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Mammographic density, plasma vitamin D levels, and risk of breast cancer in postmenopausal women

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Abstract

Mammographic density is a strong risk factor for breast cancer, but the underlying biology for this association is unknown. Studies suggest that vitamin D may reduce breast cancer risk and dietary vitamin D intake has been associated with reduced breast density. We conducted a case-control study nested within the Nurses' Health Study cohort consisting of 463 and 497 postmenopausal cases and controls, respectively. We examined the association between mammographic density and plasma levels of 25-hydroxyvitamin D [25(OH)D] and 1,25-dihydroxyvitamin D [1,25(OH)₂D]. We assessed whether plasma vitamin D metabolites modify the association between breast density and breast cancer. Percent mammographic density was measured from digitized film mammograms. Generalized linear models were used to determine mean percent breast density per quartile of vitamin D metabolite. Logistic regression models were used to calculate relative risks and confidence intervals. All models were adjusted for matching variables and potential confounders. We found no cross-sectional association between circulating levels of 25(OH)D or 1,25(OH)₂D with mammographic density. Women in the highest tertile of mammographic density and lowest tertile of plasma 25(OH)D had 4 times greater risk of breast cancer than women with the lowest mammographic density and highest plasma 25(OH)D levels (RR=3.8; 95% CI: 2.0-7.3). The overall interaction between mammographic density and plasma 25(OH)D was non-significant (p-het=.20). These results indicate that the association between mammographic density and breast cancer is independent of plasma vitamin D metabolites in postmenopausal women. Further research examining vitamin D, mammographic density and breast cancer risk is warranted.

Keywords

mammographic density; breast cancer; vitamin D; 25-hydroxyvitamin D

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This paper suggests that the link between mammographic density and breast cancer is independent of plasma vitamin D metabolites among postmenopausal women. This is the first research article to our knowledge that also assesses the interaction between vitamin D and mammographic density on breast cancer risk.

Introduction

Mammographic density is one of the strongest risk factors for breast cancer¹. Women with greater than or equal to 75% breast density are at a 4- to 6-fold greater risk of breast cancer than women with no mammographic density^{1, 2}. Mammographic density refers to the amount of epithelial cells and connective tissue in the breast which are radiodense and appear light on a film screen mammogram. Fat, the other major component of the breast, is radiolucent and appears dark on a mammogram.

Mammographic density is argued to be a surrogate marker for use in breast cancer risk prediction models and as an endpoint in prevention intervention trials³; however, the biological mechanism by which it influences the risk of breast cancer remains unknown. Mammographic density is hypothesized to represent the pool of mammary cells and to be positively correlated with the number of mammary stem cells^{4, 5}. Thus, women with higher mammographic density may have more cells susceptible to mutagenesis, thereby increasing their risk of developing breast cancer.

Epidemiologic, ecologic, and biologic studies suggest that vitamin D may be associated with a reduced risk of breast cancer⁶⁻⁹. Both diet and sun exposure influence circulating levels of plasma vitamin D metabolites¹⁰⁻¹⁶. Previtamin D formed from sunlight and dietary intake of vitamin D is hydroxylated in the liver into 25-hydroxyvitamin D [25(OH)D]. 25(OH)D is further hydroxylated to the biologically active metabolite 1,25-dihydroxyvitamin D [1,25(OH)₂D] primarily in the kidney nephrons and possibly also in breast tissue^{17, 18}. Although 1,25(OH)₂D is the biologically active metabolite, 25(OH)D is more sensitive to dietary intake and sun exposure and is considered a better biomarker of vitamin D status^{19, 20}. *In vivo* and *in vitro* studies have shown that 1,25(OH)₂D and its receptor can induce differentiation and inhibit cellular proliferation in breast tumor tissue, suggesting that the vitamin D pathway is a potential mediator of the mammographic density-breast cancer relationship^{21, 22}.

Previous studies have examined the association between vitamin D and mammographic density, but results have been inconsistent²³⁻²⁷. Three studies have shown an inverse association between intake of vitamin D from diet or supplements and mammographic density^{23, 24, 27}. A study conducted within the Minnesota Breast Cancer Family cohort, however, found no association between dietary intake of vitamin D and breast density²⁸. Only one study by Knight et al. has previously examined the cross-sectional relationship between plasma levels of 25(OH)D and mammographic density and found no evidence of an association²⁶.

In the current study we assessed the association between plasma levels of 25(OH)D and 1,25(OH)₂D and mammographic density in postmenopausal women from the Nurses' Health Study (NHS) cohort. In addition, using a nested case-control study design, we assessed the influence of varying levels of plasma vitamin D metabolites on the association between mammographic density and breast cancer risk. No other study to our knowledge has examined this possible interaction.

Materials and Methods

Study Design and Population

The Nurses' Health Study was initiated in 1976 when 121,700 US registered nurses ages 30 to 55 years returned an initial questionnaire. Information on body mass index (BMI), reproductive history, age at menopause, and postmenopausal hormone (PMH) 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 published²⁹. In brief, 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 1996.

We conducted a nested case-control study among the subcohort of women who were postmenopausal and had no history of cancer at the time of blood sample. This nested case-control study has been described in detail⁶. We restricted our analyses to postmenopausal subjects because of limited information on mammographic density and vitamin D metabolite levels among the premenopausal women. Eligible cases were women who were diagnosed with breast cancer after blood collection but before June 1, 1996⁶. Medical record reviews were used to confirm breast cancer diagnoses and to identify tumor location, histologic type, subtype, and invasiveness. Controls were matched to cases by age (± 2 years), month of blood collection, time of day that blood was drawn (± 2 hours), fasting status at the time of blood collection (≥ 10 hours since a meal versus < 10 hours or unknown), and PMH use (yes/no).

Laboratory Analyses

Plasma 25(OH)D assays were conducted in three batches. The first and second batches were assayed at Dr. Holick's laboratory at the Boston University School of Medicine (Boston, MA) between November 1993 and July 1994 and October 1999 and June 2000, respectively. The third batch was assayed at Dr. Hollis' laboratory at the Medical University of South Carolina (Charleston, SC) between June and September 2003. The methods used to assay 25(OH)D have been described in detail previously^{30, 31}. The mean coefficients of variation for 25(OH)D were 17.6%, 16.4%, and 8.7% for batches one, two, and three, respectively. Among postmenopausal women at blood draw, 25 (OH)D measurements were available on 537 cases and 547 controls. Plasma 1,25(OH)₂D was analyzed in a single batch by Dr. Hollis between March and July 2001 by radioimmunoassay using radioiodinated tracers³¹. Among postmenopausal women at blood draw, 1,25(OH)₂D measurements were available on 499 cases and 502 controls. The mean coefficient of variation for 1,25(OH)₂D was 7.3%. For all assays, case-control pairs were analyzed together, but were randomly ordered within each pair to mask the disease status from laboratory technicians.

Mammographic Density Measurements

Collection of mammograms in this nested case-control study has been described in detail previously³². For all consenting women, we attempted to obtain the mammograms as close as possible to the date of blood collection. Overall, we had mammographic density measurements on 85.0% of cases and 86.8% of controls with vitamin D measurements. We excluded an additional 4 cases and 3 controls who were not postmenopausal at the time of their mammogram. In total, there were 463 cases and 497 controls with both vitamin D and mammographic density measurements contributing to this analysis. The median time between mammography and blood draw was 5 months (interquartile range: 20 months before blood draw to 1 month after). Women for whom we obtained mammograms were similar to those who we were unable to get mammograms with respect to age, BMI, and levels of circulating vitamin D metabolites. This study was approved by the Committee on the Use of Human Subjects in Research at Brigham and Women's Hospital.

To assess mammographic density, the craniocaudal views of both breasts were digitized at 261 microns/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 Toronto³³. 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 0.93³⁴. 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 measurements are used³⁵.

Covariate Information

. Postmenopausal status was assessed through a supplemental questionnaire administered at the time of blood collection. Women were considered postmenopausal if 1) her natural menstrual periods had ceased permanently 12 or more months before blood draw, 2) she had a bilateral oophorectomy, or 3) she had a hysterectomy with at least 1 ovary remaining and was aged ≥ 56 years (nonsmokers) or ≥ 54 years (smokers). These are the ages at which 90% of the Nurses' Health Study participants who had a natural menopause were postmenopausal. All other covariates were assessed from biennial questionnaires before blood draw including age at mammography, alcohol intake, 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. Covariates were considered potential confounders in the analysis of vitamin D metabolites and mammographic density if there was *a priori* evidence in published literature that the factor was related to mammographic density and plasma vitamin D metabolite levels. Covariates associated with breast density or plasma vitamin D metabolites and breast cancer risk were considered potential confounders in the case-control analysis.

Data Analysis

We conducted an analysis of the association between plasma vitamin D metabolites and mammographic density among the 493 eligible control subjects. We calculated partial Spearman correlations adjusted for potential confounders, as well as laboratory batch for 25(OH)D, to determine the association between circulating levels of vitamin D metabolites and mammographic density among controls. There were some differences in distribution of 25(OH)D concentrations between laboratory batches. The quality control samples included in each batch had variability similar to that of the control samples, suggesting that the differences were due to batch-to-batch variability and were not true differences in vitamin D metabolite levels between batches. There were 108 cases/120 controls in batch 1, 180 cases/180 controls in batch 2, and 164 cases and 169 controls in batch 3. We created quartiles of 25(OH)D based on batch-specific cut points and controlled for batch in all analyses with continuous 25(OH)D measures.

Generalized linear models were used to evaluate the impact of each vitamin D metabolite as a predictor of percent breast density and to determine the mean percent breast density per quartile of vitamin D metabolite. We considered covariates which have been reported to be associated with breast density as potential confounders in this analysis. We presented a model adjusted for body mass index since this was the only variable that altered the results. However, for illustrative purposes we have also shown the results of the full model which included all potential confounders. The full multivariate model was adjusted for matching factors and the following additional covariates: BMI (continuous, kg/m²), family history of breast cancer (yes/no), duration of PMH use (continuous), alcohol intake (0, <5, 5 to <15, ≥ 15 g/d), parity/age at first birth (nulliparous, age at first birth <25 years, age at first birth

25-29 years, age at first birth ≥ 30 years, and missing), and age at menarche (<12 , 12, 13, or >13 years). Women with dense breasts are more likely to be diagnosed with BBD, and therefore, we did not include history of BBD because it may be a partial surrogate measure for mammographic density^{36, 37}. Additional adjustment for circulating α -carotene levels did not alter results, and were therefore not included in the final multivariate models.

Despite prior research that suggests breast density may vary with season²⁵, we did not find any significant variation in mammographic density level across season or month of mammography in our study population and therefore, did not include time of mammogram in the analysis. Secondary analyses were conducted stratified by levels of BMI (<25 , 25-30, ≥ 30), dietary calcium intake (low/high), circulating levels of insulin-like growth factor (IGF) and IGF binding protein, and PMH use (never, current, and ever users).

To evaluate whether plasma vitamin D metabolites modified the mammographic density-breast cancer association, we examined the association between cross-classified tertiles of mammographic density and tertiles of 25(OH)D and 1,25(OH)₂D and subsequent breast cancer risk. Cutpoints for tertiles for both metabolites were based on the distribution in the control group. To account for batch-to-batch variability, we created tertiles of 25(OH)D based on batch-specific cut points in the controls.

Using unconditional logistic regression, we estimated the relative risk of breast cancer across tertiles of mammographic density and vitamin D metabolites compared to the referent group (highest tertile of vitamin D metabolite/lowest tertile of mammographic density) using odds ratios and we calculated 95% confidence intervals. We conducted a simple analysis adjusted only for matching factors, and also multivariate-adjusted analyses. We considered covariates which have been reported to be associated with breast cancer as potential confounders in this analysis. In addition to the matching factors, our final multivariate model included age at menarche (<12 , 12, 13, or >13 years), BMI (continuous, kg/m²), family history of breast cancer (yes/no), duration of PMH use (continuous), alcohol intake (0, <5 , 5 to <15 , ≥ 15 g/d), parity/age at first birth (nulliparous, age at first birth <25 years, age at first birth 25-29 years, age at first birth ≥ 30 years, and missing), and age at menopause (<46 , 46 to <50 , 50 to <55 , or ≥ 55 years). Women with dense breasts are more likely to be diagnosed with BBD, and therefore, we did not include history of BBD because it may be a partial surrogate measure for mammographic density^{36, 37}.

We used a Wald test to evaluate the presence of a linear trend across tertiles of vitamin D metabolite within each level of mammographic density. In the test for trend, we modeled each plasma vitamin D metabolite as a continuous variable in the regression model. We used the likelihood ratio test to determine if there was evidence of a multiplicative interaction between plasma vitamin D metabolite levels and mammographic density.

Results

There were 463 cases and 497 matched controls in this study. Compared to controls, cases had similar circulating levels of 1,25(OH)₂D and lower mean circulating levels of 25(OH)D. The median age of controls in this study at the time of mammography was 61.0 years. Among control subjects, women in the highest quartile of circulating levels of 25(OH)D were more likely to be leaner, current users of postmenopausal hormones, have a higher percentage of mammographic density, and have a higher dietary intake of vitamin D and calcium compared to women in the lowest quartiles of 25(OH)D (Table 1). There was little variation in age at menopause, age at first birth, age at menarche, parity, and alcohol consumption according to quartiles of circulating vitamin D metabolite levels.

We found no significant association between circulating levels of plasma 25(OH)D or 1,25(OH)₂D with mammographic density among controls in this study (Table 2). In addition, the association between vitamin D metabolites and mammographic density did not vary across categories of BMI, dietary calcium intake, IGF levels, or PMH use (data not shown).

We observed an inverse association between circulating 25(OH)D and breast cancer risk in the current study population, consistent with what was observed in our previous study [6]. Women in the highest tertile of 25(OH)D had a significant 34% reduction in breast cancer risk relative to women in the lowest tertile (OR=0.66, 95% CI 0.47-0.93). Adjustment for percent mammographic density did not change these estimates. As has been shown previously, percent mammographic density was a significant predictor of breast cancer risk in this study. Women in the highest tertile of breast density were at a 3-fold increased risk of breast cancer compared with women in the lowest quintile (OR=2.9, 95% CI 2.0-4.3). Additional adjustment for 25(OH)D did not alter this association (OR=2.9, 95% CI 2.0-4.3). These results suggest that the associations between circulating 25(OH)D and breast cancer and that of mammographic density and breast cancer are independent of one another. α

In our case-control analysis, women in the highest tertile of mammographic density and lowest tertile of plasma 25(OH)D had approximately 4 times greater risk of breast cancer than women with the lowest mammographic density and highest level of plasma 25(OH)D (RR = 3.8; 95% CI, 2.0-7.3; Table 3). Among women in the highest tertile of mammographic density, there was a 48% reduced risk of breast cancer in women with a high versus low level of plasma 25(OH)D (OR=0.5; 95% CI, 0.3-0.9, p-trend=0.10). Although there is some suggestion that the mammographic density-breast cancer relationship may vary by level of plasma 25(OH)D, the overall interaction was non-significant (p-heterogeneity= 0.20). Across tertiles of plasma 1,25(OH)₂D, we found no clear variation in the association between mammographic density and breast cancer risk.

Discussion

In this study we found no evidence of an association between plasma levels of 25(OH)D or 1,25(OH)₂D and mammographic density in postmenopausal women. In addition, the associations between 25(OH)D and breast cancer and mammographic density and breast cancer appear to be independent of one another. The results of this study suggest that the biological mechanism by which mammographic density increases the risk of breast cancer is independent of plasma vitamin D metabolite levels. These results are consistent with a study by Knight et al. which found no cross-sectional relationship between plasma levels of 25(OH)D and mammographic density in a study of 487 premenopausal and postmenopausal women from the Minnesota Breast Cancer Family Study ²⁶.

We found that a low level of plasma 25(OH)D and high mammographic density was associated with an increased risk of breast cancer, which was consistent with our original hypothesis. Although the interaction between plasma vitamin D metabolites and mammographic density was not significant, our study suggests that the association between breast density and the risk of breast cancer may vary by level of plasma 25(OH)D. To our knowledge, no other study has examined the influence of varying levels of circulating vitamin D metabolites on the association between mammographic density and breast cancer risk. If confirmed in a larger study, our results would suggest that increasing plasma levels of 25(OH)D may reduce the risk of breast cancer in postmenopausal women with a high percent mammographic density.

We did not observe any clear associations between circulating 1,25(OH)₂D and breast cancer risk or mammographic density throughout this study. This is not surprising, however, because 1,25(OH)₂D is tightly regulated by homeostasis and is not considered a useful biomarker of time-integrated vitamin D status, despite it being the biologically active metabolite^{10, 20, 38}.

A limitation of the study is measurement error in both vitamin D metabolites and mammographic density. While the CVs for the 25(OH)D assay indicate good reproducibility there is still measurement error in this assay. Because all cases and controls were measured at the same time and the laboratory was blinded to case-control status, this error would be nondifferential and would result in an attenuation of the true association. Similarly, the reliability measures for mammographic also indicate that it is measured well although with some random error. This random error will also lead to an attenuation of the true association between mammographic density and breast cancer risk. Another issue which may have reduced our power to detect an association between vitamin D levels and mammographic density is that the blood collection and mammograms were not conducted on the same date. We attempted to obtain mammograms that were closest in time to the time of blood collection. Although 90% of mammograms were within one year of the blood collection date, the variability in the lag between the two measures could have reduced our ability to detect an association. While this study was adequately powered to observe differences in mammographic density that are believed to be biologically relevant (e.g., 5% point difference), we had limited power to detect smaller differences in association. Similarly, we had limited power to detect an interaction between mammographic density and vitamin D metabolites in relation to breast cancer risk in the current study.

Another limitation of this study is that it is only generalizable to postmenopausal women. Previous studies suggest that the relationship between vitamin D and mammographic density, as well as vitamin D and the risk of breast cancer, may differ among women who are premenopausal versus postmenopausal^{6, 23}. Diorio et al found that dietary intake of vitamin D was associated with premenopausal breast density primarily among women with high circulating IGF1 levels³⁹. These results are in line with laboratory studies that indicate that vitamin D inhibits the proliferative effects of the IGF1 pathway⁴⁰⁻⁴³. However, we did not find any interaction between vitamin D metabolites and IGF1 in relation to mammographic density among postmenopausal women. Consistent with the results in the current study, Knight et al found no association between breast density and plasma levels of vitamin D in both premenopausal and postmenopausal women; however, the number of premenopausal women in this study was low (n=133). Additional studies in premenopausal women are necessary to determine if menopausal status influences the association between vitamin D metabolites and breast density.

A study strength was its nested case-control design. In addition, since data on baseline characteristics were collected prospectively, we were able to adjust for many breast cancer risk factors in our analysis.

In summary, we found no relationship between plasma vitamin D metabolites and mammographic density. Thus, our study indicates that vitamin D metabolites are not a mediator of the mammographic density-breast cancer risk relationship. Our results suggest a high plasma level of 25(OH)D may be associated with a reduced risk of breast cancer among postmenopausal women with high mammographic density. Further studies are necessary to confirm these suggestive findings in a larger study and to determine possible implications for breast cancer prevention. In addition, studies to examine this association in premenopausal women would also be of importance.

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Age and age-adjusted characteristics at the time of mammography according to quartiles of Vitamin D metabolites among control subjects: Nurses' Health Study, 1990-1996

Table 1

	1,25(OH) ₂ D ^a				25 (OH)D ^b			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
N=	110	108	110	114	118	115	124	112
Means								
Age, yrs	60	60	60	59	59	60	59	59
Age at menarche, yrs	12	13	13	13	13	12	13	13
Age at first birth ^c , yrs	25	25	25	25	25	25	25	25
Age at menopause, yrs	50	50	50	50	49	51	51	50
Body mass index, kg/m ²	26	26	25	25	27	26	25	24
Parity	3.1	3.0	3.2	3.5	3.1	3.1	3.4	3.1
Alcohol consumption, g/d	6.1	6.0	7.7	7.7	5.9	6.9	5.5	8.4
Duration of PMH use ^d , months	38	61	51	51	52	55	53	45
% mammographic density	24	27	24	27	23	24	26	29
Absolute mammographic density	29	33	27	33	32	30	29	32
Dietary intake of vitamin D ^e , IU/day	397	397	371	406	311	377	417	440
Dietary intake of calcium ^e , mg/day	1259	1221	1184	1141	999	1176	1224	1348
Frequency, %								
Family history of breast cancer	12	12	11	15	7	13	14	11
Benign breast disease	43	37	38	38	39	45	33	44
Nulliparous	12	11	10	7	17	11	4	9
Current PMH user	42	44	50	50	37	42	45	56

^aFor 1,25(OH)₂D, quartile cut points were 13.0-29.1, 29.2-33.1, 33.2-37.3, 37.4-56.2 ng/mL.

^bFor 25(OH)D, quartile cut points for batch 1 were: 9.0-23.0, 24.0-29.0, 30.0-38.0, 39.0-76.0 ng/mL; for batch 2: 9.0-31.0, 32.0-38.0, 39.0-47.0, 48.0-84.0 ng/mL; and for batch 3: 6.2-22.2, 22.4-28.4, 28.5-35.1, 36.0-62.9.

^cAmong parous women

^d Among ever users of PMH
^e total intake from food and supplements

Table 2
Mean percentage of breast density by quartiles of vitamin D among postmenopausal women

Model Parameters	Mean % Density 25 (OH) D				Spearman correlation coefficient	P-value ^a
N=	118	115	124	112		
Model 1 ^b	23.4	25.2	25.6	28.2	0.14	0.003
Model 1+BMI	27.1	25.7	24.3	25.3	0.007	0.89
Full Model ^c	26.3	25.6	24.8	25.7	0.02	0.69
1,25(OH)₂ D						
N=	110	108	110	114		
Model 1 ^b	24.0	27.1	24.4	26.7	0.05	0.29
Model 1+BMI	25.6	27.7	23.2	25.8	0.003	0.96
Full Model ^c	25.5	27.6	23.3	25.8	0.01	0.78

^a P-value from spearman correlation

^b Model 1: age at blood draw (continuous), month of blood draw (Feb-April, May-July, Aug-Oct, Nov-Jan), fasting status (<10 hours or unknown, ≥ 10 hours since last meal) and time of day (1am- 6:59am, 7:00am- 12:59pm, 1pm-6:59pm, 7pm-12:59am), PMH use (yes/no).

^c Full model: Includes covariates in Model 1, BMI , family history of breast cancer, duration of PMH, alcohol consumption, age at first birth/parity, and age at menarche.

Table 3

Multivariate^a relative risk of breast cancer among postmenopausal women according to mammographic density and circulating vitamin D levels.

Tertiles of vitamin D ^d	Tertiles of mammographic density ^c			P-Heterogeneity
	T1 (low)	T2	T3 (high)	
25 (OH) D				LRT
T1 (low)	1.0 (0.5-1.9) 46/65	2.1 (1.1-4.1) 60/48	3.8 (2.0-7.3) 84/41	0.20
T2	0.9 (0.5-1.7) 32/52	1.0 (0.5-2.0) 37/60	2.4 (1.3-4.6) 65/49	
T3 (high)	1.0 (Ref) 27/42	1.3 (0.7-2.4) 39/55	2.1 (1.1-4.0) 62/57	
P-trend ^b	0.21	0.39	0.10	
1,25(OH)₂ D				0.95
T1 (low)	0.7 (0.4-1.3) 36/60	1.7 (0.9-3.4) 39/36	2.2 (1.2-4.0) 59/50	
T2	0.9 (0.5-1.8) 29/44	1.3 (0.7-2.5) 48/61	3.3 (1.7-6.1) 68/41	
T3 (high)	1.0 (REF) 32/47	1.1 (0.6-2.2) 38/54	2.8 (1.5-5.2) 72/49	
P-trend ^b	0.49	0.27	0.23	

^a Multivariate adjusted for: age at blood draw, month of blood draw, fasting status and time of day, PMH use, BMI, family history of breast cancer, duration of PMH, alcohol consumption, age at first birth/parity, and age at menarche.

^b P-for trend based on the inclusion of the continuous level of the vitamin D metabolite adjusted for batch

^c Cutpoints for tertiles of mammographic density 0-<13.4, 13.4-<32.6, 32.6+ %.

^d Cutpoints for tertiles of vitamin D: For 1,25(OH)₂D, quartile cut points were 13.0-29.1, 29.2-33.1, 33.2-37.3, 37.4-56.2 ng/mL; For 25(OH)D, quartile cut points for batch 1 were: 9.0-23.0, 24.0-29.0, 30.0-38.0, 39.0-76.0 ng/mL; for batch 2: 9.0-31.0, 32.0-38.0, 39.0-47.0, 48.0-84.0 ng/mL; and for batch 3: 6.2-22.2, 22.4-28.4, 28.5-35.1, 36.0-62.9.