REPORT

Plasma Sex Steroid Hormone Levels and Risk of Breast Cancer in Postmenopausal Women

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Background: **A positive relationship has generally been observed between plasma estrogen levels and breast cancer risk in postmenopausal women, but most of these studies have been small and few have evaluated specific estrogen fractions (such as percent bioavailable estradiol). In addition, few studies have evaluated plasma androgen levels in relation to breast cancer risk, and their results have been inconsistent. We prospectively evaluated relationships between sex steroid hormone levels in plasma and risk of breast cancer in postmenopausal women by use of a case–control study nested within the Nurses' Health Study.** ***Methods:*** **Blood samples were collected during the period from 1989 through 1990. Among postmenopausal women not using hormone replacement therapy at blood collection (n = 11 169 women), 156 women were diagnosed with breast cancer after blood collection but before June 1, 1994. Two control subjects were selected per case subject and matched with respect to age, menopausal status, month and time of day of blood collection, and fasting status at the time of blood collection.** ***Results:*** **From comparisons of highest and lowest (reference) quartiles, we observed statistically significant positive associations with risk of breast cancer for circulating levels of estradiol (multivariate relative risk [RR] = 1.91; 95% confidence interval [CI] = 1.06–3.46), estrone (multivariate RR = 1.96; 95% CI = 1.05–3.65), estrone sulfate (multivariate RR = 2.25; 95% CI = 1.23–4.12), and dehydroepiandrosterone sulfate (multivariate RR = 2.15; 95% CI = 1.11–4.17). We found no substantial associations with percent free or percent bioavailable estradiol, androstenedione, testosterone, or dehydroepiandrosterone. The positive relationships were substantially stronger among women with no previous hormone replacement therapy.** ***Conclusion:*** **Our data, in conjunction with past epidemiologic and animal studies, provide strong evidence for a causal relationship between postmenopausal estrogen levels and the risk of breast cancer. [J Natl Cancer Inst 1998;90: 1292–9]**

Substantial indirect evidence supports a central role for endogenous hormones in breast cancer development *(1).* Reproductive factors such as early age at menarche, late age at menopause, and nulliparity are associated with an increased risk of breast cancer. The rate of increase in age-specific breast cancer incidence rates slows at menopause, a time when endogenous estrogen levels decrease dramatically. In postmenopausal women, obesity *(2)* and use of postmenopausal hormone therapy *(3),* both positively related to plasma estrogen levels, also are positively related to breast cancer risk. Estrogens also induce mammary tumors in animals *(4).* Androgens may influence breast cancer risk either directly *(5)* or indirectly, through their conversion to estradiol *(6,7).*

The relationships in postmenopausal women between hormone levels in plasma and the risk of breast cancer have been evaluated in six previous prospective studies *(8–13).* For estrogens, the overall evidence supports a positive association *(14).* However, in most studies, only one or two of the major circulating estrogens have been evaluated and, with one exception *(8),* the studies have been small, containing only 15–71 case subjects with breast cancer. For plasma androgens, the data are more limited and the results inconsistent.

To evaluate these relationships in detail, we conducted a prospective, nested case–control study within the Nurses' Health Study cohort. We evaluated the levels of circulating estrogens and androgens in relation to the risk of breast cancer. We also calculated estimates of effect that accounted for laboratory measurement error and the random within-person fluctuation in hormone levels over time *(15).*

Subjects and Methods

Study Population

The Nurses' Health Study cohort was established in 1976 when 121 700 female registered nurses, 30–55 years of age, completed and returned a mailed questionnaire. The cohort continues to be followed every 2 years by questionnaire to update exposure status and to identify cases of newly diagnosed disease. Data have been collected on many breast cancer risk factors, including height, weight, age at

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See ''Notes'' following ''References.''

menarche and menopause, age at birth of first child, parity, postmenopausal hormone use, diagnosis of benign breast disease, and family history of breast cancer.

During the period from 1989 through 1990, blood samples were collected from 32 826 cohort members (27% of the original cohort) who were 43–69 years of age when blood was collected. Details regarding the blood collection methods have been published *(16)*. Briefly, each woman arranged to have her blood drawn and then shipped, via overnight courier and with an ice pack to keep the sample cool, to our laboratory, where it was processed and separated into plasma, red blood cell, and white blood cell components. Within 26 hours of being drawn, 97% of the samples were received in our laboratory. The stability of estrogens and androgens in whole blood for 24–48 hours has been documented previously *(17)*. Since collection, samples have been archived at −130 °C or colder in continuously monitored liquid nitrogen freezers. As of 1994, the follow-up rate among women who gave a blood sample was 98%. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Both case and control subjects in this analysis are women who, at blood collection, were postmenopausal and had not used postmenopausal hormones for at least 3 months. The participants were defined as postmenopausal if they reported having a natural menopause or a bilateral oophorectomy. Women who reported a hysterectomy with either one or both ovaries remaining were defined as postmenopausal when they were 56 years old (if a nonsmoker) or 54 years old (if a current smoker), ages at which natural menopause had occurred in 90% of the respective cohorts.

Case subjects were women who had reported no cancer diagnosis before blood collection and who were diagnosed with breast cancer after blood collection but before June 1, 1994. Overall, 156 cases of breast cancer (140 invasive and 16 *in situ*) were reported from among the 11 169 women eligible at baseline. (The other 21 657 women were not eligible because they were premenopausal, were postmenopausal but were using postmenopausal replacement hormones, were of uncertain menopausal status, or had a prior cancer diagnosis.) All cases of breast cancer were confirmed by medical record review with one exception, in which the nurse confirmed the diagnosis but the medical record was unavailable; because of the high confirmation rate (99%) upon medical record review, this case subject was kept in the analysis. The time from blood collection to diagnosis ranged from less than 1 month to 57 months (mean [standard deviation] = 28.7 [15.8] months). Two control subjects were matched per case subject by age (±2 years), month of blood collection, time of day that blood was drawn (±2 hours), and fasting status at the time of blood collection (≥10 hours since a meal versus <10 hours or unknown). Ninety-three percent of control matches were exact; the most relaxed match was within ±5 years of age, ±3 months of blood collection from case subjects, and ±7 hours for time of blood collection.

Laboratory Analyses

With the exception of estrone sulfate, all analyses were performed by the Nichols Institute (San Juan Capistrano, CA). Plasma samples were extracted with hexane–ethyl acetate (4 : 1, vol/vol), and the extract was applied to celite columns (celite in ethylene glycol). The steroids were eluted from the columns in the following fractions: fraction 1, 3.5 mL of *iso*-octane (androstenedione); fraction 2, 3.5 mL of *iso*-octane containing 10% ethyl acetate (dehydroepiandrosterone [DHEA] and testosterone); fraction 3, 3.0 mL of *iso*-octane containing 15% ethyl acetate (estrone); and fraction 4, 5.0 mL of *iso*-octane containing 40% ethyl acetate (estradiol). Fractions 1–4 were then assayed by radioimmunoassay *(18–21)*. Dehydroepiandrosterone sulfate (DHEAS) was assayed by radioimmunoassay without a prior separation step *(22)*. Percent free estradiol (i.e., percent nonprotein bound) was assayed by use of equilibrium dialysis *(23,24)*; the percent dialyzable estradiol was calculated as described by Vermuelen et al. *(24)*. The percent bioavailable estradiol (i.e., percent free plus percent albumin-bound estradiol) was assayed by use of an ammonium sulfate precipitation *(25,26)*. All case–control–control triplet samples were assayed together; the samples were ordered randomly within a triplet and labeled so that the laboratory could not identify the case–control status. Although all members of a triplet were analyzed at the same time, the triplets were analyzed in up to three different batches (sent in 1992, 1993, and 1996).

For estrone sulfate, the first two batches of samples were assayed at the laboratory of Dr. C. Longcope at the University of Massachusetts Medical Center, Worcester, and the third batch was assayed at the Nichols Institute. In each laboratory, after extraction of estrone, estrone sulfate was assayed by radioimmunoassay of estrone, after enzyme hydrolysis, organic extraction, and separation by column chromatography *(27)*.

In each batch of samples, we interspersed plasma replicates (one replicate per 10 case and/or control samples) that were labeled to preclude their identification by the assaying laboratory; these replicate samples were used to assess laboratory precision. Within-batch laboratory coefficients of variation ranged from 6% (percent bioavailable estradiol) to 13.6% (DHEA).

The assay detection limit was 2 pg/mL for estradiol, 0.5% for both percent free estradiol and percent bioavailable estradiol, 10 pg/mL for estrone, 50 pg/mL for estrone sulfate (in each laboratory), 3 ng/dL for androstenedione, 1 ng/dL for testosterone, 3 ng/dL for DHEA, and 5 μg/dL for DHEAS. When plasma hormone values were reported as less than the detection limit, we set the value to half this limit (which occurred only for estrone [n = 6], estrone sulfate [n = 2], and DHEAS [n = 2]).

Reproducibility Study

Three hundred ninety Nurses' Health Study participants who gave a first blood sample during the period from 1989 through 1990 were asked to provide two additional samples that were collected during the following 2 years. The women were postmenopausal, had not used postmenopausal hormones for at least 3 months, and had no previous diagnosis of cancer (except nonmelanoma skin cancer); these criteria were applied at each sample collection. Of the 390 women, 186 (48%) sent two additional samples. A random sample of 80 of these women who had all three samples drawn between 6 AM and 12 noon was sent for hormone analysis, at the same laboratories used for the main study, and forms the basis of the reproducibility study. Additional details regarding this study are provided elsewhere *(15)*.

Data Analyses

We used quartile categories, with cut points based on the distribution in the control subjects, for the purpose of summarizing breast cancer risk according to plasma hormone level. For most of the hormones, the mean and standard deviation of both the control values and the quality-control replicates were very similar across batches; thus, quartile cut points were made according to the distribution in the control subjects overall. The lowest quartile was used as the referent in all analyses. For estrone, estrone sulfate, and DHEA, the median value for the control subjects varied by as much as 40% between batches, so that quartile cut points based on all control subjects combined resulted in uneven batch-specific distributions (e.g., the lowest quartile of estrone contained 12% of the control subjects from the first two batches but 41% of the control subjects from the third batch). Because the mean value of the quality-control replicates in each of the datasets varied in the same manner for these three assays, much (if not all) of this difference appeared due to laboratory drift rather than to true differences in hormone levels between the batches. Thus, for these three hormones, we defined batch-specific quartile cut points. In addition, in all analyses, we controlled for batch. For several hormones (e.g., estradiol), the control distribution was unequal across quartiles because of multiple identical hormone values.

One matched set was removed from the analysis because the case subject's estrogen values were in the premenopausal range (estradiol = 411 pg/mL). Individual values more than 2.5-fold higher than the normal range according to the assaying laboratory also were removed; this resulted in the removal of two testosterone values only. In addition, several women did not have a sufficient volume of plasma for all assays. The final number of case and control samples available for each hormone analysis is provided in Table 1.

To test for differences in hormone levels between case and control subjects, we used mixed-effects regression models for clustered data to adjust for possible confounding due to the matching factors and for any residual correlation between case and control subjects within the matched set *(28)*. To compare proportions between case and control subjects, we used the Mantel–Haenszel test *(29)*. We used conditional logistic regression analyses to estimate relative risks (RRs) (odds ratios) and 95% confidence intervals (CIs) *(30)*. In analyses stratified by prior postmenopausal hormone use, however, we used unconditional logistic regression, controlling for the matching factors, to maximize our sample size. We conducted tests for trend by modeling the natural logarithm of the hormone level as a continuous variable and calculating a Wald statistic *(31)*. All *P* values are based on two-sided tests. The regression calibration method was used to correct RRs and 95% CIs for laboratory measurement error and random within-person variability *(32–35)*. The within-person variance was calculated from the reproducibility study and the between-person variance from the current case–control study. (Thus, intraclass correlation coefficients are slightly different from the

Table 1. Median and range* of plasma hormone levels for case and matched control subjects

Hormone and unit of measure	Case subjects		Control subjects		
	No. of subjects	Median level (range)	No. of subjects	Median level (range)	P†
Estradiol, pg/mL	154	8.0 (4–16)	306	7.0 (4–14)	.04
Free estradiol, %	152	1.60 (1.33–1.85)	303	1.55 (1.33–1.82)	.12
Bioavailable estradiol, %	154	23.5 (13.9–40.6)	305	23.0 (13.5–37.2)	.26
Estrone, pg/mL	154	31 (20–51)	306	28 (17–45)	.02
Estrone sulfate, pg/mL	144	232 (102–593)	288	192 (97–420)	.02
Androstenedione, ng/dL	147	62 (35–99)	296	57 (30–103)	.13
Testosterone, ng/dL	147	23 (13–44)	299	22 (12–37)	.05
Dehydroepiandrosterone, ng/dL	139	210 (97–434)	272	205 (99–366)	.48
Dehydroepiandrosterone sulfate, μg/dL	153	87 (42–200)	298	79 (34–163)	.01

*Range given is from median of the bottom quartile (12.5%) to median of the top quartile (87.5%).

†*P* values are from the mixed-effects regression model, controlling for matching factors. All *P* values are two-sided.

previously published values.) In these analyses, hormone levels were log transformed to lessen the influence of a small number of high or low values. Because the measurement error correction methods require that the relationship between disease and exposure be linear on a logistic scale, restricted cubic spline models *(36)* for breast cancer incidence in relation to each log-transformed hormone value were fit to the data. With this technique, as well as formal significance testing criteria for nonlinearity, with just one exception (DHEA), none of the hormones showed substantial evidence of departure from a linear relation on the logarithmic RR scale. DHEA was modeled on its original scale.

Results

The women in this analysis ranged in age from 46 years to 69 years (mean age = 62 years) and had been menopausal for at least 1 year and up to 40 years (mean = 12 years). Compared with control subjects, case subjects had an earlier mean age at menarche (12.4 years versus 12.7 years) and a later mean age at the birth of their first child (26.0 years versus 25.3 years) and were more likely to have reported a family history of breast cancer (19% versus 15%), although none of these differences were statistically significant. We observed that case subjects, when compared with control subjects, had significantly higher plasma levels of estradiol, estrone, estrone sulfate, testosterone, and DHEAS but no substantial difference in levels of the other steroid hormones (Table 1).

In the simple conditional models, women in the top quartile of plasma estrone and estrone sulfate levels had an approximately twofold increase in breast cancer risk, which was statistically significant (for estrone, RR = 1.77 [95% CI = 1.01–3.11]; for estrone sulfate, RR = 2.12 [95% CI = 1.21–3.71]). For DHEAS, women in the top 75% of levels appeared to have an increase in breast cancer risk compared with women with the lowest levels. Modest, and generally nonsignificant, positive associations were noted for percent free estradiol, androstenedione, and testosterone and breast cancer risk. We observed little association with either percent bioavailable estradiol or DHEA. When we evaluated absolute levels of free and bioavailable estradiol, the associations were similar to those for total estradiol.

When a number of established breast cancer risk factors were controlled for statistically (Table 2), the relationships tended to strengthen somewhat, primarily because of control for age at birth of first child and body mass index at age 18 years. The association with estradiol was statistically significant (RR = 1.91; 95% CI = 1.06–3.46). Body mass index at age 18 was included in these models because it is inversely related to postmenopausal breast cancer risk *(2);* thus, we expected it could be a confounder. In contrast, when we included body mass index at the time of blood collection in each of the models, RRs for the estrogens were modestly attenuated, because, in postmenopausal women, body mass index is a major determinant of estrogen levels *(16)*. For example, when the top quartile is compared with the bottom quartile, the RR decreased from 1.91 to 1.69 (95% CI = 0.83–3.42) for estradiol and from 1.96 to 1.75 (95% CI = 0.90–3.38) for estrone.

When we assessed the relationships between plasma hormones and the risk of breast cancer after excluding *in situ* breast cancer cases (n = 16), we observed nearly identical RRs. We also evaluated these relationships after excluding data from the 30 breast cancer cases that had been diagnosed within 1 year of blood collection, to assess whether the positive associations might be due to an influence of the breast cancer itself on hormone levels. With the exception of percent free and percent bioavailable estradiol, where the relationships were slightly strengthened (comparison of the top quartile with the bottom quartile, 1.69 [95% CI = 0.86–3.32] and 1.50 [95% CI = 0.79–2.84], respectively), results again did not differ materially.

We next evaluated the relationships between hormone levels and the risk of breast cancer according to postmenopausal hormone use before blood collection (i.e., never versus past use) (Table 3). We hypothesized that our single hormone measure would best reflect long-term endogenous hormone exposure among the never users and, therefore, we might see stronger associations in this group. Because of the small number of cases in each of the groups, we included in the statistical models only the matching factors and other most important covariates (Table 3). Among those who had never used postmenopausal hormones, the relationships with the estrogens, particularly estradiol and estrone sulfate, were markedly strengthened (comparison of the top quartile with the bottom quartile: for estradiol, RR = 3.53 [95% CI = 1.55–8.03]; for estrone sulfate, RR = 4.34 [95% CI = 1.87–10.1]). The association with DHEAS also was stronger. In contrast, the relationships among past hormone users were weak (or null) and not statistically significant, although the 95% CIs were wide.

Most of the steroid hormones are positively correlated. For example, the Spearman correlations for estradiol with estrone, testosterone, and DHEAS were .67, .45, and .27, respectively. Therefore, we evaluated the independent association of each of the hormones with breast cancer risk, among all case and control subjects combined, when estradiol also was included in the statistical model. The RRs for testosterone were substantially attenuated (comparison of the top quartile with the bottom quartile: RR = 1.08 [95% CI = 0.52–2.25]), whereas the RRs for estrone (RR = 1.50 [95% CI = 0.64–3.54]), DHEAS (RR = 1.90 [95% CI =

Table 2. Relative risk (RR) of breast cancer (and 95% confidence intervals [CI]) by category of plasma hormone levels among postmenopausal women in the Nurses' Health Study

Plasma hormone level	Quartile categories				*P* value for trend*
	1	2	3	4	
Estradiol, pg/mL	⩽5	6–7	8–11	⩾12	
No. case subjects/No. control subjects	41/97	33/69	36/78	44/62	
Simple RR†	1.0 (referent)	1.12	1.09	1.73	.04
Multivariate RR‡ (95% CI)	1.0 (referent)	1.17 (0.64–2.15)	1.12 (0.62–2.03)	1.91 (1.06–3.46)	.03
Free estradiol, %	⩽1.43	1.44–1.55	1.56–1.70	⩾1.71	
No. case subjects/No. control subjects	38/74	29/79	38/75	47/75	
Simple RR†	1.0 (referent)	0.70	0.98	1.23	.14
Multivariate RR‡ (95% CI)	1.0 (referent)	0.71 (0.37–1.34)	1.05 (0.55–1.98)	1.48 (0.81–2.72)	.05
Bioavailable estradiol, %	⩽17.38	17.39–23.0	23.1–31.38	⩾31.39	
No. case subjects/No. control subjects	40/76	35/77	31/76	48/76	
Simple RR†	1.0 (referent)	0.84	0.78	1.19	.26
Multivariate RR‡ (95% CI)	1.0 (referent)	0.84 (0.45–1.54)	0.79 (0.43–1.44)	1.27 (0.72–2.26)	.28
Estrone, pg/mL§					
No. case subjects/No. control subjects	27/70	36/77	38/79	53/79	
Simple RR†	1.0 (referent)	1.24	1.28	1.77	.02
Multivariate RR‡ (95% CI)	1.0 (referent)	1.46 (0.77–2.77)	1.42 (0.74–2.75)	1.96 (1.05–3.65)	.01
Estrone sulfate, pg/mL§					
No. case subjects/No. control subjects	29/73	27/71	28/70	60/74	
Simple RR†	1.0 (referent)	0.93	1.04	2.12	.02
Multivariate RR‡ (95% CI)	1.0 (referent)	1.01 (0.51–2.00)	1.14 (0.58–2.26)	2.25 (1.23–4.12)	.01
Androstenedione, ng/dL	⩽40	41–57	58–77	⩾78	
No. case subjects/No. control subjects	26/73	37/76	45/73	39/74	
Simple RR†	1.0 (referent)	1.33	1.74	1.50	.14
Multivariate RR‡ (95% CI)	1.0 (referent)	1.25 (0.70–2.29)	1.88 (1.00–3.54)	1.46 (0.77–2.76)	.10
Testosterone, ng/dL	⩽15	16–22	23–31	>31	
No. case subjects/No. control subjects	33/75	38/79	37/78	39/67	
Simple RR†	1.0 (referent)	1.12	1.10	1.34	.05
Multivariate RR‡ (95% CI)	1.0 (referent)	1.12 (0.60–2.10)	1.07 (0.57–2.00)	1.40 (0.73–2.70)	.04
Dehydroepiandrosterone, ng/dL§					
No. case subjects/No. control subjects	43/73	25/68	33/65	38/66	
Simple RR†	1.0 (referent)	0.62	0.90	0.99	.36
Multivariate RR‡ (95% CI)	1.0 (referent)	0.64 (0.33–1.24)	0.74 (0.40–1.36)	1.08 (0.59–1.98)	.31
Dehydroepiandrosterone sulfate, μg/dL	⩽48	49–78.5	79–124	⩾125	
No. case subjects/No. control subjects	23/73	48/76	37/75	45/74	
Simple RR†	1.0 (referent)	2.15	1.68	2.10	.01
Multivariate RR‡ (95% CI)	1.0 (referent)	2.20 (1.12–4.29)	1.62 (0.84–3.14)	2.15 (1.11–4.17)	.01

**P* value for trend from model with the logarithm of hormone level entered as a continuous variable. All *P* values are two-sided.

†Conditional model controlling for matching factors only.

‡Conditional model additionally controlling for body mass index at age 18 years (<21, 21–22.9, 23–24.9, or ⩾25 kg/m^2), history of breast cancer (no family history or history in mother or sister), age at menarche (<12, 12, 13, or ⩾14 years), parity/age at first birth (nulliparous, 1–4 children/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, ⩾5 children/age at first birth <25 years, or ⩾5 children/age at first birth ⩾25 years), age at menopause (<45, 45–49, 50–55, or >55 years or missing), and past postmenopausal hormone use (continuous in years).

§For estrone, cut points for batches 1 and 2 were <25, 25–32, 33–42, and >42 pg/mL; for batch 3, they were <18, 18–23, 24–30, and >30 pg/mL. For estrone sulfate, cut points for batch 1 were ⩽118, 119–164, 165–227, and >227 pg/mL; for batches 2 and 3, they were ⩽141, 142–205, 206–299, and >299 pg/mL. For dehydroepiandrosterone, cut points for batch 1 were ⩽114, 115–162, 163–252, and >252 pg/mL; for batches 2 and 3, they were ⩽159, 160–223, 224–320, and >320 pg/mL.

0.96–3.77]), and estradiol itself were only modestly reduced. When estrone and estrone sulfate were included in the same statistical model, neither was attenuated, although the 95% CIs for each widened considerably.

We next corrected the associations for laboratory error and random within-person variability; in these analyses, hormone levels were modeled as continuous variables (Table 4). The RR (based on a contrast in hormone levels from the 12.5 to the 87.5 percentiles of the distribution, corresponding to the medians of the bottom quartile and the top quartile, respectively, as shown in Table 1) for estradiol strengthened considerably, increasing from 1.77 to 2.42. Similarly, the relationships with each of the other hormones strengthened somewhat, although only the relationships with estrone, estrone sulfate, percent free estradiol, DHEAS, and testosterone were statistically significant. As in the categorical analyses, the association with testosterone was substantially attenuated after we controlled for estradiol.

Discussion

We observed positive associations between circulating levels of estradiol, estrone, estrone sulfate, and DHEAS and risk of breast cancer in postmenopausal women. In contrast, we found no substantial associations for percent bioavailable estradiol, androstenedione, or DHEA in relation to breast cancer. The positive relationships were considerably stronger among women with no previous use of hormone replacement therapy after menopause.

Table 3. Multivariate relative risk* of breast cancer by plasma hormone level, according to use of postmenopausal hormones before blood collection

Hormone	Multivariate RR by quartile				95% confidence interval‡	*P*§
	1†	2	3	4		
No use of postmenopausal hormones before blood collection‖						
Estradiol	1.0	2.07	1.52	3.53	1.55–8.03	.003
Free estradiol, %	1.0	0.52	1.27	1.47	0.67–3.23	.04
Bioavailable estradiol, %	1.0	0.76	0.77	1.80	0.83–3.93	.11
Estrone	1.0	0.82	1.57	2.85	1.23–6.61	.002
Estrone sulfate	1.0	1.33	1.36	4.34	1.87–10.1	.002
Androstenedione	1.0	1.35	1.73	1.77	0.72–4.32	.27
Testosterone	1.0	0.62	1.10	1.32	0.56–3.11	.12
Dehydroepiandrosterone	1.0	0.77	0.48	1.11	0.48–2.60	.92
Dehydroepiandrosterone sulfate	1.0	3.65	3.57	4.15	1.57–11.0	.005
Use of postmenopausal hormones before blood collection¶						
Estradiol	1.0	0.78	1.00	1.39	0.50–3.84	.33
Free estradiol, %	1.0	0.92	0.67	1.31	0.52–3.25	.42
Bioavailable estradiol, %	1.0	0.97	1.24	1.33	0.53–3.38	.40
Estrone	1.0	1.70	1.28	0.87	0.33–2.27	.85
Estrone sulfate	1.0	0.82	1.19	1.08	0.43–2.71	.23
Androstenedione	1.0	1.66	1.83	1.08	0.40–2.90	.70
Testosterone	1.0	1.56	1.23	1.61	0.59–4.37	.13
Dehydroepiandrosterone	1.0	0.64	0.96	1.03	0.42–2.53	.29
Dehydroepiandrosterone sulfate	1.0	1.73	0.68	1.01	0.38–2.65	.53

*Unconditional logistic regression analyses using same category cut points as in Table 2 and controlling for the matching factors, body mass index at age 18 years, age at first birth, and parity, with categories as described in Table 2.

†Referent.

‡95% confidence interval for top versus bottom quartile comparison.

§*P* value for trend from model with logarithmic hormone level entered as a continuous variable. All *P* values are two-sided.

‖From 71 to 83 case subjects and from 168 to 190 control subjects, depending on the specific hormone.

¶From 65 to 71 case subjects and from 105 to 118 control subjects, depending on the specific hormone.

Table 4. Correction of multivariate relative risk (RR)* estimates and 95% confidence intervals (CIs) for random within-person measurement error

Hormone	ICC†	RR (95% CI)	
		Uncorrected	Corrected
Estradiol	.66	1.77 (1.06–2.93)	2.42 (1.10–5.35)
Free estradiol, %	.79	1.69 (1.03–2.80)	1.97 (1.03–3.77)
Bioavailable estradiol, %	.87	1.30 (0.82–2.06)	1.36 (0.80–2.31)
Estrone	.77	1.91 (1.15–3.16)	2.35 (1.20–4.58)
Estrone sulfate	.83	1.80 (1.14–2.85)	2.04 (1.17–3.58)
Androstenedione	.64	1.51 (0.89–2.58)	1.95 (0.82–4.63)
Testosterone	.84	1.65 (1.00–2.71)	1.83 (1.01–3.32)
Dehydroepiandrosterone	.53	1.34 (0.89–2.02)	1.75 (0.79–3.85)
Dehydroepiandrosterone sulfate	.81	1.94 (1.17–3.24)	2.29 (1.21–4.34)

*RR based on comparing median hormone level in top quartile to median level in bottom quartile (*see* Table 1 for range in values).

†ICC = intraclass correlation coefficient.

Strengths of our study include that it was prospective and relatively large. In addition, we were able to evaluate nine steroid hormones or hormone fractions, all of which were assayed with good precision. By using multiple hormone measures from a subset of study participants, we were able to correct our RR estimates for the random (and largely biologic) variation in hormone levels that cannot ordinarily be captured by a single hormone measurement.

Evidence from our study, in conjunction with that from other recent prospective studies *(8–12)*, supports a strong predictive role for plasma estradiol levels in relation to breast cancer risk among postmenopausal women. In only one small prospective study *(13)* has a positive association not been observed. Although considerably larger RRs have been reported for contrasts in levels generally similar to ours *(11,12)*, these two studies had sample sizes of only 24 and 61 case subjects, respectively; thus, their confidence limits broadly overlap ours. Moreover, some of the heterogeneity in RRs between studies may be due to various prevalences of past postmenopausal hormone use in study subjects. The magnitude of the associations also might be expected to vary because of different sensitivities and specificities of the laboratory assays used in the studies *(37,38);* this limitation makes the comparison of results between studies difficult and estimation of the increase in disease risk per unit increase in estradiol levels (as is done with plasma cholesterol level and heart disease risk) currently infeasible.

Free estradiol or bioavailable estradiol is hypothesized to be readily available to the breast tissue and thus is considered to be the most biologically active estrogen fraction(s) *(39)*. As such, compared with total estradiol, a stronger relationship between one of these fractions and breast cancer risk might be expected. However, the epidemiologic evidence has not been consistent *(8,9,40–43)*. We noted only a marginally significant positive relationship with percent free estradiol. We also observed no substantial relationship between percent bioavailable estradiol and risk, in contrast to the only previous large prospective study of this issue (compari-

son of the top quartile with the bottom quartile, RR = 4.4) *(8)*. These differences seem unlikely to be due to confounding or to different levels of measurement error. Our laboratory coefficient of variation was small, and measurement error correction did not increase the estimates appreciably. We previously documented that our blood collection methods did not alter levels of percent free estradiol *(17)*, suggesting that a change in the bioavailable fraction also is unlikely. The average age (59 years versus 62 years) and lengths of follow-up times (5 years versus 2.5 years) of the populations in the study by Toniolo et al. *(8)* and in our study also were similar. In addition, although the percent bioavailable estradiol values varied substantially between the two studies, the results of the two assays are highly correlated. We sent 112 of our control samples for analysis to the laboratory used by Toniolo et al.; the Spearman correlation between the percent bioavailable estradiol assays from the two laboratories was .91. To our knowledge, these estrogen fractions have not been evaluated in any other large prospective studies; thus, additional assessments are needed.

Estrone sulfate is the most abundant circulating estrogen in postmenopausal women *(44,45)* and a major component of some postmenopausal hormone preparations. Although Dorgan et al. *(10)*, in the only other prospective study to examine this hormone, observed little association with breast cancer risk, they were unable to rule out an approximately twofold increase in risk, such as we observed among women in the top 25% of the distribution compared with those in lower exposure categories.

Androgens have been hypothesized to increase breast cancer risk either directly by increasing the growth and proliferation of breast cancer cells *(5)* or indirectly by their conversion to estrogen *(6,7)*. Testosterone has been positively associated with breast cancer in most *(10–12,46–49)* but not all *(50,51)* previous studies. However, the positive association has tended to weaken after controlling for total estradiol (or another estrogen fraction) *(12,46)*, similar to our findings, suggesting that increased levels of testosterone may have a modest, but indirect, association with breast cancer through its conversion to estradiol.

DHEA and DHEAS are adrenal androgens that decrease substantially with increasing age and have little documented physiologic role *(52)*. DHEA administered to rodents can decrease the risk of spontaneous and chemically induced cancers *(53)*. However, in postmenopausal women, DHEA has been proposed to act like an estrogen in stimulating cell growth *(52)*, in part because of the estrogenic effect of its major metabolite, 5-androstenediol *(54)*.

DHEAS has been evaluated in relation to breast cancer risk in five previous prospective studies; with one exception *(55)* (21 case subjects), nonsignificant positive associations have been reported *(10,11,46,56)*, although in one of these studies *(46)* the weak positive association became inverse after controlling for estradiol. We observed a positive association that was essentially independent of estradiol. In the two previous assessments of DHEA and breast cancer *(10,56)*, a statistically significant positive association was observed. We found no statistically significant association but cannot rule out a modest positive relationship. As a whole, these findings should serve to caution against the increasing use of pharmacologic doses of DHEA as an ''anti-aging'' agent. DHEA and DHEAS are metabolically interconvertible, and after oral administration of DHEA, circulating levels of DHEAS rise substantially *(57)*. Certainly, epidemiologic evidence does not support a decreased risk of breast cancer with increasing levels of these androgens and, in fact, suggests a possible positive association. In addition, DHEA supplementation may increase levels of plasma insulin-like growth factor-I *(58)*, a hormone that has recently been associated with risk of breast cancer *(59,60)* and prostate cancer *(61)*.

Estrogen (and some androgen) levels in normal breast tissue are generally much higher than levels in plasma, and levels in malignant tissue are higher than those in normal breast tissue *(62–64)*. These differences may be due to enzyme activities in normal and malignant breast cells that result in the local conversion of androgens to estrogens, estrone sulfate to estrone, and estrone to estradiol *(6,63,65)*. Although several reports *(62–64,66,67)* have indicated that there is little if any correlation between plasma and tissue steroid levels, these studies were all small ($n = \leqslant 14$ women) and the correlations were not provided. Given our findings and those of others described above, it seems unlikely that these levels are entirely uncorrelated. A low correlation would suggest, however, that the relationships between tissue hormone levels and breast cancer risk may be stronger than those observed with our plasma surrogates.

Our data, in conjunction with past epidemiologic *(1–3,8–12)* and animal *(4)* studies, provide strong evidence for a causal relationship between postmenopausal plasma estrogen levels and risk of breast cancer *(68)*. However, additional studies are needed before conclusions can be made as to whether total estradiol or other specific fractions are most important to risk. Testosterone most likely has a modest, indirect influence on risk through its conversion to estradiol, and increasing evidence suggests a positive relationship between DHEAS and the risk of breast cancer. Although higher estrogen levels may have both beneficial *(69)* and adverse effects, reducing the levels or activity of endogenous estrogens may be a promising means for preventing breast cancer in postmenopausal women.

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Notes

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