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TITLE: Total xenoestrogen body burden in relation to mammographic density, a marker of breast cancer risk

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CONTRACTING ORGANIZATION: University of Wisconsin

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14. ABSTRACT
Humans are exposed to a large number of environmental chemicals which have estrogenic activity ("xenoestrogens") and therefore may raise breast cancer risk. This study is evaluating the association of total xenoestrogen burden with mammographic breast density, which is a strong intermediate marker of breast cancer risk. All study procedures and manuals of operation for this study are finalized, and IRB approval obtained. Subject recruitment is complete. Breast density measurements on all participants have been completed. Analysis of xenoestrogen levels in the blood samples is currently ongoing. Data analysis will ensue upon completion of the analysis of xenoestrogen levels in blood samples. Since no statistical analyses have been conducted, no scientific knowledge has been produced yet.

15. SUBJECT TERMS
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INTRODUCTION

Breast cancer risk increases with higher endogenous estrogen levels and with use of pharmaceutical estrogens. Humans are also exposed to a large number of environmental chemicals which have estrogenic activity ("xenoestrogens"). Previous studies have focused on the relation between single xenoestrogen chemicals and breast cancer risk, with little evidence to support an association. The recent development of an assay to measure the sum estrogenic activity of xenoestrogens in biological samples presents a novel opportunity to evaluate total xenoestrogen exposure in relation to breast cancer risk. This study will evaluate the association of total xenoestrogen burden with mammographic breast density, which is a strong intermediate marker of breast cancer risk. To accomplish this aim, 200 healthy postmenopausal women receiving their regularly scheduled screening mammogram at a clinic in Madison, Wisconsin, will provide a blood sample and complete a questionnaire regarding established breast cancer risk factors and potential sources of xenoestrogen exposure, including diet, occupation, and lifestyle factors. The blood samples will be analyzed for total serum xenoestrogen burden, and breast density will be measured from participants' mammograms as continuous percent density. Statistical analyses will be used to identify important predictors of total xenoestrogen burden and to measure the association between total xenoestrogen burden and breast density. This study will describe current xenoestrogen exposure levels, assess their relation to breast density, and provide direction to future studies of the potential health effects of these ubiquitous compounds.

BODY

The approved Statement of Work for this grant includes:

Task 1. Finalize procedures, Months 1-6:

a. Finalize manual of operations for blood collection, processing, and temporary storage

Progress report: All study procedures and manuals of operation for this study have been finalized.

b. Obtain IRB/Human Subjects and HIPAA regulatory approvals

Progress report: Final human subjects protection approval for this study was obtained February 18, 2008 from the University of Wisconsin Institutional Review Board.

c. Pilot test questionnaire

Progress report: The study questionnaire was piloted and finalized in February 2008 (see Appendix).

d. Pilot E-Screen bioassay on 5-10 anonymous samples

Progress report: Anonymous blood samples were obtained from UW Hospital Clinics and used for piloting of the E-Screen bioassay. Use of these blood samples revealed quality control issues that have just recently (October 2009) been resolved.

e. Pilot quantitative density measurement on 5-10 anonymous mammograms at Group Health (Seattle)

Progress report: Anonymous mammograms were delivered in November 2008 to collaborators at Group Health and used for piloting of the density measurement process.

f. Finalize Microsoft Access database to track participant recruitment, questionnaires, blood samples, mammograms, and mammogram reports

Progress report: A Microsoft Access database was finalized in June 2008 to track participant recruitment, questionnaires, blood samples, mammograms, and mammogram reports.

Task 2. Recruit participants, Months 7-12:

a. Recruitment of 200 women obtaining screening mammograms at the UW Health West-Madison Clinic

Progress report: In March of 2009 we completed recruitment of 200 subjects for the study. Recruitment of an additional 70 subjects was completed by July 2009 using ancillary funding from the Susan Komen Foundation. Recruitment took longer than anticipated due to delays in obtaining IRB approval and slow subject accrual during the recruitment period. The addition of a second study site in September 2008 enhanced the rate of subject accrual and allowed us to reach our goal.

b. Obtain signed permission for release of radiology report corresponding to the screening mammogram from participants

Progress report: Signed permission has been obtained for all recruited subjects.

c. Collect blood sample and questionnaire from participants at the Clinic

Progress report: Blood samples and questionnaires have been obtained for all recruited subjects.

d. Implement questionnaire data entry and quality control measures

Progress report: Data entry from all questionnaires has been completed, with double-data-entry on a sample of questionnaires for quality control.

e. Submit annual progress report to the DOD

Progress report: An annual progress report was submitted in 2008 and 2009. This constitutes the third annual progress report.

Task 3. Analyze blood samples and mammograms, Months 13-18:

a. Transport blood samples from Office of Clinical Trials to the Wisconsin State Laboratory of Hygiene (both located in Madison, WI)

Progress report: Blood samples for the recruited subjects have been transported to the WI State Laboratory of Hygiene.

b. Perform E-Screen blood sample analysis for total xenoestrogen burden

Progress report: We have encountered extensive delays in the measurement of serum xenoestrogen levels by the Wisconsin State Laboratory of Hygiene. Unfortunately, a number of issues arose during the assay validation and quality control procedures for the planned xenoestrogen E-Screen assay. This assay combines advanced chemistry techniques with a cell-based bioassay. The chemistry techniques (solid phase extraction, HPLC, etc.) used to isolate the xenoestrogens appeared to introduce contaminants to the sample that interfered with the cell-based bioassay measuring xenoestrogen activity. After exhaustive efforts to remedy this contamination, we decided in April 2010 that the challenges to using this assay were insurmountable. After extensive discussions with our collaborators at the Wisconsin State Laboratory of Hygiene, we decided to pursue alternative methods to assay xenoestrogen activities in the samples. Rather than using the combined HPLC/cell based assay to measure total xenoestrogen activity, we decided to perform direct measurement of individual xenoestrogens via mass spectroscopy. Sufficient serum and funding is available for all study subjects to perform these measurements. While assessment of total combined xenoestrogen activity was our primary goal, direct measurement of a wide variety of individual xenoestrogens appeared to be the most feasible approximation of this goal. In May 2010 we developed a list of molecules which we wished to measure, which included a number of phytoestrogens, alkylphenols, parabens, phthalates, and other compounds found in personal care products. The assays were successfully developed, validated, and quality control tested between June 2010 and August 2010. In September 2010, measurement of xenoestrogen levels in study samples began. Completion of sample analysis is anticipated by the end of November 2010. We have requested a no cost extension so that this analysis of the blood samples can be completed.

c. Deliver mammogram copies from UW-Madison to Group Health (Seattle) for quantitative measurement

Progress report: All study mammograms have been delivered to Group Health.

d. Interpretation of mammograms for quantitative density measurements

Progress report: Quantitative density assessment has been completed on all study mammograms.

Task 4. Data analysis and communication of results, Months 19-24:

a. **Conduct statistical analysis of potential sources of xenoestrogen exposure**
b. **Conduct statistical analysis of relation between total xenoestrogen burden and mammographic density**
c. **Prepare manuscripts and final report to the DOD**

Progress report: No statistical analyses regarding xenoestrogen burden have been conducted yet, due to delays in the lab assays. No publications have been prepared. These tasks will be conducted upon completion of the serum xenoestrogen analyses. However, data analyses and dissemination of results have been performed for ancillary studies (see "Reportable Outcomes" below).

KEY RESEARCH ACCOMPLISHMENTS

- All study procedures finalized
- IRB approval obtained
- Recruitment completed
- Questionnaire data entered
- Mammographic breast density assessment completed

REPORTABLE OUTCOMES

- Poster presentation of the study design at the 2008 DOD Era of Hope Conference.[1]
- Funding has been obtained from the Susan Komen Foundation for an ancillary study of sex hormones and breast density in this study population. The Komen Foundation is providing funds to analyze sex hormone levels in the blood samples obtained in this study and for recruitment of 70 additional subjects. We presented a poster at the 2010 Annual Meeting of the Society for Epidemiologic Research describing preliminary findings regarding the association between sex hormone levels and breast density (attached).[2] We have also drafted a manuscript describing these results, which is currently under review at the journal "Hormones and Cancer".
- Funding has been obtained from the National Institutes of Health to support an ancillary study of the vitamin D pathway in relation to breast density in this study population. NIH is providing funds to analyze serum levels of vitamin D, parathyroid hormone, calcium, IGF1, IGFBP3, and retinol. We presented a poster describing preliminary findings at the 2010 Annual Meeting of the American Society of Preventive Oncology (attached).[3] A manuscript describing these results is currently in preparation.

CONCLUSION

All study procedures and manuals of operation for this study have been finalized. Final human subjects' protection approval for this study was obtained February 18, 2008 from the University of Wisconsin Institutional Review Board. In June 2008 we began recruitment of subjects for the study. Recruitment of 200 subjects was completed in March 2009. All questionnaire data has been entered and all quantitative breast density assessment has been completed. Extensive delays were encountered in the measurement of serum xenoestrogen levels by the Wisconsin State Laboratory of Hygiene. Challenges in quality control for the originally planned assay have forced the lab to utilize a different assay for the measurement of xenoestrogen levels in the study samples. These assays are currently being conducted. Statistical data analysis of the study aims will ensue upon completion of the assessment of blood xenoestrogen levels. Since no analyses have been conducted yet regarding xenoestrogen levels, no scientific knowledge has been produced yet for our specific aims.

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1. Sprague BL, Trentham-Dietz A, Sisney GA, Hemming J, Buist DSM. Total xenoestrogen body burden in relation to mammographic breast density, a marker of breast cancer risk. Presented at "Era of Hope: the Department of Breast Cancer Research Program Meeting", June 25-28, 2008; Baltimore, MD.
2. Sprague BL, Trentham-Dietz A, Gangnon RE, Buist DSM, Burnside ES, Aiello Bowles EJ, Sisney GS. Circulating sex hormones and mammographic breast density. Presented at the Annual Meeting of the Society for Epidemiologic Research, 2010; Seattle, Washington. *American Journal of Epidemiology* 171:S1, 2010.
3. Sprague BL, Trentham-Dietz A, Skinner HG, Buist DSM, Burnside ES, Aiello Bowles EJ, Gangnon RE, Sisney GS. The vitamin D pathway and mammographic breast density. Presented at the Annual Meeting of the American Society of Preventive Oncology, 2010; Bethesda, Maryland.

APPENDICES

1. Poster regarding sex hormones and breast density presented at the 2010 Annual Meeting of the Society for Epidemiologic Research.
2. Poster regarding vitamin D and breast density presented at the 2010 Annual Meeting of the American Society of Preventive Oncology.

SUPPORTING DATA

None

Circulating Sex Hormones and Mammographic Breast Density

Brian L. Sprague, Amy Trentham-Dietz, Ronald E. Gangnon, Diana S.M. Buist, Elizabeth S. Burnside, Erin J. Aiello Bowles, and Gale S. Sisney

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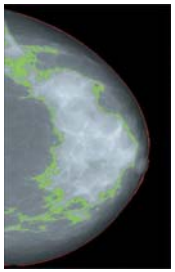
Introduction

- Mammographic breast density refers to the appearance of breast tissue on a mammogram and is determined by the relative amount of radiodense epithelial and stromal tissues compared with radiolucent fat tissue (Figure 1).
- High breast density is associated with a 4-fold increased risk of breast cancer, and is also associated with many known breast cancer risk factors (parity, postmenopausal hormone use, etc.).
- These characteristics have led many to consider the use of breast density as an intermediate marker of breast cancer risk.



$$\% \text{ density} = \frac{\text{area of dense tissue}}{\text{total breast area}}$$

Figure 1. Measuring mammographic breast density. Computer software is used to identify the percent of breast area which is high density (white area).

Study Aims

Most established breast cancer risk factors are believed to influence breast cancer risk through sex hormone pathways – most notably, estrogen exposure. We sought to determine whether breast density is associated with circulating sex hormone levels.

Methods

Population

Women (N=266) were recruited from screening mammography clinics in Madison, WI, between June 2008 and July 2009. Eligible women were postmenopausal, aged 55-70, with no history of postmenopausal hormone use, and no previous diagnosis of breast cancer.

Data Collection

- A blood sample was obtained for the analysis of sex hormone levels.
- Percent breast density was measured from each subject's screening mammogram as a continuous variable using a computer-assisted thresholding technique (Cumulus software).
- A study questionnaire was administered to assess body weight and other potential confounders.

Analyses

Multivariable linear regression was used to assess the association of serum measurements with percent density (square root transformed) in age- and multivariable-adjusted models.

Results

Characteristics of the study population

- Mean percent breast density was 15.6%, with range 0.5-71.2.
- 68% of subjects were overweight or obese.

Table 1. Distribution of circulating hormone levels in study subjects (N=266).

Hormone	Mean	Standard Deviation	Q1	Median	Q3	Range
Estradiol (pg/mL)	11.9	13.6	6.4	9.4	13.2	2.8, 156.0
Free estradiol (pg/mL)	0.31	0.30	0.14	0.24	0.36	0.03, 3.0
Bioavailable estradiol (pg/mL)	7.8	7.6	3.6	6.0	9.0	0.7, 77.0
Estrone (pg/mL)	30.8	16.8	19.8	26.6	39.6	6.0, 117.8
Estrone sulfate (ng/mL)	0.74	0.31	0.54	0.68	0.89	0.21, 2.00
Testosterone (ng/dL)	23.0	11.7	14.4	19.8	28.0	4.0, 81.6
Free testosterone (pg/mL)	4.7	2.6	2.9	4.1	6.1	0.8, 16.2
Bioavailable testosterone (ng/dL)	11.6	6.3	7.1	10.1	14.9	1.9, 39.8
Progesterone (pg/mL)	52.1	47.0	33.3	45.6	61.1	8.0, 181.1
Sex hormone binding globulin (nmol/L)	45.2	23.7	29.7	42.4	54.8	5.2, 197.0

The association between sex hormones and mammographic breast density

- In age-adjusted analyses, many sex hormones were associated with breast density.
- These associations were largely eliminated in the multivariable-adjusted model.
  - ➢Progesterone was the only hormone significantly associated with breast density.

Table 2. The associations between serum measurements and breast density.

Hormone	Mean Percent Density by Hormone Quartile Q1	Q2	Q3	Q4	P_{trend}
Estradiol					
Age-adjusted	16.8 (14.2, 19.7)	14.0 (11.4, 16.8)	13.7 (11.2, 16.5)	8.5 (6.5, 10.8)	**<0.0001**
Multivariable-adjusted*	13.6 (11.4, 16.1)	13.8 (11.4, 16.4)	13.6 (11.3, 16.1)	11.9 (9.6, 14.5)	0.38
Free estradiol					
Age-adjusted	19.2 (16.2, 22.4)	12.8 (10.4, 15.4)	12.8 (10.4, 15.5)	9.0 (7.0, 11.3)	**<0.0001**
Multivariable-adjusted*	15.0 (12.4, 17.8)	12.5 (10.3, 14.9)	12.9 (10.7, 15.3)	12.6 (10.2, 15.2)	0.30
Bioavailable estradiol					
Age-adjusted	19.6 (16.6, 22.8)	12.4 (10.1, 15.0)	12.8 (10.4, 15.5)	9.0 (7.0, 11.3)	**<0.0001**
Multivariable-adjusted*	15.4 (12.8, 18.2)	12.1 (10.0, 14.5)	12.9 (10.7, 15.3)	12.5 (10.2, 15.1)	0.24
Estrone					
Age-adjusted	16.2 (13.4, 19.2)	12.0 (9.6, 14.7)	14.3 (11.6, 17.1)	10.6 (8.3, 13.1)	**0.02**
Multivariable-adjusted*	13.7 (11.4, 16.2)	11.7 (9.6, 14.0)	14.2 (11.9, 16.7)	13.3 (11.0, 15.9)	0.80
Estrone sulfate					
Age-adjusted	14.3 (11.6, 17.3)	14.4 (11.7, 17.3)	12.9 (10.5, 15.6)	11.3 (9.0, 13.9)	0.09
Multivariable-adjusted*	13.2 (10.9, 15.8)	13.5 (11.3, 16.0)	13.3 (11.0, 15.7)	12.7 (10.4, 15.1)	0.71
Testosterone					
Age-adjusted	12.9 (10.3, 15.7)	15.3 (12.5, 18.4)	13.8 (11.1, 16.7)	11.1 (8.8, 13.7)	0.27
Multivariable-adjusted*	12.7 (10.5, 15.2)	13.7 (11.4, 16.3)	13.9 (11.6, 16.5)	12.5 (10.3, 14.9)	0.91
Free testosterone					
Age-adjusted	16.4 (13.6, 19.6)	14.3 (11.6, 17.1)	12.5 (10.0, 15.2)	10.1 (7.9, 12.6)	**0.001**
Multivariable-adjusted*	14.7 (12.2, 17.5)	13.0 (10.8, 15.4)	13.7 (11.4, 16.1)	11.7 (9.5, 14.0)	0.13
Bioavailable testosterone					
Age-adjusted	16.6 (13.7, 19.7)	14.1 (11.5, 17.0)	12.5 (10.0, 15.2)	10.1 (7.9, 12.6)	**<0.001**
Multivariable-adjusted*	14.8 (12.4, 17.6)	12.9 (10.7, 15.3)	13.7 (11.4, 16.1)	11.7 (9.5, 14.0)	0.12
Progesterone					
Age-adjusted	10.6 (8.4, 13.0)	11.5 (9.2, 14.1)	14.8 (12.1, 17.7)	16.6 (13.7, 19.7)	**<0.001**
Multivariable-adjusted*	12.2 (10.1, 14.6)	11.6 (9.6, 13.9)	14.1 (11.8, 16.6)	15.0 (12.6, 17.6)	**0.05**
Sex hormone binding globulin					
Age-adjusted	10.4 (8.2, 12.9)	12.5 (10.1, 15.2)	12.1 (9.8, 14.7)	18.2 (15.3, 21.5)	**<0.001**
Multivariable-adjusted*	13.0 (10.7, 15.6)	15.1 (12.7, 17.7)	10.3 (8.4, 12.5)	14.6 (12.2, 17.4)	0.88

Mean percent density displayed is reverse transformed from model of square root density.
*Adjusted for age, body mass index, age at menarche, parity, vigorous physical activity, smoking, and family history of breast cancer.

Discussion

We found little evidence for a strong relation between circulating estrogens and mammographic breast density among postmenopausal women with no history of postmenopausal hormone use.

However, there was a positive association between progesterone and breast density, and the suggestion of an inverse association between free or bioavailable testosterone and breast density.

These findings provide evidence that the known association between breast density and breast cancer risk may be through pathways independent of circulating estrogen levels (Figure 2).

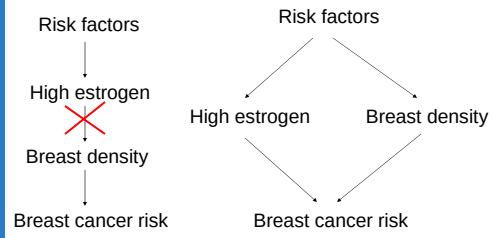


Figure 2. Schematic of potential role of breast density in relation to breast cancer risk, risk factors, and estrogen exposure.

Limitations

The interpretation of these results is limited by:

- The cross-sectional nature of the data.
- A single blood draw for each subject to measure circulating hormone levels..
- Limited power to detect small associations between sex hormones and breast density.

Acknowledgements

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The Vitamin D Pathway and Mammographic Breast Density

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Introduction

• Vitamin D has a number of chemopreventive properties.

• These properties may be mediated or modified by other molecules in the Vitamin D pathway (Figure 1).

• There is very little epidemiologic data exploring the effects of Vitamin D on breast cancer risk in the context of these other molecules.

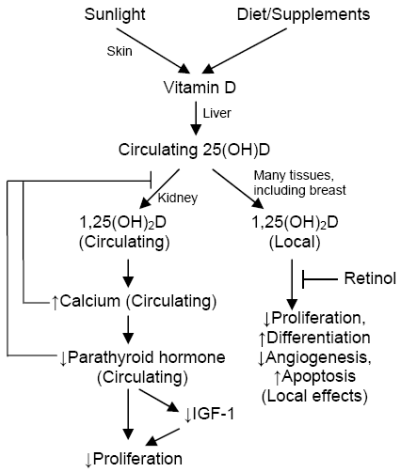

Figure 1. Hypothesized pathway by which Vitamin D may influence breast carcinogenesis.

Study Aims

A cross-sectional study of mammographic breast density was conducted. Breast density reflects the amount of proliferating breast epithelial cells and is considered an intermediate marker of breast cancer risk. We investigated the following aims:

1)To examine the association between blood levels of Vitamin D and mammographic breast density.

2)To examine the association between breast density and parathyroid hormone (PTH) and IGF-1.

3)To determine if the relation between Vitamin D and breast density is modified by blood levels of retinol and/or calcium.

Methods

Population

Women (N=269) were recruited from screening mammography clinics in Madison, WI between June 23, 2008, and July 3, 2009. Eligible women were postmenopausal, aged 55-70, with no history of postmenopausal hormone use, and no previous diagnosis of breast cancer.

Data Collection

• A blood sample was obtained for the analysis of serum levels of 25-hydroxy vitamin D [25(OH)D], parathyroid hormone (PTH), insulin-like growth factor-1 (IGF1), retinol, and calcium.

• Percent breast density was measured from each subject's screening mammogram as a continuous variable using a computer-assisted thresholding technique (Cumulus software).

• A study questionnaire was administered to assess body weight and other potential confounders.

Analyses

Multivariable linear regression was used to assess the association of serum measurements with percent density (square root transformed), while adjusting for age, body mass index, and month of blood draw.

Results

Characteristics of the study population

• Mean percent breast density was 15.6%, with range 0.5-71.2.

• 6.0% of subjects were Vitamin D deficient (25(OH)D < 20 ng/mL) and 26.7% were insufficient (25(OH)D between 20-29 ng/mL).

• 68% of subjects were overweight or obese.

There was no association between breast density and circulating 25(OH)D, PTH, or IGF1 after adjusting for age, season of blood draw, and body mass index (Table 1, Figure 2).

Table 1. Adjusted least-square means of percent breast density by quartile of selected molecules.

	N	Mean Percent Density*	95% CI*	Mean Percent Density†	95% CI†
25(OH)D (ng/mL)					
<27.6	62	11.2	8.9, 13.9	14.1	11.7, 16.8
27.6-33.8	62	13.4	10.8, 16.3	13.6	11.3, 16.1
33.9-40.5	62	13.2	10.5, 16.1	12.3	10.1, 14.7
>40.5	61	15.2	12.4, 18.2	13.1	10.8, 15.6
		P_{trend} = 0.06		P_{trend} = 0.44	
PTH (pg/mL)					
<27.1	61	15.4	12.5, 18.5	14.0	11.6, 16.6
27.1-36.8	61	13.4	10.7, 16.3	14.2	11.8, 16.8
36.9-51.2	61	13.1	10.5, 15.9	12.4	10.2, 14.8
>51.2	60	11.3	8.8, 14.0	12.7	10.4, 15.3
		P_{trend} = 0.06		P_{trend} = 0.33	
IGF1 (ng/mL)					
<102.0	61	12.5	10.0, 15.3	14.6	12.1, 17.3
102.0-132.9	61	13.8	11.1, 16.7	13.7	11.4, 16.2
133.0-160.9	62	11.7	9.3, 14.5	11.5	9.4, 13.8
>160.9	63	14.8	12.1, 17.8	13.5	11.2, 16.0
		P_{trend} = 0.43		P_{trend} = 0.33	

*Adjusted for age and season.
†Adjusted for age, season, and body mass index.

Stratified by Body Mass Index

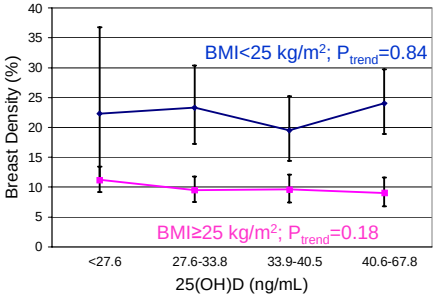

Figure 2. Breast density according to 25(OH)D quartile, stratified by body mass index and adjusted for age, season of blood draw, and body mass index.

Results

The relation between 25(OH)D and breast density was not substantially modified by retinol or calcium levels (Table 2).

Table 2. Beta coefficients describing the relation between 25(OH)D and the square root of breast density.

	N	β coefficient*	95% CI*	P*
All women	243	-0.0068	-0.0238, 0.0101	0.43
Retinol ≤ 0.72 ng/mL	125	0.0036	-0.0213, 0.0284	0.78
Retinol > 0.72 ng/mL	118	-0.0145	-0.0393, 0.0103	0.25
		$P_{interaction}$ = 0.34		
Calcium ≤ 10.23 mg/dL	122	-0.0035	-0.0265, 0.0195	0.77
Calcium > 10.23 mg/dL	121	-0.0090	-0.0360, 0.0179	0.51
		$P_{interaction}$ = 0.90		

*Adjusted for age, season, and body mass index.

Discussion

Consideration of the other molecules in the Vitamin D pathway did not reveal a strong association between Vitamin D and mammographic breast density.

The interpretation of these results is limited by the cross-sectional nature of the data. There was also limited power to detect small associations between Vitamin D and breast density, particularly among subgroups. Further, the limited geographic range of participants likely restricted variation in Vitamin D exposure.

It remains possible that Vitamin D influences breast cancer risk independent of an effect on breast density.

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