Associations among Circulating Sex Hormones, Insulin-Like Growth Factor, Lipids, and Mammographic Density in Postmenopausal Women

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Abstract

Objective: **Hormone therapy use has been positively associated with mammographic density in several studies. However, few studies have examined the association between endogenous hormone levels and mammographic density. Therefore, we evaluated the relationship of endogenous sex hormones, insulin-like growth factor (IGF), and lipids with mammographic density in 88 overweight, postmenopausal women not taking hormone therapy.**

Methods: **Percent density and dense area were evaluated as continuous measures using a computer-assisted program. We used multiple linear regression to evaluate the associations of sex hormones, IGF, and cholesterol with mammographic density, adjusting for confounders, including adiposity. We evaluated stratification by history of hormone therapy use (former versus never) and hormone therapy latency (<5 versus ≥5 years).**

Results: **Among former hormone therapy users, mammographic density was inversely associated with circulating levels of estrone (P = 0.01), estradiol (P = 0.003), free estradiol (P = 0.004), testosterone (P = 0.04), free testosterone (P = 0.02), androstenedione (P < 0.001), dehydroepiandrosterone (P = 0.01), and the ratio of IGF-I to its binding protein (IGF-I/IGFBP-3; P = 0.04). We found similar associations when we limited the analyses to women who had used hormone therapy within the past 5 years. We also noted positive associations of mammographic density with total cholesterol (P = 0.03) and low-density lipoprotein (P = 0.03) among former hormone therapy users. No associations were noted among women who had never used hormone therapy.**

Conclusions: **These results suggest that there is an inverse relationship between endogenous sex hormones and mammographic density in postmenopausal women among former users of hormone therapy. This is not consistent with the hormone therapy literature and should be confirmed in larger studies.** (Cancer Epidemiol Biomarkers Prev 2005;14(6):1411–7)

Introduction

Mammographic density refers to the proportion of white-appearing areas on a mammogram that are thought to represent glandular and stromal tissue (1). High levels of mammographic density have been associated with a 4- to 6-fold increase in breast cancer risk (2-6). However, the pathway through which this occurs is not clearly defined. One hypothesis is that increased endogenous sex hormone levels may play a role, as several studies have noted that the use of exogenous hormones, particularly estrogen plus progesterone therapy, is associated with an increase in mammographic density (7-13) and breast cancer risk (14-16). We are aware of only two studies that have examined the association of blood or urine levels of estradiol and estrone with mammographic density (17, 18); however, the reported results were inconsistent. Meyer et al. found that the hormone levels were not strongly associated with Wolfe mammographic patterns in 110 postmenopausal women (17), whereas Boyd et al. found that mammographic density was inversely associated with free estradiol and positively associated with sex hormone binding globulin (SHBG) levels in 189 postmenopausal women after accounting for age and waist circumference (18).

Because cholesterol is the precursor to androgen and estrogen formation, serum cholesterol and lipoproteins may also be associated with mammographic density. Boyd et al. reported that mammographic density was positively associated with high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in 273 premenopausal women (19). Several studies have also found that breast cancer patients are more likely to have higher levels of total cholesterol compared with women with benign breast diseases or healthy controls (20-22). However, studies of HDL and LDL and their association with breast cancer have been inconsistent (22-25).

A third hypothesis suggests that growth factors may influence stromal cell proliferation and subsequently affect mammographic density. Several studies have examined the association between mammographic density and insulin-like growth factor (IGF-I; refs. 18, 26-28) and IGF binding protein-3 (IGFBP-3; refs. 18, 26, 28). Three studies found positive correlations between mammographic density and IGF-I, with

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the strongest associations occurring among premenopausal women (18, 26, 27). Predominantly inverse associations have been observed between mammographic density and IGFBP-3 (18, 26, 28).

In this study, we examined the relationship of mammographic density with circulating hormone levels, cholesterol, and IGF concentrations in overweight, postmenopausal women who were not current users of hormone therapy. The results from this study may provide insight on the biological mechanisms that contribute to increased mammographic density and breast cancer risk.

Materials and Methods

Study Participants. Participants were from the Physical Activity for Total Health study, a randomized clinical trial designed to test the effect of a yearlong moderate-intensity exercise intervention versus stretching control group on body fat and circulating sex hormone concentrations (29, 30). The present study was a cross-sectional examination of baseline mammographic density and hormone levels among the participants enrolled in this trial.

The study population consisted of 173 postmenopausal women. Recruitment and eligibility criteria have been described in detail elsewhere (29, 31). Briefly, women were ages 50 to 75 years, not currently taking hormone therapy, nonsmokers, sedentary, and overweight (body mass index $\geq$25.0 or between 24.0 and 25.0 kg/m^2, with percent body fat >33.0). Ninety-two women had a mammogram within 12 months before enrollment or 1 month after enrollment and were eligible for the present study. Informed consent was obtained following requirements of the Fred Hutchinson Cancer Research Center Institutional Review Board.

Data Collection. We collected information on mammographic density, plasma hormone levels, anthropometric measures, and demographic variables (30). Reproductive and medical history information, including hormone therapy use, and other demographic variables were collected via a self-administered questionnaire. Participants underwent a dual-energy X-ray absorptiometry scan to determine percent body fat.

Mammograms were collected from individual providers specified by each woman. Each film was digitized using an Epson 1680 scanner (Epson America, Inc., Long Beach, CA). Films were read for percent density, dense area (measured in thousands of pixels), and total area (measured in thousands of pixels) by one of the authors (E.J.A.) using Cumulus 108, a computer-assisted mammogram-reading program developed at the University of Toronto. This method has been described in detail elsewhere (32). Briefly, the reader uses a sliding scale to outline the breast edge and then the dense area based on pixel brightness. Percent density is the proportion of dense area relative to the total area of the breast. We randomly selected 10% of the films to be reread as quality control films. Intraclass correlations were 0.88 for both percent density and dense area.

Both right and left craniocaudal films were measured for density and the average of the two views was used as the final measurement. Six women had either a right or a left craniocaudal film that was of poor quality and unreadable; therefore, the density for the other film was included as the final measurement in the analysis. Four women, for whom both right craniocaudal and left craniocaudal films were unreadable, were not included in the analyses, resulting in a final sample size of 88.

All sex hormone assays were done at the Reproductive Endocrine Research Laboratory (University of Southern California) and the methods have been described previously (33, 34). Briefly, estrone, estradiol, testosterone, dehydroepiandrosterone (DHEA), and androstenedione were quantified by sensitive and specific RIAs following organic solvent extraction and Celite column partition chromatography (35, 36). SHBG, follicle-stimulating hormone, and DHEA sulfate were quantified via an immunometric assay using an Immulite Analyzer (Diagnostic Products Corp., Los Angeles, CA). Free estradiol and free testosterone were calculated using measured estradiol or testosterone concentrations, respectively, SHBG concentrations, and an assumed constant for albumin (37, 38). IGF-I was quantified via a two-site chemiluminescence immunoassay using the Nichols Advantage Specialty System from Nichols Diagnostics Institute (San Juan Capistrano, CA). IGFBP-3 was quantified via a sensitive and specific competitive protein-binding RIA using the IGFBP-3 100T kit (Nichols Diagnostic Institute). Lipid assays were done at the Northwest Lipid Research Laboratories (University of Washington, Seattle, WA) under the direction of Dr. Santica Marcinova. Triglyceride and total cholesterol concentrations in serum were determined enzymatically on a Hitachi 917 autoanalyzer (Tokyo, Japan) using Boehringer Mannheim reagent (Mannheim, Germany). We assayed HDL-cholesterol using methods standardized to the Centers for Disease Control and Prevention Reference Methods (39) and removed LDL and VDL by precipitation (40). We measured HDL-cholesterol in the supernatant enzymatically on a Hitachi 917 autoanalyzer. We computed LDL-cholesterol using the Friedewald equation (41): LDL-cholesterol = total cholesterol $-$ very low density lipoprotein-cholesterol $-$ HDL-cholesterol, where very low density lipoprotein-cholesterol is triglycerides/5. We excluded four women from the analyses of HDL and LDL because they either had HDL measured using a different assay or their triglycerides were >400 ng/dL (thus making the Friedewald equation inaccurate). All coefficients of variation were <18% (33).

Statistical Analyses. We used multiple linear regression to evaluate serum marker levels by quartiles of mammographic density and, secondarily, by a log-transformed continuous measure of mammographic density. All biomarkers, except IGF-I and IGFBP-3, were log transformed in the analyses to remove skewness and improve the normality assumption of the distribution. We evaluated crude and adjusted associations between hormones individually and both percent mammographic density and dense area. We examined stratification by prior hormone therapy use (never versus former) and hormone therapy latency (<5 versus $\geq$5 years before randomization) among former hormone therapy users. All models were adjusted for *a priori* specified covariates, including age (continuous), ethnicity (non-Hispanic White versus other), years since menopause (continuous), and percent body fat (continuous). We calculated statistical significance for trend across quartiles of percent density by including the quartiles as a single equally spaced continuous term in the linear regression model. We evaluated the statistical significance of the continuous measure of mammographic density using the Wald test. We considered $P < 0.05$ to be statistically significant. All analyses were conducted using Stata7SE (StataCorp, College Station, TX).

Results

We analyzed data for a total of 88 women, 45 of whom had never used hormone therapy and 43 who were former users (Table 1). The majority of study participants were Caucasian and the average age was 61 years. The average percent mammographic density for the population was low (6.1% overall). Both percent density and dense area were slightly greater among women who had never used hormone therapy compared with former users; however, these

Table 1. Demographic and hormone characteristics of 88 postmenopausal women stratified by history of hormone therapy use

Baseline characteristics	Never users (n = 45)	Former users		
		All former users (n = 43)	Used <5 y ago (n = 20)	Used ≥5 y ago (n = 19)
	n (%)	n (%)	n (%)	n (%)
Ethnicity				
Non-Hispanic White	36 (80.0)	41 (95.3)	18 (90.0)	19 (100.0)
Other	9 (20.0)	2 (4.7)	2 (10.0)	0 (0)
	Mean (SD)	*Mean (SD)*	*Mean (SD)*	*Mean (SD)*
Age (y)	60.5 (7.4)	60.6 (6.3)	58.1 (5.1)	62.5 (6.6)
Percent mammographic density (%)	6.7 (6.1)	5.8 (4.6)	6.6 (4.6)	4.7 (4.2)
Dense breast area (1,000 pixels)	27.4 (24.1)	22.9 (14.9)	24.8 (13.2)	19.5 (15.3)
Total breast area (1,000 pixels)	427.9 (100.0)	427.3 (76.5)	415.4 (82.5)	448.6 (71.2)
Percent fat mass (dual-energy X-ray absorptiometry; %)	43.4 (2.7)	43.7 (3.1)	43.7 (3.5)	43.7 (3.1)
Estrone (pg/mL)	48.0 (12.4)	48.4 (19.9)	50.0 (20.8)	48.5 (205)
Estradiol (pg/mL)	18.9 (5.9)	18.7 (7.9)	18.6 (8.7)	19.5 (7.9)
Free estradiol (pg/mL)	0.51 (0.18)	0.51 (0.25)	0.52 (0.28)	0.53 (0.23)
Testosterone (pg/mL)	237.4 (89.3)	230.7 (106.3)	249.8 (132.3)	226.7 (77.4)
Free testosterone (pg/mL)	5.1 (2.0)	5.0 (2.4)	5.6 (3.2)	4.8 (1.2)
Androstenedione (ng/mL)	613.2 (225.1)	583.4 (251.6)	617.3 (310.7)	582.9 (192.0)
DHEA (ng/mL)	2.7 (1.3)	2.7 (1.6)	3.0 (2.0)	2.5 (1.2)
DHEA sulfate (μg/mL)	67.1 (41.7)	80.9 (67.1)	96.3 (89.7)	68.3 (33.9)
SHBG (nmol/L)	42.3 (29.3)	38.2 (19.7)	35.8 (14.1)	39.4 (21.3)
Follicle-stimulating hormone (mIU/L)	68.0 (24.3)	63.8 (19.9)	67.7 (23.1)	59.5 (17.6)
IGF-I (ng/mL)	106.6 (31.5)	111.7 (28.6)	111.5 (32.1)	110.9 (27.0)
IGFBP-3 (μg/mL)	3.9 (1.0)	4.1 (1.0)	4.2 (0.9)	3.8 (0.9)
IGF-I/IGFBP-3 ratio	28.8 (12.1)	28.5 (8.7)	26.9 (5.3)	30.3 (10.8)
Total cholesterol (mg/dL)*	228.0 (38.2)	239.6 (49.8)	246.5 (65.1)	237.6 (28.6)
LDL (mg/dL)†	147.5 (35.6)	160.2 (52.3)	170.4 (69.1)	155.3 (26.8)
HDL (mg/dL)†	55.2 (12.9)	52.5 (14.1)	49.9 (13.8)	54.6 (14.6)
LDL/HDL ratio†	2.8 (1.0)	3.4 (2.1)	3.6 (2.9)	3.1 (1.0)
Total cholesterol/HDL ratio†	4.4 (1.3)	5.0 (2.3)	5.5 (3.1)	4.6 (1.2)

*n = 43 never users and 40 former users.
†n = 41 never users and 40 former users.

differences were not significantly different. All endogenous biomarker levels were very similar between former users and never users.

We did not observe any relationships between serum biomarkers and mammographic density among women who had never used hormone therapy (Table 2). We noted statistically significant inverse associations between mammographic density and almost all sex hormones among women who were former users of hormone therapy. We observed consistently inverse associations for estrone, estradiol, and free estradiol with mammographic density (P for trend = 0.01, 0.003, and 0.004, respectively). We found similar results when estrogen users were limited to women who had used estrogen <5 years before randomization. Among the androgens, the mean hormone levels of androstenedione at the two highest quartiles of percent density were significantly lower than the lowest density quartile (P = 0.01 for third quartile and P < 0.001 for fourth quartile). Free testosterone and DHEA were significantly inversely associated with mammographic density (P for trend = 0.02 and 0.01, respectively). These trends were observed among women who were more recent users of hormone therapy but not among women who had used hormone therapy ≥5 years before the study. SHBG and follicle-stimulating hormone were not associated with mammographic density.

We noted a slight inverse association between IGF-I and density in former hormone therapy users, although this association may be limited to women who had stopped using hormone therapy in the past 5 years (Table 3). Although we did not find any associations between density and IGFBP-3, the IGF-I/IGFBP-3 ratio significantly decreased with increasing quartiles of percent density in former hormone therapy users (P for trend = 0.04 for all former users and 0.05 for recent former users).

Total cholesterol and LDL were positively associated with mammographic density among all former hormone therapy users (P for trend = 0.03 for each) (Table 4). However, these associations did not remain statistically significant when restricting to women who had used hormone therapy within the last 5 years. We did not note any other important associations between density and cholesterol.

When we evaluated mammographic density as a continuous variable, we observed similar but weaker relationships compared with the categorical models among all former hormone therapy users (data not shown). Among the hormones, the only associations that remained statistically significant at P < 0.05 were those between mammographic density and estrone (P = 0.014), free testosterone (P = 0.049), androstenedione (P = 0.013), DHEA (P = 0.046), and DHEA sulfate (P = 0.040). The associations between continuous mammographic density and IGF measures also were similar to the categorical models (data not shown). However, the association between continuous mammographic density and lipid measures varied slightly between the linear and categorical analyses. Mammographic density was inversely related to total cholesterol and LDL in women who had never used hormone therapy (P = 0.053 and 0.047, respectively) and positively associated in women who were former users of hormone therapy (P = 0.044 and 0.071, respectively; data not shown). We noted the same patterns in the data when we analyzed former hormone therapy users by time since last use, with stronger relationships among continuous mammogram density, hormone levels, and lipids among recent former users (data not shown).

Table 2. Adjusted means of sex hormones by quartiles of percent density stratified by history of hormone therapy use

	Never used hormone therapy (n = 45)	Former users of hormone therapy (n = 43)	Used hormone therapy <5 y ago (n = 20)	Used hormone therapy ≥5 y ago (n = 19)
	Adjusted hormone levels by quartiles of percent density* (95% CI)	Adjusted hormone levels by quartiles of percent density* (95% CI)	Adjusted hormone levels by quartiles of percent density† (95% CI)	Adjusted hormone levels by quartiles of percent density† (95% CI)
Estrone (pg/mL)	43.7 (35.9-53.2)	52.6 (44.2-62.5)	66.4 (46.1-95.8)	47.9 (36.2-63.3)
	51.2 (43.5-60.3)	50.6 (41.0-62.6)	46.6 (31.2-69.7)	59.1 (38.1-91.4)
	44.7 (37.3-53.6)	39.8 (32.6-48.6)‡	38.3 (26.7-55.0)‡	39.6 (25.7-61.1)
	46.0 (38.1-55.6)	34.6 (28.8-41.7)‡	33.5 (23.8-47.1)‡	39.5 (25.7-60.8)
P for trend	0.96	0.01‡	0.01‡	0.17
Estradiol (pg/mL)	16.8 (13.9-20.2)	20.2 (17.1-23.8)	23.6 (16.9-33.3)	19.6 (15.2-25.3)
	18.9 (16.2-22.1)	18.7 (15.3-22.9)	15.2 (10.5-22.0)	24.1 (16.1-35.9)
	17.9 (15.1-21.3)	15.8 (13.1-19.1)	15.5 (11.1-21.6)	14.9 (10.0-22.1)
	19.6 (16.4-23.5)	14.0 (11.8-16.7)‡	12.9 (9.4-17.5)‡	18.1 (12.1-26.7)
P for trend	0.35	0.003‡	0.025‡	0.24
Free estradiol (pg/mL)	0.40 (0.32-0.50)	0.53 (0.44-0.65)	0.68 (0.47-0.98)	0.50 (0.38-0.67)
	0.53 (0.45-0.64)	0.51 (0.40-0.64)	0.40 (0.27-0.60)	0.66 (0.43-1.0)
	0.47 (0.39-0.58)	0.45 (0.36-0.56)	0.43 (0.30-0.62)	0.46 (0.30-0.71)
	0.52 (0.43-0.65)	0.36 (0.30-0.45)‡	0.34 (0.24-0.48)‡	0.51 (0.33-0.78)
P for trend	0.21	0.004‡	0.023‡	0.10
Testosterone (pg/mL)	188 (145-243)	264 (210-331)	327 (204-523)	249 (174-357)
	260 (209-323)	223 (169-296)	186 (110-312)	253 (143-444)
	225 (177-286)	188 (144-244)	215 (135-342)	179 (102-312)
	206 (161-265)	164 (129-210)‡	166 (107-258)‡	168 (97-293)
P for trend	0.93	0.04‡	0.07	0.77
Free testosterone (pg/mL)	3.5 (2.7-4.6)	5.6 (4.4-7.0)	7.8 (5.1-12.0)	5.0 (3.6-6.9)
	6.0 (4.9-7.5)‡	4.9 (3.7-6.4)	3.8 (2.4-6.2)‡	5.6 (3.3-9.4)
	4.8 (3.8-6.1)	4.4 (3.4-5.8)	4.9 (3.2-7.5)	4.7 (2.8-7.8)
	4.4 (3.4-5.7)	3.3 (2.6-4.3)‡	3.5 (2.3-5.2)‡	3.9 (2.3-6.4)
P for trend	0.55	0.02‡	0.03‡	0.28
Androstenedione (ng/mL)	444 (351-560)	708 (576-871)	851 (550-1,315)	674 (484-941)
	633 (521-770)‡	674 (523-868)	557 (344-900)	764 (454-1,288)
	561 (452-696)	466 (368-592)‡	507 (329-780)	465 (278-778)
	636 (507-797)‡	397 (318-494)‡	366 (244-550)‡	421 (252-704)
P for trend	0.09	<0.001‡	0.01‡	0.46
DHEA (ng/mL)	1.8 (1.3-2.6)	3.0 (2.2-4.1)	4.4 (2.4-8.0)	2.6 (1.6-4.1)
	2.7 (2.0-3.6)	2.9 (2.0-4.3)	2.0 (1.0-3.9)	4.5 (2.2-9.2)
	2.2 (1.6-3.1)	2.1 (1.4-3.0)	1.6 (0.9-2.9)‡	2.6 (1.3-5.4)
	2.5 (1.8-3.6)	1.6 (1.1-2.2)‡	1.3 (0.7-2.3)‡	1.6 (0.8-3.2)
P for trend	0.35	0.01‡	0.01‡	0.19
DHEA sulfate (μg/mL)	45.4 (29.6-69.7)	76.4 (51.1-114.2)	101.9 (49.6-209.4)	64.3 (35.2-117.4)
	55.8 (37.7-82.7)	65.0 (40.9-103.3)	42.1 (18.9-93.6)	104.4 (43.1-252.7)
	53.9 (35.3-82.2)	82.7 (53.5-127.9)	68.5 (33.1-141.6)	104.4 (44.4-245.4)
	51.6 (34.0-78.2)	38.4 (25.3-58.5)‡	26.5 (13.4-52.2)‡	35.0 (12.5-97.5)
P for trend	0.77	0.10	0.04‡	0.24
SHBG (nmol/L)	46.1 (34.3-61.8)	36.5 (28.2-47.4)	28.8 (18.8-44.0)	40.6 (23.3-56.1)
	30.0 (23.5-38.4)‡	35.7 (26.0-49.0)	39.1 (24.5-62.4)	35.0 (21.1-58.2)
	36.4 (27.8-47.8)	28.3 (21.0-38.2)	31.7 (20.8-8.2)	23.4 (14.2-38.7)
	35.4 (26.6-47.1)	38.7 (29.4-51.1)	37.5 (25.2-55.7)	31.2 (18.9-51.5)
P for trend	0.48	0.63	0.48	0.12
Follicle-stimulating hormone (mIU/L)	63.1 (51.2-77.8)	52.7 (43.8-63.4)	46.2 (34.3-62.3)	55.9 (44.5-70.2)
	68.5 (57.5-81.6)	58.3 (46.4-73.1)	60.3 (43.4-83.8)	54.9 (38.4-78.4)
	72.5 (59.8-88.0)	72.8 (58.8-90.1)‡	85.8 (63.1-114.0)‡	66.6 (46.8-94.9)
	55.8 (45.5-68.3)	63.3 (52.0-77.1)	75.8 (57.4-100.0)‡	51.4 (36.1-73.1)
P for trend	0.48	0.06	0.01‡	0.05

*Quartiles of percent density: first quartile: 0.2% to 2.75% (n = 9 never users and 13 former users); second quartile: 2.8% to 4.9% (n = 14 never users and 8 former users); third quartile: 5.1% to 7.5% (n = 11 never users and 11 former users); and fourth quartile: 7.5% to 26.1% (n = 11 never users and 11 former users). Adjusted for age, ethnicity, years since menopause, and percent body fat.

†Quartiles of percent density: first quartile: 0.2% to 2.75% (n = 4 users <5 years and 8 users ≥5 years); second quartile: 2.8% to 4.9% (n = 4 users <5 years and 4 users ≥5 years); third quartile: 5.1% to 7.5% (n = 6 users <5 years and 4 users ≥5 years); and fourth quartile: 7.5% to 26.1% (n = 6 users <5 years and 3 users ≥5 years). Adjusted for age, ethnicity, years since menopause, and percent body fat.

‡P < 0.05, compared with first quartile.

Discussion

We examined associations between mammographic density and a variety of plasma biomarkers in postmenopausal women. The majority of estrogens and androgens and IGF-I/IGFBP-3 were inversely associated with mammographic density among former hormone therapy users. Total cholesterol and LDL levels were positively associated with mammographic density among former hormone therapy users. We did not observe any associations between any biomarker and mammographic density among women who had never used hormone therapy.

Despite the vast array of literature showing that mammographic density is positively associated with exogenous estrogen levels (7-13), our results do not support a positive association between endogenous hormone levels and mammographic density. In fact, we observed strong associations in the opposite direction of what we had expected, specifically among women who were recent, former users of hormone therapy. Our results were similar to Meyer et al. who noted inverse associations between mammographic density (assessed using the Wolfe criteria) and estrogens in 110 premenopausal women, but these were not statistically significant (17). Boyd et al. also found a inverse association with free estradiol among 189 postmenopausal women who were not current hormone users (18). However, Boyd et al. found a positive association between SHBG and density, which we did not observe. A third study found that mammogram density increased with increasing serum estrone levels in postmenopausal women randomized to receive combined hormone therapy (42). However, this study concluded that estrone alone does not increase mammogram density, because there was no association between estrone and mammogram density in women randomized to receive estrogen only.

One possible reason that we failed to observe the same positive associations that are observed in studies of hormone therapy use could be that exogenous hormones generate a steroidal environment that affects mammographic density differently than endogenous hormones. The most common estrogen used in the United States is Premarin (43), which contains many estrogens, including horse estrogens and some testosterone. It is possible that the effect of these estrogens is different from endogenous estrogens. Estrogen metabolites, such as 2-hydroxyestrone and 16α-hydroxyestrone, have been shown to be biologically active (44), and their concentrations are considerably higher following oral exogenous estrogen administration (45). The effect of hormone therapy on breast biology may linger even after discontinuation of hormone therapy use. Differences in estrogen metabolism between hormone therapy users and nonusers may be part of the reason that we generally saw significant inverse associations with mammographic density among former users and no associations among women who had never used hormone therapy. In never users, body fat may be a more important predictor of mammographic density than endogenous estrogen levels. Differences in estrogen metabolism may also help explain why we saw inverse associations in women who used hormone therapy <5 years before the study but not in women who stopped using ≥5 years prior. This is supported by data suggesting that the increased risk conferred by using hormone therapy returns to baseline levels ~5 years after hormone discontinuation (46). However, this hypothesis needs to be confirmed in other studies.

In addition, the positive association between mammographic density and hormone therapy use shown in previous studies may have been due to an association between progesterone and mammographic density and not an association between estrogen and mammographic density. This hypothesis is supported by the fact that several studies have shown strong positive associations between estrogen plus progestin therapy and mammographic density and much weaker associations between estrogen-only therapy and mammographic density (9, 47, 48). We did not measure endogenous progesterone levels in our study and are unable to test this hypothesis; however, Boyd et al. did observe a positive association between progesterone and mammographic density in postmenopausal women (18).

Table 3. Adjusted means of IGF-I and IGFBP-3 by quartiles of percent density stratified by history of hormone therapy use

	Never used hormone therapy (n = 45)	Former users of hormone therapy (n = 43)	Used hormone therapy <5 y ago (n = 20)	Used hormone therapy ≥5 y ago (n = 19)
	Adjusted hormone levels by quartiles of percent density* (95% CI)	Adjusted hormone levels by quartiles of percent density* (95% CI)	Adjusted hormone levels by quartiles of percent density† (95% CI)	Adjusted hormone levels by quartiles of percent density† (95% CI)
IGF-I (ng/mL)	94.1 (73.6-114.6)	115.5 (97.4-133.7)	127.0 (91.2-162.9)	115.2 (87.8-142.6)
	111.1 (93.9-128.2)	116.3 (94.1-138.5)	118.6 (79.0-158.1)	117.4 (74.5-160.3)
	117.4 (98.5-136.4)	110.9 (90.1-131.8)	105.0 (69.5-140.5)	115.2 (72.8-157.7)
	105.2 (85.4-125.1)	101.4 (82.1-120.7)	97.5 (64.1-131.0)	100.8 (58.5-143.1)
P for trend	0.40	0.19	0.18	0.50
IGFBP-3 (μg/mL)	3.8 (3.1-4.4)	3.7 (3.1-4.3)	3.7 (2.6-4.8)	3.7 (2.9-4.5)
	4.4 (3.9-5.0)	4.1 (3.3-4.8)	4.7 (3.5-5.9)	3.4 (2.1-4.7)
	3.4 (2.8-4.0)	3.9 (3.2-4.6)	4.4 (3.3-5.5)	3.7 (2.4-5.0)
	4.1 (3.5-4.8)	4.4 (3.8-5.1)	4.3 (3.3-5.3)	4.0 (2.7-5.3)
P for trend	0.90	0.29	0.53	0.91
IGF-I/IGFBP-3	24.8 (20.5-30.1)	30.8 (26.0-36.4)	32.8 (25.3-42.5)	31.2 (25.6-3.81)
	24.1 (20.5-28.3)	28.6 (23.2-35.2)	25.0 (18.8-33.2)	34.7 (25.4-47.3)
	36.3 (30.4-43.3)‡	8.9 (23.8-35.1)	3.8 (18.4-30.8)	1.8 (23.4-43.2)
	25.1 (20.9-30.2)	23.1 (19.3-27.7)‡	22.7 (17.8-28.8)‡	25.3 (18.6-34.4)
P for trend	0.25	0.04‡	0.05‡	0.44

*Quartiles of percent density: first quartile: 0.2% to 2.75% (n = 9 never users and 13 former users); second quartile: 2.8% to 4.9% (n = 14 never users and 8 former users); third quartile: 5.1% to 7.5% (n = 11 never users and 11 former users); and fourth quartile: 7.5% to 26.1% (n = 11 never users and 11 former users). Adjusted for age, ethnicity, years since menopause, and percent body fat.

†Quartiles of percent density: first quartile: 0.2% to 2.75% (n = 4 users <5 years and 8 users ≥5 years); second quartile: 2.8% to 4.9% (n = 4 users <5 years and 4 users ≥5 years); third quartile: 5.1% to 7.5% (n = 6 users <5 years and 4 users ≥5 years); and fourth quartile: 7.5% to 26.1% (n = 6 users <5 years and 3 users ≥5 years). Adjusted for age, ethnicity, years since menopause, and percent body fat.

‡P < 0.05, compared with first quartile.

Table 4. Adjusted means of cholesterol measures by quartiles of percent density stratified by history of hormone therapy use

	Never used hormone therapy (n = 43)	Former users of hormone therapy (n = 40)	Used hormone therapy <5 y ago (n = 19)	Used hormone therapy ≥5 y ago (n = 18)
	Adjusted hormone levels by quartiles of percent density* (95% CI)	Adjusted hormone levels by quartiles of percent density* (95% CI)	Adjusted hormone levels by quartiles of percent density† (95% CI)	Adjusted hormone levels by quartiles of percent density† (95% CI)
Total cholesterol (mg/dL)	235 (207-265)	217 (193-244)	216 (175-266)	208 (180-241)
	228 (206-253)	228 (200-261)	223 (182-272)	204 (164-254)
	229 (206-258)	245 (216-278)	268 (224-321)	274 (221-340)‡
	211 (186-239)	248 (220-281)	264 (223-313)	271 (219-336)
P for trend	0.25	0.03‡	0.08	0.64
LDL (mg/dL)	155 (128-188)	137 (114-165)	142 (102-196)	127 (101-160)
	146 (123-173)	141 (115-174)	133 (97-182)	120 (86-169)
	142 (118-171)	162 (133-197)	187 (141-248)	199 (143-279)‡
	133 (109-161)	170 (140-205)	183 (141-239)	188 (134-262)
P for trend	0.24	0.03‡	0.12	0.62
HDL (mg/dL)	51.5 (43.6-60.8)	51.1 (43.7-59.9)	48.7 (35.6-66.6)	50.0 (40.1-62.3)
	57.8 (49.8-67.2)	50.1 (41.9-60.0)	48.6 (36.0-65.7)	54.2 (39.0-75.2)
	58.0 (49.4-68.1)	49.2 (41.5-58.3)	47.4 (36.2-62.2)	45.6 (33.1-63.0)
	48.3 (40.8-57.1)	51.4 (43.5-60.6)	55.7 (43.2-71.8)	49.8 (36.1-68.7)
P for trend	0.62	0.65	0.45	0.44
LDL/HDL	3.0 (2.3-4.0)	2.7 (2.1-3.5)	2.9 (1.9-4.5)	2.5 (1.9-3.5)
	2.5 (2.0-3.2)	2.8 (2.1-3.8)	2.7 (1.8-4.2)	2.2 (1.4-3.5)
	2.4 (1.9-3.2)	3.3 (2.5-4.4)	4.0 (2.7-5.8)	4.4 (2.8-6.9)
	2.7 (2.1-3.6)	3.3 (2.5-4.4)	3.3 (2.3-4.7)	3.8 (2.4-5.9)
P for trend	0.61	0.21	0.50	0.39
Total cholesterol/ HDL	4.6 (3.7-5.6)	4.3 (3.5-5.2)	4.4 (3.2-6.2)	4.2 (3.3-5.3)
	4.0 (3.3-4.8)	4.6 (3.6-5.7)	4.6 (3.3-6.3)	3.8 (2.7-5.3)
	4.0 (3.2-4.9)	5.0 (4.0-6.2)	5.6 (4.2-7.5)	6.0 (4.3-8.4)
	4.4 (3.5-5.4)	4.8 (3.9-6.0)	4.7 (3.6-6.2)	5.4 (3.9-7.6)
P for trend	0.76	0.36	0.66	0.33

*Quartiles of percent density: first quartile: 0.2% to 2.75% (n = 9 never users and 11 former users); second quartile: 2.8% to 4.9% (n = 14 never users and 8 former users); third quartile: 5.1% to 7.5% (n = 10 never users and 11 former users); and fourth quartile: 7.5% to 26.1% (n = 10 never users and 10 former users). Adjusted for age, ethnicity, years since menopause, and percent body fat.

†Quartiles of percent density: first quartile: 0.2% to 2.75% (n = 4 users <5 years and 7 users ≥5 years); second quartile: 2.8% to 4.9% (n = 4 users <5 years and 4 users ≥5 years); third quartile: 5.1% to 7.5% (n = 6 users <5 years and 4 users ≥5 years); and fourth quartile: 7.5% to 26.1% (n = 6 users <5 years and 3 users ≥5 years). Adjusted for age, ethnicity, years since menopause, and percent body fat.

‡$P < 0.05$, compared with first quartile.

Our results showing a positive association between LDL and density were similar to Boyd et al., who found a positive association among premenopausal women (49). Boyd et al. also noted a positive association with HDL, which we did not see. It is interesting that we noted a positive association between LDL and density but inverse associations between most estrogens and androgens and mammographic density. In our data, unadjusted correlations showed that LDL was inversely associated with estrogen and androgen levels. We are not aware of any other studies that have examined cholesterol or lipoprotein levels with mammographic density; however, our results are also consistent with studies that have shown an increase in total cholesterol (20-22) or LDL levels (22) in breast cancer patients compared with controls.

Our data were not in agreement with several studies that have reported positive associations between mammographic density and IGF-I, IGFBP-3, or IGF-I/IGFBP-3 (18) primarily among premenopausal women, whereas our study population was exclusively postmenopausal (26, 27). A fourth study noted no association between mammographic density and IGF-I in premenopausal women but found an inverse association with IGFBP-3 among women with a body mass index <25 kg/m^2 (28).

As the majority of previous studies have examined premenopausal women, it is possible that our results differ because the association between density and IGF-I is modified by menopausal status. Studies by Boyd et al. and Byrne et al. examined both premenopausal and postmenopausal women separately (18) and found different results between the two populations (26). Because our study was limited to overweight, postmenopausal women, the average percent density was much lower in our population than in either of these prior studies. It is possible that the association between IGF-I and density is different at higher levels of density or that there was an insufficient range of mammographic density for some predictors to be associated. Neither of these previous studies reported whether prior hormone therapy use modified the associations.

Our study had several limitations. We conducted this study on a select population of women who were overweight or obese at the time of data collection and had a mammogram within 12 months before or 1 month after study enrollment. This homogeneity resulted in a small average percent density with very little variation. In addition, serum sex hormone levels are much lower than hormone levels in breast tissue. It is possible that hormone levels in breast tissue are high enough to affect mammographic density differently, but serum levels do not adequately reflect tissue levels. However, this study also had several strengths. Although our sample size was small, we still noted very consistent results among the various biomarkers and when using different measures of mammographic density (percent density, dense area, continuous

measures, and categorical measures). In addition, measuring mammographic density as a continuous scale may provide more information than the Breast Imaging Reporting and Data System or Wolfe categorical measures (50), which most previous mammographic density studies have used. We noted similar results when we analyzed percent density as a continuous or categorical measure and when we used dense area; therefore, we only presented the categorical analyses using quartiles of percent density for ease of interpretation.

In conclusion, our results showed a strong inverse relationship among hormone levels, IGF-I, and mammographic density in postmenopausal women who were former users of hormone therapy. We also noted positive associations among total cholesterol, LDL, and mammographic density in this subgroup. These results should be interpreted in light of the highly selective population and should be confirmed in larger, more diverse studies. Further research on hormones and mammographic density is also needed in postmenopausal women currently taking hormone therapy, as this is a strong positive predictor of mammographic density, and the relationship between endogenous hormone levels and mammographic density may be very different.

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