

Circulating Sex Hormones and Mammographic Breast Density among Postmenopausal Women

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Abstract The use of breast density as an intermediate or predictive marker of breast cancer risk is limited by an incomplete understanding of the etiology of breast density. High blood levels of endogenous estrogens and androgens are associated with increased risk of breast cancer among postmenopausal women. We sought to examine whether these hormones are also associated with breast density. The Wisconsin Breast Density Study enrolled 257 postmenopausal women, ages 55–70 years, with no history of postmenopausal hormone use, from mammography clinics in Madison, Wisconsin. Subjects provided a blood sample for sex hormone analysis, and breast density was measured from subjects' screening mammograms using a computer-assisted thresholding method. Numerous sex hormones were associated with breast density in age-adjusted analyses. However, further adjustment for body mass index and other potentially confounding factors substantially attenuated or eliminated

these associations. In the fully adjusted model, there remained a positive association between percent breast density and serum progesterone ($P=0.03$), with percent density rising from 11.9% (95% CI: 9.8, 14.1%) among women in the lowest quartile of serum progesterone to 15.4% (12.9, 18.2%) among women in the highest quartile. There was also a positive association between sex hormone binding globulin and percent breast density ($P=0.06$). In contrast, there were no independent associations between percent breast density and estradiol (total, free, or bioavailable), estrone, estrone sulfate, or testosterone (total, free, or bioavailable). These results suggest that breast density has a hormonal etiology; however, it may differ in important ways from that of breast cancer risk.

Keywords Mammographic breast density · Estrogens · Androgens · Progesterone · Breast cancer

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Introduction

Mammographic breast density is one of the strongest known risk factors for breast cancer [1–4]. A recent meta-analysis estimated that women with density in 75% or more of the mammogram had a 4.6-fold increase in breast cancer risk compared to women with density in less than 5% [5]. Breast density is also associated with many of the same factors that are associated with breast cancer risk, including parity, age at first birth, and use of postmenopausal hormones [2, 4, 6].

These characteristics have led many to consider the use of mammographic breast density as an intermediate marker of breast cancer risk [7–9]. The use of breast density as a surrogate endpoint could enable studies of breast cancer risk that are smaller, less expensive, and of shorter duration [10]. However, certain features of breast density challenge this framework. Body mass index (BMI), a known breast cancer risk factor, is inversely associated with breast density [11, 12]. Additionally, recent evidence suggests that breast density does not mediate the relations between body mass index or postmenopausal hormone use and breast cancer risk [13, 14]. This suggests that the influence of certain risk factors on breast cancer risk may operate through biological pathways independent of breast density.

Most established breast cancer risk factors are believed to influence breast cancer risk through sex hormone pathways [15, 16]. High circulating sex hormone levels have consistently been associated with elevated breast cancer risk [17–19]. Menstrual, reproductive, and lifestyle risk factors have all been associated with increased estrogen exposure and changes in estrogen metabolism [20–22]. While postmenopausal hormone use has been associated with increased breast density [23, 24], the relation between circulating endogenous sex hormone levels and breast density is unclear [8]. To improve our understanding of the etiology of breast density, we examined the relation between circulating endogenous sex hormones and breast density in a population of postmenopausal women. Since exogenous hormone use could obscure this relation, the study was restricted to women who had never used postmenopausal hormones.

Materials and Methods

The Wisconsin Breast Density Study is a cross-sectional study of postmenopausal women receiving a screening mammogram in Madison, WI. This study was approved by the University of Wisconsin Health Sciences Institutional Review Board. All subjects provided written informed consent.

Study Population

Eligible participants for this study were postmenopausal women, aged 55–70 years, attending the UW Health West-Madison Clinic or UW Health Breast Center to receive a screening mammogram. Postmenopausal status was defined as having no menstrual cycles within the past 12 months. Women who reported a previous diagnosis of breast cancer, breast implants, or having ever used postmenopausal hormone therapy were not eligible for participation in the study. All participants were included in study analyses regardless of the results of their screening mammogram.

A letter of invitation for this research study was sent through the mail to women aged 55–70 years who were due for their annual mammogram at the UW Health West-Madison Clinic or UW Health Breast Center. In the letter, women were informed of the study and provided with the contact information for the University of Wisconsin Office of Clinical Trials (UW OCT) research specialist coordinating recruitment. Additionally, pamphlets inviting women to participate were provided at check-in to all women attending the screening mammography clinics whose electronic medical record data did not meet any of the exclusion criteria. During the screening mammogram, the technician inquired with each potentially eligible woman to determine if she was interested in participating in the study and whether she met the eligibility criteria. For interested and eligible women, a research specialist then provided a written description of the general nature of study and the study requirements, confirmed that the woman met the eligibility criteria, and answered any questions. Signed informed consent and Health Insurance Portability and Accountability Act forms were obtained by the research specialist. As a recruitment incentive, participants were offered \$25 which was paid upon completing the study.

A total of 268 subjects were recruited between June 25, 2008 and July 3, 2009. During the recruitment period, 7,611 women attended the clinics for screening mammograms. Of these, 3,201 (42.1%) were between the ages of 55 and 70 years. Of these age-eligible women, 1,302 (40.7%) were deemed potentially eligible based on a review of their electronic medical record. Of these, 268 were enrolled (20.6%). The number of women who were truly eligible is unknown; many women may have determined that they were ineligible after receiving the pamphlet (e.g., if they were premenopausal or had previously taken postmenopausal hormones not included in their UW Health medical record).

Data Collection

Following the subject's mammogram and consent to participate in the study, a questionnaire was administered to the subject and a blood sample was obtained.

Table 1 Characteristics of study participants ($N=257$), Madison, Wisconsin, 2008–2009

	<i>N</i>	Percent	Age-adjusted mean% density ^a (95% CI)	Multivariable-adjusted ^b mean% density ^a (95% CI)
Age				
55–59	133	51.8	15.3 (13.3, 17.4) ^c	14.8 (13.2, 16.6)
60–64	71	27.6	11.1 (8.9, 13.6) ^c	11.7 (9.7, 13.9)
65–70	53	20.6	11.3 (8.7, 14.2) ^c	11.5 (9.3, 14.1)
			$P_{\text{trend}}=0.01$	$P_{\text{trend}}=0.01$
Body mass index (kg/m²)				
<18.5	2	0.8	31.0 (13.6, 15.5)	28.9 (12.5, 51.9)
18.5–24.9	78	30.4	21.4 (18.7, 24.3)	20.7 (18.1, 23.5)
25.0–29.9	81	31.5	12.0 (10.0, 14.1)	12.4 (10.5, 14.5)
≥30.0	94	36.6	8.7 (7.2, 10.4)	8.8 (7.3, 10.5)
Missing	2	0.8	–	–
			$P_{\text{trend}}<0.0001$	$P_{\text{trend}}<0.0001$
Age at menarche				
≤11	61	23.7	10.9 (8.6, 13.6)	12.4 (10.1, 14.8)
12–13	149	58.0	12.8 (11.2, 14.6)	13.0 (11.5, 14.5)
14+	47	18.3	18.1 (14.7, 22.0)	15.4 (12.6, 18.5)
			$P_{\text{trend}}=0.002$	$P_{\text{trend}}=0.13$
Parity				
0	65	25.3	15.5 (12.7, 18.6)	14.7 (12.4, 17.3)
1	33	12.8	13.5 (9.9, 17.6)	15.4 (12.1, 19.2)
2	92	35.8	12.5 (10.4, 14.8)	12.3 (10.5, 14.3)
3+	67	26.1	12.0 (9.6, 14.7)	12.1 (10.1, 14.4)
			$P_{\text{trend}}=0.06$	$P_{\text{trend}}=0.05$
Age at first birth^d				
<24	73	38.0	10.5 (8.4, 12.9)	11.8 (9.8, 14.0)
24–29	69	35.9	14.5 (11.9, 17.4)	13.4 (11.2, 15.7)
30	50	26.0	12.6 (9.8, 15.7)	12.0 (9.5, 14.8)
			$P_{\text{trend}}=0.20$	$P_{\text{trend}}=0.82$
Age at menopause				
<50	67	26.1	13.0 (10.5, 15.8)	13.5 (11.3, 15.9)
50–54	137	53.3	13.4 (11.6, 15.4)	13.3 (11.8, 15.0)
55+	48	18.7	13.3 (10.3, 16.6)	12.7 (10.2, 15.6)
Missing	5	2.0	–	–
			$P_{\text{trend}}=0.86$	$P_{\text{trend}}=0.70$
Breastfeeding duration^d				
Never	52	2.1	10.5 (8.0, 13.3)	11.9 (9.6, 14.4)
≤1 year	79	27.1	12.0 (9.8, 14.4)	11.7 (9.8, 13.7)
>1 year	57	41.2	15.1 (12.1, 18.3)	14.0 (11.6, 16.7)
Missing	4	29.7	–	–
			$P_{\text{trend}}=0.03$	$P_{\text{trend}}=0.25$
Oral contraceptive use				
Never	52	20.2	13.9 (10.9, 17.2)	13.6 (11.0, 16.5)
<10 years	149	58.0	12.7 (11.0, 14.5)	13.2 (11.7, 14.7)
≥10 years	55	21.4	14.0 (11.2, 17.2)	13.1 (10.7, 15.7)
Missing	1	0.4	–	–
			$P_{\text{trend}}=0.92$	$P_{\text{trend}}=0.79$
Alcohol consumption (drinks/week)				
None	93	36.2	13.0 (10.8, 15.3)	13.3 (11.4, 15.3)

Table 1 (continued)

	<i>N</i>	Percent	Age-adjusted mean% density ^a (95% CI)	Multivariable-adjusted ^b mean% density ^a (95% CI)
<5 per week	122	47.5	13.6 (11.7, 15.7)	13.2 (11.6, 15.0)
≥5 per week	31	12.1	13.1 (9.4, 17.3)	13.2 (10.0, 16.8)
Missing	11	4.3	–	–
			$P_{\text{trend}}=0.83$	$P_{\text{trend}}=0.95$
Vigorous physical activity (h/week)				
0–1.0	76	29.6	11.5 (9.3, 13.9)	12.4 (10.4, 14.5)
1.1–4.0	90	35.0	12.3 (10.3, 14.6)	12.3 (10.4, 14.2)
>4.0	91	35.4	15.8 (13.4, 18.3)	15.1 (13.0, 17.2)
			$P_{\text{trend}}=0.01$	$P_{\text{trend}}=0.07$
Pack years of smoking				
None	156	60.7	15.0 (13.2, 16.8)	14.3 (12.8, 15.9)
1–15	47	18.3	11.7 (8.9, 14.8)	12.8 (10.2, 15.6)
>15	44	17.1	9.6 (7.0, 12.5)	10.3 (8.0, 13.0)
Missing	10	3.9	–	–
			$P_{\text{trend}}=0.002$	$P_{\text{trend}}=0.01$
First degree family history of breast cancer				
No	197	76.7	14.1 (12.6, 15.8)	13.9 (12.6, 15.3)
Yes	60	23.4	10.6 (8.2, 13.2)	11.1 (9.0, 13.4)
			$P_{\text{trend}}=0.02$	$P_{\text{trend}}=0.04$
Education				
High school	46	17.9	10.1 (7.5, 13.1)	12.4 (9.8, 15.4)
Some college	58	22.6	11.6 (9.1, 14.4)	13.4 (11.0, 16.1)
College diploma	75	29.2	15.2 (12.6, 17.9)	14.3 (12.1, 16.6)
Advanced degree	77	30.0	14.4 (12.0, 17.1)	12.6 (10.5, 14.9)
Missing	1	0.4	–	–
			$P_{\text{trend}}=0.01$	$P_{\text{trend}}=0.93$

CI, confidence interval

^a Mean percent density displayed is reverse transformed from model of square root density

^b Adjusted for age (continuous), body mass index (continuous), parity (0, 1, 2, 3+), vigorous physical activity (0–1.0, 1.1–4.0, >4.0 h/week), pack years of smoking (None, 1–15, >15), and family history of breast cancer (No, Yes)

^c Mean density values by age group are not age-adjusted

^d Among parous women only

Questionnaire The questionnaire included items known or suspected to be associated with breast cancer or mammographic density, including age, height, weight, age at menarche, age at first pregnancy, parity, age at menopause, first degree family history of breast cancer, alcohol consumption, physical activity, lactation history, smoking, and education level. Alcohol consumption was ascertained as the number of bottles or cans of beer, glasses of wine, and drinks of hard liquor consumed per day, week, or month. Physical activity was assessed as the average number of hours per week spent doing physically vigorous activities that cause large increases in heart rate or breathing, such as participating in sports activities, climbing stairs, heavy gardening, or lifting/carrying heavy objects. To assess smoking history, women reported whether they

had smoked more than 100 cigarettes in their lifetime. For those reporting more than 100 cigarettes, we recorded the age she started smoking, the age she stopped (if applicable), and the average number of cigarettes or packs of cigarettes she smoked per day over the entire time she was smoking.

Blood Analyses A trained phlebotomist from the UW OCT obtained a 30-mL whole blood sample from the participant via venipuncture, collected in three uncoated glass 10-mL red top vacutainer tubes (no anticoagulant; Fisher Scientific, Pittsburgh, Pennsylvania). Blood samples were allowed to clot for 30 min, then spun down in a centrifuge at 2,500 rpm for 20 min. Serum was aliquoted in 2-mL aliquots frozen at -70°C . Sex hormone analyses were

conducted by Dr. Frank Stanczyk at the Reproductive Endocrine Research Laboratory at the University of Southern California. Progesterone, testosterone, estrone, and estradiol were quantified by validated, previously described radioimmunoassays (RIAs) [25–27]. Estrone sulfate was measured by direct RIA using a commercial kit (Beckman Coulter Diagnostic Systems Laboratories, Webster, Texas). Sex hormone binding globulin (SHBG) was quantified by direct chemiluminescent immunoassay using the Immulite analyzer (Siemens Medical Solutions Diagnostics, Malvern, Pennsylvania). The inter-assay coefficients of variation ranged between 7% and 12%. The assay sensitivities for progesterone, testosterone, estrone, estradiol, SHBG, and estrone sulfate are 0.02 ng/ml, 1.5 ng/dL, 4 pg/ml, 2 pg/ml, 1 nM, and 0.025 ng/ml, respectively. Free and bioavailable (non-SHBG-bound) testosterone and estradiol were calculated using the measured total testosterone and estradiol levels, SHBG concentrations, and an average assumed concentration for albumin [28, 29]. This method has been found to have high validity, with Pearson's correlation coefficients ≥ 0.80 between free testosterone or free estradiol values calculated using this method and the values measured by dialysis-based methods [30].

Mammographic Density Participants underwent their normally scheduled screening mammogram. All women received a digital mammogram conducted on either a Senographe 2000D (GE Medical Systems) or a Clearview CSm2 CR (Fujifilm Corporation) machine. Digital copies of the craniocaudal view of the left breast were obtained for the quantitative analysis of mammographic breast density using a computer-assisted thresholding method. "Cumulus" software [31] was used to measure percent breast density as a continuous variable. All density measurements were done

by the same reader (EJAB), who was blinded in respect to serum hormone levels and all identifying information. A 10% sample was read twice for quality control purposes. Reliability for the continuous density measure was assessed by the intraclass correlation coefficient [32]. We found excellent reliability, with intraclass correlation coefficients of 0.98, 0.99, and 0.92 for percent density, total breast area, and dense area, respectively. Lower 95% confidence limits for these measures were 0.96, 0.99, and 0.86, respectively.

Statistical Analyses

Three women had insufficient serum available for hormone analyses. There were eight women (all under 60 years old) with estradiol levels greater than 35 pg/mL, suggesting that they were not truly postmenopausal. Exclusion of these 11 women left a total of 257 samples available for analysis. Testosterone levels were missing for three women and certain covariates were missing data for a small fraction of subjects (see Table 1). Multiple imputation was used to impute missing testosterone levels and covariate data. Ten imputations were conducted using the Markov Chain Monte Carlo method [33]. The imputation model contained percent breast density and all variables listed in Tables 1 and 2, except for free and bioavailable estradiol/testosterone values, as these are derived from total estradiol/testosterone and SHBG values. For subsequent analyses, each model was fit separately to the ten imputed datasets and their results combined for statistical inferences using the methods of Rubin [34].

Spearman correlation coefficients were calculated to assess the association between each serum sex hormone and body mass index. Multivariable linear regression was used to assess the association between serum sex hormone

Table 2 Distribution of circulating hormone levels in study participants ($N=257$), Madison, Wisconsin, 2008–2009

	Mean	Standard deviation	Q1	Median	Q3	Range	Correlation ^a with BMI (P value)
Estradiol (pg/mL)	10.1	5.8	6.0	8.8	12.4	2.8, 33.9	0.49 (<0.0001)
Free estradiol (pg/mL)	0.27	0.17	0.14	0.23	0.34	0.03, 0.98	0.57 (<0.0001)
Bioavailable estradiol (pg/mL)	6.8	4.4	3.6	5.9	8.7	0.7, 24.8	0.57 (<0.0001)
Estrone (pg/mL)	29.3	14.2	19.1	26.1	36.9	6.0, 86.4	0.37 (<0.0001)
Estrone sulfate (ng/mL)	0.73	0.29	0.54	0.68	0.88	0.21, 2.00	0.18 (0.004)
Testosterone (ng/dL) ^b	22.8	11.5	14.4	19.7	28.0	4.0, 81.6	0.01 (0.84)
Free testosterone (pg/mL) ^b	4.7	2.5	2.9	4.1	6.0	0.8, 16.2	0.24 (<0.001)
Bioavailable testosterone (ng/dL) ^b	11.5	6.1	7.2	10.2	14.8	1.9, 39.8	0.24 (<0.001)
Progesterone (pg/mL)	48.5	23.5	33.0	44.7	59.2	8.0, 181.1	-0.18 (0.004)
Sex hormone binding globulin (nmol/L)	44.6	23.3	29.7	40.1	54.2	5.2, 197.0	-0.54 (<0.0001)

Q1 first quartile; Q3 third quartile; BMI body mass index

^a Spearman correlation coefficient

^b Testosterone values were missing for three subjects

Table 3 The association between sex hormones and mammographic breast density (N=257), Madison, Wisconsin, 2008–2009

Hormone	Linear regression of square root percent breast density on hormone levels		P ^b	Mean percent density by hormone quartile ^a				P _{trend} ^b
	β coefficient	SE (β)		Q1	Q2	Q3	Q4	
Estradiol (pg/mL)								
Age-adjusted	-0.0715	0.0158	<0.0001	16.7 (14.1, 19.6)	13.9 (11.4, 16.8)	13.7 (11.2, 16.4)	8.2 (6.1, 10.5)	<0.0001
Age and BMI adjusted	-0.0100	0.0169	0.55	13.8 (11.6, 16.3)	13.6 (11.3, 16.2)	14.1 (11.8, 16.7)	11.1 (8.7, 13.8)	0.25
Multivariable-adjusted ^c	-0.0102	0.0167	0.54	13.9 (11.6, 16.3)	13.6 (11.3, 16.1)	13.9 (11.6, 16.4)	11.3 (8.9, 13.9)	0.26
Free estradiol (pg/mL)								
Age-adjusted	-2.50	0.523	<0.0001	19.1 (16.2, 22.3)	12.7 (10.4, 15.3)	12.7 (10.4, 15.4)	8.8 (6.7, 11.1)	<0.0001
Age and BMI adjusted	-0.25	0.584	0.67	15.4 (12.8, 18.3)	11.9 (9.8, 14.3)	13.6 (11.3, 16.1)	12.1 (9.6, 14.8)	0.22
Multivariable-adjusted ^c	-0.35	0.574	0.54	15.4 (12.8, 18.1)	12.0 (9.9, 14.2)	13.6 (11.3, 16.0)	12.1 (9.7, 14.8)	0.22
Bioavailable estradiol (pg/mL)								
Age-adjusted	-0.0985	0.0206	<0.0001	19.5 (16.6, 22.6)	12.3 (10.0, 14.9)	12.7 (10.4, 15.3)	8.8 (6.7, 11.1)	<0.0001
Age and BMI adjusted	-0.0099	0.0230	0.66	15.8 (13.1, 18.6)	11.6 (9.5, 13.9)	13.6 (11.3, 16.1)	12.0 (9.5, 14.7)	0.17
Multivariable-adjusted ^c	-0.0137	0.0226	0.54	15.7 (13.2, 18.5)	11.7 (9.6, 13.9)	13.6 (11.3, 16.0)	12.0 (9.6, 14.7)	0.17
Estrone (pg/mL)								
Age-adjusted	-0.0174	0.00661	0.01	16.1 (13.4, 19.1)	12.0 (9.6, 14.6)	14.2 (11.6, 17.0)	10.5 (8.2, 13.2)	0.02
Age and BMI adjusted	0.0047	0.00652	0.47	13.7 (11.4, 16.2)	11.2 (9.2, 13.5)	14.6 (12.2, 17.2)	13.6 (11.0, 16.4)	0.60
Multivariable-adjusted ^c	0.0041	0.00638	0.52	13.6 (11.4, 16.0)	11.5 (9.5, 13.8)	14.4 (12.1, 16.9)	13.5 (11.0, 16.2)	0.66
Estrone sulfate (ng/mL)								
Age-adjusted	-0.447	0.325	0.17	14.2 (11.5, 17.2)	14.6 (11.9, 17.5)	12.7 (10.2, 15.4)	11.6 (9.2, 14.3)	0.11
Age and BMI adjusted	-0.049	0.292	0.87	13.0 (10.7, 15.5)	14.5 (12.1, 17.1)	12.8 (10.6, 15.2)	12.7 (10.4, 15.2)	0.65
Multivariable-adjusted ^c	-0.070	0.284	0.81	13.4 (11.1, 15.9)	14.1 (11.8, 16.6)	12.6 (10.5, 15.0)	12.9 (10.6, 15.3)	0.56
Testosterone (ng/dL)								
Age-adjusted	-0.00974	0.00823	0.24	12.6 (10.1, 15.4)	15.1 (12.3, 18.1)	14.0 (11.4, 17.0)	11.5 (9.1, 14.1)	0.46
Age and BMI adjusted	-0.00810	0.00730	0.27	13.0 (10.8, 15.5)	14.1 (11.7, 16.8)	14.0 (11.7, 16.6)	11.9 (9.7, 14.3)	0.51
Multivariable-adjusted ^c	-0.00403	0.00719	0.58	12.6 (10.4, 14.9)	14.1 (11.7, 16.7)	13.9 (11.6, 16.5)	12.5 (10.3, 14.9)	0.96
Free testosterone (pg/mL)								
Age-adjusted	-0.0971	0.0384	0.01	15.9 (13.1, 19.1)	14.5 (11.9, 17.5)	12.5 (10.0, 15.2)	10.3 (8.1, 12.9)	0.003
Age and BMI adjusted	-0.0387	0.0349	0.27	14.3 (11.8, 17.0)	14.2 (11.8, 16.7)	13.2 (11.0, 15.7)	11.4 (9.2, 13.7)	0.08
Multivariable-adjusted ^c	-0.0253	0.0340	0.46	13.7 (11.3, 16.2)	14.1 (11.8, 16.5)	13.6 (11.4, 16.1)	11.7 (9.5, 14.0)	0.23
Bioavailable testosterone (ng/dL)								
Age-adjusted	-0.0396	0.0156	0.01	16.1 (13.2, 19.2)	14.4 (11.7, 17.3)	12.5 (10.0, 15.2)	10.3 (8.1, 12.9)	0.002
Age and BMI adjusted	-0.0158	0.0142	0.27	14.5 (12.0, 17.1)	14.0 (11.6, 16.6)	13.2 (11.0, 15.7)	11.4 (9.2, 13.7)	0.07
Multivariable-adjusted ^c	-0.0103	0.0139	0.46	13.8 (11.5, 16.3)	13.9 (11.6, 16.4)	13.6 (11.4, 16.1)	11.7 (9.5, 14.0)	0.21
Progesterone (pg/mL)								
Age-adjusted	0.0138	0.0040	0.0006	10.5 (8.4, 13.0)	11.6 (9.3, 14.2)	14.7 (12.1, 17.6)	17.1 (14.0, 20.4)	<0.001

Table 3 (continued)

Hormone	Linear regression of square root percent breast density on hormone levels		Mean percent density by hormone quartile ^a				P_{trend}^b	
	β coefficient	SE (β)	P^b	Q1	Q2	Q3		Q4
Age and BMI adjusted	0.0096	0.0036	0.007	11.7 (9.7, 14.0)	11.7 (9.6, 14.0)	14.2 (11.9, 16.7)	15.9 (13.3, 18.7)	0.01
Multivariable-adjusted ^c	0.0075	0.0035	0.03	11.9 (9.8, 14.1)	12.0 (9.9, 14.3)	14.1 (11.8, 16.6)	15.4 (12.9, 18.2)	0.02
Sex hormone binding globulin (nmol/L)								
Age-adjusted	0.0192	0.00390	<0.0001	10.8 (8.5, 13.3)	12.4 (10.1, 15.1)	12.2 (9.8, 14.9)	18.3 (15.3, 21.6)	0.001
Age and BMI adjusted	0.0068	0.00400	0.09	13.9 (11.4, 16.5)	14.6 (12.2, 17.2)	10.5 (8.4, 12.7)	14.3 (11.7, 17.1)	0.70
Multivariable-adjusted ^c	0.0073	0.00389	0.06	13.5 (11.1, 16.1)	14.5 (12.2, 17.0)	10.6 (8.7, 12.8)	14.6 (12.1, 17.3)	0.98

SE standard error; BMI body mass index

^a Mean percent density displayed is reverse transformed from model of square root density

^b P refers to the statistical test of the continuous hormone variable; P_{trend} refers to the test for the ordinal variable representing hormone quartile in a separate model

^c Adjusted for age (continuous), body mass index (continuous), parity (0, 1, 2, 3+), vigorous physical activity (0–1.0, 1.1–4.0, >4.0 h/week), pack years of smoking (None, 1–15, >15), and family history of breast cancer (No, Yes)

levels and the square root of percent breast density (measured continuously), while adjusting for age, body mass index, and other variables. Percent density was square root transformed to improve the normality of the data, as the distribution of percent density was positively skewed. Potential confounders which were considered included all variables listed in Table 1. A reverse stepwise procedure was used to select variables for inclusion in the final model. Beginning with a full multivariable model including all potential confounders listed in Table 1, covariates were sequentially removed based on the largest P value. All remaining covariates significantly associated with percent density at the $P < 0.10$ level were selected for inclusion in the final multivariable model. These included age, body mass index, parity, vigorous physical activity, pack years of smoking, and family history of breast cancer. These covariates were parameterized in the models as displayed in Table 1, with the exception of age and body mass index which were included as continuous variables. In separate models, adjusted least-squares mean levels of square root percent density were calculated according to quartiles of each sex hormone level [35]. These mean values were reverse transformed for display purposes. Tests of trends across hormone quartile groups were conducted by inclusion of hormone quartile as an ordinal term in the regression models. Wald tests for effect modification by body mass index (measured continuously) and interactions between hormones were conducted by including cross-product interaction terms in the regression models. All statistical analyses were performed using SAS Statistical Software (Version 9; SAS Institute, Inc., Cary, North Carolina).

Results

Selected characteristics of the study subjects are shown in Table 1. The mean age of participants was 60.7 years (standard deviation, 4.4). Eighty-one percent of the participants reported a natural menopause, whereas 15% reported a surgical menopause (3% reported other causes). Approximately 32% of participants were overweight ($25 \leq \text{BMI} < 30 \text{ kg/m}^2$) and 37% were obese ($\text{BMI} \geq 30 \text{ kg/m}^2$). Median percent breast density among the study subjects was 11.3% (mean 15.6, standard deviation 13.1, range 0.4–71.2). Age, BMI, parity, pack years of smoking, and a family history of breast cancer were inversely associated with percent density in the multivariable-adjusted model.

The distribution of sex hormone concentrations is displayed in Table 2. Given the strong association between BMI and breast density, we examined the correlation of each hormone with BMI. There were strong positive correlations between BMI and total, free, and bioavailable

estradiol ($r=0.49-0.57$), and a strong negative correlation between BMI and SHBG ($r=-0.54$). Moderate positive correlations were observed for estrone, estrone sulfate, and free and bioavailable testosterone ($r=0.18-0.37$) and a moderate inverse correlation for progesterone ($r=-0.18$). There was no correlation between total testosterone and BMI ($r=0.01$).

Table 3 displays the relation between each hormone and percent breast density. In age-adjusted analyses, breast density was lower among women with higher estradiol, free estradiol, bioavailable estradiol, estrone, free testosterone, and bioavailable testosterone levels ($P<0.05$). Breast density was higher among women with higher progesterone and SHBG levels ($P<0.001$). Additional adjustment for BMI largely attenuated these associations, with progesterone the only hormone which remained significantly associated with percent density ($P=0.007$). Further adjustment for other potential confounders had a relatively minor effect on the regression coefficients. In the final multivariable-adjusted model, mean percent density was 15.4% among women in the highest quartile of progesterone, compared to 11.9% among women in the lowest quartile. This positive association did not appear to vary by body mass index ($P_{\text{interaction}}=0.92$). There was also a positive association between SHBG and percent density in the multivariable-adjusted model, though it was of borderline statistical significance ($P=0.06$). There was no clear pattern in percent density when women were categorized by quartile of SHBG ($P_{\text{trend}}=0.98$).

Since combined progesterone and estrogen hormone therapy is known to raise breast cancer risk, we examined the potential interaction between progesterone and estrogen levels in their association with breast density. Breast density was most elevated among women with high progesterone and low total or free estradiol levels (Table 4). There was no statistically significant interaction between progesterone and total or free estradiol ($P_{\text{interaction}}>0.8$). Within each quartile of total or free estradiol concentration, the magnitude and direction of the association between progesterone and breast density was similar.

Discussion

We found that all examined hormones, with the exception of total testosterone and estrone sulfate, were associated with percent breast density in our cohort of postmenopausal women with no history of postmenopausal hormone use. These associations were largely eliminated after adjusting for body mass index and other confounding variables. However, after full adjustment, there remained a positive association between progesterone and percent breast density, and the suggestion of a positive association between percent breast density and SHBG. These findings provide evidence that breast density is related to endogenous hormone levels, though this etiology may differ in important ways from that of breast cancer.

Our observations of age-adjusted associations between circulating sex hormones and percent breast density in

Table 4 Percent breast density according to estrogen and progesterone levels ($N=257$), Madison, Wisconsin, 2008–2009

	Linear regression of square root percent breast density on progesterone levels			Mean percent density ^{a,b} by progesterone quartile			
	β coefficient ^b	SE (β)	P	<33.0 pg/mL	33.0–44.6 pg/mL	44.7–59.1 pg/mL	≥ 59.2 pg/mL
Estradiol quartile							
<6.0 pg/mL	0.0055	0.0102	0.59	13.7 (11.7, 15.8)	12.2 (10.2, 14.2)	14.1 (11.8, 16.6)	19.9 (16.3, 23.8)
6.0–8.7 pg/mL	0.0109	0.0073	0.14	11.7 (9.6, 14.0)	13.8 (11.6, 16.1)	14.3 (11.8, 17.2)	15.5 (12.8, 18.5)
8.8–12.3 pg/mL	0.0083	0.0069	0.23	9.8 (7.8, 12.0)	13.5 (10.2, 17.1)	15.0 (13.0, 17.2)	16.4 (14.0, 19.0)
≥ 12.4 pg/mL	0.0118	0.0054	0.03	10.6 (8.2, 13.4)	9.2 (7.3, 11.4)	12.0 (9.2, 15.3)	12.6 (10.4, 14.9)
$P_{\text{interaction}}=0.84$							
Free estradiol quartile							
<0.14 pg/mL	0.0083	0.0080	0.30	14.5 (12.2, 16.9)	14.5 (12.0, 17.1)	15.3 (12.9, 17.8)	20.2 (16.8, 23.8)
0.14–0.22 pg/mL	0.0102	0.0101	0.31	10.4 (8.6, 12.4)	11.3 (9.4, 13.4)	13.3 (10.8, 16.0)	14.7 (12.0, 17.6)
0.23–0.33 pg/mL	0.0057	0.0083	0.49	11.0 (8.8, 13.4)	12.9 (10.5, 15.6)	14.2 (12.0, 16.5)	15.6 (13.1, 18.2)
≥ 0.33 pg/mL	0.0104	0.0054	0.05	10.6 (8.3, 13.3)	9.9 (7.9, 12.2)	13.7 (11.0, 16.7)	13.3 (11.1, 15.8)
$P_{\text{interaction}}=0.85$							

^a Percent density displayed is reverse transformed from model of square root density

^b Adjusted for age (continuous), body mass index (continuous), parity (0, 1, 2, 3+), vigorous physical activity (0–1.0, 1.1–4.0, >4.0 h/week), pack years of smoking (None, 1–15, >15), and family history of breast cancer (No, Yes)

postmenopausal women are similar to those reported by the majority of previous studies [36–39]. However, as is typically observed [8], body mass index was strongly associated with both breast density and most sex hormones in our study population. We found that further adjustment for body mass index substantially attenuated or eliminated most of the associations between sex hormones and breast density. These results are consistent with the findings of a recent review by Becker and Kaaks [8] of eight previous studies in postmenopausal women, which reported that the majority of studies have observed no relation between percent density and serum estrogens, testosterone, estrone, and estrone sulfate after adjustment for BMI.

Relatively few studies have examined the relation between progesterone and breast density. Whereas three studies reported a null association [36, 37, 39], Greendale et al. [40] found a positive association, with an absolute difference in percent breast density of about 5 percentage points between the lowest and highest quintiles of progesterone. We observed a difference of about 3.5 percentage points between the lowest and highest quartiles, and about 4.5 percentage points between the lowest and highest quintiles. Each of the previous studies included former users of postmenopausal hormones, which may potentially account for variation in their findings. Multiple lines of evidence suggest that progesterone may promote breast epithelial cell proliferation and carcinogenesis. Proliferative activity in normal breast tissue is highest in the luteal phase of the menstrual cycle when progesterone levels are highest [20]. *In vitro* studies have reported a variety of effects of progestins on mammary cells [41], including increased cell proliferation in cultured cells from mouse mammary tumors [42]. Finally, the Women's Health Initiative randomized trials have demonstrated that addition of progestin to estrogen in postmenopausal hormone formulations is associated with elevated breast cancer risk [43], whereas estrogen alone was not associated with increased risk [44]. Few studies have directly examined the relation between circulating progesterones and breast cancer risk in postmenopausal women. In the Nurses' Health Study, baseline progesterone levels were not associated with breast cancer risk among postmenopausal women in a nested case–control study [18].

A number of previous studies have reported a positive association between SHBG and percent breast density after adjusting for BMI [36, 38–40, 45], though null relations have also been reported [37, 46, 47]. We observed a borderline significant positive association between percent breast density and SHBG. Notably, however, there did not appear to be a clear dose–response pattern in percent density according to quartile of SHBG. Additionally, there was no relation between bioavailable (non-SHBG bound) estradiol or testosterone and breast density. Thus, the

mechanism by which SHBG may influence breast density is unclear.

A number of limitations should be considered in the interpretation of this study. Since all women were recruited at screening mammography clinics in Madison, Wisconsin, the generalizability of the results may be limited. Ninety-seven percent ($N=249$) of subjects reported white race. A single blood draw for each subject was used for the measurement of sex hormone levels. The hormone level at the time of enrollment in the study may not be representative of long-term exposure to that hormone. However, the Nurses Health Study has found intraclass correlation coefficients of 0.7–0.8 for most sex hormones measured up to 3 years apart on the same women [48]. Single measures of sex hormone levels in postmenopausal women have been adequate to reveal an association with breast cancer risk [17]. Further, a single measurement is likely more representative of long-term hormone levels among women, such as those in our study, who have never used postmenopausal hormones. Nonetheless, misclassification due to a single measure would tend to bias our results towards a null finding. Thus, this is unlikely to explain the positive association we observed between progesterone and breast density. Our study has a relatively small sample size, and power was insufficient to rule out potential interactions or detect small differences in breast density according to sex hormone levels. However, the power of the study was maximized by using a continuous measure of breast density with high reliability, and there was sufficient power to detect clinically significant differences in breast density. Furthermore, to increase our sensitivity to differences in endogenous hormone levels, the study population was restricted to women who had never used postmenopausal hormones.

Multiple imputations were used to impute the small numbers of missing testosterone and covariate data. Sensitivity analyses in which subjects with missing testosterone values were excluded revealed a negligible impact upon the results.

Our results suggest that the hormonal etiology of breast density may differ from that of breast cancer. Increased levels of circulating estrogens and androgens have consistently been associated with elevated breast cancer risk [17]. For instance, women in the highest quartile of total or free estradiol levels have a twofold increased risk of breast cancer compared to women in the lowest quartile [17]. Yet, our data adds further evidence that circulating endogenous androgens and estrogens are not strongly related to breast density. Interestingly, Tamimi et al. [49] recently reported that the association between circulating sex hormone levels and breast cancer risk was not attenuated by adjustment for mammographic breast density, suggesting that they are independent risk factors. Thus, it appears that breast density

may not be an appropriate surrogate marker for studying breast cancer risk factors that operate through estrogen or androgen pathways.

In summary, our results indicate that the age-adjusted associations between most circulating sex hormones and breast density are eliminated after adjustment for BMI and other potential confounders. After adjustment for BMI, only progesterone and SHBG were associated with mammographic breast density among our population of postmenopausal women who had never used postmenopausal hormones. Continued research will be necessary to understand the biological mechanisms through which sex hormones and breast density influence breast cancer risk. As progesterone was the only hormone associated with breast density in this study, our results suggest that increased attention to this hormone in particular is warranted.

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Conflict of Interest The authors declare that they have no conflict of interest.

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