Endogenous Sex Hormone Levels and Mammographic Density among Postmenopausal Women

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Abstract

Background: **Mammographic density is one of the strongest predictors of breast cancer risk. The mechanism by which breast density increases breast cancer risk is unclear although it has been hypothesized that breast density reflects cumulative exposure to estrogens.**

Methods: **To evaluate this hypothesis, we conducted a cross-sectional study among 520 postmenopausal women in the Nurses' Health Study that examined the relation between circulating sex hormones and mammographic density. Women were postmenopausal and not taking exogenous hormones at the time of blood collection and mammogram. Percent breast density was measured from digitized mammograms using a computer-assisted method. Circulating estrone, estradiol, androstenedione, testosterone, DHEA, DHEA sulfate, sex hormone–binding globulin, progesterone, and prolactin were measured in plasma.**

Results: **In contrast to the prior hypothesis, circulating estrogens were inversely related to percent mammographic density. The mean percent mammographic density was 25.6% among women in the lowest quartile of circulating estradiol compared with 14.4% among women in the highest quartile [Spearman correlation (r) = −0.22, P < 0.0001]. Circulating estrogens alone explained 1% to 5% of the variation of mammographic density. Body mass index was positively associated with circulating estradiol levels (r = 0.45, P < 0.0001) and inversely related to percent mammographic density (r = −0.51, P < 0.0001). After adjustment for body mass index, there was no association between estradiol and breast density (r = 0.01, P = 0.81). Likewise, there was no relation between the other sex hormones measured or prolactin and mammographic density after adjustment for body mass index.**

Conclusion: **These findings indicate that in postmenopausal women, mammographic density is independent of circulating sex hormone levels.** (Cancer Epidemiol Biomarkers Prev 2005;14(11):2641–7)

Introduction

Mammographic density is one of the strongest predictors of breast cancer risk (1). Women with ≥75% breast density are at a 4- to 6-fold greater risk of breast cancer than women with no density (1, 2). The radiographic appearance of the breast on a mammogram varies depending on the composition of the breast. Fat is radiolucent and appears dark on a film screen mammogram. In contrast, epithelial cells and connective tissue are radiodense. They appear light on a mammogram and are considered to be ''mammographically dense.''

The mechanism by which mammographic density increases breast cancer risk is unclear although it has been hypothesized that breast density represents increased cellular proliferation. There is evidence that use of exogenous hormones increases mammographic density (3-9), and combined formulations of estrogen and progestin may have the most pronounced effects (6, 8, 9). Clinical trials have shown that treatment with tamoxifen, a selective estrogen receptor modulator with antiestrogenic effects in the breast, reduces breast density (10-13). The associations of exogenous hormones and tamoxifen with mammographic density suggest that endogenous estrogens may influence breast density and that the positive association between mammographic density and breast cancer is due to elevated levels of circulating estrogens. Based on these data, it has been hypothesized that breast density represents cumulative exposure to estrogens (14). The purpose of the current study was to directly examine the relation between endogenous estrogens, androgens, progesterone, prolactin, and sex hormone–binding globulin (SHBG) and mammographic density among postmenopausal women not currently on exogenous hormones.

Materials and Methods

Study Design and Population. The Nurses' Health Study was initiated in 1976 when 121,700 U.S. registered nurses ages 30 to 55 years returned an initial questionnaire (15). Information on body mass index (BMI), reproductive history, age at menopause, and postmenopausal hormone use as well as diagnosis of cancer and other diseases are updated every 2 years through questionnaires. During 1989 and 1990, blood samples were collected from 32,826 women. Detailed information regarding blood collection methods has been published (16). In general, blood samples were returned within 26 hours of blood draw; immediately centrifuged; aliquoted into plasma, RBC, and buffy coat fractions; and stored in liquid nitrogen freezers. The follow-up rate among women who provided blood samples was 99% through 1998.

We conducted a cross-sectional analysis among controls from a breast cancer case-control study nested within the Nurses' Health Study cohort. This nested case-control study examined plasma sex steroid hormones and breast cancer risk and included breast cancer cases diagnosed after blood collection but before June 1, 1998, and matched controls (17). There were 655 potential postmenopausal controls who had no

Received 7/27/05; revised 8/19/05; accepted 9/1/05.

Grant support: USPHS grants CA087969, CA049449, and CA075016; Specialized Programs of Research Excellence in Breast Cancer grant CA089393 from the National Cancer Institute, NIH, Department of Health and Human Services and Breast Cancer Research Fund; and American Cancer Society Cissy Hornung Clinical Research Professorship (G.A. Colditz).

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doi:10.1158/1055-9965.EPI-05-0558

history of cancer and were not taking postmenopausal hormones at the time of blood collection or during the 3 months prior. At the time of mammography collection, 631 participants were alive and eligible to receive letters for participation in this study. Five hundred sixty-two (89.1%) women gave permission to obtain mammograms, whereas 4% refused, 4% reported never having a mammogram, 2% did not respond, and 1% could not recall if they had a mammogram. For all consenting women, we attempted to obtain the mammograms taken before and as close to the date of blood collection as possible. We successfully obtained mammograms from 540 controls (96.1% of those consenting). The median time between mammography and blood draw was 8 months (interquartile range, 2.5 years before to 1 month after). Women for whom we obtained mammograms were very similar to those whom we were unable to get mammograms with respect to age, BMI, and circulating hormone levels. We excluded five women whose mammograms were not film mammograms. Because menopausal status and postmenopausal hormone use are associated with both hormone levels and mammographic density, we restricted all analyses to women who were postmenopausal and not taking exogenous hormones at the time of both mammography and blood collection ($n = 520$). This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital.

Laboratory Analyses. Estrone, estradiol, non-SHBG–bound estradiol, androstenedione, testosterone, DHEA, and DHEA sulfate were all assayed at the Nichols Institute of Quest Diagnostics (San Juan Capistrano, CA). Assays were conducted in up to five batches. The first batch of estrone sulfate was assayed at Dr. Longcope's laboratory at the University of Massachusetts Medical Center (Worcester, MA). The remaining batches were assayed at the Nichols Institute. The first two batches of SHBG and prolactin were assayed in Dr. Longcope's laboratory; the third and fourth batches were assayed in the Reproductive Endocrinology Unit Laboratory at Massachusetts General Hospital (Boston, MA). All batches of progesterone were assayed at the same time at Quest Diagnostics.

The methods used to assay these hormones have been described in detail (16-18). Briefly, samples were extracted with hexane-ethyl acetate, the steroids were eluted from celite columns, and the fractions were assayed by RIA (19-23). DHEA sulfate was assayed by RIA without a prior separation step. To quantify estrone sulfate levels, unconjugated estrone was first extracted from the plasma and the sulfate bond was enzymatically cleaved from estrone sulfate to release estrone. The estrone was extracted by an organic solvent and subjected to chromatography and RIA (24). Free estradiol was calculated according to the law of mass action described by Sodergard et al. (25). Non-SHBG–bound estradiol was only measured through the 1996 follow-up cycle and was assayed by use of an ammonium sulfate precipitation (25, 26). Prolactin was measured using a microparticle enzyme immunoassay using the IMx system (Abbott Laboratory, Abbott Park, IL) in Dr. Longcope's laboratory and the AxSYM Immunoassay system (Abbott Laboratory) at Massachusetts General Hospital.

Plasma samples were placed in random order in boxes sent to the laboratory. To assess laboratory precision, masked samples from two plasma pools (in a ratio of 1:10 study samples) were randomly interspersed and labeled. Within-batch coefficients of variation ranged from 6% (DHEA sulfate) to 15% (progesterone).

The assay detection limit was 2 pg/mL for estradiol, 10 pg/mL for estrone, 40 pg/mL for estrone sulfate, 3 ng/dL for androstenedione, 1 ng/dL for testosterone, 3 ng/dL for DHEA, 5 μg/dL for DHEA sulfate, 3 ng/dL for progesterone, and 0.6 ng/mL for prolactin. Values were below the detectable limit for estrone in 15 samples, for estrone sulfate in 2 samples, for androstendione in 1 sample, for DHEA in 1 sample, for DHEA sulfate in 5 samples, for progesterone in 176 samples, and for testosterone in 2 samples. When plasma hormone values were reported as less than the detectable limit, we set the value to half the detectable limit.

We used the extreme Studentized deviate many-outlier procedure to determine outlying hormone values by batch (27). This process resulted in the exclusion of one estrone value, two estradiol values, one estrone sulfate value, three androstenedione values, and one testosterone value. There was insufficient plasma for all subjects to have measurements on the all hormones. Final numbers are presented in Table 3A-C.

Mammographic Density Measurements. To assess mammographic density, the craniocaudal views of both breasts were digitized at 261 μm/pixel with a Lumysis 85 laser film scanner, which covers a range of 0 to 4.0 absorbance. The software for computer-assisted thresholding was developed at the University of Toronto (28). The film screen images were digitized and viewed on the computer screen. For each image, the observer set one threshold level to define the edge of the breast and a second threshold delineating the dense area of the breast within the original threshold region. The Cumulus software calculated the total number of pixels within the entire region of interest and within the area identified as dense. Using these values, the software program calculated the percentage of the breast area that was dense. This measure of mammographic breast density was highly reproducible within this study. The within-person intraclass correlation coefficient is equal to 0.93 (29). We used the average percentage density of both breasts for this analysis. Previous studies have shown similar results when the breast density of a random side (right or left) or the average of the two are used (30). In this study, we also evaluated the association of sex hormones with the absolute area of mammographic density; however, because results were similar and percent breast density has consistently been a stronger predictor of breast cancer risk, we present the results of percent mammographic density only.

Covariate Information and Control of Confounding. Postmenopausal status and use of postmenopausal hormones at blood draw were assessed through a supplemental questionnaire administered at the time of blood collection. Women were considered postmenopausal if they reported no menstrual periods within the 12 months before blood collection with natural menopause, bilateral oophorectomy or hysterectomy with one or more ovaries retained, and were ≥54 years if a smoker or ≥56 years if a nonsmoker. These are the ages at which 90% of the Nurses' Health Study participants who had a natural menopause were postmenopausal.

Menopausal status and postmenopausal hormone use at the time of the mammogram was assessed using data from biennial questionnaires before the date of the mammogram. All other covariates were assessed from biennial questionnaires before the blood collection. Age at mammography, BMI, alcohol consumption, smoking status, family history of breast cancer, personal history of benign breast disease, age at menarche, parity, age at first birth, alcohol consumption, use of postmenopausal hormones, and age at menopause were all evaluated as potential predictors of mammographic density. Covariates were considered potential confounders if there was *a priori* evidence in the published literature that the factor was related to both circulating hormone levels and mammographic percentage density.

Statistical Analysis. Age-adjusted means and frequencies were calculated according to previously published categories of mammographic density among postmenopausal women (<10%, 10-25%, and >25%). Partial Spearman correlations were calculated between BMI and circulating hormones and mammographic density was adjusted for age and laboratory batch.

There were some differences in the distribution of hormone concentrations between laboratory batches. The quality control samples included in each batch showed variability similar to that of the control samples, suggesting that these differences are due to batch-to-batch variability and are not true differences in concentrations. Therefore, we created quartiles of hormones based on batch-specific cut points and controlled for batch in all analyses with continuous hormone measures.

Generalized linear models were used to evaluate the effect of each hormone as a predictor of percent breast density and to determine the mean percent of breast density per hormone quartile adjusted for age at blood draw (continuous) and laboratory batch in initial models. BMI (continuous) was the only covariate that resulted in appreciable changes in estimates. We also created a model including the following additional covariates that exhibited an association with mammographic density (Table 1): age at first birth (nulliparous, age at first birth <25, age at first birth 25-29, age at first birth 30+, and missing), alcohol consumption (none,<5 g/d, 5-14.9 g/d, 15+ g/d, and missing), history of benign breast disease (yes/no), family history of breast cancer (yes/no), and past use of postmenopausal hormones (yes/no). Partial Spearman correlation coefficients between continuous hormone measures and percent mammographic density were calculated with adjustment for laboratory batch and potential confounders. All P values presented are two-sided tests of statistical significance. R^2 values were obtained from regression models of continuous hormone measurements regressed on square root–transformed percent breast density. Square root transformation of mammographic density was necessary to improve the normality of the data.

Results

The mean age of women in this study (n = 520) was 61.4 years at blood draw (range, 46-69 years). Among women with a natural menopause, the mean time between menopause and blood draw was 10.9 years. In this population of postmenopausal women, the mean percent breast density was 19.4% (range, 0-73.5%). Women with mammographic density >25% had a later age at first birth; earlier age at menopause; were more likely to be lean, nulliparous, have a personal history of benign breast disease, and to be past users of postmenopausal hormones; and were less likely to be current smokers than women with lower percentage of mammographic density (Table 1).

Table 1. Age and age-adjusted characteristics of the study population at blood draw according to categories of mammographic density, Nurses' Health Study (1989-1990)

	Mammographic density (%)		
Range	<10	10.0-25.0	>25.0
No. women	188	175	157
Mean			
Age at blood draw, y	61.3	61.8	61.3
Age at mammogram, y	63.0	63.0	62.7
Age at menarche, y	12.6	12.5	12.8
Age at first birth, y	24.7	25.4	25.9
Age at menopause*, y	50.6	50.9	49.8
Time since menopause*, y	12.2	12.8	14.0
Parity†	3.7	3.5	3.1
Alcohol consumption, g	2.4	2.9	2.8
BMI, kg/m^2	28.7	26.1	23.4
Frequency (%)			
Nulliparous	4.3	6.9	11.5
History of benign breast disease	30.6	34.6	39.0
Past postmenopausal hormone use	34.0	38.9	46.2
Current smoker	14.2	12.9	11.5
Family history of breast cancer	12.4	14.4	15.8
Natural menopause	74.3	72.4	66.2
Bilateral oophorectomy	9.1	8.3	12.7

*Among those with a natural menopause.
†Among parous women only.

Table 2. Partial Spearman correlations between endogenous hormones, mammographic density, and BMI (Nurses' Health Study)

	BMI	
	r	P
Hormones		
Estrone	0.34	<0.0001
Estradiol	0.45	<0.0001
Free estradiol	0.52	<0.0001
Non-SHBG–bound estradiol	0.54	<0.0001
Estrone sulfate	0.29	<0.0001
DHEA	−0.03	0.57
DHEA sulfate	0.01	0.79
Testosterone	0.02	0.64
Free testosterone	0.24	<0.0001
Androstenedione	0.07	0.10
SHBG	−0.40	<0.0001
Progesterone	−0.07	0.14
Prolactin	0.005	0.93
Mammographic density		
Percent density	−0.51	<0.0001
Absolute density	−0.26	<0.0001

NOTE: Data are adjusted for age at blood draw and laboratory batch.

In postmenopausal women, the ovaries cease to produce estrogen and estrogen production occurs primarily in adipose tissue (31). There was a strong positive correlation between BMI and circulating estradiol levels (r = 0.45, P < 0.0001; Table 2) and inverse correlation with circulating SHBG levels (r = −0.40, P < 0.0001; Table 2). BMI was inversely related to percent mammographic density (r = −0.51, P < 0.0001; Table 2). We also examined the relation between both waist-to-hip ratio and weight change since age 18, and circulating hormones and mammographic density. We found similar correlations with these other measures of adiposity, although the relationships were slightly attenuated.

In general, circulating estrogens were inversely related to percent mammographic density when adjusted for age and laboratory batch (Table 3A). The mean percent mammographic density was 25.6% among women in the lowest quartile of circulating estradiol compared with 14.4% among women in the highest quartile [Spearman correlation (r) = −0.22, P < 0.0001; Table 3A]. The partial Spearman correlation coefficient with percent mammographic density was strongest for free estradiol (r = −0.26, P < 0.0001) and non-SHBG bound estradiol (r = −0.28, P < 0.0001). After adjustment for BMI, there was no association between estradiol and breast density; the mean percent mammographic density was 21.6% among women in the lowest quartile of circulating estradiol compared with 20.4% among women in the highest quartile (r = 0.01, P = 0.81). Similar results were observed for other circulating estrogens and percent mammographic density when adjusted for BMI. Inclusion of other predictors of mammographic density did not further affect these results (Table 3A).

We observed no association between circulating testosterone, androstenedione, DHEA, and DHEA sulfate and mammographic density (Table 3B). Free testosterone was inversely associated with percentage mammographic density (r = −0.12, P = 0.008; Table 3B), although adjustment for BMI eliminated this association. SHBG was positively associated with mammographic density (r = 0.21, P < 0.0001; Table 3C). Women in

the lowest quartile of SHBG had a mean breast density of 14.1% compared with women in the highest quartile with a mean mammographic density of 25.6%. Adjustment for BMI dramatically attenuated this relation ($r = 0.008$, $P = 0.86$). There was no relation between circulating progesterone and mammographic density (Table 3C). Prolactin was slightly positively

Table 3.

Model variables	Mean mammographic percent density				Spearman correlation coefficient	P*	$R^{2\dagger}$
	Q1	Q2	Q3	Q4			
A. Mean percent of breast density by quartiles of circulating estrogens among postmenopausal women not on exogenous hormones, Nurses' Health Study (1989-1990)							
Estrone							
Age and batch (n = 505)	23.8	20.2	18.5	15.3	−0.15	0.0005	0.03
+BMI	20.8	18.8	17.9	20.6	0.03	0.55	0.25
+MV‡	20.2	18.7	18.4	20.6	0.05	0.30	0.31
Estradiol							
Age and batch (n = 515)	25.6	19.8	17.8	14.4	−0.22	<0.0001	0.05
+BMI	21.6	18.3	17.4	20.4	0.01	0.81	0.25
+MV‡	21.2	18.4	17.5	20.6	0.02	0.61	0.30
Free estradiol§							
Age and batch (n = 485)	26.6	20.9	16.1	13.9	−0.26	<0.0001	0.06
+BMI	22.0	18.7	16.7	19.7	0.007	0.88	0.25
+MV‡	21.6	18.5	17.3	19.8	0.02	0.68	0.30
Non-SHBG–bound estradiol							
Age and batch (n = 370)	26.5	23.0	16.8	14.6	−0.28	<0.0001	0.06
+BMI	21.8	21.1	17.7	20.3	0.007	0.90	0.26
+MV‡	21.1	21.1	18.2	20.3	0.02	0.71	0.35
Estrone sulfate							
Age and batch (n = 501)	22.6	18.9	20.1	15.4	−0.10	0.02	0.01
+BMI	19.7	18.6	19.6	19.5	0.05	0.22	0.25
+MV‡	19.5	18.8	19.9	19.1	0.06	0.22	0.30
B. Mean percent of breast density by quartiles of circulating androgens among postmenopausal women not on exogenous hormones, Nurses' Health Study (1989-1998)							
DHEA							
Age and batch (n = 488)	19.7	18.8	19.2	18.6	0.04	0.39	0.009
+BMI	20.0	18.7	19.1	18.6	0.03	0.50	0.25
+MV‡	19.9	18.8	18.8	18.8	0.04	0.37	0.29
DHEA sulfate							
Age and batch (n = 512)	19.8	20.2	18.2	19.6	0.009	0.83	0.008
+BMI	20.5	19.8	18.3	19.3	0.02	0.69	0.26
+MV‡	20.0	20.0	18.7	19.3	0.03	0.56	0.31
Testosterone							
Age and batch (n = 505)	20.4	18.3	20.4	17.7	−0.009	0.85	0.007
+BMI	19.4	18.9	20.2	18.3	0.002	0.96	0.25
+MV‡	18.7	19.3	20.2	18.6	0.01	0.83	0.30
Free testosterone§							
Age and batch (n = 485)	21.8	22.3	16.7	16.0	−0.12	0.008	0.01
+BMI	19.5	21.2	17.3	18.8	0.006	0.90	0.25
+MV‡	19.3	20.6	18.2	18.7	0.01	0.75	0.30
Androstenedione							
Age and batch (n = 501)	19.2	19.3	21.4	16.7	−0.013	0.77	0.008
+BMI	19.1	18.4	21.6	17.5	0.03	0.52	0.25
+MV‡	18.9	18.8	21.0	17.9	0.04	0.36	0.30
C. Mean percent of breast density by quartiles of circulating SHBG, progesterone, and prolactin among postmenopausal women not on exogenous hormones, Nurses' Health Study (1989-1998)							
SHBG							
Age and batch (n = 500)	14.1	18.0	20.9	25.6	0.21	<0.0001	0.04
+BMI	18.7	18.2	19.0	22.4	0.008	0.86	0.26
+MV‡	18.3	18.4	18.8	22.7	0.01	0.81	0.30
Progesterone							
Age and batch (n = 441)	19.4	20.7	19.2	19.9	0.02	0.69	0.01
+BMI	19.7	21.6	19.4	19.1	−0.02	0.64	0.29
+MV‡	19.5	20.6	19.4	19.6	−0.001	0.98	0.33
Prolactin							
Age and batch (n = 493)	16.6	19.9	20.3	21.2	0.07	0.11	0.007
+BMI	16.8	19.9	19.9	21.4	0.09	0.04	0.26
+MV‡	17.1	20.0	20.0	20.8	0.07	0.12	0.31

*P value for partial Spearman correlation between hormone and percent mammographic density.
†R^2 based on regression models of continuous hormone measurements regressed on square root–transformed average percent breast density.
‡Adjusted for the following variables at the time of blood collection: age (continuous), laboratory batch, alcohol consumption (none, <5, 5-14.9, and 15+ g/d), age at first birth (nulliparous, age at first birth <25, 25-29, and 30+ years), BMI (continuous), family history of breast cancer (yes/no), history of benign breast disease (yes/no), and prior use of postmenopausal hormones (yes/no).
§Calculated using Sodergard's method.

related to breast density ($r = 0.09$, $P = 0.04$; Table 3C), after adjustment for BMI, although further adjustment for potential confounders attenuated this association ($r = 0.07$, $P = 0.12$; Table 3C). Because use of combined exogenous hormones have been associated with mammographic density to a greater extent than estrogen alone, we also examined the relation between cross-classified levels of estradiol and progesterone and percentage density and observed no consistent relation (data not shown).

The circulating estrogens alone explained 1% to 6% of the variation of mammographic breast density. Circulating androgens, progesterone, and prolactin each explained no more than 1% of the variation of mammographic density. SHBG accounted for 4% of the variation in percentage breast density. Including estradiol, testosterone, SHBG, progesterone, and prolactin all in the same model explained only 9% of the variation in mammographic density. In contrast, BMI alone explained 24% of the variation in mammographic density. Inclusion of BMI in the models with circulating hormones, age, and batch increased the R^2 to 25% to 29% (Table 3A-C). Additional inclusion of other predictors of mammographic density only increased R^2 values to 29% to 35% (Table 3A-C).

We observed similar associations when analyses were limited to women ($n = 271$) with mammograms and blood draw within 1 year of each other. We also examined the association between circulating hormones and absolute area of density (data not shown). The results were less striking for area of density than those with percentage breast density, but the overall interpretation of the results was similar.

Discussion

In contrast to our *a priori* hypothesis, the circulating estrogens measured in this cross-sectional study were inversely related to breast density. After adjustment for BMI, these associations were no longer present. Because the primary source of endogenous estrogens in postmenopausal women is through the aromatization of androgens in adipose tissue, BMI is a strong positive predictor of circulating estrogen levels. In addition, BMI is strongly but inversely correlated with percent breast density, suggesting that the initial inverse correlation between hormone levels and breast density is a reflection of the negative correlation between BMI and breast density. Likewise, we found no significant association between circulating androgens, SHBG, progesterone, or prolactin with mammographic density in postmenopausal women, after adjusting for BMI and other potential confounders.

Only two other smaller studies have examined the association between circulating sex hormones and mammographic density (32, 33). Boyd et al. found an inverse relation between free estradiol and mammographic density (regression coefficient = −0.28, $P \leq 0.001$) among 189 postmenopausal women, which was substantially attenuated after adjustment for age and waist circumference (regression coefficient = −0.09, $P = 0.03$). Aiello et al. (33) conducted a cross-sectional study among 88 obese, postmenopausal women, and also reported inverse associations between endogenous sex hormones and mammographic density among former users of hormone therapy ($n = 43$).

We observed a nonsignificant positive association between circulating prolactin and mammographic density. These results are consistent with the positive association reported by Boyd et al. (32) among postmenopausal women only. Circulating prolactin levels have been associated with modest increases in postmenopausal breast cancer risk (18).

The inverse or lack of association between endogenous hormones and breast density does not parallel the results from numerous studies that examined the effect of hormone replacement therapy on mammographic density (3-9). Combined estrogen and progestin therapies are more likely to be associated with increases in breast density (6, 8, 9) than estrogen alone therapies (9). The Postmenopausal Estrogen/Progestin Intervention Trial, a large randomized trial of hormone replacement therapies, reported a mean change in mammographic percent density over a 1-year period of 5.1% (95% confidence interval, 2.5-6.7) for women taking cyclic conjugated estrogens and progestin, 1.34% (95% confidence interval, −0.05-2.63%) for women on conjugated estrogens only, and −0.50% (95% confidence interval, −1.4-0.5%) for women assigned to placebo (6). Because the Postmenopausal Estrogen/Progestin Intervention study was a large randomized trial, women of different body sizes should be evenly distributed among the treatment arms, and the differences in mammographic density reflect the effect of hormone therapies independent of BMI. A separate study showed that the increase in mammographic density observed after 6 months of combined estrogen and progestogen therapy was associated with concurrent increases in circulating estradiol, estrone, and SHBG ($r = 0.5$, $P < 0.01$; ref. 34).

One potential explanation for the discrepancy between exogenous hormone use and endogenous hormone levels as they relate to mammographic density is that the levels of circulating estrogens in postmenopausal women not on hormone therapy are too low to exhibit an effect. In postmenopausal women, the circulating levels of estrogens are appreciably lower than in premenopausal women (35). The circulating estradiol levels among postmenopausal women taking exogenous hormones are significantly higher than those in postmenopausal women not on hormone replacement therapy (34, 36-38). After administration of hormone therapies, circulating estradiol levels are reported to increase 3- to 6-fold and estrone 5- to 10-fold (38). If a threshold effect exists between sex hormone levels and mammographic density, we may be unable to detect an association in this study. However, the range of endogenous estrogens measured in these postmenopausal women has been associated with at least a 2-fold increase in breast cancer risk (16, 17).

The results of the current study suggest that the biological mechanism by which mammographic density increases breast cancer risk is independent of postmenopausal endogenous estrogen levels. This hypothesis is supported by a recent study reporting that high mammographic density was strongly related to both estrogen receptor−positive breast cancer (adjusted hazard ratio, 2.21; 95% confidence interval, 1.64-3.04) and estrogen receptor−negative breast cancer (adjusted hazard ratio, 2.21; 95% confidence interval, 1.16-4.18; ref. 39). This is in contrast with the relation between circulating estrogens (17), BMI (40), and postmenopausal hormone use (40), all of which increase the risk of estrogen receptor−positive, but not estrogen receptor−negative, breast cancer. A second line of evidence supporting this hypothesis comes from a study reporting that bone mineral density, believed to be a marker of cumulative estrogen exposure (41, 42), was not correlated with Breast Imaging Reporting and Data System breast density or percent mammographic density (43). The study also found that mammographic density was predictive of breast cancer risk independent of bone mineral density, indicating that breast density may influence breast cancer risk through estrogen-independent pathways (43). Clearly, factors other than estrogens may influence breast density.

One potential alternative mechanism mediating the relation between mammographic density and breast cancer is through the insulin-like growth factor or other growth factor pathways. Tamoxifen reduces mammographic density (10-13). Moreover, although it has primarily antiestrogenic effects, it also reduces circulating levels of insulin-like growth factor-I (13). Biopsies from women with dense breasts had greater staining for insulin-like growth factor-I and tissue inhibitor of metalloproteinase-3 (a stromal matrix regulatory protein) than those

with low densities (44). These differences were more pronounced among women younger than 50 years. Similarly, insulin-like growth factor-I levels are positively associated with breast density among premenopausal women in several (30, 32), but not all, studies (45).

A potential limitation of the current study is that we are using circulating levels of hormones as a proxy for the more biologically relevant breast tissue levels. The local estrogen concentrations in benign and malignant breast tissue are higher than plasma levels (46-52); however, the correlation between circulating and tissue has only been reported from a few, small studies with inconsistent results (51, 53, 54). It has been consistently observed that circulating hormone levels in postmenopausal women are predictive of breast cancer risk, suggesting that at least some correlation must exist (14). It is also possible that postmenopausal percent mammographic density may be more reflective of premenopausal circulating levels than postmenopausal levels.

To our knowledge, this large cross-sectional study among postmenopausal women in the Nurses' Health Study is the first study to examine such an extensive list of sex hormones and mammographic density. Because of the prospectively collected data, we were able to control for a number of potential confounding factors.

We found an inverse association between circulating estrogens and mammographic density among postmenopausal women, which was completely explained after adjustment for BMI. Thus, this study indicates that circulating sex hormones and mammographic density act to increase breast cancer risk through independent pathways.

Acknowledgments

We thank participants of the Nurses' Health Study for their outstanding dedication and commitment to the study.

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Cancer Epidemiol Biomarkers Prev 2005;14:2641-2647.