American Journal of Epidemiology
Vol. 162, No. 9
DOI: 10.1093/aje/kwi286
Advance Access publication September 21, 2005

Original Contribution

The Association of Endogenous Sex Steroids and Sex Steroid Binding Proteins with Mammographic Density: Results from the Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study

Gail A. Greendale[1], Shana L. Palla[2], Giske Ursin[3], Gail A. Laughlin[4], Carolyn Crandall[5], Malcolm C. Pike[3], and Beth A. Reboussin[2]

[1] Division of Geriatrics, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA.
[2] Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC.
[3] Department of Preventive Medicine/Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine, Los Angeles, CA.
[4] Department of Family and Preventive Medicine, University of California at San Diego, La Jolla, CA.
[5] Division of General Internal Medicine and Health Sciences Research, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, CA.

Received for publication October 13, 2004; accepted for publication June 1, 2005.

Mammographic density is an independent risk factor for breast cancer. In postmenopausal women, higher levels of endogenous sex steroids are associated with an increased risk of breast cancer. Limited prior data suggest that endogenous sex steroids either are not associated (total estradiol and progesterone) or are negatively associated (free estradiol) with higher mammographic density. To analyze the associations between endogenous sex steroids and mammographic density, the authors conducted a 1998–2005 cross-sectional analysis of baseline clinical trial data from the Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial for US women who had not used hormone therapy for at least 3.1 months prior to baseline. In models adjusted for age, body mass index, parity, prior use of hormone therapy, time since last use of hormone therapy, and the interaction between prior hormone therapy use and time since last hormone therapy use, higher levels of estrone ($\beta = 0.0013$, $p = 0.014$), estradiol ($\beta = 0.0009$, $p = 0.009$), and bioavailable estradiol ($\beta = 0.0021$, $p = 0.018$) were statistically significantly related to greater mammographic density. (Beta coefficients express the increment in mammographic density per-unit increment (pg/ml) of each hormone.) These results suggest that some sex steroids may increase the risk of breast cancer by stimulating breast epithelial or stromal proliferation, which appears on a mammogram as higher density.

breast neoplasms; mammography; menopause; receptors, steroid; risk factors

Abbreviations: BDL, below detectable limits; BMI, body mass index; PEPI, Postmenopausal Estrogen/Progestin Interventions; SHBG, sex hormone-binding globulin.

Higher mammographic density is an independent risk factor for breast cancer, and the magnitude of risk associated with mammographic density is greater than that associated with almost all other known clinical risk factors for breast cancer (1–5). Moreover, if mammographic density were causally related to breast cancer, higher mammographic percent density (defined as >50 percent of the breast being composed of dense tissue) would account for roughly a third of all cases of breast cancer (4, 6, 7).

On breast radiographs, fat appears dark (''nondense''), whereas connective and epithelial tissues appear white (''dense''). Mammographic density can be assessed

Correspondence to Dr. Gail A. Greendale, Division of Geriatrics, David Geffen School of Medicine at UCLA, 10945 Le Conte Avenue, Suite 2339, Los Angeles, CA 90095-1687 (e-mail: ggreenda@mednet.ucla.edu).

categorically (8, 9) or as a continuous measure (percent density, which quantifies the proportion of the breast area that consists of dense tissue) (10–12).

One proposed explanation for the increased risk of breast cancer conferred by denser breasts is that the radiologic appearance of higher mammographic density corresponds to greater proliferation of breast epithelial and/or stromal tissues (2). Consonant with this thesis, the vast majority of studies (13–20) that compared findings on mammographic density with breast tissue histology reported that higher stromal or epithelial proliferation was associated with greater degrees of mammographic density. More cellular proliferation may be one pathway to cancer causation because cells that turn over at a faster rate are more subject to errors in DNA replication (21, 22).

Our interest in studying the relation between endogenous sex steroids and mammographic density stems from the known proliferative effects of estrogens and progestins on mammary tissues (23) and the theory that endogenous sex steroids are implicated in the etiology of breast cancer (24, 25). We analyzed data from the Postmenopausal Estrogen/Progestin Interventions (PEPI) Mammographic Density Study (1998–2005) (26) to address the following question: Among postmenopausal women, is there an association between mammographic percent density and serum levels of endogenous sex steroids and sex hormone-binding globulin (SHBG)?

MATERIALS AND METHODS

The PEPI study enrolled 875 postmenopausal women, aged 45–64 years, at seven clinical centers in the United States; the design of PEPI, the parent study for this mammographic density substudy, has been detailed previously (27). Briefly, exclusion criteria were any major contraindication for use of estrogen or progestin treatment (including history of breast cancer), any cancer other than basal cell skin cancer within the previous 5 years, insulin-dependent diabetes mellitus, body mass index (BMI) greater than 40 kg/m^2, severe menopausal symptoms, or current use of estrogens or progestins. (Women could elect to stop hormone use for at least 2 months prior to the first screening visit, referred to herein as having ''quit hormones for PEPI.'') Exclusions for gynecologic reasons included last menses occurring less than 12 months or more than 10 years prior to random assignment, last menstrual period occurring prior to age 44 years, hysterectomy less than 2 months prior to the first screening visit, bilateral oophorectomy before age 44 years or more than 10 years prior to random assignment, or a follicle-stimulating hormone level of less than 40 mIU/ml.

The purpose of the PEPI Mammographic Density Study was to investigate the associations between hormones (endogenous and exogenous) and mammographic density. The PEPI mammographic density study used demographic, medical, behavioral, and laboratory data already collected for PEPI (27) and attempted to retrieve all baseline and 1-year follow-up mammograms. We were able to retrieve baseline mammograms for 603 of the 875 PEPI participants; 272 were unavailable. Seven films were excluded because of breast implants, and two films were excluded because of inadequate mammographic technique, yielding a total baseline study sample of 594 (68 percent of the original PEPI participants). The relation between endogenous sex steroid hormones and mammographic density could be obscured by the effect of recent hormone use on either mammographic density or endogenous sex steroid levels (26, 28). Therefore, we conducted initial analyses stratified by recent hormone use, defined as having quit hormones to join PEPI (173 women quit hormones to join PEPI; among them, sex steroid values were available for 171). We found no relation between mammographic density and endogenous hormones among women who quit hormones to join PEPI (data not shown). Thus, this article focuses on the results for the 404 Mammographic Density Study participants who did not have to quit hormones to participate in PEPI. The mean duration since last hormone therapy use among the 404 women in the analytic sample was 44.3 months (range, 3.1–311 months).

Demographics, medical history, lifetime use of cigarettes, prior hormone therapy use, physical activity, and alcohol consumption were assessed by using standardized questionnaires (29–31). Height and weight were measured with participants wearing light clothing and no shoes. BMI was calculated as body weight in kilograms divided by the square of height in meters.

Fasting blood samples were drawn between 7 a.m. and 10 a.m. Assays were run by B. R. Hopper in the Reproductive Endocrinology Research Laboratory at the University of California at San Diego. Estrone, total estradiol, and total testosterone were measured by radioimmunoassay after organic solvent extraction and celite column chromatography; progesterone was measured by radioimmunoassay after organic solvent extraction (32). Bioavailable levels of estradiol and testosterone were determined by a modified ammonium-sulfate precipitation method (33). SHBG was measured by using a modified Rosner method (34). Sensitivity and intra- and interassay coefficients of variation were, respectively, 4.0 pg/ml, 6 percent, and 9 percent for estrone; 3.0 pg/ml, 6 percent, and 7 percent for estradiol; 1.5 pg/ml, 6 percent, and 8 percent for bioavailable estradiol; 20 pg/ml, 4 percent, and 5 percent for testosterone; 6 pg/ml, 7 percent, and 11 percent for bioavailable testosterone, 5 nmol/liter, 6 percent, and 8 percent for SHBG, and 0.05 ng/ml, 6 percent, and 9 percent for progesterone.

Serum estrone levels were below detectable limits (BDL) in one participant, total estradiol levels were BDL in 32 participants, and bioavailable estradiol levels were BDL in 33 participants. In regression models that imputed hormone values, a value one unit below the lower limit of detection was substituted for BDL readings.

Craniocaudal mammogram films of the left breast were scanned by using software and hardware that have been described previously (26). We studied only the left breast because we (35) and others (36) have shown virtually complete concordance between right and left breast density readings. One of the authors (G. U.) performed all density assessments by using a previously described, computer-assisted, quantitative method (12).

In a 10 percent random sample, test-retest reliability for breast density, percent density, and total breast area was

calculated separately for mammograms rated by this same author (G. U.) as not difficult to read and for those judged difficult to read. For nondifficult-to-read films ($n = 104$), the intraclass correlation coefficients were greater than 0.95 for total breast density, percent density, and total breast area. For 16 films considered difficult to read, the intraclass correlation coefficients were 0.93 for breast density, 0.91 for percent density, and greater than 0.95 for total breast area (26).

We used *t* tests and chi-squared tests to examine pairwise differences in baseline characteristics between the 404 participants in our analytic sample, the 190 participants excluded from the analytic sample (because of recent hormone use ($n = 171$), missing baseline endogenous serum sex steroid measures ($n = 17$), or poor mammogram quality that precluded density reading ($n = 2$)), and the 281 original PEPI study participants who were not part of the PEPI Mammographic Density Study. Two-sample *t* tests were used to compare endogenous sex steroid and SHBG levels in the analytic sample and in those who were recent hormone users in two ways: first, by excluding cases for whom the values of estrone, estradiol, or bioavailable estradiol were BDL; and second, by using interpolated values for cases whose levels were BDL. Because hormone values were not normally distributed, we estimated the Spearman correlation coefficients between the endogenous sex steroids and SHBG.

Linear regressions were performed to examine the relations between baseline mammographic density and endogenous sex steroid levels. For ease of interpretation, all models presented in this paper use raw rather than log-transformed values of each hormone; models using log-transformed hormone values yielded the same results (data not shown). Regression models were constructed as follows. First, age-adjusted models with mammographic density as the outcome variable and each hormone as the primary exposure were estimated. Second, a set of covariates that may affect mammographic density, hormone levels, or both, was added to the age-adjusted model; these covariates were cigarette smoking (current, former, or never), physical activity (tertiles, based on the PEPI physical activity scale), alcohol consumption (tertiles, based on the PEPI food frequency questionnaire), and parity. These models were then reduced by stepwise elimination of covariates that did not substantively affect the relation between each hormone and mammographic density; only parity and age were retained at this step. Third, to examine the influence of BMI, BMI was added to the parity- and age-adjusted models. Next, ever use of hormone therapy, time since last use of hormone therapy, and an interaction between these two variables were added to the age-, parity,- and BMI-adjusted models; this constituted our final models. Quadratic relations between sex steroid and SHBG levels and mammographic density were explored, but no quadratic terms remained in the models. To assess for a potential interaction between BMI and sex steroid level, we stratified models by BMI quartile as well as checked for a multiplicative interaction between hormone and BMI.

Final models were rerun by excluding hormone outliers detected by examining *dfBeta* values (37). In addition, for the estrogen exposures, we excluded interpolated values for cases whose levels were BDL. Because model results were not affected by the inclusion or exclusion of either the statistical outliers or the interpolated estrogen values, we included these values in the models that we present as our primary results. Because of the interrelatedness of some sex steroid exposures, we fit selected models to predict mammographic density by using more than one hormone. The sample of 56 non-Caucasians was too small to permit analysis by race-ethnicity; models were rerun restricted to Caucasians, and the results were not affected. All tests of hypotheses and *p* values were two sided. Analyses were conducted by using STATA software (38). The protocol was approved by the institutional review boards at each participating center.

RESULTS

The characteristics of the 404 PEPI Mammographic Density Study participants whose data were used to study the relation between endogenous hormones and mammographic density are summarized in table 1. Women in the analytic sample were generally similar to the Mammographic Density Study participants included in this analysis, except that the latter had a lower mean BMI, less current cigarette use, slightly higher average parity, and greater mean baseline mammographic density (table 1). Also shown in table 1 are characteristics of the 281 original PEPI study participants who were not part of the Mammographic Density Study.

Table 2 summarizes the average baseline values and distributions of the sex steroid and binding globulin determinations for the analytic sample and for the Mammographic Density Study subjects not included in relational analyses because they quit hormones to join PEPI. Compared with the values for the analytic sample, those for women who quit hormones to join PEPI were lower for mean levels of testosterone and bioavailable testosterone and were slightly higher for SHBG. Estrone, estradiol, bioavailable estradiol, and progesterone levels were similar in these two groups. The mean values of estrone, estradiol, and bioavailable estradiol in the analytic sample and in the recent hormone therapy user sample, and the between-sample statistical comparisons of these means, were not substantively affected by inclusion or omission of interpolated values (table 2).

Correlations between each of the sex steroids and SHBG values are shown in table 3. The three estrogen compounds were most highly correlated with each other, with *r* values of 0.61–0.88. The testosterone analytes were also strongly correlated ($r = 0.65$).

In age-, parity-, and BMI-adjusted models, with the exception of bioavailable testosterone, each of the measured hormones and SHBG were positively related to mammographic density (table 4, model 2). The addition of prior use of hormone therapy, time since last use of hormone therapy, and an interaction term between prior use and time since last use (model 3, table 4) had virtually no impact on the estimated effect size for each hormone. In models adjusted for age, BMI, parity, prior use of hormone therapy, time since last use of hormone therapy, and the interaction between prior use of hormone therapy and time since last use of hormone therapy, higher serum levels of estrone

TABLE 1. Characteristics of PEPI* Mammographic Density Study participants in the analytic sample compared with Mammographic Density participants not included in the analysis and with original PEPI study participants not in the Mammographic Density Study, United States, 1998–2005

Characteristic†	Analytic sample ($n = 404$)	Remainder of the Mammographic Density Study participants‡ ($n = 190$)	*p* value§	PEPI participants not in the Mammographic Density Study ($n = 281$)	*p* value§
Mean age in years (SD*)	56.0 (4.4)	56.0 (4.1)	0.9769	56.2 (4.3)	0.6246
Mean years since menopause (no. (SD))	5.6 (2.8)	5.6 (2.5)	0.9859	5.7 (2.8)	0.8955
Mean body mass index (kg/m^2 (SD))	26.6 (4.7)	25.2 (4.1)	0.0003	25.6 (4.3)	0.0026
Parity (no. (mean))	3.3 (1.9)	2.9 (1.5)	0.0067	3.0 (1.7)	0.0624
Prior use of HT* (no. (%))	146 (36.1)	183 (96.3)	<0.0001	165 (58.7)	<0.0001
Mean recency of HT use (no. of months (SD))	49.5 (61.1)	6.6 (12.9)	<0.0001	27.4 (56.8)	0.0024
Quit hormones for PEPI (no. (%))	0 (0)	173 (91.1)	<0.0001	89 (31.7)	<0.0001
Smoking status (no. (%))					
Current	64 (15.8)	13 (6.8)		41 (14.6)	
Former	143 (35.4)	82 (43.2)		100 (35.6)	
Never	197 (48.8)	95 (50.0)	0.0062	140 (49.8)	0.9009
Educational status (no. (%))					
High school or less	78 (19.8)	37 (19.5)		45 (16.0)	
Some college or college	202 (51.5)	99 (52.1)		151 (53.7)	
Postgraduate	118 (29.2)	54 (28.4)	0.9806	85 (29.4)	0.5429
Physical activity level (no. (%))					
Low	144 (35.6)	55 (28.9)		94 (33.5)	
Medium	133 (32.9)	68 (35.8)		81 (28.8)	
High	127 (31.4)	67 (35.3)	0.2693	106 (37.7)	0.2182
Non-White race-ethnicity (no. (%))	56 (13.9)	16 (8.4)	0.0581	28 (10.0)	0.1261
Adherence to treatment assignment (no. (%))	355 (87.9)	170 (89.5)	0.5697	230 (81.9)	0.0282
Mean baseline mammographic % density (SD)	0.2321 (0.18)	0.2704 (0.18)	0.0164	N/A*	0.3046 (N/A)

* PEPI, Postmenopausal Estrogen/Progestin Interventions; SD, standard deviation; HT, hormone therapy; N/A, not applicable.

† Sample sizes for mean years since menopause were 417 (Mammographic Density Study) and 184 (PEPI participants not in the Mammographic Density Study). Women who had had a hysterectomy but had one remaining ovary could not respond to the question about years since menopause. Recency of HT use refers to the number of months since the participant last used postmenopausal HT. Sample sizes for recency of use were 301 (Mammographic Density Study) and 139 (PEPI participants not in the Mammographic Density Study). The recency-of-use question did not apply to never users of HT and was missing for 28 former users. Quit hormones for PEPI refers to participants who were using HT prior to the initial screening visit and who opted to discontinue HT use to be screened for PEPI. Physical activity was coded as tertiles based on the PEPI physical activity scale (see reference 29).

‡ Of the 190 Mammographic Density Study participants who were not included in the analytic sample, 173 quit hormone use to join PEPI, 11 were missing data on recent hormone use, six did not have endogenous hormone measurements, and, for two, mammograms were technically unsatisfactory for mammographic density assessment.

§ *p* value for *t* test of means or chi-squared test of proportions comparing the current analytic sample with PEPI Mammographic Density Study participants not included in this analysis or with original PEPI Study participants not in the PEPI Mammographic Density Study.

($\beta = 0.0013$, $p = 0.014$), estradiol ($\beta = 0.009$, $p = 0.009$), and bioavailable estradiol ($\beta = 0.0021$, $p = 0.018$) were statistically significantly related to greater mammographic density. (Beta coefficients express the increment in mammographic density (one percentage unit) per-unit increment (1 pg/ml) of each hormone.) SHBG and progesterone were also positively associated with mammographic density, with magnitude effect sizes similar to those for the estrogens but at borderline levels of statistical significance. Quadratic terms (i.e., hormone term squared) were explored for each model but were not statistically significant.

Hormone values for 89 participants were classified (based on *dfBeta* values) as outliers in at least one model. Among these statistical outliers, nine were for women for whom an interpolated hormone value was used. Values for four participants were outliers in all eight models, one was considered an outlier in two models, and four were outliers in only one model. In models with outliers removed, the magnitude

TABLE 2. Mean values of hormones and SHBG* at baseline for PEPI* Mammographic Density Study participants in the analytic sample and for those women excluded because they were recent postmenopausal hormone users,† United States, 1998–2005

Hormone (unit of measurement)	Participants included in the analysis of endogenous hormones and mammographic density ($n = 404$)			Recent hormone users not included in the analysis of endogenous hormones and mammographic density ($n = 171$)			p value‡
	No. whose values were BDL*	Mean no. (SD*), BDL values omitted	Mean no. (SD), BDL values substituted§	No. whose values were BDL	Mean no. (SD), BDL values omitted	Mean no. (SD), BDL values substituted	
Estrone (pg/ml)	1	18.9 (16.8)	18.9 (16.8)	1	17.4 (12.0)	17.4 (12.3)	0.2465
Estradiol (pg/ml)	32	10.4 (25.5)	9.7 (24.6)	13	8.9 (16.8)	8.7 (16.8)	0.5054
Bioavailable estradiol (pg/ml)	33	4.6 (10.0)	4.3 (9.7)	14	3.7 (6.0)	3.6 (6.0)	0.2519
Testosterone (pg/ml)	0	N/A*	150.6 (70.8)	0	N/A	135.8 (746.4)	0.0254
Bioavailable testosterone (pg/ml)	0	N/A	33.3 (20.8)	0	N/A	27.7 (22.0)	0.0036
Progesterone (ng/ml)	0	N/A	0.3 (1.1)	0	N/A	0.2 (0.3)	0.4140
SHBG ($\times 10^8$ M)	0	N/A	4.4 (2.3)	0	N/A	4.8 (2.4)	0.0406

* SHBG, sex hormone-binding globulin; PEPI, Postmenopausal Estrogen/Progestin Interventions; BDL, below detectable limits; SD, standard deviation; N/A, not applicable.

† Recent hormone users were 173 women who quit hormones for PEPI (i.e., they were using hormone therapy at the initial PEPI screening visit and opted to discontinue HT use to enroll). Of these 173 women, two did not have baseline hormone measures.

‡ If the hormone level was below the limit of assay detection (applies to estrone and estradiol only), a value 1 pg/ml lower than the assay detection limit was substituted to calculate the mean value shown here (refer to the text for further details). Eight women in the PEPI Mammographic Density Study had serum progesterone levels of $\geq$0.05 ng/ml.

§ p value for t test comparing mean values of hormones and binding globulins for women in the analytic sample with those for women excluded because of recent hormone use. Means were calculated and statistical comparisons were made by omitting BDL values. However, when interpolated values (one unit below the limit of detection of the hormone assays) were included in the calculations, p values comparing mean levels of estrone, estradiol, and bioavailable estradiol in those in the analytic sample and those excluded from the study because of recent hormone use were not materially different from those shown in this table (data not shown).

of the associations between each hormone and mammographic density (estimated by the hormone β coefficient) was approximately the same as the magnitude of the association in models that used the entire sample, with the exception of progesterone, which had a sevenfold higher effect size in the model that excluded outliers (data not shown). The three estrogen models were also rerun by excluding all interpolated values for cases with BDL estrogen levels; exclusion of the 32 participants who had nondetectable total estradiol levels and the 33 who had nondetectable bioavailable estradiol levels (32 of these were the same women as those with the nondetectable total estradiol levels) had no material effect on the results (data not shown).

Because the magnitude of the associations between sex steroids and mammographic density may be difficult to apprehend from the β coefficients (table 4), we present the

TABLE 3. Spearman correlation coefficients between each of the sex steroid hormones and SHBG* for study participants ($n = 404$),† United States, 1998–2005

Hormone	Estrone	Estradiol	Bioavailable estradiol	Testosterone	Bioavailable testosterone	SHBG	Progesterone
Estrone	1.000	0.729 (0.000)	0.614 (0.000)	0.487 (0.000)	0.472 (0.000)	−0.131 (0.000)	0.440 (0.000)
Estradiol		1.000	0.884 (0.000)	0.372 (0.000)	0.476 (0.000)	−0.294 (0.000)	0.280 (0.000)
Bioavailable estradiol			1.000	0.253 (0.000)	0.578 (0.000)	−0.542 (0.000)	0.270 (0.000)
Testosterone				1.000	0.650 (0.000)	0.148 (0.003)	0.350 (0.000)
Bioavailable testosterone					1.000	−0.523 (0.000)	0.257 (0.000)
SHBG						1.000	0.047 (0.341)
Progesterone							1.000

* SHBG, sex hormone-binding globulin.

† p value for each pair of correlations is shown in parentheses. Values for correlations and p values were not affected by inclusion of interpolated values or by deletion of observations for cases in whom estrone ($n = 1$), estradiol ($n = 32$), or bioavailable estradiol ($n = 33$) were below the limit of assay detection. Calculations presented in the table include the interpolated values for observations below detectable limits.

TABLE 4. Endogenous hormones and percent mammographic density in study participants ($n = 365$),*† United States, 1998–2005

Hormone (unit of measurement)	Model 1‡		Model 2§		Model 3¶	
	β coefficient, hormone term	*p* value, hormone term	β coefficient, hormone term	*p* value, hormone term	β coefficient, hormone term	*p* value, hormone term
Estrone (pg/ml)	0.0003	0.579	0.0013	0.013	0.0013	0.014
Estradiol (pg/ml)	0.0006	0.105	0.0009	0.008	0.0009	0.009
Bioavailable estradiol (pg/ml)	0.0007	0.528	0.0021	0.017	0.0021	0.018
Testosterone (pg/ml)	0.0002	0.208	0.0002	0.171	0.0002	0.178
Bioavailable testosterone (pg/ml)	−0.0012	0.006	−0.0002	0.710	−0.0002	0.710
Progesterone (ng/ml)	0.0087	0.274	0.0137	0.062	0.0139	0.059
Sex hormone binding globulin ($\times 10^8$ M)	0.0087	0.000	0.0072	0.076	0.0072	0.079

* Information on parity was missing for 39 participants, reducing the multivariable model sample size from 404 to 365.

† Beta coefficients were calculated per one unit of measurement for each hormone.

‡ Adjusted for age and parity.

§ Adjusted for age, parity, and body mass index.

¶ Adjusted for age, parity, body mass index, prior use of hormone therapy (yes/no), time since last use of hormone therapy (days), and the interaction of prior use of hormone therapy and time since last use of hormone therapy.

adjusted least-squares mean estimates of mammographic density by quintile of estrone, progesterone, and SHBG, adjusted for age, parity, BMI, former use of hormone therapy, duration since last use of hormone therapy, and an interaction between the latter two terms (figure 1). We found an absolute increment in mammographic percent density of approximately 5 percent when moving from the bottom to the top quintile for each case.

Some of the sex steroid hormone exposures considered in this study are biologically and/or statistically interrelated, which makes it difficult to understand their actions when they are measured at a single time point and without manipulation. On the basis of a priori biologic hypotheses, we investigated four selected models to predict mammographic density by using pairs of hormones or hormone plus binding globulin; all models were adjusted for age, parity, BMI, former hormone therapy use, time since last hormone therapy use, and an interaction between the latter two variables. To facilitate comprehension of our model choices, the rationale for each follows. First, testosterone is metabolized to estradiol; thus, one could ask whether the observed borderline effect of total testosterone on mammographic density derives from its role as a substrate for estradiol. In the joint model, the β coefficients (*p* values) for total estradiol and total testosterone were 0.0008 ($p = 0.015$) and 0.0001 ($p = 0.347$), respectively. Next, we modeled SHBG and total estradiol and SHBG and total testosterone together, because SHBG is the major carrier protein for estradiol and testosterone. The β coefficients (*p* values) for the total estradiol and SHBG models were 0.0008 ($p = 0.016$) and 0.0058 ($p = 0.157$), respectively. For the SHBG and total testosterone model, the β coefficients (*p* values) were 0.0064 ($p = 0.123$) and 0.0001 ($p = 0.291$), respectively. Overall, the effect sizes for these joint models were similar to those for the single hormone exposure models (table 4). Lastly, because the effects of progesterone may depend on exposure to estradiol, we investigated, but did not find evidence for, an interaction between total estradiol and progesterone (*p* for interaction = 0.184).

DISCUSSION

This study of 404 postmenopausal women who were not recent postmenopausal hormone users found a statistically significant, positive association between mammographic percent density and endogenous serum levels of estrone, estradiol, and bioavailable estradiol. Serum levels of progesterone and SHBG were associated with higher mammographic density, with similar magnitudes of effect but at borderline statistical significance levels. The average increment in mammographic percent density associated with being in the lowest versus the highest quintile for each of these hormones or binding globulin was substantial, an absolute difference of about 5 percent.

Mammographic density is a strong, independent predictor of breast cancer risk (1–4, 6, 11). Observational studies estimate that the risk of breast cancer increases 1.5–2 percent for each percent increment in endogenous mammographic density (density not influenced by exogenous hormones) (4, 5, 39). Therefore, mammographic density is a promising surrogate endpoint for breast cancer risk (4, 40, 41). Understanding the biologic basis of the variation in mammographic density (in this case, sex steroid hormone profiles) may be one window into breast cancer causation and, ultimately, prevention (6, 7, 40, 42, 43).

The hypothesis that endogenous sex steroids affect breast density stems from the theory that sex steroids are implicated in the etiology of breast cancer (24). Early menopause is a protective factor for breast cancer (44), and, although the number of breast cancer cases increases with chronological age, the *rate* of increase in breast cancer declines after menopause (45). The Endogenous Hormones and Breast Cancer Collaborative Group found that the risk of breast cancer was positively related to higher quintiles of each sex steroid available for analysis: estradiol, free estradiol, non-SHBG-bound estradiol, estrone, estrone sulfate, androstenedione, dehydroepiandrosterone, and total testosterone (25). The relative risk of breast cancer associated with a doubling of each of the hormone concentrations was

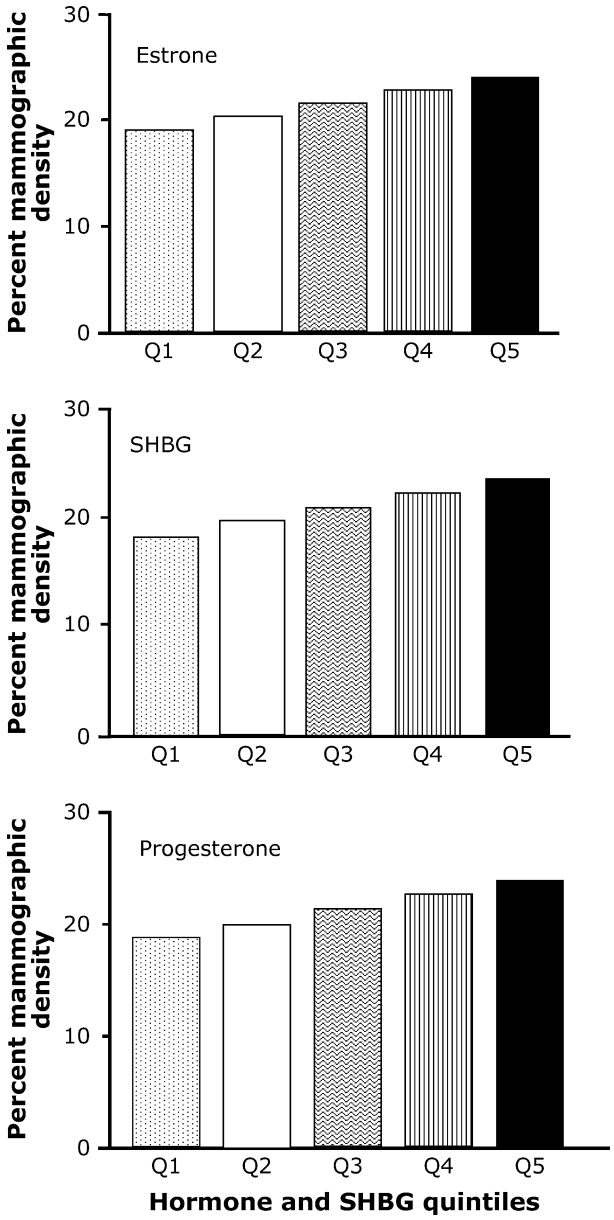

FIGURE 1. Adjusted least-squares mean estimates of percent mammographic density by quintile (Q) of estrone, sex hormone-binding globulin (SHBG), and progesterone, adjusted for age, parity, body mass index, prior use of hormone therapy (yes/no), time since last use of hormone therapy (days), and the interaction of prior use of hormone therapy and time since last use of hormone therapy ($n = 365$ because of 49 missing values for parity), Postmenopausal Estrogen/Progestin Interventions Mammographic Density Study, United States, 1998–2005.

roughly the same for each hormone, about 20–40 percent. All hormones were highly correlated; the authors inferred causality for estrogen owing to its mitogenic effect on breast tissue (21, 22) and found reduced breast cancer risk when selective estrogen receptor modulators are used (46–48). Correspondingly, our study found a positive association between mammographic density and endogenous estrone, total estradiol, bioavailable estradiol, and total testosterone. We also identified a positive relation between mammographic density and progesterone, which the Collaborative Group did not study.

The results of one published study of the relation between mammographic density and sex steroid hormones in postmenopausal women were dissimilar from ours: Boyd et al. (42) found an *inverse* relation between mammographic density and non-SHBG-bound estradiol, both before and after adjustment for age and waist circumference. In their unadjusted analyses, total estradiol and progesterone were also negatively related to breast density, but these associations did not persist after adjustment for SHBG, age, and waist circumference (42). The divergent findings do not stem from disparities in participant characteristics: both studies used similar definitions of menopause, excluded recent hormone users (the Boyd et al. study used a 6-month cutpoint), and had similar mean values and standard deviations for age, hormone levels, and mammographic density. The studies' choice of cutpoint for exclusion of recent hormone therapy users is not a likely explanation for the discrepancy in results for estradiol: PEPI analyses were adjusted for time since last hormone therapy use, and our shorter cutpoint would lead to a null rather than a systematic bias. Unlike our study, that of Boyd et al. did not report that any of the postmenopausal participants had nondetectable total estradiol measures; their total estradiol assay may have been more sensitive (the lower limit of its detection was not specified). Alternatively, the lower tail of their distribution of total estradiol values may have been slightly higher than ours. However, these possible distributional or assay differences cannot explain the disagreements in the estrogen findings: in PEPI, excluding participants who had unmeasurable estrogen levels did not change our model results. Mammographic density measurement techniques and repeatability statistics were also alike. Boyd et al. adjusted for waist circumference while this study adjusted for BMI, but our findings were not altered when we adjusted for waist circumference (data not shown).

We observed higher mammographic density in relation to higher SHBG, as did Boyd et al. (42). The direction of this association is opposite to that observed in breast cancer studies, where higher SHBG predicts lower risk (25). Hormonal theories of breast cancer causation also predict an inverse association between SHBG and cancer risk because SHBG-bound estrogen does not readily enter cells (21, 22). However, SHBG has recently been shown to regulate steroid action through a mechanism involving steroid signaling at the cell membrane (49) in both normal and cancerous breast tissue (50). Downstream effects can be agonistic or antagonistic. For example, SHBG receptor signaling inhibits estradiol-induced growth of human breast carcinoma cells (51) and enhances both estradiol- and dehydrotestosterone-mediated growth of human prostate cancer cells (52). To our knowledge, the role of the SHBG cell-membrane-associated receptor system has not been studied in normal breast tissue; however, it is plausible that the positive association of SHBG with mammographic density may be due to a cell-membrane-associated, SHBG-receptor-mediated effect.

Strengths of our study include its use of a continuous measure of mammographic density, high reproducibility of density readings, and sensitive hormone assays targeted to the postmenopausal range. Shortcomings must also be considered. First, the cross-sectional design limits causal inference. Second, all hormone exposures were single measures; in the short term, measured levels of estradiol vary in postmenopausal women, likely because of assay differences (53). However, short-term imprecision should result in nondifferential misclassification—making associations more difficult to observe. Third, we chose to retain the original PEPI design and use 3 months as our cutpoint for excluding recent hormone therapy users. This approach could be criticized because the amount of time it takes for exogenous hormone therapy to wash out is not known. We hypothesized that the breast density of recent hormone therapy users would be affected for some period of time after having used hormone therapy, because a trophic effect would not wear off immediately upon hormone therapy discontinuation. However, there was no extant literature to serve as an a priori guide to how long this breast tissue effect might persist. Therefore, we relied on the original trial design, which specified a 3-month hormone therapy washout, recognizing that this duration was chosen with cardiovascular risk factor outcomes, not mammographic density, in mind. The bias from a too-short washout period would be null—the effects of endogenous hormones would be less evident when the breast tissue is still under the influence of exogenous hormone therapy. Similarly, how long it takes for hormone levels to return to their native state after hormone therapy cessation is unknown. Although a between-groups comparison cannot directly address the question of how long a within-woman hormone washout takes, table 2 shows that estradiol, estrone, and bioavailable estradiol do not differ significantly between women who had to quit hormone therapy to join PEPI and those who did not. Finally, the models presented here include terms for ever use of hormone therapy, recency of hormone therapy use, and an interaction between ever use of hormone therapy and recency of hormone therapy use.

We found that higher endogenous estrone, total estradiol, bioavailable estradiol, progesterone, and SHBG levels were associated with greater mammographic density, providing one plausible pathway by which endogenous hormones affect the risk of breast cancer: higher hormone levels leading to higher density leading to higher cancer risk. These results are exciting. The associations between sex steroids and mammographic density line up in the same way as the associations between sex steroids and breast cancer, offering further biologic support to the notion that mammographic density is a surrogate marker for breast cancer risk—sorely needed given the long preclinical stage of this disease. These findings also invite further exploration of whether other hormones and/or genetic polymorphisms in hormone metabolic and receptor pathways explain variation in endogenous breast density. Knowledge of factors that predict endogenous density may provide a stepping-stone toward identifying and testing interventions designed to favorably modify those factors, perhaps ultimately reducing breast cancer risk.

ACKNOWLEDGMENTS

Conflict of interest: none declared.

REFERENCES

1. Saftlas AF, Szklo M. Mammographic parenchymal patterns and breast cancer risk. Epidemiol Rev 1987;9:146–74.
2. Oza AM, Boyd NF. Mammographic parenchymal patterns: a marker of breast cancer risk. Epidemiol Rev 1993;15: 196–208.
3. Warner E, Lockwood G, Math M, et al. The risk of breast cancer associated with mammographic parenchymal patterns: a meta-analysis of the published literature to examine the effect of method of classification. Cancer Detect Prev 1992; 16:67–72.
4. Boyd NF, Lockwood GA, Byng JW, et al. Mammographic densities and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1998;7:1133–44.
5. Ursin G, Ma H, Wu AH, et al. Mammographic density and breast cancer in three ethnic groups. Cancer Epidemiol Biomarkers Prev 2003;12:332–8.
6. Byrne C, Schairer C, Wolfe J, et al. Mammographic features and breast cancer risk: effects with time, age and menopause status. J Natl Cancer Inst 1995;87:1622–9.
7. Boyd NF, Dite GS, Stone J, et al. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 2002;347:886–94.
8. Wolfe JN. Breast patterns as an index of risk for developing breast cancer. AJR Am J Roentgenol 1976;126:1130–9.
9. American College of Radiology. Breast imaging reporting and data system: BI-RADS atlas. 4th ed. Reston, VA: American College of Radiology, 2003.
10. Wolfe JN, Saftlas AF, Salane M. Mammographic parenchymal patterns and quantitative evaluation of mammographic densities: a case-control study. AJR Am J Roentgenol 1987;148: 1087–92.
11. Boyd NF, Byng JW, Jong RA, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. J Natl Cancer Inst 1995;87:670–5.
12. Ursin G, Astrahan MA, Salane M, et al. The detection of changes in mammographic densities. Cancer Epidemiol Biomarkers Prev 1998;7:43–7.
13. Bright RA, Morrison AS, Brisson J, et al. Relationship between mammographic and histologic features of breast tissue in women with benign biopsies. Cancer 1988;61:266–71.
14. Urbanski S, Jensen HM, Cooke G, et al. The association of histological and radiological indicators of breast cancer risk. Br J Cancer 1988;58:474–9.
15. Bartow SA, Pathak DR, Mettler FA. Radiographic microcalcification and parenchymal pattern as indicators of histologic ''high-risk'' benign breast disease. Cancer 1990;66:1721–5.
16. Boyd NF, Jensen HM, Cooke G, et al. Relationship between mammographic and histological risk factors for breast cancer. J Natl Cancer Inst 1992;84:1170–95.
17. Wellings SR, Wolfe JN. Correlative studies of the histological and radiographic appearance of the breast parenchyma. Radiology 1978;129:299–306.
18. Fisher ER, Palekar A, Kim WS, et al. The histopathology of mammographic patterns. Am J Clin Pathol 1978;69:421–6.
19. Moskowitz M, Gartside P, McLaughlin C. Mammographic patterns as markers for high-risk benign breast disease and incident cancers. Radiology 1980;134:293–5.

20. Arthur JE, Ellis IO, Flowers C, et al. The relationship of "high risk" mammographic patterns to histological risk factors for development of cancer in the human breast. Br J Radiol 1990; 63:845–9.
21. Pike MC, Bernstein L, Spicer DV. Exogenous hormones and breast cancer risk. In: Niederhuber JE, ed. Current therapy in oncology. St. Louis, MO: BC Decker, Mosby-Year Book, Inc, 1993.
22. Pike MC, Spicer DV, Dahmoush L, et al. Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk. Epidemiol Rev 1993;15:17–35.
23. Söderqvist G. Effects of sex steroids on proliferation in normal mammary tissue. Ann Med 1998;30:511–24.
24. Henderson BE, Ross RK, Bernstein L. Estrogens as a cause of human cancer: the Richard and Hinda Rosenthal Foundation Award Lecture. Cancer Res 1988;48:246–53.
25. Key T, Appleby P, Barnes I, et al. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. J Natl Cancer Inst 2002;94: 606–16.
26. Greendale GA, Reboussin BA, Slone S, et al. Postmenopausal hormone therapy and change in mammographic density. J Natl Cancer Inst 2003;95:30–7.
27. Espeland MA, Bush TL, Mebane-Sims I, et al. Rationale, design, and conduct of the PEPI Trial. Postmenopausal Estrogen/ Progestin Interventions. Control Clin Trials 1995;16(4 suppl): 3S–19S.
28. Colacurci N, Fomaro F, De Franciscis P, et al. Effects of a short-term suspension of hormone replacement therapy on mammographic density. Fertil Steril 2001;76:451–5.
29. Greendale GA, Bodin-Dunn L, Ingles S, et al. Leisure, home and occupational physical activity and cardiovascular risk factors in postmenopausal women: the postmenopausal estrogen/progestins intervention (PEPI) study. Arch Intern Med 1996;156:418–24.
30. Greendale GA, James MK, Espeland MA, et al. Can we measure postmenopausal estrogen/progestin use? The Postmenopausal Estrogen/Progestin Interventions Trial. Am J Epidemiol 1997;146:763–70.
31. Block G, Hartman AM, Dresser CM, et al. A data-based approach to diet questionnaire design and testing. Am J Epidemiol 1986;124:453–69.
32. Anderson DC, Hopper BR, Lasley BL, et al. A simple method for the assay of eight steroids in small volumes of plasma. Steroids 1976;28:179–96.
33. Tremblay RR, Dube JY. Plasma concentrations of free and non-TeBG bound testosterone in women on oral contraceptives. Contraception 1974;10:599–605.
34. Rosner W. Isolation and characterization of the testosterone-estradiol-binding globulin from human plasma. Use of a novel affinity column. Biochemistry 1975;14:4813–20.
35. Greendale GA, Reboussin BA, Sie A, et al. Effects of estrogen and estrogen-progestin on mammographic parenchymal density. Ann Intern Med 1999;130:262–9.
36. Byng JW, Boyd NF, Little L, et al. Symmetry of projection in the quantitative analyses of mammographic images. Eur J Cancer Prev 1996;5:319–27.
37. Belsley DA, Kuh E, Welsch RE. Regression diagnostics. New York, NY: John Wiley & Sons, 1980.
38. Stata Corporation. Stata statistical software, release 7.0. College Station, TX: Stata Corporation, 2001.
39. Spicer DV, Ursin G, Parisky YR, et al. Changes in mammographic densities induced by a hormonal contraceptive designed to reduce breast cancer risk. J Natl Cancer Inst 1994; 86:431–6.
40. Vachon CM, Kuni CC, Anderson K, et al. Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States). Cancer Causes Control 2000;11:653–62.
41. Chlebowski RT, McTiernan A. Biological significance of interventions that change breast density. (Editorial). J Natl Cancer Inst 2003;95:4–5.
42. Boyd NF, Stone J, Martin LJ, et al. The association of breast mitogens with mammographic densities. Br J Cancer 2002; 87:876–82.
43. Byrne C, Colditz GA, Willett WC, et al. Plasma insulin-like growth factor (IGF) I, IGF-binding protein 3, and mammographic density. Cancer Res 2000;60:3744–8.
44. Trichopoulos D, MacMahon B, Cole P. The menopause and breast cancer. J Natl Cancer Inst 1972;48:605–13.
45. Tracy RE. Sex difference in coronary disease: two opposing views. J Chronic Dis 1966;19:822–33.
46. Tamoxifen for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. Lancet 1998;351:1451–67.
47. Fisher B, Powles TJ, Pritchard KJ. Tamoxifen for the prevention of breast cancer. Eur J Cancer 2000;36:142–50.
48. Cummings SR, Browner WS, Bauer D, et al. Endogenous hormones and the risk of hip and vertebral fractures among older women. Study of Osteoporotic Fractures Research Group. N Engl J Med 1998;339:733–8.
49. Kahn SM, Hryb DJ, Nakhla AM, et al. Beyond carrier proteins: sex hormone-binding globulin is synthesized in target cells. J Endocrinol 2002;175:113–20.
50. Frairia R, Fortunati N, Berta L, et al. Sex steroid binding protein (SBP) receptors in estrogen sensitive tissues. J Steroid Biochem Mol Biol 1991;40:805–12.
51. Fortunati N, Fissore F, Fazzari A, et al. Sex steroid binding protein (SHBG) exerts a negative control on estradiol action in MCF-7 cells (human breast cancer) through cAMP and PKA. Effect on estradiol induced cell proliferation. Endocrinology 1996;137:686–92.
52. Nakhla AM, Rosner W. Stimulation of prostate cancer growth by androgens and estrogens through the intermediacy of sex hormone-binding globulin. Endocrinology 1996;137:4126–9.
53. Cauley JA, Gutai JP, Kuller LH, et al. Reliability and interrelations among sex hormones in postmenopausal women. Epidemiology 1991;133:50–7.