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ARTICLE

Sex Hormone Levels and Risk of Breast Cancer With Estrogen Plus Progestin

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Background Although high endogenous sex hormone levels and estrogen plus progestin (E+P) therapy are associated with increased breast cancer risk, it is unknown whether pretreatment levels of sex hormones modify E+P effect on breast cancer.

Methods We conducted a nested case–control study within the Women's Health Initiative randomized clinical trial of E+P. The trial enrolled 16608 postmenopausal women aged 50 to 79 years with intact uterus and no breast cancer history. During a mean of 5.6 years of follow-up, 348 incident breast cancer case subjects were identified and matched with 348 control subjects. Case and control subjects had their sex hormone levels measured at baseline (estrogens, testosterone, progesterone, and sex hormone–binding globulin [SHBG]) and year 1 (estrogens and SHBG) using sensitive assays. All statistical tests were two-sided.

Results Statistically significant elevations in breast cancer risk were seen with greater pretreatment levels of total estradiol (P_{trend} = .04), bioavailable estradiol (P_{trend} = .03), estrone (P_{trend} = .007), and estrone sulfate (P_{trend} = .007). E+P increased all measured estrogens and SHGB at year 1 (all P < .001). The effect of E+P on breast cancer risk was strongest in women whose pretreatment levels of total estradiol, bioavailable estradiol, and estrone were in the lowest quartiles. For example, the odds ratio for E+P relative to placebo was 2.47 (95% confidence interval [CI] = 1.28 to 4.79) in the lowest total estradiol quartile, compared with 0.96 (95% CI = 0.44 to 2.09) in the highest total estradiol quartile; $P_{interaction}$ = .04).

Conclusions Women with lower pr-treatment endogenous estrogen levels were at greater risk of breast cancer during E+P therapy compared with those with higher levels. Further studies are warranted to confirm these findings.

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Higher circulating levels of endogenous sex hormones are associated with increased breast cancer risk among postmenopausal women (1–6). A meta-analysis of nine prospective studies observed a twofold increase in breast cancer risk in women with estradiol levels in the highest, relative to the lowest, quintile, with similar associations noted for estrone, estrone sulfate, and testosterone (6).

In the Women's Health Initiative (WHI) randomized trial, combined estrogen plus progestin (E+P) increased both breast cancer incidence and breast cancer mortality relative to placebo (7–9). There has been ongoing interest in determining whether reproductive hormone levels can serve as predictive markers for breast cancer risk in hormone-based chemopreventive interventions. Two prior chemoprevention trials of raloxifene and tamoxifen have explored this question and yielded mixed results (10,11). In the Multiple Outcomes of Raloxifene Evaluation (MORE) trial, women with higher baseline estradiol levels had the greatest risk reduction in breast cancer risk associated with raloxifene use (10). However, in an ancillary study within the National Surgical Adjuvant Breast and Bowel Project (NSABP) Breast Cancer Prevention Trial (P-1), the effect of tamoxifen on breast cancer did not vary by estradiol level (11). Whether pretreatment levels of endogenous sex hormones modify the effect of E+P on breast cancer risk is unknown.

In a nested case–control study within the WHI E+P trial, we investigated the extent to which the effect of combined hormone therapy on breast cancer risk was modified by pretreatment levels of endogenous sex hormones (estradiol, estrone, estrone sulfate, testosterone, and progesterone) and sex hormone–binding globulin (SHBG). In addition, we assessed the association between pretreatment sex hormone levels and overall breast cancer risk. We hypothesized that women with lower levels of sex hormones have a lower overall risk of breast cancer but experience a greater increase in breast cancer during E+P use compared with women with higher levels.

Methods

Participants

Participants were enrolled in the WHI randomized, placebo-controlled trial evaluating E+P therapy. Detailed recruitment methods and eligibility criteria have been published (12). The trial included postmenopausal women aged 50 to 79 years who were recruited at 40 US clinical centers. A woman was considered postmenopausal if she had experienced no vaginal bleeding for 6 months (12 months for the 50 to 54 years age group), had a hysterectomy, or had ever used postmenopausal hormones. Excluded were women with prior hysterectomy, prior breast cancer, or condition precluding 3-year survival. Baseline mammogram and clinical breast cancer examination not suggestive of cancer were required. Women using hormone therapy were eligible after 3-month washout. The institutional review board at each clinical center approved the study protocol, and written informed consent was obtained from all participants.

The trial randomly assigned 16 608 women to receive conjugated equine estrogens (0.625 mg/day) plus medroxyprogesterone acetate (2.5 mg/day) in a single tablet (Prempro; Wyeth-Ayerst, Collegeville, Pennsylvania) or an identical-appearing placebo. Women were contacted at 6-month intervals to collect clinical outcome information and annually for clinic visits.

This article reports results of a nested case–control study conducted within the WHI E+P trial, which included 348 incident case subjects with invasive breast cancer identified during a mean of 5.6 years of follow-up (the period of active intervention; standard deviation = 1.3 years) and 348 control subjects, matched by age at screening (within 1 year), race/ ethnicity (white, black, Hispanic, other), and date of randomization (within 30 days). Of the case subjects, 199 were on E+P and 149 were on placebo; of the control subjects, 166 were on E+P and 182 were on placebo. Control selection was done using risk set sampling. Potential control subjects were excluded for prior coronary heart disease or stroke or invasive breast cancer at any time during the trial. Case subject and potential control subjects with insufficient banked serum at baseline were excluded. Sex hormone levels (total and bioavailable estradiol, estrone, estrone sulfate, progesterone, total and bioavailable testosterone, and SHBG) were measured for case and control subjects at baseline. Measurements were repeated 1 year later for total and bioavailable estradiol, estrone, estrone sulfate, and SHGB on 665 of the 696 case and control subjects. Year 1 hormone assessments excluded case and control subjects if they had insufficient banked serum at year 1 and had an interval between randomization and year 1 blood draw of less than 270 or greater than 450 days.

Procedures

Breast cancer outcomes were ascertained twice a year through self-administered questionnaires. Self-reported breast cancer outcomes were then confirmed locally by centrally trained physician adjudicators after medical record review. Final adjudication and coding according to the National Cancer Institute's Surveillance, Epidemiology, and End Results program guidelines were conducted at the WHI Clinical Coordinating Centre (13).

Endogenous sex hormones were measured in blood samples collected at baseline and year 1 after an overnight fast of at least 12 hours (14). Serum aliquots were shipped on dry ice to the Reproductive Endocrine Research Laboratory (University of Southern California, Los Angeles, CA) for sex hormone assessment.

Total estradiol, estrone, progesterone, and total testosterone concentrations were quantified by validated radioimmunoassays after organic solvent extraction and Celite column partition chromatography (15–17). Estrone sulfate was measured by direct radioimmunoassay using a commercial kit (Beckman Coulter, Minneapolis, MN) (18). SHBG was quantified by use of a direct chemiluminescent immunoassay using the Immulite Analyzer (Siemens Medical Solutions Diagnostics, Malvern, PA). Bioavailable estradiol and testosterone concentrations, which quantify the hormone that is not bound to SHBG and include both free and albumin-bound levels, were calculated by a validated algorithm. The algorithm uses the measured total estradiol (or testosterone) and SHBG concentrations, an assumed constant for normal albumin concentration, and affinity constants of SHBG and albumin for estradiol (or testosterone) (19–21).

The intra-assay coefficients of variation ranged from 4.0% to 7.5% for all the compounds, whereas the interassay coefficients of variation ranged from 8.0% to 13.0%. The assay sensitivities for estradiol, estrone, estrone sulfate, progesterone, testosterone, and SHBG were 2 pg/mL, 4 pg/mL, 0.02 ng/mL, 50 pg/mL, 1.5 ng/dL, and 1 ng/dL, respectively.

At baseline, questionnaires were used to collect information on demographic characteristics, menstrual and reproductive history, medical history, and lifestyle and dietary habits. Weight was measured on a balance beam scale while subjects were wearing indoor clothing. Height was measured with a fixed stadiometer. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2).

Statistical analysis

Differences in baseline characteristics and sex hormones between breast cancer case and control subjects were compared using *t* test or Wilcoxon rank sum test for continuous data and Pearson χ^2 test for categorical data. Differences in levels of sex hormones at baseline and year 1, as well as percentage changes in sex hormone concentrations were compared between the active and placebo groups.

To investigate whether baseline circulating levels of sex hormones modify the effect of E+P on breast cancer risk, conditional logistic regression models were used with a product term for treatment assignment and the continuous log-transformed sex hormone level. When statistically significant interactions were identified, the risk of breast cancer associated with combined hormone therapy was estimated for each of the sex hormone quartiles using linear contrasts and reported as odds ratios (ORs) and 95% confidence intervals (CIs).

Conditional logistic regression was also used to estimate the risk of breast cancer related to baseline levels of sex hormones (expressed as quartiles). When statistically significant interactions were identified between baseline sex hormone levels and E+P use, the associations of baseline sex hormones with breast cancer were restricted to the placebo group participants. All models were adjusted for randomization assignment and variables that were statistically significantly associated with breast cancer status: age at menarche, BMI, and family history.

In a secondary analysis, we investigated the association of absolute change in sex hormone levels from baseline to year 1 (expressed as quartiles) with breast cancer risk. Conditional logistic regression models were additionally adjusted for baseline levels of the sex hormone.

Each sex hormone model excluded women (and their matched pairs) with missing hormone levels and those with extreme values defined as greater than 40 pg/mL for estradiol, greater than 160 pg/mL for estrone, greater than 3 ng/mL for estrone sulfate, greater than 500 pg/mL for progesterone, and greater than 150 ng/dL for total testosterone (n = 28 for estradiol, 20 for estrone, 68 for estrone sulfate, 16 for progesterone, and 12 for testosterone). The cut points for extreme hormone levels indicate values beyond which hormone measurements are considered to be biological outliers, as defined by the laboratory.

All analyses were performed using SAS software version 9 (SAS Institute, Inc, Cary, NC). All statistical tests were two-sided. The level of statistical significance was set at .05.

Results

Baseline Characteristics of Case and Control Subjects

Breast cancer case subjects had higher BMI, younger age at menarche, and stronger family history of breast cancer than control subjects. No differences in time since menopause, reproductive history, smoking status, and alcohol use were observed between case and control subjects (Table 1).

Pretreatment Sex Hormone Levels and Breast Cancer Risk

Higher levels of total estradiol (P_{trend} = .04), bioavailable estradiol (P_{trend} = .03), estrone (P_{trend} = .007), and estrone sulfate (P_{trend} = 0.007) were statistically significantly associated with increased breast cancer risk. Compared with women in the lowest quartile, those in the highest quartiles of these hormones had a 2.4-fold to threefold higher risk for breast cancer. Progesterone, testosterone, and SHBG concentrations were not statistically significantly associated with breast cancer risk (Table 2). Analyses for total estradiol, bioavailable estradiol, and estrone were restricted to the placebo group because of statistically significant interactions of these hormones with E+P treatment.

Baseline and Year 1 Sex Hormone Levels in the Treatment and Placebo Groups

There were no differences in baseline sex hormone levels between the placebo and treatment groups. At year 1, there was a marked

Table 1. Baseline characteristics of invasive breast cancer case subjects and matched control subjects in the Women's Health Initiative estrogen plus progestin trial*

Characteristic	Breast cancer case subjects (n = 348)	Control subjects (n = 348)	*P*
Age, y	64.3 ± 6.8	64.3 ± 6.8	.98
Race, No. (%)			.99
White	305 (87.9)	305 (87.9)	
Black	21 (6.0)	21 (6.0)	
Other	21 (6.0)	21 (6.0)	
Age at menarche, No. (%)			.004
≤10 years	28 (8.1)	16 (4.6)	
11–14 years	288 (83.2)	273 (79.4)	
≥15 years	30 (8.7)	55 (16.0)	
Age at menopause, y	50.3 ± 4.6	50.2 ± 4.2	.75
Time since menopause, y	14.3 ± 8.4	14.2 ± 8.2	.61
Age at first term pregnancy, No. (%)			.61
No full-term pregnancy	8 (2.8)	14 (4.8)	
<20 years	44 (15.5)	40 (13.8)	
20–29 years	199 (70.1)	203 (70.2)	
≥30 years	33 (11.6)	32 (11.1)	
Body mass index, kg/m^2	29.3 ± 5.6	28.5 ± 5.7	.02
Smoking, No. (%)			.11
Never	158 (46.1)	186 (54.1)	
Past	155 (45.2)	131 (38.1)	
Current	30 (8.8)	27 (7.8)	
Alcohol drinking, No. (%)			.27
Never	41 (12.6)	36 (11.1)	
Past	57 (17.5)	51 (15.7)	
Current	228 (69.9)	237 (73.2)	
Family history of breast cancer in a female relative, No. (%)	79 (24.0)	50 (15.2)	.004
Total estradiol, pg/mL†	11.2 (8.3–15.3)	10.2 (7.3–14.2)	.004
Bioavailable estradiol, pg/mL†	7.3 (5.1–10.9)	6.8 (4.6–9.8)	.008
Estrone, pg/mL†	38.7 (28.3–51.4)	34.3 (26.7–46.2)	.003
Estrone sulfate, ng/mL†	0.8 (0.6–1.1)	0.7 (0.5–1)	.03
Progesterone, pg/mL†	61.6 (42.1–84.6)	60.6 (42.4–85.1)	.99
Total testosterone, ng/dL†	25.2 (18.3–33.8)	22.8 (17.7–33.3)	.16
Bioavailable testosterone, ng/dL†	12.7 (9–18)	11.7 (8.6–16.3)	.10
Sex hormone–binding globulin, ng/dL†	39.3 (28.7–55.9)	43 (29.2–57)	.36

* Data are mean ± standard deviation or median (interquartile range) unless otherwise noted. All statistical tests were two-sided.

† Median (interquartile range) and Wilcoxon rank sum test *P* value reported.

Table 2. Association of baseline sex hormone levels with invasive breast cancer risk

Sex hormone	Number of case subjects, number of control subjects	Odds ratio (95% confidence interval)*
Total estradiol†		
Quartile (Q)1 (2.5–7.94)	27, 60	1.00 (referent)
Q2 (7.95–10.89)	42, 41	2.07 (1.06 to 4.04)
Q3 (10.90–14.99)	37, 43	1.58 (0.77 to 3.21)
Q4 (15.00–39.20)	38, 31	2.52 (1.12 to 5.63)
P_{trend}		.04
Bioavailable estradiol†		
Q1 (1.58–4.99)	29, 57	1.00 (referent)
Q2 (5.00–7.14)	37, 43	1.58 (0.75 to 3.29)
Q3 (7.15–10.52)	37, 46	1.35 (0.66 to 2.75)
Q4 (10.53–29.30)	41, 28	2.82 (1.25 to 6.36)
P_{trend}		.03
Estrone†		
Q1 (9.05–27.86)	31, 56	1.00 (referent)
Q2 (27.87–36.78)	40, 44	1.87 (0.92 to 3.80)
Q3 (36.79–50.38)	34, 43	2.19 (1.03 to 4.66)
Q4 (50.39–151.39)	42, 34	3.01 (1.34 to 6.76)
P_{trend}		.007
Estrone sulphate		
Q1 (0.23–0.56)	71, 93	1.00 (referent)
Q2 (0.57–0.76)	70, 74	1.41 (0.83 to 2.39)
Q3 (0.77–1.034)	87, 70	2.23 (1.23 to 4.02)
Q4 (1.035–2.75)	86, 77	2.38 (1.20 to 4.72)
P_{trend}		.007
Progesterone		
Q1 (11.66–40.44)	81, 77	1.00 (referent)
Q2 (40.45–58.87)	83, 85	0.89 (0.54 to 1.45)
Q3 (58.87–83.49)	88, 84	0.94 (0.56 to 1.58)
Q4 (83.50–498.7)	88, 94	0.96 (0.56 to 1.67)
P_{trend}		.96
Total testosterone		
Q1 (3.84–16.59)	64, 71	1.00 (referent)
Q2 (16.60–22.57)	76, 95	0.84 (0.50 to 1.41)
Q3 (22.58–31.38)	96, 78	1.34 (0.79 to 2.28)
Q4 (31.39–118.42)	106, 98	1.08 (0.65 to 1.80)
P_{trend}		.35
Bioavailable testosterone		
Q1 (0.51–8.30)	72, 70	1.00 (referent)
Q2 (8.31–11.50)	70, 92	0.74 (0.44 to 1.26)
Q3 (11.51–16.05)	86, 87	1.01 (0.60 to 1.68)
Q4 (16.06–81.11)	110, 89	1.11 (0.66 to 1.86)
P_{trend}		.38
Sex hormone–binding globulin		
Q1 (6.9–27.89)	80, 77	1.00 (referent)
Q2 (27.90–39.54)	94, 69	1.45 (0.87 to 2.42)
Q3 (39.55–54.99)	77, 104	0.79 (0.48 to 1.30)
Q4 (55.00–159.00)	91, 92	1.05 (0.62 to 1.79)
P_{trend}		.54

* Adjusted for treatment assignment, age at menarche, body mass index, and family history of breast cancer. Results were obtained using conditional logistic regression models. All statistical tests were two-sided.

† Total estradiol, bioavailable estradiol, and estrone estimates were obtained from the placebo group, due to their statistically significant interactions with estrogen + progestin treatment.

increase in levels of total and bioavailable estradiol, estrone, estrone sulfate, and SHBG in the E+P group compared with the placebo group (all $P < .001$). The largest percentage increase was observed for estrone (median change of approximately 230%). In the placebo group, there was a modest decrease in sex hormone levels between baseline and year 1 and a slight increase in SHBG level of 3% (Table 3).

Pretreatment Sex Hormone Levels and Effect of E+P Therapy on Breast Cancer Risk

Statistically significant interactions were observed between E+P use and pretreatment levels of total estradiol ($P_{interaction} = .04$), bioavailable estradiol ($P_{interaction} = .02$), and estrone ($P_{interaction} = .02$). The highest increase in breast cancer risk with combined hormone therapy was observed in women whose baseline levels of total estradiol, bioavailable estradiol, and estrone were in the lowest quartiles (lowest total estradiol quartile: OR for E+P vs placebo = 2.47, 95% CI = 1.28 to 4.79; lowest bioavailable estradiol quartile: OR = 2.35, 95% CI = 1.20 to 4.64; lowest estrone quartile: OR = 3.06, 95% CI = 1.52 to 6.17) (Table 4).

Women whose levels were in the second and third quartiles of these sex hormones had a non-statistically significant increased risk of breast cancer risk with E+P therapy. However, there was no evidence that E+P influenced breast cancer risk for women with total and bioavailable estradiol and estrone in the highest quartile (highest total estradiol quartile: OR for E+P vs placebo = 0.96; 95% CI = 0.44 to 2.09; $P_{interaction} = .04$) (Table 4). Levels of estrone sulfate ($P_{interaction} = .93$), progesterone ($P_{interaction} = .27$), total testosterone ($P_{interaction} = .69$), bioavailable testosterone ($P_{interaction} = .24$), and SHBG ($P_{interaction} = .14$) did not statistically significantly modify the effect of E+P on breast cancer risk (data not shown).

Baseline to Year 1 Absolute Change in Sex Hormone Levels and Breast Cancer Risk

Absolute changes in sex hormone levels (total and bioavailable estradiol, estrone, estrone sulfate, and SHBG) between baseline and year 1 were not statistically significantly associated with breast cancer risk (Table 5).

Discussion

Consistent with prior studies (1,2,4,6), higher baseline levels of total estradiol, bioavailable estradiol, estrone, and estrone sulfate were associated with higher breast cancer risk in postmenopausal women. In addition, women who had the lowest pretreatment levels of total estradiol, bioavailable estradiol, and estrone had the greatest increase in breast cancer risk during E+P therapy. To our knowledge, this study is the first to evaluate whether the effect of E+P on breast cancer risk is dependent on baseline sex hormone levels.

In women with low baseline estradiol and estrone concentrations, E+P therapy elevated the relative risk of breast cancer to the level observed in women with the highest pretreatment concentrations of sex hormones (eg, OR for E+P vs placebo in lowest total estradiol quartile was 2.47, 95% CI = 1.28 to 4.79; OR for highest total estradiol quartile vs lowest quartile was 2.52, 95% CI = 1.12 to 5.63 in the placebo group]. In addition, E+P had limited or no effect on breast cancer risk in women whose pretreatment levels of total and bioavailable estradiol and estrone fell in the highest quartile. These findings suggest that it may be possible to identify subgroups of women at differential risk for breast cancer with E+P therapy by measuring sex hormone levels.

Table 3. Sex hormone levels at baseline and year 1 and percentage changes in sex hormones in estrogen plus progestin and placebo group participants*

Sex hormone	Estrogen plus progestin (n = 365)	Placebo (n = 331)	*P*
Baseline level			
Total estradiol, pg/mL	10.9 (7.8–14.8)	10.6 (7.7–14.4)	.71
Bioavailable estradiol, pg/mL	7.0 (4.9–10.6)	7.0 (4.8–10.0)	.87
Estrone, pg/mL	36.2 (27.6–48.4)	36.2 (27.4–49.9)	.99
Estrone sulfate, ng/mL	0.77 (0.56–1.09)	0.77 (0.56–1.03)	.76
Total testosterone, ng/dL	24.0 (17.8–33.5)	23.8 (17.8–33.8)	.81
Bioavailable testosterone, ng/dL	12.3 (8.6–17.3)	12.5 (9.1–16.7)	.73
Progesterone, pg/mL	61.0 (43.5–85.5)	60.8 (40.1–84.9)	.69
SHBG, ng/dL	40.7 (28.3–57.1)	41.3 (29.4–54.9)	.93
Year 1 level			
Total estradiol, pg/mL	23.2 (15.0–32.0)	9.1 (6.3–13.5)	<.0001
Bioavailable estradiol, pg/mL	10.1 (6.6–14.3)	6.0 (4.0–9.4)	<.0001
Estrone, pg/mL	124.4 (72.6–185.5)	34.5 (25.6–50.4)	<.0001
Estrone sulfate, ng/mL	1.98 (1.18–2.74)	0.74 (0.53–0.96)	<.0001
SHBG, ng/dL	98.1 (64.7–141.0)	41.5 (29.8–57.3)	<.0001
Baseline to Year 1 percentage change			
Total estradiol	101.1 (17.9–217.4)	−13.5 (−35.3 to 14.4)	<.0001
Bioavailable estradiol	35.5 (−8.1–106.9)	−12.8 (−37.1 to 12.4)	<.0001
Estrone	228.2 (72.6–423.6)	−4.8 (−22.7 to 21.7)	<.0001
Estrone sulfate	132.4 (35.1–262.6)	−11.0 (−29.0 to 13.9)	<.0001
SHBG	133.6 (61.3–227.1)	3.3 (−12.8 to 21.0)	<.0001

* Results were obtained using conditional logistic regression models. All statistical tests were two-sided. Data are median (interquartile range). SHBG = sex hormone–binding globulin.

If confirmed by other studies, measurement of sex hormone levels to characterize breast cancer risk associated with E+P could inform risk–benefit ratio discussions, potentially providing a personalized approach to clinical decision making. However, additional studies are needed before this could be recommended in clinical practice. In the WHI E+P trial, besides breast cancer, the risks of myocardial infarction, stroke, and pulmonary emboli were all increased, whereas risks for hip fracture and colorectal cancer were decreased (7,22). Although pretreatment sex hormone levels failed to modify E+P influence on fracture risk (23), it is unknown whether they may modify the effects of therapy on other postmenopausal health outcomes. Additionally, the assays employed in this study (24) are more sensitive than those generally available in clinical practice, and studies are needed to evaluate whether the latter assays can yield similar results.

The relationship between sex hormone levels and breast cancer risk has been examined among women already using estrogen alone or combined with progestin in a nested case–control study within the Nurse's Health Study. Women using hormone therapy had higher estrogen and SHBG levels than nonusers. In addition, higher levels of free testosterone and SHBG were statistically significantly associated with higher subsequent breast cancer risk, whereas estradiol or testosterone levels were not (25). Because all hormone therapy results were based on women who were already using hormones, pretreatment sex hormone level influence on breast cancer risk was not evaluated.

Our results regarding associations between baseline estrogen levels and subsequent breast cancer confirm previous findings. In the 2002 meta-analysis of nine prospective cohort studies (6), strong associations were observed between higher levels of estradiol, estrone, and estrone sulfate and the overall breast cancer risk.

Table 4. Estrogen plus progestin and breast cancer risk by quartiles of baseline sex hormones

Sex hormone	Odds ratio (95% confidence interval)*	$P_{interaction}$†
Total estradiol, pg/mL		.04
Quartile (Q)1(2.5–7.94)	2.47 (1.28 to 4.79)	
Q2 (7.95–10.89)	1.34 (0.68 to 2.65)	
Q3 (10.90–14.99)	1.73 (0.85 to 3.51)	
Q4 (15.00–39.20)	0.96 (0.44 to 2.09)	
Bioavailable estradiol, pg/mL		.02
Q1 (1.58–4.99)	2.35 (1.20 to 4.64)	
Q2 (5.00–7.14)	1.58 (0.80 to 3.11)	
Q3 (7.15 – 10.52)	1.98 (0.96 to 4.05)	
Q4 (10.53–29.30)	0.75 (0.34 to 1.64)	
Estrone, pg/mL		.02
Q1 (9.05–27.86)	3.06 (1.52 to 6.17)	
Q2 (27.87–36.78)	1.12 (0.56 to 2.23)	
Q3 (36.79–50.38)	1.95 (0.96 to 3.97)	
Q4 (50.39–151.39)	0.95 (0.45 to 2.03)	

* Adjusted for age at menarche, body mass index, and family history for breast cancer. Odds ratios compare estrogen plus progestin treatment to placebo in each hormone quartile. Results were obtained using conditional logistic regression models. All statistical tests were two-sided.

† *P* value for interaction between hormone therapy and sex hormone level (continuous scale).

Most (1–4), but not all (11), recent studies report similar associations, particularly for estrogen receptor–positive tumors. In this study, testosterone level was not associated with breast cancer risk. Although results are mixed (11), because the association of testosterone with breast cancer risk may vary by estrogen receptor status

Table 5. Association of absolute change in sex hormone levels from baseline to year 1 with breast cancer risk in the Women's Health Initiative estrogen plus progestin trial

Sex hormone	Number of case subjects, number of control subjects	Odds ratio (95% confidence interval)*
Change in total estradiol, pg/mL		
Quartile (Q)1 (<−2.39)	75, 73	1.00 (referent)
Q2 (−2.4 to 1.99)	68, 89	0.70 (0.40 to 1.28)
Q3 (2.00–13.29)	76, 67	1.23 (0.65 to 2.30)
Q4 (13.30–150.30)	80, 70	1.14 (0.67 to 2.30)
P_{trend}		.39
Change in bioavailable estradiol, pg/mL		
Q1 (<−19.4)	76, 65	1.00 (referent)
Q2 (−19.5 to 0.27)	64, 83	0.81 (0.44 to 1.49)
Q3 (0.28–4.21)	82, 67	1.29 (0.69 to 2.39)
Q4 (4.22–91.14)	70, 74	0.95 (0.50 to 1.80)
P_{trend}		.84
Change in estrone, pg/mL		
Q1 (<−3.89)	76, 67	1.00 (referent)
Q2 (−3.90 to 13.69)	63, 97	0.62 (0.30 to 1.14)
Q3 (13.70–97.19)	82, 75	0.77 (0.39 to 1.51)
Q4 (97.20–715.62)	81, 63	0.78 (0.36 to 1.71)
P_{trend}		.63
Change in estrone sulfate, ng/mL		
Q1 (<−0.14)	57, 67	1.00 (referent)
Q2 (−0.15 to 0.13)	58, 72	0.78 (0.40 to 1.54)
Q3 (0.14–1.17)	70, 63	1.33 (0.69 to 2.58)
Q4 (1.18–9.81)	78, 61	1.38 (0.62 to 3.08)
P_{trend}		.29
Change in sex hormone–binding globulin, ng/dL		
Q1 (<−0.2)	63, 79	1.00 (referent)
Q2 (−0.3 to 12.24)	67, 84	0.93 (0.53 to 1.64)
Q3 (12.25–61.89)	96, 77	1.37 (0.71 to 2.62)
Q4 (61.90–251.00)	79, 65	1.63 (0.77 to 3.43)
P_{trend}		.22

* Adjusted for treatment assignment, baseline sex hormone level, age at menarche, body mass index, and family history of breast cancer. Results were obtained using conditional logistic regression models. All statistical tests were two-sided.

(1), our testosterone findings could be related to examinations limited to overall breast cancer risk.

One year after initiation of combined hormone therapy, serum levels of total estradiol, estrone, estrone sulfate, and SHBG increased by two- to threefold above their baseline concentrations. Levels of bioavailable estradiol also increased statistically significantly but to a lesser extent than the other hormones. The magnitudes of the increase in hormone levels were similar to those previously reported (26,27). Changes in sex hormone levels beyond the first year of hormone therapy will be investigated in future work within our study.

One-year changes in sex hormone levels were not statistically significantly associated with breast cancer risk in this study. It can be speculated that an increase in hormone levels after E+P use may only be relevant for women with low pretreatment levels; however, our sample size is insufficient to reliably test this hypothesis.

At present, certain clinically identifiable groups are known to have higher sex hormone levels. In a cross-sectional reanalysis of 13 studies of breast cancer risk factors and circulating sex hormones, hormone levels were higher in obese women, smokers of 15 or more cigarettes per day, and drinkers of 20 or more grams of alcohol per day (28). However, subgroup analyses in the WHI E+P trial did not find that the effect of combined hormone therapy on breast cancer was statistically significantly influenced by BMI, smoking, or alcohol use (8). Thus, it is unlikely that common clinical characteristics or findings can be used as a proxy for sex hormone levels in predicting the effects of hormone therapy on breast cancer risk.

Two prior studies have examined associations of hormone-targeted interventions and breast cancer risk by sex hormone levels in the context of randomized trials evaluating agents with chemoprevention potential. In the MORE trial, 7290 postmenopausal women with osteoporosis underwent measurement of estradiol levels before being randomized to raloxifene or placebo. Women with higher baseline estradiol levels were at higher breast cancer risk and had the greatest risk reduction associated with raloxifene use (10). In contrast, in an ancillary case–control study in the NSABP Breast Cancer Prevention Trial (P-1), a randomized placebo-controlled chemoprevention trial of tamoxifen in women at increased risk of breast cancer, breast cancer risk was not associated with baseline sex hormone levels in the placebo group and estradiol levels did not identify a group with particular risk reduction benefit from tamoxifen use (11). The findings from our study align more closely with the MORE results, as we also found higher estradiol levels to be associated with higher subsequent breast cancer risk, and, using sex hormone levels, we identified a population at increased risk for breast cancer after a hormonal intervention.

Study strengths include a large, well-characterized, and ethnically diverse study population, the underlying randomized, double-blind, placebo-controlled trial, central adjudication of breast cancers, and serial assessment of sex hormones determined in a central laboratory using sensitive assays. Study limitations include lack of power to examine influence by hormone receptor status and an observational study design, which precludes causal inference. In addition, this analysis evaluated the effect of conjugated equine estrogen with medroxyprogesterone acetate, administered in one dose and schedule; thus, findings cannot be generalized to different combined hormone therapy regimens. Also, results do not apply to estrogen-alone use (30) because in the WHI randomized clinical trial, a lower breast cancer incidence was associated with estrogen-alone use (31–33).

In conclusion, the risk of breast cancer associated with E+P therapy is greatest for postmenopausal women who have the lowest circulating estradiol and estrone levels. Thus, pretreatment determination of estradiol and estrone levels may be helpful in identifying women at particularly elevated risk for breast cancer with combined hormone therapy. Further studies are warranted to confirm these findings and extend them to other clinical conditions under E+P influence.

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