, GA, Seattle/Puget Sound, and five counties in central New Jersey [17]. The Seattle/Puget Sound site included a blood collection substudy that is the focus of the present analysis. The study protocol was approved by the Institutional Review Board at the Fred Hutchinson Cancer Center and the National Cancer Institute. Written informed consent was obtained from all study subjects.

Seattle/Puget Sound interview case–control study

Cases were all women 20–44 years of age in Seattle/Puget Sound who were newly diagnosed with *in situ* or invasive breast cancer during the period May 1, 1990 to December 31, 1992. Cases were identified through rapid ascertainment systems and hospital records of eligible patients were abstracted to document details on the clinical and pathologic characteristics of the diagnosed breast cancers. Controls were ascertained through random digit dialing techniques. To select a sample of control women that approximated the expected age distribution of the cases, a telephone screening interview was used to obtain information on household composition at each number drawn from a Seattle/Puget Sound sampling frame. A 90.5% response rate to the telephone screener was obtained from 5722 telephone numbers. From the screener information, a stratified random sample of controls in five year age groups was selected for inclusion.

Structured in-person interviews were obtained from 644 of 712 eligible cases (90.4%) and 610 of 748 eligible control subjects (81.6%). Reasons for interview non-response include refusal (58 cases; 110 controls), death (2 cases; 1 control), language problem (1 case; 8 controls), and subject moved (7 cases; 19 controls). The overall response rate (the product of the telephone screener and interview response rates) was 73.8%.

The in-person interview collected information about demographic factors, reproductive and menstrual history, contraceptive behavior, use of exogenous hormones, medical history, use of cigarettes, physical activity, anthropometry, family history of cancer, and recent alcohol consumption. All information on risk factors was truncated at the date of diagnosis for cases or the date of completion of the telephone screening call for controls (the reference date). In addition, height, weight and other anthropometric measures were taken following the interview.

Seattle/Puget Sound blood collection substudy

Blood collection efforts were initially restricted to cases diagnosed at hospitals within a 45 miles radius of the research staff located in the Seattle office, with the focus on obtaining blood specimens prior to treatment, including surgery involving general anesthesia. If it was not possible to collect blood prior to surgery involving general anesthesia, blood collection was attempted six weeks or more after surgery. To achieve a sufficient number of study subjects, blood collection efforts were later expanded to areas outside the Seattle radius. Blood collection was attempted on a random sample of 67% of the interviewed control subjects from within the 45-mile radius of Seattle, whereas controls for the cases from outlying areas were randomly selected from the same town/city. As a result of the sampling procedure described above, 25 cases and 208 control subjects were not targeted for participation.

On the basis of review of data from the in-person interview and a short blood screening questionnaire, subjects were also excluded from the blood substudy for any of the following reasons: already initiated adjuvant therapy (290 cases), naturally menopausal or had a prior hysterectomy or bilateral oophorectomy (64 cases, 58 controls), presence of a clotting disorder or other blood-related problem (3 controls), less than six weeks since surgery (17 cases), pregnant or less than six months postpartum (8 cases, 25 controls), less than six months post-lactation (2 cases, 16 controls), use of oral contraceptives within past six months (17 cases, 29 controls) or non-contraceptive estrogens within past six months (3 cases, 4 controls), refusal to participate (7 cases, 52

controls), prior cancer (5 cases, 5 controls), (37 cases, 15 controls) or other reason (28 cases, 7 controls). The final blood substudy dataset was comprised of 169 cases and 195 control subjects.

Among the 169 breast cancer cases, 44 had no major surgery involving general anesthesia (26.0%), 97 had blood collected before major surgery involving general anesthesia (57.3%), and 28 had their blood collected six or more weeks after major surgery involving general anesthesia (16.6%). Among the 28 cases with blood collection 6 weeks or more after major surgery, 32.1, 32.1, and 35.8% had blood collected from 50 to 67, 68 to 138, and 139 to 377 days after surgery, respectively. A total of 32.5% (n = 55), 45.0% (n = 76), 21.3% (n = 36) and 1.2% (n = 2) of the breast cancer cases were diagnosed with *in situ*, local, regional and unknown stage, respectively.

Approximately 15 ml of serum was collected from each woman using standard procedures. Blood specimens were transported on dry ice to a processing laboratory where they were centrifuged and processed within 4 h of collection. The serum was immediately stored at −70 °C for subsequent biochemical analyses.

Laboratory analyses

Blood samples were analyzed at Nichols Institute, Inc. (San Juan Capistrano, CA). Levels of estradiol, androstenedione, testostosterone and DHEA were measured by an in-house method of radioimmunoassay after extraction and separation by celite chromatography [18]. Progesterone concentration was determined using in-house procedures of radioimmunoassay after organic extraction of progesterone and other steroids. A commercially available radioimmunoassay kit manufactured by Diagnostic Systems Laboratories, Inc., Webster TX was used to measure sex-hormone binding globulin (SHBG).

An equal number of cases and controls were randomly distributed across eight laboratory batches. Laboratory personnel were blinded to case or control status of the samples. Masked quality control blood samples from two women external to the study (one each in the follicular and luteal phase of the menstrual cycle) were assayed twice in each of eight batches along with blood samples from study subjects. Total CVs (between- and within-batch) were as follows: estradiol (9.6% [follicular control], 8.6% [luteal control], DHEA (19.5, 5.7%), testosterone (10.7, 11.4%), progesterone (10.5, 22.7%), androstenedione (12.1, 11.5%), and SHBG (6.1, 8.7%), respectively.

Statistical analysis

Date of the onset of the last menstrual period was obtained at the time of the blood draw, and levels of serum estradiol and progesterone were plotted by number of days since onset of the last period (Figure 1). Characteristic fluctuations in levels of serum progesterone during the normal menstrual cycle were present. As expected, levels of serum estradiol were initially low, and rose to a peak around day 10. The characteristic trough followed by the second peak in serum estradiol was more difficult to discern, possibly because of between person variation in timing of ovulation and misclassification caused by errors in reporting date of last menses. Based on our empirical data and published data [19], subjects were classified as follows: (1) early follicular: 0–5 days since onset of menses; (2) late follicular: 6–14 days since onset of menses; and (3) luteal: 15–33 days since onset of menses. A total of 4 cases and 7 control subjects whose menstrual period onset was more

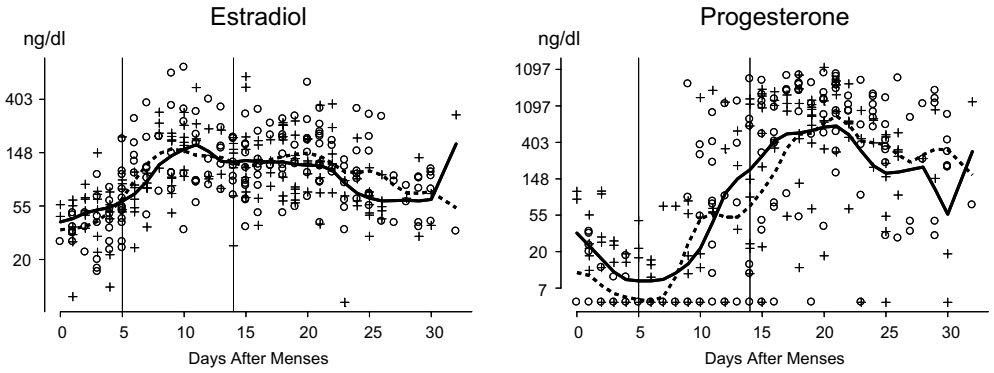

Figure 1. Serum levels of estradiol and progesterone according to number of days since onset of menses. Open circles and a dashed line are used to identify controls. Crosses and a solid line are used to identify cases.

than 33 days before were excluded from analyses for estradiol and progesterone.

Concentrations of hormones and SHBG were logarithmically transformed, and mean hormone concentrations are presented as geometric means. Associations between hormone concentrations were examined using Pearson partial correlation coefficients. Geometric mean concentrations of the hormones in cases and controls were calculated using linear regression.

Logistic regression was used to derive age-adjusted and fully adjusted relative risk (RR) estimates and 95% confidence intervals. RR estimates are shown adjusted for established risk factors including age at menarche, age at first livebirth, family history of breast cancer, personal history of benign breast biopsy, body mass index and alcohol intake. Further adjustment for ethnicity, area (*i.e.*, Seattle radius *versus* outlying area), or oral contraceptive use did not materially change the results. For estradiol and progesterone, associations are presented separately by menstrual phase; additional adjustments for day of the menstrual cycle within phase did not change the interpretation of the findings.

Serum levels of androgens, particularly DHEA and androstenedione, decline over the day [20]. For example, mean serum DHEA levels in ng/dl among control subjects in this study were 664.5, 513.1, 480.4, 394.0, 364.6, 282.4, 248.4, and 252.4 at eight time intervals from 7:00–8:00 am to 9:00–10:00 pm, respectively. Comparable figures for serum androstenedione in ng/dl were 123.5, 117.8, 111.9, 99.6, 92.6, 73.4, 81.3, and 82.3, respectively. Logistic regression analyses focusing on androgens were therefore additionally adjusted for time at blood collection as a continuous variable.

Categorical hormone cut-points for estradiol were based upon its tertile distribution among controls for separate phases of the menstrual cycle (*i.e.*, early follicular, late follicular, luteal). Only levels of luteal progesterone are presented because levels tended to be low during other phases, as expected. For SHBG and hormones that do not fluctuate markedly across the menstrual cycle (*i.e.*, androstenedione, DHEA, testosterone), categorical hormone exposure variables were based upon their distribution among controls.

Results

Cases and controls had a mean age of 39.3 (SD = 3.8) and 38.6 years (SD = 4.0), respectively. A total of 89.9, 4.1, and 5.9% of the cases were white, black and other races, respectively. Comparable figures in the controls were 84.6, 5.6 and 9.7%. Other characteristics of the study subjects who donated blood are summarized in Table 1. Cases were more likely than controls to be nulliparous, to have a later age at first birth, to be thin, to have had a prior benign breast biopsy, and to have a family history of breast cancer in a first-degree relative. Alcohol intake was similar in cases and controls. With the exception of alcohol, these results generally mirror those for the complete set of study subjects from Seattle/Puget Sound.

The geometric mean levels of sex hormones among cases and controls are presented in Table 2. Cases had a slightly higher mean serum level of estradiol in the earlier follicular phase than controls but this difference was not statistically significant. The mean serum estradiol level in the late follicular phase was lower in cases than controls ($p \leq 0.05$). There were no differences in mean serum levels of estradiol in the luteal phase, SHBG, androstenedione, or testosterone between cases and controls. Cases had a higher mean serum DHEA level than controls but this difference was not statistically significant. The mean serum progesterone level was similar in cases than controls.

In an analysis of control subjects only, serum sex hormones, particularly the androgens, were found to be correlated, as expected. Correlations between androgens ranged from 0.32 for testosterone and DHEA to 0.65 for testosterone and androstenedione after adjustment for time of blood collection; all were statistically significant. Statistically significant correlations were also observed for estrogens and SHBG (ranging from 0.32 to 0.41), estrogen and progesterone in the luteal phase (0.28), testosterone and progesterone (−0.27), and SHBG and progesterone (0.23).

Table 3 presents RRs for breast cancer by quartile of serum estradiol, SHBG and progesterone. Among women in the early follicular phase, the fully adjusted RR for the highest tertile of estradiol, as compared to the lowest, was notably elevated. Comparable RRs among women in the late follicular and luteal phase of their menstrual cycles were below the null value, although not statistically significant. A higher serum SHBG level was associated with a minimal increase in risk but this association reversed after adjustment for established breast cancer risk factors; the risk reversal was largely due to adjustment for body mass index. Relative to women in the lowest quartile, women in the upper tertile of progesterone were at decreased risk of breast cancer, but there was no overall pattern of decreasing risk with increasing serum level of progesterone.

Table 4 shows the RRs associated with the androgens. Serum DHEA was associated positively with breast cancer risk. After adjustment for known breast cancer risk factors, the RRs associated with increasing serum

Table 1. Distribution of established risk factors and associated RR for breast cancer among cases and control subjects younger than 45 years of age, Seattle, Washington

Risk factor	Subjects with blood only			All study subjects		
	Cases (n = 169)	Controls (n = 195)	RR[a] (95% CI)	Cases (n = 644)	Controls (n = 610)	RR[a] (95% CI)
Age at first birth (year)						
<20	15	30	1.0	71	102	1.0
20–24	28	39	1.44 (0.6–3.3)	160	155	1.50 (1.0–2.2)
25–29	34	48	1.35 (0.6–3.0)	129	135	1.38 (0.9–2.1)
≥30	31	30	1.80 (0.8–4.2)	118	85	1.96 (1.2–3.0)
Nulliparous	61	48	2.57 (1.2–5.6)	166	133	1.76 (1.2–2.6)
BMI (kg/m^2)						
<22	60	37	1.0	191	140	1.0
22–24.5	47	56	0.61 (0.3–1.1)	163	156	0.76 (0.6–1.1)
24.6–29	35	51	0.48 (0.3–0.9)	148	152	0.75 (0.5–1.0)
≥29.1	26	47	0.40 (0.2–0.8)	121	139	0.66 (0.5–0.9)
Missing	1	4		21	23	
History of breast biopsy						
No	153	189	1.0	593	581	1.0
Yes	16	6	3.15 (1.1–8.7)	51	29	1.67 (1.0–2.7)
Family history of breast cancer						
No	141	184	1.0	544	572	1.0
Yes	28	11	2.91 (1.3–6.3)	100	38	2.71 (1.8–4.0)
Age at menarche						
≥14	31	25	1.0	126	111	1.0
13	53	59	0.76 (0.4–1.5)	184	195	0.56(0.6–1.1)
<12	85	110	0.59 (0.3–1.1)	334	302	0.96 (0.7–1.3)
Missing		1			2	
Recent alcohol intake						
Never drinker	42	59	1.0	189	201	1.0
1–6.9 drinks/week	103	113	1.02 (0.6–1.7)	354	335	1.02 (0.8–1.3)
7–13 drinks/week	14	16	0.84 (0.4–2.0)	59	46	1.33 (0.8–2.1)
14+ drinks/week	10	7	1.15 (0.2–3.8)	41	25	1.67 (1.0–2.9)
Missing				1	3	

[a] Adjusted for continuous age, and all other factors in the table; RR = relative risk; CI = confidence interval; Alcohol intake within the 5 year period up to the reference date.

level of DHEA were 1.0, 1.81, 1.33 and 2.42, respectively. After adjustment for known risk factors, risk did not vary by quartiles of serum testosterone or androstenedione.

As shown in Table 5, when we restricted analyses to 53 cases with *in situ* cancer, age-adjusted RRs with increasing quartiles of DHEA were 1.0, 1.51, 1.26 and 1.11. For the 105 cases with localized/regional disease, the comparable RRs were 1.0, 1.73, 0.99, and 2.76, respectively. Serum testosterone and androstenedione were unrelated to risk of *in situ* or localized/regional breast cancer. Sparse numbers of study subjects precluded stage-specific analyses by menstrual cycle phase for serum estradiol and progesterone. Stage-specific analyses for SHBG did not reveal substantial differences in patterns of risk.

Discussion

Serum estradiol has been weakly related to premenopausal breast cancer risk in case–control studies nested in prospective cohorts [4–7]. Wysowski and colleagues [4] found that the mean level of serum estradiol was 16% lower in 17 cases than in 68 control subjects with specimens obtained throughout the cycle. With results resembling our own, Helzlsouer and colleagues [5] reported a 17% higher follicular estradiol but a 29% lower luteal estradiol concentration in 22 cases than in control subjects. In a study involving 79 breast cancer cases, Rosenberg and colleagues [6] reported similar unadjusted mean estrogen concentrations in cases and controls but a 1.5–2.0-fold risk for women in the upper two tertiles of estradiol relative to women in the lowest

Table 2. Mean serum hormone levels for cases and controls

	Cases			Controls		
	n	Mean	95% CI	n	Mean	95% CI
E2[a]	35	50.2	41.3–61.0	39	43.6	37.1–51.3
E2[b]	51	118.8	99.6–141.6	51	143.8	120.5–171.6
E2[c]	78	100.9	86.2–118.0	98	110.8	98.2–125.0
T[d]	160	25.3	23.8–26.9	174	26.2	24.7–27.8
A[d]	158	93.0	87.2–99.3	174	91.4	86.0–97.3
DHEA[d]	160	383.3	349.4–420.4	174	338.3	309.5–369.4
SHBG	168	39.7	37.0–42.6	193	38.2	35.6–41.0
P	75	363.3	244.2–540.4	97	368.0	260.0–521.7

T – Testosterone (ng/dl), A – Androstenedione (ng/dl), DHEA – Dehydroepiandrosterone (ng/dl), SHBG – Sex-hormone binding globulin (nmol/l), P – Progesterone (ng/dl).

[a] E2, Estradiol (pg/ml), early follicular.

[b] E2, Estradiol (pg/ml), late follicular.

[c] E2, Estradiol (pg/ml), luteal.

[d] Adjusted for time of day at blood collection.

Table 3. RR estimates and 95% CIB for estradiol, SHBG and progesterone

Risk factor	Cases (n = 169)	Controls (n = 195)	Age-adjusted RR (95% CI)	Fully adjusted[a] RR (95% CI)
Estradiol (pg/ml)				
Early follicular (0–5 days)				
≤36	8	13	1.0	1.0
37–50	7	13	0.97 (0.3–3.5)	0.78 (0.2–3.7)
>50	20	13	2.62 (0.8–8.2)	3.10 (0.8–12.7)
Late follicular (6–14 days)				
≤100	22	17	1.0	1.0
98–193	16	17	0.68 (0.3–1.8)	0.47 (0.1–1.5)
>193	13	17	0.58 (0.2–1.5)	0.54 (0.2–1.7)
Luteal (15–33 days)				
≤87	34	34	1.0	1.0
87–144	22	32	0.67 (0.3–1.4)	0.66 (0.3–1.5)
>144	22	33	0.70 (0.3–1.4)	0.60 (0.3–1.4)
SHBG (nmol/l)				
≤24	34	46	1.0	1.0
29–42	58	55	1.41 (0.8–2.5)	1.26 (0.7–2.4)
43–54	34	42	1.11 (0.6–2.1)	0.84 (0.4–1.7)
>54	42	50	1.15 (0.6–2.1)	0.81 (0.4–1.6)
Progesterone (ng/dl)				
Luteal (15–33 days)				
≤287	22	29	1.0	1.0
288–1127	34	32	1.31 (0.6–2.8)	1.56 (0.7–3.7)
>1127	19	36	0.64 (0.3–1.4)	0.55 (0.2–1.4)

[a] Adjusted for age, age at menarche, age at first birth, body mass index, alcohol intake, prior benign biopsy, and family history of breast cancer.

( ) days since start of last period.

tertile, after innovative adjustments for day of cycle by spline modeling. In the Guernsey cohort [7], the mean estradiol concentrations was 75% higher in cases than controls based on seven case subjects in the midcycle phase. Results from ten case–control studies published prior to 1988 (with 3–36 premenopausal breast cancer cases) were also inconsistent [3]. Serum estradiol levels were higher in cases than controls in two of three more recently published case–control studies [9–11]. The failure to observe a consistent pattern of results across

Table 4. RR estimates and 95% CIs for DHEA, testosterone, and androstenedione

Risk factor	Cases (n = 161)[a]	Controls (n = 174)[a]	Age-Adjusted[b] RR (95% CI)	Adjusted[c] RR (95% CI)
DHEA (ng/dl)				
<219	22	47	1.0	1.0
220–319	35	41	1.58 (0.8–3.3)	1.81(0.8–4.0)
320–457	34	45	1.14 (0.5–2.4)	1.33 (0.6–2.9)
>457	69	41	2.19 (1.1–4.5)	2.42 (1.1–5.2)
Missing	1	0		
Testosterone (ng/dl)				
≤19	33	41	1.0	1.0
20–24	39	40	0.90 (0.5–1.8)	1.08 (0.5–2.3)
25–31	38	44	0.74 (0.4–1.5)	0.89 (0.4–1.9)
>31	50	49	0.92 (0.5–1.8)	1.12 (0.6–2.5)
Missing	1	0		
Androstenedione (ng/dl)				
≤69	33	42	1.0	1.0
70–90	38	47	0.91 (0.5–1.8)	1.0 (0.5–2.1)
91–111	28	43	0.67 (0.3–1.4)	0.88 (0.4–1.9)
>111	59	42	1.18 (0.6–2.3)	1.33 (0.6–2.8)
Missing	3	0		

[a] Excludes 8 cases and 21 controls with missing time of blood collection.
[b] Adjusted for age, and time of blood collection.
[c] Adjusted for age, time of blood collection, age at menarche, age at first birth, body mass index, alcohol intake, prior benign biopsy and family history of breast cancer.

Table 5. RR[a] estimates and 95% CIs for androgens by extent of disease

	In situ		Local/regional	
	Cases (n = 53)	RR (95% CI)	Cases (n = 106)	RR (95% CI)
DHEA (ng/dl)				
<219	10	1.0	12	1.0
220–319	14	1.51 (0.6–3.9)	21	1.73 (0.7–4.2)
320–457	15	1.26 (0.5–3.3)	19	0.99 (0.4–2.5)
>457	16	1.11 (0.5–3.1)	53	2.76 (1.2–6.4)
Missing			1	
Testosterone (ng/dl)				
<20	16	1.0	17	1.0
20–24	11	0.59 (0.2–1.5)	28	1.29 (0.5–2.9)
25–31	15	0.71 (0.3–1.7)	23	0.83 (0.4–1.9)
>31	13	0.50 (0.2–1.3)	37	1.33 (0.6–2.9)
Missing			1	
Androstenedione (ng/dl)				
<69	14	1.0	19	1.0
70–90	13	0.75 (0.3–1.9)	25	1.0 (0.5–2.2)
91–111	13	0.74 (0.3–1.9)	15	0.60 (0.3–1.4)
>111	14	0.65 (0.2–1.8)	45	1.44 (0.7–3.1)
Missing	1		2	

[a] Adjusted for age and time of blood collection.

various studies may reflect inadequate sample sizes further complicated by cyclic, intrasubject or laboratory variation in estrogen levels [21]. Alternatively, tissue estrogen levels, individual susceptibility, or hormones other than estrogens may be more important than currently recognized.

The relation between serum SHBG levels and premenopausal breast cancer risk is unclear [5, 7].

Helzlsouer and colleagues [5] reported nearly identical blood levels of SHBG in cases and controls, and Thomas and colleagues [7] reported 8% higher blood level of SHBG in cases than controls. After adjustment for other risk factors, particularly body mass index, we found higher levels of SHBG were associated with a slight reduction in breast cancer risk. The majority of case–control studies of premenopausal women have not reported substantial case–control differences in SHBG levels [3, 9].

Based on findings from laboratory and animal studies [22–26], DHEA is hypothesized to increase breast cancer risk in a low estrogen milieu, such as postmenopausal women, but to decrease risk in a high-estrogen milieu, such as premenopausal women. Epidemiologic studies have consistently linked higher blood levels of DHEA with risk of breast cancer in postmenopausal women [1] but data are sparse for premenopausal women. In a nested case–control study consisting of 15 premenopausal breast cancer cases and 29 control subjects, Helzlsouer and colleagues [12] reported a RR of 0.4 (95% CI = 0.04–4.4) in the highest compared to the lowest tertile of blood levels of DHEA. A case–control study totaling fewer than 10 premenopausal breast cancer cases reported a similar inverse association [16]. Because DHEA is secreted by the adrenal cortex, the higher levels of DHEA in cases than controls that we observed may have been induced by surgery, anesthesia, or breast cancer. Blood levels of DHEA have been reported to decrease after major surgery but levels are reported to return to preoperative levels within a few weeks [27]. In an effort to address this potential bias, we purposely collected blood prior to major surgery, or six weeks or more after major surgery. In analyses excluding 28 cases with blood collection after surgery, the association between risk and DHEA was not substantially changed (RRs = 1.0, 1.85, 1.15, 2.65 for increasing quartiles of serum DHEA) [data not shown]. A positive association between DHEA and premenopausal breast cancer risk was not present in our study for the subset of women with *in situ* disease. These findings may indicate that the association observed in the present study is disease-related. An alternative explanation is that our risk estimates stratified by extent of disease at diagnosis were less stable due to small numbers. In view of the limitations of existing studies, including our own, and the rising sale of DHEA supplements [28], large studies, especially those using prediagnostic serum levels of DHEA, are of priority.

We observed no clear association between levels of serum testosterone and breast cancer risk. Results for premenopausal breast cancer from existing cohort studies support our observation [4, 5], although some small clinical case–control studies have reported a positive association [13–15]. Serum levels of androstenedione were also unrelated to risk in our study. Helzlsouer and colleagues [5] reported an increased risk among premenopausal women with higher prediagnostic serum levels of androstenedione (RR = 5.6; 95% CI = 0.6–52.7 for the highest *versus* lowest tertile). By contrast, Wysowski and colleagues [4] reported that prediagnostic serum levels of androstenedione were 9% lower in 17 cases than 64 controls. Serum androstenedione level was not associated with risk in a case–control study of premenopausal women [22].

Serum progesterone levels were 9–12% lower in cases compared to controls in two prospective cohort studies of premenopausal women [4, 7] whereas the third study found progesterone levels to be 97% higher in cases than controls [5]. A number of case control studies have observed lower levels of serum progesterone in premenopausal cases than controls [3, 9].

The epidemiologic data from our case–control study must be interpreted cautiously because comparisons of blood levels of sex hormones in breast cancer cases and control subjects may be influenced by clinical cancer or other disease-related factors. In this regard, we note that sex hormone associations observed in postmenopausal women differed little between cases diagnosed more than two years after blood collection and cases diagnosed earlier [1]. Furthermore, several methodologic factors enhance the potential validity of our findings, including the larger sample size, restriction to individuals who had blood drawn prior to surgery or six or more weeks later, and exclusion of individuals who received adjuvant therapy. Although the proportion of subjects from the main study available for this substudy was relatively low, it is also reassuring that the pattern of results for established breast cancer risk factors based on the subset of women who participated in the blood collection substudy was generally similar to those from the larger Seattle case–control interview study.

In summary, we did not find a convincing association between serum estradiol, testosterone or androstenedione and premenopausal breast cancer risk but there was a suggestion of reduced risk among women with higher levels of luteal progesterone. We also found serum levels of DHEA to be higher in premenopausal breast cancer cases than controls, a finding that should be investigated further in large prospective studies.

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