Endogenous Estrogen, Androgen, and Progesterone Concentrations and Breast Cancer Risk Among Postmenopausal Women

Stacey A. Missmer, A. Heather Eliassen, Robert L. Barbieri, Susan E. Hankinson

Background: **Levels of endogenous hormones have been associated with the risk of breast cancer among postmenopausal women. Little research, however, has investigated the association between hormone levels and tumor receptor status or invasive versus *in situ* tumor status. Nor has the relation between breast cancer risk and postmenopausal progesterone levels been investigated. We prospectively investigated these relations in a case-control study nested within the Nurses' Health Study.** ***Methods:*** **Blood samples were prospectively collected during 1989 and 1990. Among eligible postmenopausal women, 322 cases of breast cancer (264 invasive, 41 *in situ*, 153 estrogen receptor [ER]-positive and progesterone receptor [PR]-positive [ER^+/PR^+], and 39 ER-negative and PR-negative [ER^-/PR^-] disease) were reported through June 30, 1998. For each case subject, two control subjects (n = 643) were matched on age and blood collection (by month and time of day). Endogenous hormone levels were measured in blood plasma. We used conditional and unconditional logistic regression analyses to assess associations and to control for established breast cancer risk factors.** ***Results:*** **We observed a statistically significant direct association between breast cancer risk and the level of both estrogens and androgens, but we did not find any (by year) statistically significant associations between this risk and the level of progesterone or sex hormone binding globulin. When we restricted the analysis to case subjects with ER^+/PR^+ tumors and compared the highest with the lowest fourths of plasma hormone concentration, we observed an increased risk of breast cancer associated with estradiol (relative risk [RR] = 3.3, 95% confidence interval [CI] = 2.0 to 5.4), testosterone (RR = 2.0, 95% CI = 1.2 to 3.4), androstenedione (RR = 2.5, 95% CI = 1.4 to 4.3), and dehydroepiandrosterone sulfate (RR = 2.3, 95% CI = 1.3 to 4.1). In addition, all hormones tended to be associated most strongly with *in situ* disease.** ***Conclusion:*** **Circulating levels of sex steroid hormones may be most strongly associated with risk of ER^+/PR^+ breast tumors. [J Natl Cancer Inst 2004;96:1856–65]**

Epidemiologic data now provide strong evidence for an influence of plasma steroid hormones on the risk of breast cancer in postmenopausal women *(1)*—a long proposed, but previously poorly supported, hypothesis. The associations between the risk of breast cancer and the level of estrogens and androgens (with relative risks [RRs] for breast cancer ranging from 2.0 to 2.5 when comparing the top 20% with the bottom 20% of hormone levels) are strong compared with those of most other breast cancer risk factors. However, few studies have investigated these associations as stratified by tumor receptor status or by invasive versus *in situ* disease. In addition, studies of the effect of postmenopausal hormone use suggest that formulations containing estrogen and progestin are associated with a greater increase in breast cancer risk than those with estrogen only *(2–5).* However, the influence of endogenous progesterone levels remains unknown.

Affiliations of authors: Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (SAM, AHE, SEH); Department of Epidemiology, Harvard School of Public Health, Boston, MA (SAM, AHE, SEH); Department of Obstetrics, Gynecology, and Reproductive Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA (SAM, RLB).

Correspondence to: Dr. Stacey Missmer, Channing Laboratory, Brigham and Women's Hospital, 181 Longwood Avenue, Boston, MA 02115 (e-mail: stacey.missmer@channing.harvard.edu).

See "Notes" following "References."

DOI: 10.1093/jnci/djh336

Within the large, prospective Nurses' Health Study cohort, we previously investigated *(6)* the relation between endogenous estrogens and androgens and breast cancer risk among postmenopausal women (156 cases of breast cancer with follow-up from 1990 through 1994) and found. To explore the association between endogenous hormone levels and breast cancer risk in greater detail than was previously possible, we conducted a second nested case-control study that extends the follow-up through 1998 and increases the total number of incident cases of breast cancer to 322. We evaluated the associations between endogenous hormone levels and breast cancer risk overall and assessed whether the associations varied by stratification by other breast cancer risk factors, by tumor receptor status, or by invasive versus *in situ* disease.

Materials 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 most known breast cancer risk factors including height, weight, age at menarche and menopause, age at first birth, postmenopausal hormone use, and family history of breast cancer.

During 1989 and 1990, blood samples were collected from 32 826 cohort members, who were 43–69 years of age at blood collection and formed the blood cohort. Details regarding the blood collection methods have been previously published *(6,7)*. Briefly, each woman arranged to have her blood drawn and then shipped, via overnight courier and with an ice-pack, to our laboratory, where it was processed and separated into plasma, red blood cell, and white blood cell components. Samples have been stored in continuously monitored liquid nitrogen freezers since collection. As of 1998, the follow-up rate among the women who provided blood samples was 99%.

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. Of the blood cohort, 11 169 women met these criteria; case and control subjects were selected from this sub-cohort. We defined a postmenopausal participant in this study as a woman who reported having a natural menopause or a bilateral oophorectomy or as a woman who reported having a hysterectomy with either one or both ovaries remaining when she was 56 years old (if a nonsmoker) or 54 years old (if a current smoker), ages at which natural menopause had occurred in 90% of these respective groups.

Case subjects in this analysis are women with no reported cancer diagnosis (other than non-melanoma skin cancer) before blood collection and who were diagnosed with breast cancer after blood collection but before June 1, 1998. Overall, 322 cases of breast cancer (264 invasive, 41 *in situ,* 153 estrogen receptor (ER)- and progesterone receptor (PR)-positive [ER^+/PR^+], and 39 ER-negative and PR-negative [ER^-/PR^-] disease) were reported from among the 11 169 women eligible at baseline. All cases of breast cancer were confirmed by and tumor details (receptor status and invasive versus *in situ* tumors) were obtained from a medical record review, with one exception. A single nurse confirmed the diagnosis of breast cancer, but the medical record was unavailable. Because of the high confirmation rate upon medical record review (99%) in the Nurses' Health Study, we kept this case subject in the analysis. However, 17 cases were not included in the invasive versus *in situ* case sub-analyses because the pathology report was unclear as to whether the tumor was invasive or because the information was missing. Time from blood collection to diagnosis ranged from less than 1 month to 106 months (median = 52 months; 5^{th} percentile-95^{th} percentile = 4–96 months). Two control subjects (total n = 643) were matched per case subject by age (year), month of blood collection, time of day that blood was drawn ($\pm$2 hours), and fasting status at the time of blood collection ($\geq$10 hours since a meal versus $<$10 hours or unknown). Ninety-four percent of control matches were exact; the most relaxed matches were within $\pm$6 years of age, $\pm$14 months of blood collection from case subjects, and $\pm$11 hours for time of blood collection. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

Laboratory Analyses

Analyses were conducted by three different laboratories. For estrone, estradiol, androstenedione, testosterone, dehydroepiandrosterone (DHEA), and dehydroepiandrosterone sulfate (DHEAS), all batches were assayed at Quest Diagnostic's Nichols Institute (San Juan Capistrano, CA). For estrone sulfate, the first batch was assayed at the University of Massachusetts Medical Center's Longcope Steroid Radioimmunoassay Laboratory (Worcester); the remaining batches were assayed at Nichols. The first two batches of sex hormone-binding globulin (SHBG) were assayed at the Longcope Laboratory; the third and fourth batches were assayed at Massachusetts General Hospital's Reproductive Endocrinology Unit Laboratory (Boston). All batches of progesterone were assayed at the same time at Quest Diagnostics.

Hormone assay methods have been described previously in detail *(6)*. Endogenous hormone levels were measured in blood plasma. In brief, samples were extracted with a mixture of hexane and ethyl acetate (4:1, vol/vol) and applied to a celite column, the steroids were eluted from the column (celite in ethylene glycol), and the fractions were subjected to radioimmunoassay *(8–12)*. DHEAS was assayed by radioimmunoassay without a prior separation step *(13)*. To quantify estrone sulfate levels, estrone was first extracted from the plasma, and then the estrone sulfate bond was enzymatically cleaved to release estrone, which was then extracted from the plasma by an organic solvent and was subjected to chromatography and then radioimmunoassay *(14)*. Free and percent free estradiol were calculated by the law of mass action according to the method described by Sodergard et al. *(15)*.

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 five different batches (sent in 1992, 1993, 1996, 1998, and 2001). To assess laboratory precision, replicates of 10% of all samples assayed were randomly interspersed and labeled to preclude their iden-

tification. Within-batch laboratory coefficients of variation ranged from 6% (DHEAS) to 15% (progesterone).

The detection limits of the assays were as follows: 2 pg/mL for estradiol, 10 pg/mL for estrone, 40 pg/mL for estrone sulfate (in each laboratory), 3 ng/dL for androstenedione, 1 ng/dL for testosterone, 3 ng/dL for DHEA, 5 μg/dL for DHEAS, and 3 ng/dL for progesterone. When plasma hormone values were reported as less than the detection limit, we set the value to half this limit. Values were less than the detection limit of estrone in 22 samples, estrone sulfate in three samples, androstenedione in one sample, testosterone in two samples, DHEA in one sample, DHEAS in five samples, and progesterone in 274 samples.

Covariate Data

We obtained information on other breast cancer risk factors from one or more of the biennial NHS questionnaires. Age at menarche and height were asked on the 1976 questionnaire. Age at first birth and parity were asked on the 1976 questionnaire and updated until the 1984 questionnaire. Family history of breast cancer was asked on the 1976 questionnaire and updated on the 1982 and 1988 questionnaires. Weight at age 18 years was asked on the 1980 questionnaire; current weight was obtained from the questionnaire completed at blood collection. Menopausal status and postmenopausal hormone use was asked on all biennial questionnaires, and this information was updated until diagnosis of breast cancer when case subjects were identified and matched to control subjects.

Statistical Analyses

We used quartile cut points to divide the data into fourths, with cut points based on the distribution in the control subjects. For most of the hormones, we chose quartile cut points according to the distribution in the control subjects overall and used the lowest fourth as the referent in all analyses.

For estrone, estrone sulfate, testosterone, estradiol, and DHEA, the median value for the control subjects varied in such a way that quartile cut points that were based on all control subjects combined resulted in uneven batch-specific distributions (between batch differences in medians ranged from ≤2% up to a maximum of 30%-60% depending on the hormone). Because the mean value of the quality-control replicates in each of the datasets varied in the same manner for these five assays, much (if not all) of this difference appeared to be caused by laboratory drift rather than by true differences in hormone levels between the batches. When the fifth batch was sent for assay of estrone, estrone sulfate, testosterone, and DHEA, we included approximately 10 samples from each of the previous batches to assess laboratory drift. Using the mean percent change between each of the first four batches and the fifth batch, we recalibrated the earlier hormone values to the fifth batch scale. Thus, for these four hormones, we defined one set of batch-specific quartile cut points by the recalibrated values combined with the fifth batch values. For estradiol, the median value for the control subjects in the fifth batch varied from the first four batches by 50%, but we had not included samples from earlier batches to allow recalibration. Thus, for estradiol, we defined two quartile cut points: one that was based on the first four batches combined and the other that was based on the fifth batch. We also controlled for batch in all analyses. When statistical analyses were repeated with batch-specific cut points for all hormones, rather than the recalibrated data, results were nearly identical.

We removed two matched sets of a case subject and two control subjects from the analysis, because the case subject's estrogen values were in the premenopausal range, dropping the total number of case subjects from 324 to 322. We used the extreme Studentized deviate Many-Outlier procedure *(16,17)* to assess for outliers in each set of laboratory results. This procedure resulted in the removal of three estradiol values, four androgen values, two testosterone values, one DHEA value, and three progesterone values. In addition, several women did not have a sufficient volume of plasma for all assays. Therefore, from the 322 total case subjects, the final number of case and control samples available for each individual hormone analysis is shown in Table 1. Case subject and control subject distributions across the data by fourths for each individual hormone are shown in Table 2.

To test for differences in hormone levels between case subjects and control subjects, we used mixed-effects regression models for clustered data to adjust for possible confounding due to the matching factors and to adjust for any residual correlation between case subjects and control subjects within the matched set *(18)*. To maintain matched triplet integrity, we used conditional logistic regression to estimate odds ratios (referred to

Table 1. Plasma hormone levels for postmenopausal case subjects and matched control subjects

			Case definition						
	Control subjects		All case subjects		Case subjects with invasive disease		Case subjects with *in situ* disease		*P*
Hormone	No.	Median (range*)	No.	Median (range*)	No.	Median (range*)	No.	Median (range*)	value†
Estradiol, pg/mL	637	6 (4–13)	319	7 (4–15)	261	7 (4–15)	41	8 (5–17)	<.001
Free estradiol, pg/mL	605	0.10 (0.05–0.21)	301	0.11 (0.05–0.26)	247	0.11 (0.05–0.27)	39	0.10 (0.06–0.24)	<.001
Estrone, pg/mL	624	23 (14–38)	320	26 (15–43)	262	26 (15–43)	41	28 (15–45)	<.001
Estrone sulfate, pg/mL	622	280 (136–600)	313	339 (154–823)	258	339 (154–823)	39	348 (150–823)	<.001
Progesterone, ng/dL	530	4.0 (1.5–10.0)	270	4.0 (1.5–10.0)	222	4.0 (1.5–10.0)	32	4.0 (1.5–10.5)	.64
Sex hormone binding globulin, nm/L	622	48 (24–79)	310	47 (21–81)	255	44 (20–80)	39	56 (22–87)	.08
Androstenedione, ng/dL	621	57 (31–103)	312	62 (38–103)	255	61 (37–103)	41	63 (39–99)	.01
Testosterone, ng/dL	628	19 (11–33)	312	22 (12–37)	256	22 (12–37)	40	22 (14–39)	<.001
Free testosterone, ng/dL	608	0.22 (0.10–0.43)	301	0.25 (0.13–0.51)	248	0.25 (0.13–0.52)	38	0.25 (0.14–0.46)	<.001
Dehydroepiandrosterone, ng/dL	603	248 (116–473)	305	283 (127–557)	248	258 (133–564)	41	328 (122–536)	.01
Dehydroepiandrosterone sulfate, μg/dL	634	85 (35–169)	320	90 (44–205)	262	90 (43–200)	41	90 (50–205)	<.001

*Range from median of the bottom fourth (12.5%) to median of the top fourth (87.5%).

†*P* value, from the mixed-effects regression model comparing all case subjects to control subjects, controlling for matching factors; two-sided.

Table 2. Quartile ranges for plasma hormone levels among postmenopausal case subjects and matched control subjects

Plasma hormone	Quartile ranges (No. case subjects/No. control subjects)			
Estradiol, pg/mL	* (79/209)	* (82/168)	* (49/116)	* (109/144)
Free estradiol, pg/mL	<0.064 (55/149)	0.064–0.096 (81/155)	0.097–0.148 (59/147)	>0.148 (106/154)
Estrone, pg/mL	<18 (68/159)	18–23 (67/160)	24–30 (79/151)	>30 (106/154)
Estrone sulfate, pg/mL	<178 (50/155)	178–279 (76/155)	280–421 (70/157)	>421 (117/155)
Progesterone, ng/dL	<1.6 (91/191)	1.6–4.0 (49/95)	4.1–8.0 (78/139)	>8.0 (52/105)
SHBG, nm/L	<34 (88/150)	34–48 (72/166)	49–67 (75/149)	>67 (75/157)
Androstenedione, ng/dL	<43 (64/159)	43–57 (64/156)	58–78 (93/155)	>78 (91/151)
Testosterone, ng/dL	<15 (66/164)	15–19 (54/153)	20–26 (95/160)	>26 (97/151)
Free testosterone, ng/dL	<0.16 (54/156)	0.16–0.22 (71/154)	0.23–0.32 (86/145)	>0.32 (90/153)
DHEA, ng/dL	<165 (67/152)	165–247 (69/149)	248–367 (74/150)	>367 (95/152)
DHEAS, μg/dL	<52 (53/160)	52–85 (96/162)	86–135 (81/156)	>135 (90/156)

*Batch-specific quartile cut points were used to categorize estradiol. The cut points for the 1990–1992, 1992–1994, 1994–1996 batches were <6, 6–7, 8–10, and ≥11 pg/mL; the cutpoints for the 1996–1998 batch were <5, 5–6, 7–8, and ≥9 pg/mL. SHBG = sex hormone binding globulin; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate.

herein as relative risks) and 95% confidence intervals (CIs) in the total data set *(19)*. Results from simple and multivariable models were very similar. To increase statistical power, we used unconditional logistic regression, controlling for the matching factors, for all subset analyses (e.g., analyses according to prior postmenopausal hormone use or tumor receptor status or analyses that were stratified by invasive versus *in situ* cases). These analyses thus include case subjects and control subjects whose match was excluded because of outlying hormone values (as described above) or missing sub-group-defining data. These subset analyses also were conducted by conditional logistic regression, and results were similar, although less precise. We conducted tests for trend by modeling the natural logarithm of the hormone level as a continuous variable and calculating a Wald statistic *(19)*. Additionally, we calculated tests for trend by modeling the median of the fourths of each hormone. All *P* values were from two-sided tests.

To test for differences in trend across fourths of hormone level by breast cancer tumor characteristics, we used polychotomous logistic regression *(20)* with three end points for tumor invasiveness (invasive, *in situ*, and no breast cancer) and four end points for tumor receptor status (ER^+/PR^+, ER^+/PR^-, ER^-/PR^-, and no breast cancer). One and two degree of freedom tests, respectively, compared a model with separate slopes in each ER/PR group to a model with a common slope. The likelihood ratio test statistic was applied to a chi-squared distribution to obtain two-sided *P* values. Too few cases of ER^-/PR^+ disease occurred (n = 6) in the cohort for this tumor receptor pattern to be considered separately.

The interactions between hormone levels and established breast cancer risk factors were evaluated by adding cross-classified variables (e.g., estrone [medians of continuous fourths] × postmenopausal hormone use [dichotomized as never and past, which was defined by use up to time of diagnosis or control referent date]) to the logistic models; presence of an interaction was assessed with the Wald test. These analyses were conducted among all women combined.

RESULTS

Both case subjects and control subjects in this analysis ranged in age from 45 to 69 years, with a mean age of 62 years. The mean years since menopause (13.2 versus 13.5 years), body mass index at age 18 years (21.4 versus 21.6 kg/m^2), parity (3.2 versus 3.3 children), age at first birth (25.7 versus 25.4 years), and age at menopause (48.7 versus 48.4 years) did not differ between case subjects and control subjects, respectively. Case subjects, compared with control subjects, were statistically significantly more likely to have a family history of breast cancer (24.2% versus 17.1%; $P = .01$) and were younger at menarche (12.5 versus 12.7 years; $P = .03$). Circulating steroid hormone levels were statistically significantly greater among case subjects with breast cancer than among control subjects for all hormones investigated, with the exception of progesterone (Table 1). In conditional logistic regression models that were adjusted for known breast cancer risk factors (body mass index at age 18 years, family history of breast cancer, age at menarche, age at first birth, parity, age at menopause, and duration of postmenopausal hormone use), the risk of breast cancer was statistically significantly greater among the highest fourth than among the lowest fourth and was linearly associated across fourths for all hormones, except for progesterone and SHBG (Table 3). When tests for trend were modeled with the median of the fourths for each hormone, results were nearly identical, except for trends

Table 3. Risk of breast cancer by fourths of plasma hormone levels among postmenopausal women

Plasma hormone	RR (95% CI)*				*P* value†
	1	2	3	4	
Estradiol, pg/mL					
MV RR‡	1.0 (referent)	1.3 (0.9 to 1.9)	1.1 (0.7 to 1.7)	2.1 (1.5 to 3.2)	<.001
Invasive disease	1.0 (referent)	1.4 (0.9 to 2.1)	1.3 (0.8 to 2.0)	2.0 (1.3 to 3.0)	<.001
In situ disease§	1.0 (referent)	1.9 (0.7 to 4.8)	1.1 (0.4 to 3.7)	3.0 (1.2 to 7.4)	.01
Never PMH use	1.0 (referent)	1.6 (0.8 to 3.0)	1.9 (1.0 to 3.7)	3.6 (2.0 to 6.4)	<.001
Past PMH use	1.0 (referent)	1.3 (0.8 to 2.3)	0.8 (0.4 to 1.5)	1.6 (0.9 to 2.8)	.22
Free estradiol, pg/mL					
MV RR‡	1.0 (referent)	1.3 (0.9 to 2.0)	1.0 (0.7 to 1.6)	1.9 (1.2 to 2.9)	<.001
Invasive disease	1.0 (referent)	1.4 (0.9 to 2.3)	1.1 (0.7 to 1.8)	2.0 (1.3 to 3.1)	<.001
In situ disease§	1.0 (referent)	2.4 (0.9 to 6.7)	1.6 (0.5 to 4.8)	2.2 (0.8 to 6.4)	.12
Never PMH use	1.0 (referent)	1.4 (0.7 to 2.8)	1.1 (0.6 to 2.3)	2.6 (1.4 to 4.9)	<.001
Past PMH use	1.0 (referent)	1.3 (0.7 to 2.3)	1.0 (0.5 to 1.9)	1.4 (0.8 to 2.7)	.19
Estrone, pg/mL					
MV RR‡	1.0 (referent)	1.0 (0.7 to 1.6)	1.3 (0.9 to 2.0)	1.7 (1.1 to 2.6)	<.001
Invasive disease	1.0 (referent)	0.9 (0.6 to 1.4)	1.2 (0.8 to 1.8)	1.4 (0.9 to 2.2)	.003
In situ disease§	1.0 (referent)	1.5 (0.5 to 4.4)	1.8 (0.6 to 5.2)	3.0 (1.1 to 8.2)	.01
Never PMH use	1.0 (referent)	0.7 (0.3 to 1.4)	1.6 (0.9 to 3.0)	3.0 (1.7 to 5.5)	<.001
Past PMH use	1.0 (referent)	1.1 (0.6 to 1.9)	1.0 (0.6 to 1.9)	0.8 (0.4 to 1.5)	.72
Estrone sulfate, pg/mL					
MV RR‡	1.0 (referent)	1.6 (1.0 to 2.5)	1.4 (0.9 to 2.2)	2.4 (1.6 to 3.8)	<.001
Invasive disease	1.0 (referent)	1.5 (1.0 to 2.4)	1.3 (0.8 to 2.1)	2.2 (1.4 to 3.5)	<.001
In situ disease§	1.0 (referent)	2.0 (0.6 to 6.4)	2.4 (0.8 to 7.3)	3.5 (1.2 to 10.2)	.03
Never PMH use	1.0 (referent)	1.5 (0.8 to 3.0)	1.9 (1.0 to 3.7)	3.4 (1.8 to 6.3)	<.001
Past PMH use	1.0 (referent)	1.5 (0.8 to 2.7)	1.2 (0.6 to 2.2)	1.8 (1.0 to 3.2)	.06
Progesterone, ng/dL					
MV RR‡	1.0 (referent)	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	0.9 (0.6 to 1.5)	.90
Invasive disease	1.0 (referent)	0.9 (0.5 to 1.4)	1.1 (0.7 to 1.7)	0.8 (0.5 to 1.3)	.77
In situ disease§	1.0 (referent)	2.9 (1.1 to 7.6)	1.2 (0.4 to 3.5)	1.6 (0.5 to 5.0)	.67
SHBG, nm/L					
MV RR‡	1.0 (referent)	0.7 (0.5 to 1.1)	0.9 (0.6 to 1.3)	0.8 (0.6 to 1.3)	.14
Invasive disease	1.0 (referent)	0.7 (0.5 to 1.1)	0.7 (0.5 to 1.1)	0.8 (0.5 to 1.3)	.04
In situ disease§	1.0 (referent)	0.6 (0.2 to 1.9)	2.3 (1.0 to 5.7)	0.6 (0.2 to 1.9)	.76
Androstenedione, ng/dL					
MV RR‡	1.0 (referent)	1.0 (0.7 to 1.6)	1.6 (1.0 to 2.3)	1.5 (1.0 to 2.3)	.04
Invasive disease	1.0 (referent)	1.0 (0.6 to 1.5)	1.4 (0.9 to 2.1)	1.4 (0.9 to 2.2)	.08
In situ disease§	1.0 (referent)	1.6 (0.6 to 4.5)	1.9 (0.7 to 5.1)	2.3 (0.8 to 6.5)	.24
Testosterone, ng/dL					
MV RR‡	1.0 (referent)	0.9 (0.6 to 1.4)	1.5 (1.0 to 2.2)	1.6 (1.0 to 2.4)	<.001
Invasive disease	1.0 (referent)	0.7 (0.4 to 1.2)	1.4 (0.9 to 2.1)	1.4 (0.9 to 2.2)	.003
In situ disease§	1.0 (referent)	1.7 (0.5 to 5.5)	3.1 (1.0 to 9.3)	3.7 (1.2 to 11.0)	.01
Free testosterone, ng/dL					
MV RR‡	1.0 (referent)	1.4 (0.9 to 2.1)	1.6 (1.0 to 2.4)	1.7 (1.1 to 2.6)	<.001
Invasive disease	1.0 (referent)	1.3 (0.8 to 2.0)	1.5 (0.9 to 2.4)	1.8 (1.1 to 2.8)	<.001
In situ disease§	1.0 (referent)	2.4 (0.8 to 7.8)	3.9 (1.3 to 11.5)	1.7 (0.5 to 5.9)	.17
DHEA, ng/dL					
MV RR‡	1.0 (referent)	1.1 (0.7 to 1.7)	1.1 (0.7 to 1.7)	1.4 (0.9 to 2.2)	.02
Invasive disease	1.0 (referent)	1.2 (0.8 to 1.8)	1.1 (0.7 to 1.7)	1.3 (0.9 to 2.1)	.05
In situ disease§	1.0 (referent)	0.3 (0.1 to 1.2)	1.3 (0.5 to 3.1)	1.6 (0.7 to 3.8)	.22
DHEAS, μg/dL					
MV RR‡	1.0 (referent)	1.9 (1.2 to 2.9)	1.6 (1.1 to 2.5)	1.7 (1.1 to 2.7)	.003
Invasive disease	1.0 (referent)	2.1 (1.3 to 3.3)	1.7 (1.1 to 2.7)	1.8 (1.1 to 2.9)	.01
In situ disease§	1.0 (referent)	1.9 (0.7 to 5.1)	1.4 (0.5 to 3.9)	1.5 (0.5 to 4.0)	.26

*Batch-specific quartile cut points were used to categorize estradiol. The cut points for the 1990–1992, 1992–1994, 1994–1996 batches were <6, 6–7, 8–10, and ≥11 pg/mL; the cutpoints for the 1996–1998 batch were <5, 5–6, 7–8, and ≥9 pg/mL. RR = relative risk; CI = confidence interval; MV = multivariable, PMH = postmenopausal hormones; SHBG = sex hormone binding globulin; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate. Sample sizes were as follows: all women = 322 case subjects, 643 control subjects; invasive disease = 264; *in situ* disease = 41; never PMH use = 162; past PMH use = 160.

†*P* value, test for trend. The logarithm of the hormone level was entered into the model as a continuous variable; two-sided.

‡Conditional logistic regression models controlling for body mass index at age 18 years (<21, 21–22.9, 23–24.9, or ≥25 kg/m^2), family history of breast cancer (yes or no), age at menarche (<12, 12, 13, or ≥14 y), age at first birth and parity (nulliparous; 1–4 children, first birth <25 y; 1–4 children, first birth 25–29 y; 1–4 children, first birth ≥30 y; ≥5 children, first birth <25 y; or ≥5 children, first birth ≥25 y), age at menopause (<46, 46–50, 51–55, or ≥56 y), and duration of PMH use (continuous) were used in the main analyses among all women. Unconditional logistic regression, controlling for the matching factors (age [5-year groups], month of blood draw [6-month blocks], time of blood draw [4-hour blocks], fasting status [<10 versus ≥10 hours]) and the same covariates as the conditional multivariable models were used for subgroup analyses.

§Unconditional logistic regression model controlling for matching factors only.

across fourths of DHEA and DHEAS, which were attenuated and no longer statistically significant (data not shown). The simple conditional models controlling for matching factors only differed negligibly (data not shown).

When analyses were restricted to those case subjects diagnosed from July 1, 1994, through June 30, 1998 [i.e., to the case subjects added since the publication of results from the 1990–1994 analysis *(6)*], the associations between hormone levels and breast cancer risk were similar or slightly stronger than those in the initial report. The greatest differences, when comparing the highest with lowest fourths in the 1990–1994 and 1994–1998 periods, respectively, were observed for estradiol (RR = 1.9, 95% CI = 1.1 to 3.5, and RR = 2.6, 95% CI = 1.5 to 4.7), testosterone (RR = 1.4, 95% CI = 0.7 to 2.7, and RR = 2.0, 95% CI = 1.1 to 3.6), and DHEA (RR = 1.1, 95% CI = 0.6 to 2.0, and RR = 2.0, 95% CI = 1.0 to 3.8). The only association to decrease slightly between analyses was that for DHEAS (for the 1990–1994 analysis, RR = 2.2, 95% CI = 1.1 to 4.2, and for the 1994–1998 analysis, RR = 1.5, 95% CI = 0.8 to 2.7). We observed negligible differences between the two follow-up periods for estrone (for the 1990–1994 analysis, RR = 2.0, 95% CI = 1.1 to 3.7, and for the 1994–1998 analysis, RR = 1.8, 95% CI = 1.0 to 3.4), estrone sulfate (for the 1990–1994 analysis, RR = 2.3, 95% CI = 1.2 to 4.1, and for the 1994–1998 analysis, RR = 2.5, 95% CI = 1.3 to 4.5), and androstenedione (for the 1990–1994 analysis, RR = 1.5, 95% CI = 0.8 to 2.8, and for the 1994–1998 analysis, RR = 1.7, 95% CI = 0.9 to 3.2).

Among estrogen metabolites, we observed a twofold increase in the risk of breast cancer associated with estradiol, free estradiol, estrone, or estrone sulfate, when the highest and lowest fourths were compared. The association with percent free estradiol was similar (RR = 1.4, 95% CI = 0.9 to 2.1), although we did not observe a statistically significant trend (*P* value, test for trend [P_{trend}] = .11) (data not shown). When estradiol and testosterone were placed in the same multivariable model, the association with estradiol was essentially unchanged (RR = 1.9, comparing the highest with the lowest fourth of estradiol, 95% CI = 1.3 to 2.9; P_{trend} = .005), although the relative risk associated with testosterone was attenuated (RR = 1.2, comparing the highest with the lowest fourth of testosterone, 95% CI = 0.8 to 2.0; P_{trend} = .09) (data not shown). Associations of the estrogen metabolites with breast cancer risk were strongest among women who had never used postmenopausal hormones. However, statistically significant effect modification was observed for estrone and estradiol (*P* value, test for heterogeneity [$P_{heterogeneity}$]<.001 and $P_{heterogeneity}$ = .04, respectively), but associations of androgens with breast cancer risk did not vary statistically significantly when stratified by postmenopausal hormone use. We also observed that the relative risk was 50%–100% greater among case subjects with *in situ* disease than among case subjects with invasive disease for all hormones examined—except free estradiol, SHBG, free testosterone, DHEA, and DHEAS—although confidence intervals were wide and overlapped those of case subjects with invasive disease (Table 3). Polychotomous comparisons also were not statistically significant (data not shown). The associations observed among case subjects with *in situ* disease changed negligibly when case subjects with lobular (n = 3) or both lobular and intraductal (n = 4) tumors were excluded (data not shown).

We also evaluated potential effect modification of the association between endogenous hormone level and breast cancer risk by the following factors: age at blood collection (stratified at the control median = 63 years), age at cancer diagnosis (median = 67 years), time from menopause to blood collection (median = 13 years), waist-to-hip ratio (median = 0.79), and weight change from age 18 years to baseline (increase of <2 kg/m^2 compared with increase of 2 or more kg/m^2). The majority of these interactions were not statistically significant. However, the association of androgen levels with the risk of breast cancer was statistically significantly stronger among women whose weight increased <2 kg/m^2 from age 18 years to baseline (comparing the highest fourth to the lowest fourth of androgen levels). Specifically, among these subjects, we observed statistically significant associations between breast cancer risk and the level of androstenedione (RR = 2.1, 95% CI = 1.2 to 3.8; $P_{heterogeneity}$ = .06), free testosterone (RR = 2.6, 95% CI = 1.4 to 4.7; $P_{heterogeneity}$ = .03), and DHEA (RR = 2.0, 95% CI = 1.2 to 3.6; $P_{heterogeneity}$ = .03) (data not shown).

When associations between hormone levels and the risk of breast cancer were evaluated according to receptor status of the tumor, the strongest associations and most consistent dose-response relations were observed among case subjects with ER^+/PR^+ tumors for all hormones except progesterone (Table 4). For example, among those with ER^+/PR^+ tumors, comparing the highest to lowest fourth of circulating hormone levels, we observed an increased risk associated with breast cancer for estradiol (RR = 3.3, 95% CI = 2.0 to 5.4; $P_{heterogeneity}$<.001), for testosterone (RR = 2.0, 95% CI = 1.2 to 3.4; $P_{heterogeneity}$ = .009), for androstenedione (RR = 2.5, 95% CI = 1.4 to 4.3; $P_{heterogeneity}$ = .22), and for DHEAS (RR = 2.3, 95% CI = 1.3 to 4.1; $P_{heterogeneity}$ = .85). No linear trend was observed for any hormone among women with PR^- tumors regardless of ER tumor status. We also investigated whether these tumor receptor-specific associations would differ when analyses were restricted to participants who had never used postmenopausal hormones. Although sample sizes of such subjects were very small, associations further restricted to case subjects with ER^+/PR^+ tumors tended to increase in magnitude. For example, among case subjects with ER^+/PR^+ tumors who had never used postmenopausal hormones, the risk of breast cancer associated with the top fourth of estradiol levels was approximately fivefold higher (RR = 4.8, 95% CI = 2.2 to 10.9) than that associated with the bottom fourth.

Finally, we evaluated the joint effect of estradiol fourths with fourths of progesterone and testosterone associated with the risk of breast cancer (Table 5). We found statistically significant Spearman correlations between estradiol and testosterone (r = .44) and between estrogen and progesterone (r = .15). However, tests for heterogeneity did not indicate statistically significant interactions between estradiol levels and either testoserone or progesterone levels. Regardless of testosterone level, the highest fourth of circulating estradiol concentration was associated with the greatest risk of breast cancer. Although this pattern was also true for the stratification of estradiol by progesterone level, there was an indication that high levels of endogenous progesterone among women with the lowest amount of circulating estradiol were associated with a decreased risk for breast cancer (for the comparison of the lowest fourth of estradiol but the top half of progesterone levels to those with the lowest fourths of both hormones, RR

Table 4. Risk of breast cancer according to fourths of plasma hormone levels by tumor receptor status*

Plasma hormone (No. case subjects/ No. control subjects)	RR (95% CI)				P_{trend}†	$P_{heterogeneity}$‡
	1	2	3	4		
Estradiol						<.001
ER^+/PR^+ (153/637)	1.0 (referent)	1.8 (1.0 to 3.0)	1.5 (0.8 to 2.8)	3.3 (2.0 to 5.4)	<.001	
ER^-/PR^- (38/637)	1.0 (referent)	1.0 (0.4 to 2.2)	0.5 (0.2 to 1.7)	1.0 (0.4 to 2.4)	.46	
ER^+/PR^- (33/637)	1.0 (referent)	0.8 (0.3 to 2.1)	0.8 (0.3 to 2.4)	1.0 (0.4 to 2.6)	.82	
Estrone						.04
ER^+/PR^+ (153/624)	1.0 (referent)	1.2 (0.7 to 2.1)	1.6 (0.9 to 2.8)	2.4 (1.4 to 4.1)	<.001	
ER^-/PR^- (38/624)	1.0 (referent)	0.5 (0.2 to 1.3)	1.0 (0.4 to 2.4)	0.7 (0.3 to 1.7)	.92	
ER^+/PR^- (34/624)	1.0 (referent)	0.6 (0.2 to 1.7)	0.6 (0.2 to 1.6)	0.9 (0.4 to 2.3)	.92	
Estrone sulfate						.80
ER^+/PR^+ (150/622)	1.0 (referent)	1.5 (0.8 to 2.7)	1.6 (0.9 to 3.0)	2.8 (1.6 to 4.9)	<.001	
ER^-/PR^- (39/622)	1.0 (referent)	1.5 (0.6 to 4.0)	0.9 (0.3 to 2.7)	1.9 (0.7 to 4.8)	.34	
ER^+/PR^- (32/622)	1.0 (referent)	0.8 (0.3 to 2.3)	0.6 (0.2 to 1.8)	1.3 (0.5 to 3.4)	.28	
Progesterone						.22
ER^+/PR^+ (131/530)	1.0 (referent)	1.3 (0.7 to 2.3)	1.3 (0.8 to 2.2)	1.3 (0.7 to 2.4)	.38	
ER^-/PR^- (34/530)	1.0 (referent)	0.9 (0.3 to 2.4)	1.1 (0.5 to 2.6)	0.3 (0.1 to 1.3)	.17	
ER^+/PR^- (28:530)	1.0 (referent)	0.8 (0.2 to 2.5)	0.8 (0.3 to 2.3)	0.8 (0.3 to 2.7)	.82	
SHBG						.002
ER^+/PR^+ (147/622)	1.0 (referent)	0.7 (0.4 to 1.1)	0.6 (0.4 to 1.0)	0.5 (0.3 to 0.8)	.001	
ER^-/PR^- (38/622)	1.0 (referent)	0.4 (0.2 to 1.3)	0.7 (0.3 to 1.9)	1.1 (0.5 to 2.8)	.78	
ER^+/PR^- (33/622)	1.0 (referent)	1.6 (0.5 to 4.6)	0.7 (0.2 to 2.7)	1.8 (0.6 to 5.0)	.72	
Testosterone						.03
ER^+/PR^+ (149/628)	1.0 (referent)	0.9 (0.5 to 1.7)	1.8 (1.1 to 3.1)	2.0 (1.2 to 3.4)	<.001	
ER^-/PR^- (38/628)	1.0 (referent)	0.4 (0.2 to 1.1)	0.6 (0.3 to 1.6)	0.7 (0.3 to 1.6)	.35	
ER^+/PR^- (33/628)	1.0 (referent)	0.9 (0.3 to 2.9)	1.4 (0.5 to 3.9)	1.9 (0.7 to 5.0)	.12	
Androstenedione						.22
ER^+/PR^+ (148/621)	1.0 (referent)	1.5 (0.9 to 2.7)	1.9 (1.1 to 3.3)	2.5 (1.4 to 4.3)	<.001	
ER^-/PR^- (38/621)	1.0 (referent)	0.7 (0.3 to 1.9)	0.9 (0.4 to 2.4)	0.9 (0.4 to 2.4)	.73	
ER^+/PR^- (34/621)	1.0 (referent)	0.5 (0.2 to 1.6)	1.1 (0.4 to 2.6)	0.7 (0.3 to 2.0)	.43	
DHEA						.76
ER^+/PR^+ (145/603)	1.0 (referent)	1.3 (0.8 to 2.2)	1.1 (0.6 to 1.9)	1.6 (0.9 to 2.7)	.05	
ER^-/PR^- (36/603)	1.0 (referent)	1.3 (0.4 to 3.5)	1.7 (0.6 to 4.7)	1.5 (0.5 to 4.2)	.26	
ER^+/PR^- (32/603)	1.0 (referent)	0.9 (0.3 to 2.8)	1.2 (0.4 to 3.2)	1.0 (0.3 to 2.9)	.77	
DHEAS						.85
ER^+/PR^+ (153/634)	1.0 (referent)	2.4 (1.3 to 4.1)	1.9 (1.1 to 3.5)	2.3 (1.3 to 4.1)	.002	
ER^-/PR^- (38/634)	1.0 (referent)	0.9 (0.3 to 2.5)	1.0 (0.4 to 2.6)	1.4 (0.5 to 3.5)	.24	
ER^+/PR^- (33/634)	1.0 (referent)	0.5 (0.2 to 1.5)	0.7 (0.2 to 1.8)	1.1 (0.4 to 2.7)	.94	

*Unconditional logistic regression model controlling for the following matching factors only: age (5-year groups), month of blood collection (6-month blocks), time of blood collection (4-hour blocks), fasting status (<10 versus ≥10 hours). RR = relative risk; CI = confidence interval; ER = estrogen receptor; PR = progesterone receptor; SHBG = sex hormone binding globulin; DHEA = dehydroepiandrosterone; DHEAS = dehydroepiandrosterone sulfate.

†*P* value, test for trend. The logarithm of the hormone level was entered into the model as a continuous variable; two-sided.

‡*P* value, test for heterogeneity. Likelihood ratio test calculated from polychotomous logistic regression; two-sided, two degrees of freedom.

= 0.5, 95% CI = 0.2 to 1.3). When these analyses were restricted to case subjects with ER^+/PR^+ tumors, similar patterns were observed.

Discussion

Among the 322 case subjects and 643 control subjects included in this nested case-control analysis, we observed a statistically significant direct association between the endogenous levels of each steroid hormone evaluated and the risk of breast cancer, with the exceptions of the endogenous levels of progesterone and SHBG. We observed the greatest magnitudes of effect among case subjects with ER^+/PR^+ tumors. Strengths of this study include its size; prospectively collected environmental, reproductive, and biomarker data that reduced concerns of recall bias or blood sample timing relative to breast cancer diagnosis; and the collection of detailed tumor-specific data.

The relation between endogenous steroid hormones and breast cancer risk has been evaluated in nine prospective epidemiologic studies *(6,21–31)*, including our own with follow-up from the 1990–1994 analysis *(6)*. Recently, data from these studies were pooled and re-analyzed *(1)*. For all hormones evaluated, our results are consistent with those of this collaborative study *(1)*. Similarly, the results that we observed for 1990–1994 and 1994–1998 analyses were similar, suggesting that a single blood sample can predict breast cancer risk for at least 8 years after collection. This result is also consistent with the results observed by the NYU Women's Health Study, which has recently added 7 years of follow-up to their original analysis *(32)*. We observed stronger associations among women who had never used postmenopausal hormones because a single blood sample likely best reflects long-term hormone levels in these women. That the relations with breast cancer risk among women who had never used postmenopausal hormones are most apparent for estrone, estradiol, and estrone sulfate was expected, because these hormones are most affected by Premarin, the predominant postmenopausal hormone used in this population.

Table 5. Estradiol, testosterone, and progesterone in relation to breast cancer risk presented as relative risks (RRs) and 95% confidence intervals (CIs)*

	Estradiol fourths			
	1†	2†	3†	4†
Testosterone fourths				
<15 ng/dL	1.0 (referent)	1.0 (0.5 to 2.1)	1.1 (0.4 to 3.3)	3.8 (1.5 to 9.9)
15–19 ng/dL	1.0 (0.5 to 1.9)	0.9 (0.4 to 1.9)	0.9 (0.4 to 2.2)	1.5 (0.7 to 3.5)
20–26 ng/dL	1.5 (0.7 to 3.0)	2.1 (1.1 to 4.0)	1.5 (0.7 to 2.9)	2.2 (1.2 to 4.1)
>26 ng/dL	1.1 (0.4 to 2.7)	2.0 (1.0 to 3.9)	1.3 (0.6 to 2.8)	2.4 (1.4 to 4.2)
				$P_{heterogeneity} = .33$
Progesterone fourths				
<1.6 ng/dL	1.0 (referent)	0.7 (0.4 to 1.4)	0.6 (0.3 to 1.5)	1.7 (0.9 to 3.3)
1.6–4 ng/dL	1.2 (0.6 to 2.5)	0.6 (0.2 to 1.5)	1.2 (0.5 to 3.1)	1.4 (0.7 to 3.1)
4.1–8 ng/dL	0.5 (0.2 to 1.1)	1.6 (0.8 to 3.2)	1.0 (0.5 to 2.1)	1.8 (0.9 to 3.7)
>8 ng/dL	0.5 (0.2 to 1.3)	1.1 (0.5 to 2.4)	0.8 (0.3 to 2.2)	1.4 (0.7 to 2.9)
				$P_{heterogeneity} = .50$

*Unconditional logistic regression model controlling for the following matching factors only: age (5-year groups), month of blood collection (6-month blocks), time of blood collection (4-hour blocks), fasting status (<10 versus ≥10 hours).

†Batch-specific quartile cut points were use to categorize estradiol. Cut points 1–4, respectively, for the 1990–1992, 1992–1994, 1994–1996 batches were <6, 6–7, 8–10, and ≥11 pg/mL; the cut points 1–4, respectively, for the 1996–1998 batch were <5, 5–6, 7–8, and ≥9 pg/mL.

We are, to our knowledge, the first to report analyses that distinguish between endogenous levels of steroid hormones and the risk for invasive and *in situ* disease. We observed modest differences between these associations, with the associations with *in situ* cancer being generally of greater magnitude. These findings are consistent with the 50% or greater reduction in *in situ* breast cancer with tamoxifen use *(33).* It has been argued that the increased risk of *in situ* disease among postmenopausal hormone users may be a diagnostic bias reflecting more frequent and detailed examination of women exposed to exogenous hormones *(34–36).* However, our data suggest that the association is biologic, at least in part. Exclusion of case subjects with lobular disease did not measurably alter the associations that we observed; however, further study in larger case populations are needed.

In addition, our findings suggest that higher concentrations of endogenous steroids–both estrogens and androgens–are primarily associated with an increased risk of ER^+/PR^+ breast cancers. We have recently reported that both body mass index and current postmenopausal hormone use are preferentially associated with an increased risk of ER^+/PR^+ tumors in this cohort *(37).* However, in the only prior assessment of endogenous estrogens by tumor ER status *(38)*, no differences between the effect of estrogens on the risk of ER^+ versus PR^- tumors were observed, although only 53 case subjects with ER^+ tumors and 23 case subjects with ER^- tumors were evaluated.

The association with an increased risk for ER^+/PR^+ tumors and higher levels of endogenous steroid hormones is biological feasible because the presence of ERs and PRs in cancer cells is considered to provide a growth advantage, as shown by the positive association between the phenotype and high proliferative activity *(39).* ER overexpression has been associated with mammary tumors in animal models, and selective estrogen receptor modulators, such as tamoxifen, block ER activation in the breast, suggesting that ER-mediated regulation of gene expression plays a biologically important role in normal and malignant cells *(39–41).* In addition, chemoprevention trials evaluating selective estrogen receptor modulators have found a decreased incidence of ER^+ tumors associated with use of such modulators *(33,42–44)*, and it has been suggested that efficacy of such drugs may differ by the woman's underlying hormone profile *(45).* Less is known about the influence of chemopreventive agents or agonists on PRs, although the level PR action seems to be dependent upon ER action and thus is an indication of a functional ER *(46).*

To our knowledge, our study was the first to investigate whether progesterone levels are associated with breast cancer risk in postmenopausal women, and we observed no statistically significant association. Interestingly, we observed that case subjects with PR^+ tumors were statistically significantly most strongly affected by all circulating steroid hormones, except for progesterone. On the basis of largely indirect evidence, progesterone has been hypothesized to decrease breast cancer risk by opposing estrogenic stimulation of the breast *(47,48)* and to increase risk because breast mitotic rates are highest in the luteal (high progesterone) phase of the menstrual cycle *(49–51).* Results of murine studies suggest that implantation of progesterone inhibits apoptosis in the mammary gland *(52)* and that the progesterone signal contributes to mammary tumor susceptibility *(53).* It is possible that the range of progesterone concentrations among subjects in our study was not wide enough to detect a trend when comparing the highest with the lowest fourths or that the typical level of circulating progesterone among postmenopausal women is indeed too low to initiate or promote breast neoplasia. Results from epidemiologic studies of the association between endogenous progesterone and breast cancer risk in premenopausal women have been inconsistent, with non-statistically significant positive *(54,55)* and inverse *(23,56)* associations being reported.

Studies of postmenopausal hormone use have consistently shown that a greater risk of breast cancer is associated with the use of formulations containing estrogen and progestin than with the use of formulations containing only estrogen *(2–5,51).* However, even when stratified by fourths of endogenous estradiol concentration, we did not observe such an interaction between progesterone and estradiol levels. Given the lack of association that we observed with naturally circulating progesterone and the relatively strong associations observed with synthetic progestin exposure *(2–5)*, it may be that synthetic progestins have a more dramatic or metabolically different effect on breast tissue proliferation. In recent studies conducted in breast cancer cell lines, the type, dose, and regimen of progestogen used influenced growth stimula-

tion *(57–61)* and the pro- or antiapoptotic effect observed *(62,63)*.

Overall, our data confirm the important role for circulating steroid hormones in the etiology of breast cancer. We also observed that the history of postmenopausal hormone use and the receptor status of a breast tumor may modify these relations. Although we did not observe a direct association between the risk of breast cancer and progesterone levels, additional studies of this association are warranted. A key question is whether endogenous hormone levels could add substantially to the ability to predict an individual woman's risk of breast cancer beyond standard breast cancer risk factors–particularly, body mass index–to identify those who would most benefit from increased screening or chemoprevention [e.g., with tamoxifen *(42)*, raloxifene *(43)*, or aromatase inhibitors *(64)*]. Additional tumor subtype-specific analyses may further elucidate the underlying mechanisms of these relations and lead to more targeted and efficacious hormone-based prevention protocols.

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NOTES

Support for this project was from NIH grants P01 CA-87969, CA-49449, P50 CA-089393, and DAMD-17–02–1–0692. Dr. Missmer was partially supported by Training grant in cancer epidemiology 5 T32 CA090001–281 from the National Cancer Institute.

We thank Victor Pontes, Helena Judge Ellis, and Todd Reid for their expert assistance and Drs. Eric Winer and Myles Brown for their thoughtful suggestions. In addition, Drs. Graham Colditz, Bernard Rosner, and Walter Willett who contributed helpful critical comments. We also thank the participants of the Nurses' Health Study for their longstanding contributions.

Manuscript received May 10, 2004; revised September 28, 2004; accepted October 21, 2004.