Vitamin D and Calcium Intakes from Food or Supplements and Mammographic Breast Density

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Abstract

Background: **A better understanding of factors that affect breast density, one of the strongest breast cancer risk indicators, may provide important clues about breast cancer etiology and prevention. This study evaluates the association of vitamin D and calcium, from food and/or supplements, to breast density in premenopausal and postmenopausal women separately.**

Methods: **A total of 777 premenopausal and 783 postmenopausal women recruited at two radiology clinics in Quebec City, Canada, in 2001 to 2002, completed a food frequency questionnaire to assess vitamin D and calcium. Breast density from screening mammograms was assessed using a computer-assisted method. Associations between vitamin D or calcium and breast density were evaluated using linear regression models. Adjusted means in breast density were assessed according to the combined daily intakes of the two nutrients using generalized linear models.**

Results: **In premenopausal women, total intakes of vitamin D and calcium were inversely related to breast density ($\beta = -1.4$; $P = 0.004$ for vitamin D; $\beta = -0.8$; $P = 0.0004$ for calcium). In multivariate linear regression, simultaneous increments in daily total intakes of 400 IU vitamin D and 1,000 mg calcium were associated with an 8.5% (95% confidence interval, 1.8-15.1) lower mean breast density. The negative association between dietary vitamin D intake and breast density tended to be stronger at higher levels of calcium intake and vice versa. Among postmenopausal women, intakes of vitamin D and calcium were not associated with breast density.**

Conclusion: **These findings show that higher intakes of vitamin D and calcium from food and supplements are related to lower levels of breast density among premenopausal women. They suggest that increasing intakes of vitamin D and calcium may represent a safe and inexpensive strategy for breast cancer prevention.**

Introduction

The idea that vitamin D and calcium may have a role in the prevention of breast cancer is generating growing interest (1-7). Vitamin D (8) and calcium (9) have both been found to modulate cellular proliferation and differentiation in several tissues, including breast tissue. The suggestion that vitamin D or calcium may protect against breast cancer is supported by ecologic, case-control, cohort, and laboratory studies, but the evidence still remains inconclusive. For instance, ecologic data show that vitamin D (UV-B radiation and diet) is associated with a reduction in breast cancer mortality (4, 10-15) and incidence (16, 17). Protective effects of vitamin D (18, 19) or calcium (19-27) on breast cancer risk were reported in some, although not all (18, 19, 22), case-control studies, and statistical significance was reached in a subset of these (25-27). Cohort studies (28-30) have provided evidence for a possible role of vitamin D or calcium in reducing breast cancer risk. In a cohort study of the relationship between intakes of dairy products and breast cancer risk, milk was found to have a protective effect, and calcium was described as largely, but not solely, responsible for this effect (30). In the first National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (29), sunlight exposure and dietary vitamin D intake correlated with reduced breast cancer risk. Furthermore, in the Nurses' Health Study, vitamin D and calcium were inversely associated with breast cancer risk, but this effect was seen in premenopausal women only (28).

Mammographic breast density is strongly related to breast cancer risk (31-40) and has been proposed as an intermediate marker in studies of potential approaches for the prevention of breast cancer (41-52). Mammographic breast density seems to reflect extent of epithelial and nonepithelial cells in the breast (53) as well as epithelial and/or stromal proliferation (54) and this may explain, at least in part, the relation of breast density with breast cancer risk. To our knowledge, only three studies (55-57) have examined the relation of vitamin D or calcium intakes to breast density. The first report found no association between total vitamin D (food and supplements) and breast density, among premenopausal or postmenopausal women, in a historical cohort study of breast cancer families in United States (55). Calcium intake was not examined by the authors. In contrast, the two other studies (56, 57) found that vitamin D and calcium from food were both negatively associated with breast density at least in premenopausal women.

The present study aimed at clarifying the relation of individual and combined intakes of vitamin D and calcium, from food and/or supplements, to breast density in premenopausal and postmenopausal women separately.

Materials and Methods

Study Population and Recruitment Procedures. The study subjects were recruited between February 2001 and March 2002 among women who received a screening mammogram at two private radiology clinics in Quebec City (Quebec, Canada). To be eligible, women had first to be classified as premenopausal or postmenopausal according to the Nurses' Health Study criteria (58). Thus, women were considered premenopausal if they had at least one natural menstrual cycle within 12 months or were ages <48 years (if a nonsmoker) or 46 years (if a smoker) after hysterectomy without bilateral oophorectomy. They were considered as postmenopausal if they reported complete cessation of menses for at least 12 months or were ages at least 56 years (if a nonsmoker) or 54 years (if a smoker) after hysterectomy without bilateral oophorectomy or uninterrupted menses following continuous use of hormonal derivatives. To remain eligible, women should have no personal history of cancer, breast reduction or implants, diabetes mellitus, dwarfism/acromegaly, and thyroid, adrenal, or hepatic disease; have never taken tamoxifen or raloxifene; have not taken oral contraceptives or use hormone replacement therapy in the last 3 months before mammography; and not be pregnant. This study was reviewed and approved by the Research Ethics Committee of the Centre hospitalier affilié universitaire de Québec (Quebec, Quebec, Canada). Study participants provided written informed consent.

A total of 800 premenopausal and 814 postmenopausal women were identified as potentially eligible for the study. Among these women, 23 women were subsequently found to be ineligible (13 premenopausal and 10 postmenopausal women); 11 women (2 premenopausal and 9 postmenopausal women) declined participation; 17 women (8 premenopausal and 9 postmenopausal women) could not provide a blood sample, film mammograms, or the food frequency questionnaire; and 3 women (postmenopausal) were excluded because they reported extremely low (<600 kcal) or high (>5,000 kcal) daily caloric intake based on the food frequency questionnaire. Thus, the present analysis is based on data from 777 premenopausal and 783 postmenopausal women.

Data Collection. At the radiology clinic, the women's weight, height, and waist and hip circumferences were measured by a trained research nurse who also collected the blood specimen (20 mL). Known or suspected breast cancer risk factors were documented by a telephone interview and included reproductive and menstrual history, family history of breast cancer, personal history of breast biopsies, past use of contraceptives and hormone replacement therapy, smoking status, alcohol intake, education, and physical activity. The level of physical activity, expressed as metabolic equivalent hours per week (59), was assessed using the Nurses' Health Study II Activity and Inactivity Questionnaire (60).

Diet was assessed with a self-administered 161-item semiquantitative food frequency questionnaire (97GP copyrighted at Harvard University, Boston, MA). In this questionnaire, women reported their frequency of consumption for specific foods, ranging from ''never'' to ''six or more times per day,'' over the previous year. The analysis of the nutrient content of diet as assessed with the food frequency questionnaire was done at Harvard University, where dietary nutrients intakes were calculated based on the nutrient content of food derived from U.S. Department of Agriculture sources, supplemented with data from food manufacturers and personal communications with laboratories. Women provided the duration, brand, and type of multivitamins and the number of tablets taken per week, their daily dose of calcium supplements (<400, 400-900, 901-1,300, or >1,300 mg), and their use of vitamin D if taken on a regular basis (assumed to be 400 IU/d). Some brands of multivitamins available in Canada had no specific code in the Harvard database and were assigned a generic code (e.g., Generic B Complex and Generic Women's Formula). The total nutrient intake was calculated by adding the amounts from multivitamins and specific supplements to the intake from food.

Digitization of Mammograms and Assessment of Mammographic Breast Density. All mammograms were digitized using a Kodak Lumiscan85 digitizer. Assessment of breast density was done by one trained author (C.D.) without any information on women using a computer-assisted method (36, 61, 62). The extent of breast density was calculated as the proportion of the breast projection showing tissue density from one randomly selected craniocaudal view for each woman. Variability in the assessment of breast density was similar in premenopausal and postmenopausal women: the within-batch intraclass correlation coefficient was 0.98 and the between-batch coefficient of variation was 4%.

Statistical Analysis. As originally planned, all the analyses were done in premenopausal and postmenopausal women separately. Nutrient intakes from food or supplements (including multivitamins) were studied separately or combined to study their total intake.

Associations between continuous levels of vitamin D (IU/d) or calcium (mg/d) daily intakes in the past year and continuous measures of breast density were evaluated using linear regression models (Table 2). Given the different measurement units and pattern of consumption, the βs for vitamin D and calcium are not directly comparable.

In premenopausal women, multivariate-adjusted means in breast density were assessed according to categories of daily vitamin D (<100, 100-199, 200-399, and ≥400 IU) and calcium (<650, 650-999, 1,000-1,199, and ≥1,200 mg) total intakes, respectively, using ANOVA (generalized linear model); *P*s for linear contrast are reported.

In premenopausal women, multivariate-adjusted means in breast density were further assessed according to the combined daily vitamin D and calcium total intakes using generalized linear model (Table 3). Multivariate linear regression was used to study the associations between continuous levels of dietary vitamin D (IU/d) or calcium (mg/d) intakes and breast density within each of the three tertiles of intake of calcium or vitamin D, respectively. Finally, adjusted means in breast density were compared between women in the lower and those in the higher tertiles of both vitamin D and calcium total intakes using the generalized linear model procedure.

Covariates considered *a priori* as potential confounders were included in all multivariate models. The following covariates were treated as continuous variables: age (years), body mass index (kg/m^2), age at menarche (years), number of full-term pregnancies, age at first full-term pregnancy (years), duration of past use of contraceptives and hormonal replacement therapy (years), alcohol intake in the past year (drinks per week), mean daily caloric intake in the past year (kcal/d), and level of physical activity in the past year (metabolic equivalent hours per week). Other covariates included in all multivariate models were family history of breast cancer in first-degree relative (yes/no), personal history of breast biopsies (yes/no), smoking status (nonsmoker, ex-smoker, or current smoker), and education (highest completed degree: primary or less, secondary, college, or university). Models of the effect of vitamin D or calcium intakes from food on breast density were adjusted for vitamin D or calcium from supplements, respectively. Supplement use, which is recognized as being a behavioral marker for other factors related to cancer risk (63-65), was selected *post hoc* as a potential confounder. To adjust for such factors, an indicator variable for current use of vitamin D or calcium supplements (yes/no) was included in multivariate models of the effect of total vitamin D or calcium intakes on breast density.

The percentage of women with adequate daily vitamin D and calcium intakes was assessed based on the standards set by the Food and Nutrition Board of the National Academy of Sciences (66). Adequate daily intake of vitamin D for women ages 19 to 50, 51 to 70, and >70 years is 200, 400, and 600 IU, respectively. Adequate daily intake of calcium for women ages 19 to 50 and >50 years is 1,000 and 1,200 mg, respectively (66).

Statistical significance was based on two-sided *P*s. All statistical analyses were carried out using the SAS (SAS Institute, Inc., Cary, NC) software system.

Results

Study Population. Characteristics of premenopausal and postmenopausal women are described in Table 1. Briefly, age ranged between 31 and 58 years (mean, 46.7 years) and between 45 and 81 years (mean, 61.4 years) in premenopausal and postmenopausal women, respectively. As expected, several characteristics differed between the two groups. Premenopausal women were leaner and more physically active, were more likely to report a family history of breast cancer, were less likely to have used hormonal replacement therapy, and had a higher percentage of the breast with mammographic density. On the other hand, postmenopausal women had more children and at a younger age, were less likely to have completed primary school, to have used oral contraceptives, or to have consumed alcohol in the past year, and were more likely to be nonsmoker. Premenopausal and postmenopausal women reported comparable mean daily dietary vitamin D (187.6 and 202.3 IU, respectively) and calcium (856.5 and 862.9 mg, respectively) intakes. Adequate total daily vitamin D and calcium intakes were reached by 29.6% and 24.8% of premenopausal and postmenopausal women, respectively. The proportion of multivitamins users was similar in premenopausal and postmenopausal women (20.7% and 22.6%, respectively), whereas premenopausal women were less likely than postmenopausal women to report vitamin D (7.5% and 23.8%, respectively) or calcium (20.2% and 44.1%, respectively) as single supplements.

Sources of Vitamin D and Calcium Intakes. Among the foods or supplements listed in the food frequency questionnaire, a few items were identified as explaining a large part of the variability in vitamin D or calcium intakes. In premenopausal and postmenopausal women, half the variance in dietary vitamin D intake was explained by milk intake (glasses) and half by fish intake (mainly dark meat fish). Sixty percent of the variance in dietary calcium intake were explained by milk intake, whereas other dairy products (cheese and yogurt) contributed to another 30% of the variability. Multivitamins accounted for a large portion of the variability in vitamin D intake from supplements (60% and 54% in premenopausal and postmenopausal women, respectively) but to a lesser portion of the variability in calcium intake from supplements (9% and 6%, respectively).

Vitamin D and Calcium Intakes and Breast Density. In premenopausal women, daily dietary and total intakes of vitamin D or calcium were associated with lower breast density (Table 2). The first series of models (Table 2, model 1) estimate the separate association of vitamin D intakes from food and supplements with breast density without adjustment for calcium intake and vice versa. Higher levels of dietary vitamin D intake were associated with lower breast density even when intake of vitamin D from supplements was taken into consideration ($\beta = -1.8$; $P = 0.008$). Higher vitamin D intake from supplements also was associated with lower breast density when adjustments were made for dietary vitamin D, although this association failed to reach statistical significance ($\beta = -1.0$; $P = 0.16$). Finally, higher total vitamin D intakes from food and supplements were clearly related to lower breast density ($\beta = -1.4$; $P = 0.004$). Multivariate

Table 1. Characteristics of premenopausal and postmenopausal women

	Premenopausal women (n = 777)	Postmenopausal women (n = 783)
Age (y), mean ± SD	46.7 ± 4.6	61.4 ± 6.8
Body mass index (kg/m^2), mean ± SD	25.2 ± 4.5	27.1 ± 4.7
Age (y) at menarche, mean ± SD	12.8 ± 1.6	12.7 ± 1.6
Parity, mean ± SD	1.6 ± 1.1	2.1 ± 1.8
Age (y) at first birth,* mean ± SD	26.3 ± 4.2	25.2 ± 4.1
Contraceptive ever use, %	91.8	53.5
Hormonal replacement therapy ever use, %	5.8	39.5
Personal history of breast biopsies, %	14.4	16.1
Family history of breast cancer,† %	36.6	30.4
Education (highest degree completed), %		
Primary or less	6.6	26.4
Secondary	31.3	34.2
College	26.5	12.5
University	35.7	26.8
Smoking status, %		
Nonsmoker	45.6	58.9
Ex-smoker	39.5	27.8
Current smoker	14.9	13.3
Daily average intake, mean ± SD		
Calories (kcal)	1,912.0 ± 521.3	1,971.3 ± 641.8
Vitamin D from food (IU)	187.6 ± 121.8	202.3 ± 134.4
Vitamin D from supplements‡ (IU)	393.9 ± 199.3	470.4 ± 235.7
Calcium from food (mg)	856.5 ± 362.2	862.9 ± 416.9
Calcium from supplements‡ (mg)	373.2 ± 336.7	486.3 ± 337.9
Alcohol§ intake (drinks per week), mean ± SD	3.4 ± 3.8	2.5 ± 4.4
Physical activity (metabolic equivalent hours per week), mean ± SD	27.0 ± 22.3	25.7 ± 23.4
Breast density (%), mean ± SD	42.0 ± 24.3	18.5 ± 16.8

*In parous women.

†Mother, sister, and daughter.

‡In vitamin D or calcium supplements users, respectively. In premenopausal women, n = 201 and 257 for vitamin D and calcium, respectively. In postmenopausal women, n = 311 and 426, respectively.

§In all premenopausal and postmenopausal women, respectively. Complete abstainers represent 7.6% premenopausal women and 17.1% postmenopausal women.

Table 2. Relation of vitamin D or calcium intakes to breast density (percentage of the breast showing mammographic density) in premenopausal and postmenopausal women

Vitamin D and calcium intakes	Premenopausal women (n = 777), β* (P)			Postmenopausal women (n = 783), β* (P)		
	Crude models	Adjusted model 1†	Adjusted model 2‡	Crude models	Adjusted model 1†	Adjusted model 2‡
Vitamin D (IU/d)						
From food	−1.4 (0.05)	−1.8 (0.008)	−1.0 (0.27)	0.4 (0.33)	−0.4 (0.40)	−0.7 (0.19)
From supplements§	−1.2 (0.17)	−1.0 (0.16)	−0.6 (0.44)	0.4 (0.29)	0.4 (0.29)	0.3 (0.47)
Total	−0.1 (0.68)	−1.4 (0.004)	−0.7 (0.23)	0.3 (0.08)	0.1 (0.76)	0.0 (1.00)
Calcium (mg/d)						
From food	−0.5 (0.04)	−0.7 (0.005)	−0.5 (0.15)	0.3 (0.06)	0.1 (0.72)	0.2 (0.31)
From supplements§	−1.1 (0.02)	−0.7 (0.06)	−0.5 (0.23)	0.5 (0.05)	0.2 (0.46)	0.1 (0.70)
Total	−0.4 (0.06)	−0.8 (0.0004)	−0.6 (0.02)	0.3 (0.006)	0.1 (0.49)	0.1 (0.58)

*βs are estimated from linear regression analysis; vitamin D (IU) and calcium (mg) intakes are continuous variables (P). βs represent absolute mean decrease or increase in breast density (%) for increments of 100 IU vitamin D or 100 mg calcium, respectively.
†Model 1: These models are adjusted for age, body mass index, age at menarche, number of birth, age at first birth, duration of use of oral contraceptive and hormone replacement, personal history of breast biopsies, family history of breast cancer, education, alcohol and total caloric intakes, physical activity, and smoking. In addition, the βs for dietary vitamin D and calcium intakes are also adjusted for intakes of vitamin D or calcium from supplements, respectively; the βs for vitamin D and calcium from supplements are adjusted for dietary vitamin D or calcium intakes, respectively; and the βs for total vitamin D and calcium are adjusted for an indicator variable of vitamin D or calcium supplement use, respectively.
‡Model 2: These models are adjusted for the same covariates as in model 1. In addition, the βs for both dietary vitamin D and vitamin D from supplements are adjusted for calcium intake; the βs for both dietary calcium and calcium from supplements are adjusted for vitamin D intake; the β for total vitamin D is adjusted for total calcium intake and use of calcium supplements; and the β for total calcium is adjusted for total vitamin D intake and use of vitamin D supplements.
§In supplements users only.

adjustments using the season of the year the women had their mammograms as a proxy for vitamin D from sun exposure did not materially alter these estimates. Similarly, higher calcium intakes, whether from food, from supplements, or in total, were associated with lower breast density (β = −0.7, −0.7, and −0.8; P = 0.005, 0.06, and 0.0004, respectively). Means in breast density were assessed according to categories of daily vitamin D and calcium total intakes adjusting for the same factors as in model 1 of Table 2. For categories of increasing vitamin D total intake (<100, 100-199, 200-399, ≥400 IU), adjusted means in breast density (and the number of women in each category) were 45.2% (n = 140), 43.8% (n = 241), 41.5% (n = 197), and 38.7% (n = 199), respectively (P = 0.04 for linear contrast). For increasing calcium total intake (<650, 650-999, 1,000-1,199, and ≥1,200 mg/d), adjusted means in breast density were 46.8% (n = 194), 41.6% (n = 262), 43.5% (n = 103), and 38.1% (n = 218), respectively (P = 0.002 for linear contrast).

The second series of models (Table 2, model 2) estimate the association of daily vitamin D intake while adjusting for daily intake of calcium and vice versa. When calcium intake is taken into account, the strength of the relation of vitamin D to breast density was weakened. The strength of the relation of calcium to breast density was also reduced, but the association of total calcium intake with breast density remained statistically significant.

In postmenopausal women, daily vitamin D and calcium intakes, whether from food, from supplements, or in total, showed little or no association with breast density (Table 2).

The variation of breast density according to the combined daily intakes of vitamin D and calcium was examined in premenopausal women (Table 3). Because vitamin D and calcium intakes are highly correlated (Pearson correlation coefficient = 0.57; P < 0.001), many women with low vitamin D intake also have a low calcium intake and many women with high vitamin D intake also have a high calcium intake. An absolute reduction in mean breast density of 6.9% (P = 0.017) was observed among women in the top tertile of both vitamin D and calcium intakes compared with those in the bottom tertile of intakes of the two nutrients. When considering both nutrients as continuous variables together with an interaction term for the combined effect of the two in a linear regression model (adjusted for the same factors as those listed in Table 3), simultaneous increments in total intakes of 400 IU vitamin D and 1,000 mg calcium were associated with an absolute reduction in mean breast density of 8.5% (95% confidence interval, 1.8-15.1).

Discussion

Our findings support the idea that a diet with higher vitamin D and calcium intakes, whether from food and/or supplements, is associated with lower breast density in premenopausal women. These findings are consistent with those of two (56, 57) of the three (55-57) previous studies of this relation.

Given the strength of the association of breast density to breast cancer risk (31-40), the lower breast density associated with higher levels of vitamin D and calcium support the notion that high intakes of vitamin D and calcium may be associated with a reduction of beast cancer risk. Among premenopausal women, simultaneous increments of 400 IU/d vitamin D and 1,000 mg/d calcium, amounts commonly found in supplements, were associated with an 8.5% (P = 0.01) lower mean breast density in the present study. By comparison, among healthy women at high risk of developing breast cancer, those who receive 54 months of tamoxifen have an absolute reduction of 6.4% of breast density compared with placebo (13.7% among women ages ≤45 years; ref. 43); in high-risk women, tamoxifen has been shown to reduce the risk of breast cancer by 30% to 50% (67, 68). Breast density at a given age is related to long-term breast cancer risk (37, 69). Thus, lower breast density in premenopausal women may be associated with a reduction in breast cancer risk even after the menopause.

In the present study, misclassification of vitamin D and calcium intakes, which were derived from a semiquantitative food frequency questionnaire and based on U.S. nutrient database, is possible. However, the accuracy of the food and nutrient intakes from this food frequency questionnaire was assessed in several validation studies (70-72). Vitamin D intake from the food frequency questionnaire was recently found to be positively correlated (0.25; P < 0.001) with serum concentrations of 25-hydroxyvitamin D among 343 participants in the Nurses' Health Study (73). Intakes of nutrients from multivitamins are particularly susceptible to misclassification as generic codes had to be used in some instances. However, differential recall bias is unlikely because women were not

aware of the specific study objectives regarding vitamin D and calcium intakes at time of data collection. Thus, misclassification of vitamin D and calcium dietary intakes, whether from food or supplements, is likely to be random and seems unlikely to explain observed associations. Finally, sun exposure contributes to a large extent to vitamin D status, but sun exposure was not measured. However, controlling for seasonality did not alter our estimates, suggesting that this was not a major factor in this analysis.

Misclassification of breast density is also possible. However, almost all mammograms were taken in the same clinic with the same equipment that were accredited by the Canadian Association of Radiology in addition to satisfying the high-quality standards of the Quebec breast cancer screening program. Furthermore, assessment of breast density was done by one trained reader (C.D.) without any information about the subjects using a computer-assisted method. Although the density of only one breast was assessed, a preliminary validation study (n = 100 participants) showed high correlation between right and left breasts (intraclass correlation coefficient = 0.95). Thus, if present, misclassification of breast density should be relatively small and most likely random.

To control confounding, adjustments were made for factors known or suspected to be related to breast density. In addition, the inverse associations of vitamin D intake from food plus supplements to breast density were seen only when the analytic approach took into account supplement use, which is a recognized behavioral marker related to cancer risk (63-65). In the report of the only known negative study of total vitamin D (food and supplements) and breast density (55), there is no indication that the authors did this. Nevertheless, residual confounding is possible. Milk is the predominant vehicle for vitamin D fortification (74) and is a major source of calcium. Consequently, the association of vitamin D or calcium from food with breast density may be confounded by other constituents of milk. Confounding of the observed relations of vitamin D or calcium intakes from supplements to breast density is also a potential problem especially for vitamin D. Multivitamins accounted for a high proportion of the variance in vitamin D intake from supplements (60%) in premenopausal women and multivitamins may include different vitamins and minerals with potentially opposing effects on breast density. Thus, residual confounding by other constituents of multivitamins may interfere with the evaluation of the association of vitamin D from supplements with breast density. Multivitamins accounted for a much lower proportion of the variance in calcium intake from supplements (9%) and such residual confounding is likely to be less problematic in the evaluation of the association of calcium from supplements with breast density.

Our data suggest that vitamin D and calcium intakes should be considered jointly rather than individually when examining their association with breast density. Higher intake in one nutrient is related to lower breast density only in the presence of higher intake of the other nutrient. This potential interplay between vitamin D and calcium associated with lower breast density was also seen in our previous study (57). Furthermore, these two nutrients are also suspected to act jointly in decreasing colorectal adenoma recurrence (75) or colorectal cancer risk (76-78). For instance, in their recent randomized controlled trial of calcium supplementation study, Grau et al. (75) found that calcium's protection against colorectal adenoma recurrence was confined to patients with adequate vitamin D status and vitamin D's effect was confined to those receiving calcium. Their results were in agreement with those of at least three previous cohort studies (76, 78) and one case-control study (77) showing an inverse association of vitamin D with colorectal carcinoma that was stronger among subjects with higher calcium intake (76, 77) or vice versa (78). Because systemic and intracellular calcium homeostasis is regulated by vitamin D (7), this potential interaction between vitamin D and calcium is plausible and highlights the importance of considering both nutrients simultaneously in future studies.

The apparent association of higher vitamin D and calcium intakes with lower breast density seems to be stronger in premenopausal women. This possible modifying effect of menopausal status may, at least in part, be explained by the interaction of vitamin D, estrogen, and insulin-like growth factor-I (IGF-I) seen in laboratory studies. On one hand, the antiproliferative and proapoptotic activities of vitamin D at the cellular level are mediated by vitamin D receptors present in the nucleus of normal and transformed breast cells, and the activity of vitamin D receptors has been found to be promoted by estrogen and IGF-I in breast cancer cells (79, 80). On the other hand, the actions of IGF-I and estrogen seem to be restrained by vitamin D. In breast cancer cells, vitamin D has been shown to diminish the proliferative effects of IGF-I, repeal the antiapoptotic effects of IGF-I, and down-regulate the expression of IGF-I receptors (81-84). Moreover, vitamin D has been shown to suppress the proliferative activity of 17β-estradiol and down-regulate the levels of estrogen receptors (79, 82, 85). All of these different interactions suggest that, in the presence of higher levels of IGF-I and estrogen, the effects of vitamin D on breast tissue may be greater and more easily detectable. Because premenopausal women have higher circulating levels of estrogen and IGF-I (86, 87), the effects of vitamin D on breast tissue and breast density may be greater in these women.

In conclusion, this study finds that premenopausal women who have higher intakes in vitamin D and calcium, either from diet or supplements, also tend to have lower breast density. Whether women who increase their intakes of vitamin D and calcium experience reductions in their breast density prospectively still needs to be evaluated. Furthermore, the suggestion that vitamin D or calcium intakes may be associated with reduction in breast cancer risk needs to be clarified. Vitamin D and calcium have little or no side effects even at doses much

Table 3. Relation of the combined total intakes of vitamin D and calcium to breast density in premenopausal women

		Vitamin D (IU/d)			β^* (P)
		Tertile 1 (17.4-144.6)	Tertile 2 (144.7-302.9)	Tertile 3 (303.0-1,659.3)	
Calcium (mg/d)	Tertile 1 (198.1-725.2)	45.2 (165)†	45.6 (72)	45.9 (22)	−0.6 (0.62)
	Tertile 2 (725.3-1,106.0)	41.6 (77)	42.9 (114)	44.0 (68)	−0.2 (0.79)
	Tertile 3 (1,106.1-3,130.0)	47.3 (17)	36.3 (73)	38.3 (169)	−1.1 (0.06)
β^* (P)		−0.3 (0.60)	−1.4 (0.001)	−0.7 (0.03)	

*Adjusted βs (P) are estimated from linear regression analysis; vitamin D (IU) and calcium (mg) intakes are continuous variables. βs represent absolute mean decrease in breast density (%) for increments of 100 IU vitamin D or 100 mg calcium, respectively. Analysis take simultaneously into account the same factors as those listed below.

†Values in cells are means of the percentage of the breast showing mammographic density and numbers in parentheses are the number of women in this category of the combined intakes of vitamin D and calcium. Means are adjusted for age, body mass index, age at menarche, number of birth, age at first birth, duration of use of oral contraceptive and hormone replacement, personal history of breast biopsies, family history of breast cancer, education, alcohol and total caloric intakes, physical activity, smoking, and for the indicator variables of vitamin D or calcium supplement use.

higher than those commonly used for supplementation (88-90) and many women have daily intakes of these nutrients below recommended levels (74, 91, 92). Thus, the idea that increases in vitamin D and calcium intakes may represent a safe and inexpensive means for preventing breast cancer in the general population deserves much attention.

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