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Premenopausal plasma 25-hydroxyvitamin D, mammographic density, and risk of breast cancer

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Abstract

Purpose—Epidemiologic evidence for an association between plasma 25-hydroxyvitamin D [25(OH)D] and breast cancer is inconsistent. Data are especially limited for premenopausal women and for associations with mammographic density.

Methods—To test the hypothesis that plasma concentration of 25(OH)D is associated with mammographic density, we conducted a cross-sectional study among 835 premenopausal women in the Nurses' Health Studies. We measured 25(OH)D in blood samples and used multivariable linear regression to quantify the association of average percent density by quartile of plasma 25(OH)D. In a nested case-control analysis including 493 breast cancer cases, we evaluated risk of breast cancer associated with vitamin D status within tertiles of mammographic density.

Results—Women in the top quartile of plasma 25(OH)D levels had an average percent breast density 5.2 percentage points higher than women in the bottom quartile (95% confidence interval: 1.8, 8.7; *P*-trend <0.01), after adjusting for predictors of 25(OH)D and established breast cancer risk factors. Plasma 25(OH)D concentration was significantly inversely associated with breast cancer risk among women with high mammographic density (odds ratio comparing top to bottom tertile of 25(OH)D = 0.50; 95% confidence interval= 0.30, 0.83; P-trend <0.01) but not among women in lower tertiles of mammographic density (P-interaction <0.01).

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

Conclusions—These results do not support the hypothesis that vitamin D is inversely associated with percent mammographic density in premenopausal women. There was evidence that the association between premenopausal 25(OH)D and breast cancer risk varies by mammographic density, with an inverse association apparent only among women with high mammographic density.

Keywords

mammographic density; breast cancer; epidemiology; vitamin D

Introduction

Vitamin D is hypothesized to reduce breast cancer risk but epidemiologic evidence for an association between serum 25-hydroxyvitamin D [25(OH)D] levels and breast cancer is inconsistent (1–13) and data are limited for premenopausal women (6–9).

Results from a recent meta-analysis suggested significant reduction in breast cancer risk associated with increasing 25(OH)D levels based on case-control studies but no association for prospective studies (14), while other recent meta-analyses of prospective studies suggested inverse associations of 25(OH)D with breast cancer risk among postmenopausal women but not premenopausal women (10, 15). Recent analyses in the Nurses' Health Study II also found no significant association between plasma 25(OH)D levels in premenopausal women and risk of breast cancer (9, 16).

Mammographic density is one of the strongest risk factors for breast cancer, with relative risks ranging from 4–6 for women in the highest quartile of density vs. lowest (17) in both pre- and postmenopausal women, and is considered an intermediate marker of risk (17, 18). A measure of the relative amount of fibroglandular tissue in the breast which appears light on a mammogram (vs. fat, which appears dark), mammographic density also is associated with several breast cancer risk factors, including age, menopause, and body size. Because vitamin D has anti-proliferative and pro-differentiation effects in normal breast tissue (19) and therefore could have direct or indirect influences on breast tissue composition, we hypothesized that women with higher plasma 25(OH)D would have lower percent mammographic density. Prior investigations of this hypothesis have mostly reported null findings (20–28); however, these studies have been limited by fairly small sample sizes and few have included premenopausal women (20, 21, 24, 25). Understanding how vitamin D may influence mammographic density among premenopausal women may enhance our understanding of breast cancer etiology. Further, evaluating whether mammographic density might modify the association between vitamin D levels and breast cancer risk, which has only been done in a single study of postmenopausal women (23), could inform potential prevention strategies.

Methods

Study population

The Nurses' Health Study (NHS) is a prospective cohort that was established in 1976 and included 121,701 registered female nurses in the United States, ages 30 to 55 years at enrollment. Similarly, the NHSII is an ongoing prospective cohort study of 116,430 women who were ages 25 to 42 at baseline in 1989. Self-administered questionnaires are collected every two years to update information on diseases and risk factors such as weight, family history of breast cancer, age at menarche, parity, alcohol consumption, and use of oral contraceptives. Blood samples were collected from 32,826 women in the NHS during 1989–1990 (29) and from 29,611 women in the NHSII (30). Samples have been stored in liquid nitrogen freezers (<−130°C) since collection. Within these subcohorts, nested case-control studies of breast cancer were established to investigate a wide range of biomarkers as potential predictors of breast cancer risk, as described previously (1, 9, 30–32). Briefly, we identified new diagnoses of breast cancer through biennial questionnaires and regular searches of the National Death Index and confirmed diagnoses through medical record review. Eligible cases were women who were diagnosed with breast cancer after blood collection but before June 1, 2004 for NHS or before June 1, 2007 for NHSII and had no prior history of cancer (except non-melanoma skin cancer). Controls were matched to each case by age (±2 years); menopausal status; month/year of blood collection; time of day of blood draw (±2 hours); and fasting status for both cohorts (1, 9); additional matching criteria in NHSII included race/ethnicity (African-American, Asian, Hispanic, Caucasian, other) and luteal day for timed samples (date of next period-date of luteal blood draw, ±1 day) (9).

Film-screen mammograms have been collected for women included in the nested case-control studies. Screening mammograms were obtained as close as possible to the time of blood collection (median time from blood to mammogram: 5 months; interquartile range: −2 to 22 months, among controls) and we successfully obtained mammograms from approximately 80% of eligible women. Women from whom we did and did not receive mammograms were similar with regard to breast cancer risk factors, including body mass index (BMI), parity and family history of breast cancer (31, 33). We restricted all analyses to women who were premenopausal at the time of both mammography and blood collection. We also excluded a single control with missing data on age at first birth and parity. The final analytic sample consisted of 835 controls (204 from NHS and 631 from NHSII) and 493 cases (194 from NHS and 299 from NHSII).

This study was approved by the institutional review boards of the Harvard School of Public Health and Brigham and Women's Hospital. Informed consent was implied by receipt of completed questionnaires and blood samples.

Mammographic density measurements

To assess mammographic density, the mammogram films for the craniocaudal views of both breasts were digitized at 261 μm/pixel with a Lumysis 85 laser film scanner, which covers a range of 0 to 4.0 absorbance. Film screen images were digitized and viewed on the computer screen and total breast area and total dense area were assessed using Cumulus software (34).

Percent mammographic density was calculated as absolute dense area divided by total breast area. All images were read by a single reader; within NHSII, mammograms were read in two batches approximately three years apart. Although there was high reproducibility within batch (within-person intraclass correlation coefficients ≥0.90; (35)), there was evidence of batch-to-batch variability in density measurements. Therefore, for the larger case-control dataset within NHSII, we fit multivariable linear regression models to estimate the effect of mammogram batch on density measurements, adjusting for age, menopausal status, BMI, and case-control status (36). We then adjusted density measurements in the second batch by adding the coefficient for mammogram batch to the raw value to estimate the measurements that would have been obtained if the mammogram had been included in the first batch (33).

We used the average percent density of both breasts for our main analyses as this is more strongly related to breast cancer risk than absolute density phenotypes (31, 37). However, recent evidence suggests that absolute dense and non-dense area may be independently associated with breast cancer risk (35, 38–40), so we also examined these as separate outcomes in secondary analyses.

Laboratory analyses

Plasma 25(OH)D concentrations were assayed in six batches. Detailed laboratory methods have been previously described (1, 9). Briefly, we measured plasma 25(OH)D using a high-affinity protein-binding assay after ethanol extraction (41) (batch 1; 70 cases, 84 controls) or a radioimmunoassay with radioiodinated tracers after acetonitrile extraction (batches 2–6; 423 cases, 752 controls) (42). The overall coefficients of variation (CVs) from masked replicate quality control samples included in each batch ranged from 6.0 to 17.6% (9, 43). To account for batch-to-batch variation in 25(OH)D measurements, we recalibrated levels from all batches to achieve a distribution comparable to an “average” batch, independent of age, BMI, menopausal status, case-control status, and season of blood draw, according to methods outlined by Rosner et al. (36, 43) and similar to the approach described above for mammographic density measurements.

Statistical analyses

To evaluate the association between 25(OH)D levels and mammographic density, we conducted a cross-sectional analysis among the 835 controls. Quartile cut points of plasma 25(OH)D were defined overall and within season of blood draw (i.e., February – April, May – July, August – October, November – January). We fit multivariable linear regression models with percent mammographic density as the dependent variable and quartiles of plasma 25(OH)D as the independent variable to quantify the relationship between 25(OH)D and density. In secondary analyses incorporating absolute measures of dense and non-dense breast area, we applied a square-root transformation to improve normality of these outcomes. Generalized estimating equations were used to take into account the correlation between matched controls. Statistical tests for trend were from a Wald test using the median of each quartile as a continuous variable.

Multivariable models adjusted for cohort (NHS, NHSII), ages at blood collection and mammography (continuous), race (white, nonwhite), and variables related to blood

collection including season of blood draw (February – April, May – July, August – October, November – January), fasting status (>8 hours, ≤ 8 hours), luteal day (<8, ≥8, untimed collection), and time of day (12–4 am, 4–6 am, 6 am–12 pm, 12 pm–12 am). Results from these models were generally similar to those that adjusted for age alone, so age-adjusted models are not presented. The final multivariable models included additional adjustment for age at menarche (<12, 12, 13, ≥14 years), parity and age at first birth (nulliparous; 1–2 children, <25 years; 1–2 children, 25–29 years; 1–2 children, ≥30 years; 3+ children, <25 years; 3+ children, ≥25 years), family history of breast cancer (yes, no), personal history of biopsy-confirmed benign breast disease (yes, no), alcohol intake (none, <5 g/d, ≥ 5 g/d, missing), and body mass index (BMI) at blood collection (continuous). We also considered potential confounding by BMI at age 18, physical activity, waist circumference, and waist-to-hip ratio, and season of mammography but results were not substantially different; therefore, these variables were not included in final multivariable models. Risk factor information was based on information from questionnaires completed at the time of blood collection (i.e., weight) or from biennial questionnaires completed close to the time of blood collection. A missing indicator category was used to account for missing values in the categorical covariate alcohol consumption (n=87). Three individuals were missing information on weight; we assigned these individuals the mean BMI for adjustment in multivariable models.

We conducted analyses stratified by BMI (<25 vs. ≥25 kg/m^2) and tested for statistical interaction by modeling the cross-product terms of continuous BMI and quartile medians of 25(OH)D (Wald test). We also stratified by season (winter vs. summer months).

To evaluate whether mammographic density modified the association between vitamin D status and breast cancer risk, among breast cancer cases (n=493) and controls (n=835), we fit an unconditional logistic regression model, adjusting for the matching factors and covariates listed above, that incorporated an interaction term for continuous plasma 25(OH)D and continuous percent mammographic density. We calculated odds ratios and 95% confidence intervals to estimate the relative risk of breast cancer for an “average” woman (i.e., a woman with the median level of plasma 25(OH)D and median value of percent mammographic density) according to joint categories of 25(OH)D and mammographic density tertiles compared with the referent group of lowest tertile of percent mammographic density/highest tertile of plasma 25(OH)D. Tertiles were defined according to the distributions among controls. We tested for linear trend of the vitamin D-breast cancer association by modeling plasma 25(OH)D as a continuous variable within strata of mammographic density. We used a likelihood ratio test to determine if there was evidence of a multiplicative interaction between plasma 25(OH)D and mammographic density on breast cancer risk, comparing a model with an interaction term for continuous measures of 25(OH)D and mammographic density to a main-effects only model. These models also included a term for continuous percent mammographic density. We performed secondary analyses restricting to invasive breast cancers only and estrogen receptor (ER)-positive tumors only. There were too few ER-negative cases (n=85) for meaningful analysis.

Analyses were conducted with SAS version 9.3 for UNIX (SAS Institute, Cary, NC). All *P* values were based on two-sided tests and were considered statistically significant if <0.05.

Results

All women were premenopausal at blood draw and ranged in age from 32 to 58 years at blood draw (median age: 45 years among cases and 44 years among controls). Cases were more likely to have a personal history of benign breast disease or a family history of breast cancer and had higher average percent mammographic density than controls (Table 1). As expected, BMI was inversely related to 25(OH)D concentrations among controls: the age-adjusted BMI for women in the lowest quartile of 25(OH)D was 26.9 compared to 23.5 for those in the highest quartile (Table 2). There were some differences in the percentage of women with a personal history of benign breast disease or a family history of breast cancer by 25(OH)D status, but no specific trends were apparent. Women in the lowest quartile of 25(OH)D levels were more likely to be nulliparous (20.7%) compared to those with higher 25(OH)D (11.7–15.0%). Alcohol consumption was higher among women with higher 25(OH)D concentrations. Age-adjusted average percent mammographic density increased with increasing 25(OH)D level, from 37.7% in the lowest quartile to 47.6% in the highest quartile (Table 2).

In initial linear regression models controlling for age, race, season of blood draw and other variables related to blood collection, there was a significant positive cross-sectional association between 25(OH)D levels and mammographic density in controls [difference in average percent mammographic density between top and bottom quartile was 10.9 percentage points (95% CI: 7.0, 14.8; p-trend <0.01)] (Table 3, Model 1). After further adjustment for BMI, the association was attenuated but remained statistically significant (Table 3, Model 2). In our final multivariable-adjusted models including blood collection variables, BMI, age at menarche, parity and age at first birth, family history of breast cancer, personal history of benign breast disease, and alcohol consumption, women in the top quartile of 25(OH)D levels had an average percent breast density 5.2 percentage points higher than women in the bottom quartile (95% confidence interval: 1.8, 8.7; p-trend <0.01) (Table 3, Model 3). Results were similar when season-specific quartiles of plasma 25(OH)D levels were considered and when stratified by winter vs. summer months (data not shown).

Because BMI is a strong predictor of both mammographic density and plasma 25(OH)D concentration and was observed to be a confounder of the vitamin D-mammographic density association, we stratified analyses by BMI at blood draw (<25 vs. ≥25 kg/m^2). Similar positive associations between 25(OH)D levels and average percent breast density were observed within strata of BMI (Table 4) and there was no evidence of effect modification by BMI (P-interaction = 0.15). In secondary analyses considering the association between 25(OH)D levels and absolute measures of breast density, we observed a significant positive association for absolute dense breast area and a significant inverse association for absolute non-dense area, with stronger associations apparent for women with BMI ≥25 kg/m^2 (Supplementary Table).

In the case-control analysis, the association between plasma 25(OH)D and breast cancer risk varied across category of mammographic density (P-interaction <0.01) (Table 5). Specifically, an inverse association between plasma 25(OH)D and breast cancer risk was apparent only among women with high percent mammographic density (P-trend <0.01).

Women in the highest tertile of percent mammographic density and lowest tertile of 25(OH)D had a >60% increased risk of breast cancer compared to women with low mammographic density and high 25(OH)D (RR: 1.63; 95% CI: 1.15, 2.33). In contrast, the association was absent or in the opposite direction for women with lower percent mammographic density. Of note, there was an apparent reduction in breast cancer risk among women in the lowest tertiles of mammographic density and 25(OH)D compared to those with low mammographic density and high 25(OH)D (RR: 0.60; 95% CI: 0.42, 0.88; P-trend = 0.11). This finding could have been driven by adiposity, as women with low 25(OH)D and low mammographic density tended to have higher BMI compared with other groups. However, further stratification by BMI revealed similar patterns of association in lean and overweight/obese women, although sample sizes for joint categories of exposure were small (data not shown). In sensitivity analyses, results were similar when restricted to invasive breast cancers only (n=371) and ER-positive tumors only (n=346) (data not shown).

Discussion

Contrary to our initial hypothesis, we observed a positive associations between plasma 25(OH)D and percent mammographic density among premenopausal women. Much of the apparent effect, however, was explained by BMI, which is a strong negative predictor of mammographic density (44–46) and a strong negative predictor of plasma 25(OH)D (47). Likewise, Sprague et al. (22) also reported significant positive associations between 25(OH)D and mammographic density (among postmenopausal women) in age-adjusted analyses prior to adjustment for BMI. Because BMI is strongly inversely correlated with breast density (Spearman correlation coefficient: −0.56), it is difficult to disentangle these effects from observed associations between BMI and plasma 25(OH)D. We adjusted for BMI continuously in multivariable models and associations were substantially attenuated; however, residual confounding by adiposity remains a concern. In stratified analyses, positive associations were apparent for both leaner and heavier women.

The lack of evidence for an inverse association between plasma 25(OH)D and percent mammographic density in this study of premenopausal women is generally consistent with the published literature on this topic. Of eight previous investigations of 25(OH)D and mammographic density (20–27), five of which included premenopausal women (20, 21, 24, 25, 27), none reported evidence of a statistically significant association overall. However, two prior studies reported inverse associations between seasonal changes in plasma 25(OH)D and mammographic density (21, 27). Specifically, Crew et al. observed a statistically significant inverse association between 25(OH)D and breast density during the months of July – December only (21), while Brisson et al. incorporated a lag time of about 4 months to reveal a strong negative correlation between 25(OH)D levels and mammographic density (27). In our analyses, however, we observed positive associations both in summer and winter months. Knight et al. (25) additionally reported non-significantly higher percent densities among pre- and postmenopausal women with the highest levels of 25(OH)D (reviewed in (28)), while we observed a statistically significant positive association.

Further, our case-control analysis suggested that the association between vitamin D status and breast cancer risk may vary by mammographic density. Among women in the highest tertile of mammographic density, those with lower plasma 25(OH)D levels had significantly higher risk of breast cancer than those with higher levels. While the associations are smaller in magnitude, this finding is consistent with our previous report among postmenopausal women in the NHS (23) and, if confirmed in other populations with prospective data, offers a possible opportunity for breast cancer risk reduction among women at high risk due to high mammographic density.

Unexpectedly, we also observed higher risk of breast cancer among women with low mammographic density and high 25(OH)D levels. This finding could be due to chance or may reflect residual confounding by BMI, as described above. Indeed, the association was similar in magnitude to that observed among overweight and obese women in our earlier analysis of plasma 25(OH)D and breast cancer risk in the NHSII (9). Alternatively, the positive association observed between 25(OH)D and breast cancer risk among women with low mammographic density may reflect the residual positive association between 25(OH)D and percent mammographic density that we observed in these data.

There are several important limitations to this study. First, analyses of the vitamin D-mammographic density association were cross-sectional in nature. We measured 25(OH)D in a single plasma sample collected close to the time of mammogram. While a single blood measurement may not accurately reflect long-term vitamin D status, reproducibility of plasma 25(OH)D measures in NHSII is fairly good over 2–3 years (intraclass correlation coefficient = 0.72) (48). Also, we lacked data to examine change in vitamin D status and change in mammographic density over time, which may be of etiologic interest. Finally, our ability to detect an inverse association of vitamin D with mammographic density at high levels of plasma 25(OH)D may have been limited because few women had very high concentrations.

The current study is the largest study of associations between plasma 25(OH)D and mammographic density in premenopausal women to date; additional strengths of this study include quantitative assessments of percent and absolute mammographic density from screening mammograms with high intra-reader reliability and detailed information on potential confounders, including predictors of breast density and breast cancer risk factors. Further, we were able to examine the joint effect of 25(OH)D levels and mammographic density on breast cancer risk using a prospective nested case-control study design.

Taken together with the existing literature on this topic, these results do not support the hypothesis that vitamin D is inversely associated with mammographic density in premenopausal women. While we did not find evidence that higher plasma 25(OH)D levels are associated with lower percent mammographic density, there was evidence that vitamin D may modify the effect of mammographic density on breast cancer risk among premenopausal women, with inverse associations mainly apparent among women with high mammographic density (i.e., those at higher risk of developing breast cancer), after adjusting for other established risk factors for breast cancer. These results should be interpreted with caution, however, due to the possibility of residual confounding by BMI

and the relatively small sample sizes within joint exposure categories. If confirmed in larger studies, particularly in studies with prospective data, these findings suggest that increasing circulating vitamin D concentrations may reduce breast cancer risk among women at high risk due to high percent mammographic density.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Age and age adjusted characteristics at the time of blood draw among cases and controls.

	Cases (n = 493)	Controls (n = 835)
Age at blood draw, y	45.3 (4.6)	44.4 (4.6)
Age at mammography, y	46.2 (4.4)	45.6 (4.4)
Months between blood draw and mammogram	11.3 (22.8)	13.1 (25.3)
Body mass index, kg/m^2	24.7 (4.5)	25.2 (5.4)
Family history of breast cancer, %	12.9	9.4
History of benign breast disease, %	24.9	18.2
Parity and age at first birth		
Nulliparous, %	13.3	15.1
Number of children (among parous women)	2.4 (0.9)	2.5 (0.99)
Age at first birth, y (among parous women)	26.3 (4.2)	26.2 (4.2)
Alcohol consumption, g/day*	4.6 (8.4)	4.2 (7.2)
Average % mammographic density	47.6 (20.0)	41.8 (20.1)
Plasma 25(OH)D, ng/mL	26.5 (9.8)	27.2 (10.3)

Means (SD) or percentages are shown.

*Alcohol consumption missing for 87 individuals.

Table 2

Age and age adjusted characteristics at the time of blood draw according to quartiles of plasma 25(OH)D among controls.

	Quartile 1 (n = 208)	Quartile 2 (n = 208)	Quartile 3 (n = 211)	Quartile 4 (n = 208)
Cut points (ng/mL)	1.95–<19.8	19.8–<26.8	26.8–<33.3	33.3–79.6
Plasma 25(OH)D, ng/mL	14.9 (3.9)	23.6 (2.0)	29.8 (1.8)	40.6 (7.5)
Age at blood draw, y	44.8 (4.5)	44.6 (4.6)	44.2 (4.8)	44.1 (4.5)
Age at mammography, y	46.0 (4.3)	45.9 (4.5)	45.3 (4.6)	45.2 (4.3)
Body mass index, kg/m^2	26.9 (6.5)	25.9 (5.6)	24.3 (4.7)	23.5 (3.8)
Family history of breast cancer, %	5.0	10.2	13.9	6.7
History of benign breast disease, %	18.4	14.7	18.5	16.7
Parity and age at first birth				
Nulliparous, %	20.7	11.7	14.6	15.0
Number of children (among parous women)	2.3 (0.99)	2.5 (0.87)	2.4 (0.94)	2.5 (0.95)
Age at first birth, y (among parous women)	26.2 (4.1)	26.0 (4.0)	26.1 (4.3)	26.6 (4.4)
Alcohol consumption, g/day*	2.8 (5.6)	3.7 (6.0)	5.3 (8.5)	5.5 (8.0)
Average % mammographic density	37.7 (20.5)	38.7 (19.1)	45.3 (19.1)	47.6 (19.0)

Means (SD) or percentages are shown.

*Alcohol information missing for 53 controls.

Table 3

Difference in average percent mammographic density [β (95% confidence interval)] by quartile of 25(OH)D among controls

	Quartile 1 (n = 208)	Quartile 2 (n = 208)	Quartile 3 (n = 211)	Quartile 4 (n = 208)	p-trend*
Cut points (ng/mL)	1.95–<19.8	19.8–<26.8	26.8–<33.3	33.3–79.6	
Model 1	ref	1.38 (−2.34, 5.10)	7.95 (4.19, 11.70)	10.90 (7.03, 14.76)	<0.01
Model 2	ref	−1.25 (−4.51, 2.00)	2.54 (−0.80, 5.87)	3.88 (0.41, 7.36)	<0.01
Model 3	ref	−0.10 (−3.34, 3.14)	3.09 (−0.23, 6.42)	5.22 (1.76, 8.69)	<0.01

Model 1: adjusted for age at mammogram(continuous), age at blood collection (continuous), season of blood collection (February–April, May–July, August–October, November–January), luteal day (<8, ≥8, untimed collection), fasting status at blood collection (>8h/≤8h), race (white, nonwhite), time of blood collection (12–4am, 4–6am, 6am–12pm, continuous)

Model 2: additionally adjusted for body mass index at blood draw (continuous)

Model 3: additionally adjusted for age at menarche (<12, 12, 13, ≥14y), parity and age at first birth (nulliparous, 1–2 children & <25y, 1–2 children & 25–29y, 1–2 children & ≥30y, ≥3 children & <25y, ≥3 children & ≥25y), family history of breast cancer (yes/no), history of benign breast disease (yes/no), alcohol use (0, <5g/day, >=5 g/day, missing)

*Trend test based on median value of each quartile as a continuous variable in the multivariable model.

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Table 4

Difference in average percent mammographic density [β (95% confidence interval)] by quartile of 25(OH)D among controls, stratified by body mass index (BMI).

	Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-trend*
Cut points (ng/mL)	1.95–<19.8	19.8–<26.8	26.8–<33.3	33.3–79.6	
BMI <25 kg/m^2	ref	0.60 (−3.70, 4.91)	0.97 (−3.24, 5.18)	4.01 (−0.32, 8.33)	0.06
BMI ≥25 kg/m^2	ref	0.11 (−4.74, 4.96)	7.30 (2.10, 12.51)	7.01 (1.23, 12.78)	<0.01

Models are adjusted for age at mammogram (continuous), age at blood collection (continuous), season of blood collection (February–April, May–July, August–October, November–January), luteal day (<8, ≥8, untimed collection), fasting status at blood collection (>8h/≤8h), race (white, nonwhite), time of blood collection (12–4am, 4–6am, 6am–12pm, continuous), body mass index at blood draw (continuous), age at menarche (<12, 12, 13, ≥14y), Parity and age at first birth (nulliparous, 1–2 children & <25y, 1–2 children & 25–29y, 1–2 children & ≥30y, ≥3 children & <25y, ≥3 children & ≥25y), family history of breast cancer (yes/no), history of benign breast disease (yes/no), alcohol use (0, <5g/day, >=5 g/day, missing)

*Trend test based on median value of each quartile as a continuous variable in the multivariable model. Test for interaction: $P = 0.15$

Table 5

Multivariable relative risk of breast cancer among premenopausal women, according to plasma 25(OH)D and average percent mammographic density.

		Tertile of average percent mammographic density		
Tertile of 25(OH)D		**T1 (low)**	**T2**	**T3 (high)**
T1 (low)		0.60 (0.42, 0.88)	1.02 (0.75, 1.38)	1.63 (1.15, 2.33)
	cases/controls	55/130	51/81	77/70
T2		0.62 (0.44, 0.86)	0.86 (0.64, 1.16)	1.17 (0.84, 1.63)
	cases/controls	35/93	51/99	63/84
T3 (high)		1.0 (ref)	0.99 (0.98, 1.00)	1.15 (0.97, 1.36)
	cases/controls	33/55	61/99	67/124
	P-trend*	0.11	0.65	<0.01

Multivariable relative risks represent relative risks for a woman with the mean level of plasma 25(OH)D and mean mammographic density within each joint classification of exposures, based on models including an interaction term for continuous 25(OH)D by continuous average percent mammographic density and adjusted for age at mammogram (continuous), age at blood collection (continuous), season of blood collection (February–April, May–July, August–October, November–January), luteal day (<8, ≥8, untimed collection), fasting status at blood collection (>8h/≤8h), race (white, nonwhite), time of blood collection (12–4am, 4–6am, 6am–12pm, continuous), body mass index at blood draw (continuous), age at menarche (<12, 12, 13, ≥14y), parity and age at first birth (nulliparous, 1–2 children & <25y, 1–2 children & 25–29y, 1–2 children & ≥30y, ≥3 children & <25y, ≥3 children & ≥25y), family history of breast cancer (yes/no), history of benign breast disease (yes/no), alcohol use (0, <5g/day, >=5 g/day, missing).

* Trend test based on continuous 25(OH)D in multivariable models within strata of percent mammographic density.

Test for interaction: P <0.01.