Dairy, Calcium, and Vitamin D Intake and Postmenopausal Breast Cancer Risk in the Cancer Prevention Study II Nutrition Cohort

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Abstract

Background: **Calcium, vitamin D, and dairy products are highly correlated factors, each with potential roles in breast carcinogenesis. Few prospective studies have examined these relationships in postmenopausal women.**
Methods: **Participants in the Cancer Prevention Study II Nutrition Cohort completed a detailed questionnaire on diet, vitamin and mineral supplement use, medical history, and lifestyle in 1992 to 1993. After exclusion of women with a history of cancer and incomplete dietary data, 68,567 postmenopausal women remained for analysis. During follow-up through August 31, 2001, we identified 2,855 incident cases of breast cancer. Multivariate-adjusted rate ratios (RR) were calculated using Cox proportional hazards models.**
Results: **Women with the highest intake of dietary calcium (>1,250 mg/d) were at a lower risk of breast cancer than those reporting ≤500 mg/d [RR, 0.80; 95% confidence interval (95% CI), 0.67-0.95; P_{trend} = 0.02]; however, neither use of supplemental calcium nor vitamin D intake was associated with risk. Consumption starting at two or more servings of dairy products per day was likewise inversely associated with risk (RR, 0.81; 95% CI, 0.69-0.95; P_{trend} = 0.002, compared with <0.5 servings/d). The associations were slightly stronger in women with estrogen receptor–positive tumors comparing highest to lowest intake: dietary calcium (RR, 0.67; 95% CI, 0.51-0.88; P_{trend} = 0.004); dairy products (RR, 0.73; 95% CI, 0.57-0.93; P_{trend} = 0.0003), and dietary vitamin D (RR, 0.74; 95% CI, 0.59-0.93; P_{trend} = 0.006).**
Conclusions: **Our results support the hypothesis that dietary calcium and/or some other components in dairy products may modestly reduce risk of postmenopausal breast cancer. The stronger inverse associations among estrogen receptor–positive tumors deserve further study.** (Cancer Epidemiol Biomarkers Prev 2005;14(12):2898–904)

Introduction

Dairy products have been hypothesized to have opposing effects on breast cancer risk because they contain both potentially protective and deleterious compounds. Dairy foods are the major source of calcium and vitamin D, metabolically interrelated and highly correlated dietary factors that may influence breast carcinogenesis through a variety of mechanisms. Both nutrients have direct effects on cell proliferation and differentiation of several cancer cell lines *in vitro* (1, 2). In a rodent model, calcium reduces fat-induced cell proliferation by maintaining intracellular calcium concentrations (3). Vitamin D acts through the vitamin D receptor, a nuclear transcription regulating factor that signals the synthesis of proteins involved in cell cycle regulation (1, 4). Clinically, breast tumors with greater vitamin D receptor expression correlate positively with time between first diagnosis and relapse (5). The vitamin D receptor ligand 1,25,dihydroxyvitamin D [1,25$(OH)_2$D] can be directly synthesized in breast tissue (6) from 25-hydroxyvitamin D (7). 1,25$(OH)_2$D was shown to preferentially induce differentiation in estrogen receptor–positive (ER+) tumors (5) and down-regulate the ER (8, 9). Other potential anticarcinogenic mechanisms of vitamin D compounds and the vitamin D receptor include blocking the mitogenic activity of insulin and insulin-like growth factor I (10) and induction of *BRCA1* gene expression by transcriptional activation (11). Dairy foods are also rich in conjugated linoleic acid, a fatty acid with anticarcinogenic effects in experimental studies (12).

Milk also contains several hormones and growth factors (13, 14), including insulin-like growth factor I, a potent mitogen proposed to increase cancer development in the breast, colon, and prostate. In cross-sectional studies of adult men (15) and women (16), dairy products were positively associated with higher circulating insulin-like growth factor I. Thus, the net effect of dairy product consumption on breast cancer risk may reflect a balance of beneficial and detrimental factors.

Over 36 individual case-control and 10 prospective cohort studies have evaluated the relationship of dairy products and breast cancer with inconsistent findings (summarized in ref. 17). A recent pooled analysis of eight North American and Western European cohort studies on meat and dairy consumption found no association with dairy fluids or solids in postmenopausal breast cancer (18). Fewer studies have specifically evaluated dietary calcium and vitamin D in relation with breast cancer risk (19-28), particularly among postmenopausal women (21, 29). Furthermore, no studies have evaluated these dietary factors in relation to ER status.

We examined whether recent intake of dairy products, calcium, or vitamin D was related to the risk of postmenopausal breast cancer among over 68,000 women from the Cancer Prevention Study (CPS) II Nutrition Cohort. In a secondary analysis, we assessed whether these relationships were modified by ER status of the tumor.

Materials and Methods

Study Population. Women in this study were participants in the CPS II Nutrition Cohort, a prospective study of cancer incidence and mortality among 86,404 men and 97,786 women

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(30). The Nutrition Cohort was begun by the American Cancer Society in 1992 and is a subgroup of the ~1.2 million participants in CPS II, a prospective study of cancer mortality established in 1982. In brief, Nutrition Cohort participants were recruited from members of the CPS II cohort who resided in 21 states with population-based cancer registries as described in detail elsewhere (30). Participants were 50 to 74 years of age at enrollment in 1992 or 1993, when they completed a 10-page confidential, self-administered mailed questionnaire that included questions on demographic, medical, lifestyle, and dietary factors. Follow-up questionnaires were sent to cohort members in 1997, 1999, and 2001 to update exposure information and to ascertain newly diagnosed cancers. The response rate among living cohort members was 91% through 2001. The Emory University School of Medicine Institutional Review Board has approved all aspects of the CPS II Nutrition Cohort.

Case Ascertainment. Incident cancer was defined as a new diagnosis of either nonfatal or fatal breast cancer that occurred after enrollment in 1992 or 1993 and before August 31, 2001. We included all interval reported breast cancers because previous pilot work in this cohort found that the sensitivity of self-reported breast cancer was 91% (31). Ascertainment of breast cancer as a contributing cause of death among cohort members was accomplished through linkage with the National Death Index (32). We documented ER and progesterone receptor (PR) status of the tumors through medical record reports and through state cancer registries when available. ER status was verified in 53% of the cases: 1,283 tumors were ER+ and 227 were ER−.

Dietary Assessment. Calcium, vitamin D, and dairy product intakes were measured in 1992 to 1993 using a semiquantitative 68-item food frequency questionnaire (FFQ), which is a modification of the brief Health Habits and History Questionnaire developed by Block et al. (30, 33). The FFQ inquired about portion size (small, medium, or large). Questions on frequency of consumption ranged from ''never or less than once per month'' to ''two or more per day'' for foods and to ''six or more per day'' for beverages; use of low-fat foods, including ice cream and cheese, was also ascertained. The questionnaire included a section asking about frequency of use of several vitamin supplements during the past year (multivitamins and calcium among others). Individual vitamin D supplement use is not common (34) and was not included on the FFQ. Information on supplement dose was collected among participants reporting calcium supplement use (250, 500, 600, or 750 mg per tablet).

Dietary and total nutrient intakes and gram weights of foods were estimated using the Diet Analysis System version 3.8a (35). Total calcium estimates (mg/d) included contributions from diet, individual calcium supplements, and multivitamin pills (estimated at 130 mg per multivitamin pill). Vitamin D values were added to the nutrient database using U.S. Department of Agriculture sources (36). Total vitamin D (IU/d) included intake from diet and multivitamins (estimated to be 400 IU per pill). Nutrient estimates were adjusted for total energy using the residuals method (37). Gram weights for all dairy foods (whole, low-fat and skim milk, regular and low-fat cheese, yogurt, and ice cream) were converted to servings using the following weights: milk and yogurt, 240 g per serving (8 oz cup); cheese, 57 g per serving (2 oz); and ice cream, 72 g per serving (0.5 cup). We did not consider butter intake, which is not generally a recommended dairy food and is not a major contributor of calcium intake.

The FFQ was validated using four random 24-hour recalls collected over a 1-year period as the comparison measure among 441 Nutrition Cohort participants (38). Median energy-adjusted, attenuation-corrected Pearson validity correlations and 95% confidence intervals (95% CI) among women were high for dietary calcium (r = 0.66; 95% CI, 0.51-0.76) and for dairy products (r = 0.63; 95% CI, 0.45-0.77).

Analytic Cohort. We excluded from this analysis women who were lost to follow-up between enrollment in 1992/1993 and August 31, 2001 (n = 3,514), or, who, at baseline, reported a history of breast cancer (n = 6,220) or other cancer (except nonmelanoma skin cancer; n = 5,805). Because we had too few women to examine menopausal status separately, we excluded women who were premenopausal or perimenopausal at baseline (n = 4,448) or with unknown menopausal status (n = 233). We also excluded women who reported extreme values of daily energy intake (<500 or >3,500 kcal/d), or FFQs with ≥15% of the 68 questions blank (n = 7,345). Women with missing or uninterpretable responses for more than four dairy products (n = 85), calcium supplements (n = 952), or multivitamin use (n = 543), or women who reported both greater than six servings of milk per day and identical responses for two types of milk (n = 42) were also excluded. Finally, women were excluded if diagnosis date was unknown (n = 32). The final analytic cohort consisted of 68,567 women.

Statistical Analysis. We used Cox proportional hazards models to estimate the total cancer incidence rate ratio (RR) and 95% CI in relation with specific dietary factors (39). Age adjustment stratified on single year of age within each Cox model. Covariates included family history of breast cancer in a mother or sister (yes/no); history of breast cyst (yes/no); height (<63, 63 to <65, 65 to <67, ≥67 in, missing height), race (White versus non-White); weight gain since age 18 (−5-5, 6-20, 21-40, 41-60, 60+, missing or lost >5 lbs); education (<high school, high school, some college, college+, missing); age at menarche (<12, 12, 13, ≥14, unknown), age at menopause (<45, 45 to <50, 50 to <54, ≥54, unknown), age at first birth and number of live births (nulliparous and combinations of 1-2, 3+ live births and age <25, ≥25 or unknown number of live births or age at first birth); alcohol consumption (none, <1 drink/d, 1, or ≥1 drink/d, unknown); hormone replacement therapy [HRT; never, current estrogen replacement therapy, past estrogen replacement therapy, current combined replacement therapy (estrogen/progesterone), past combined replacement therapy, unknown/missing]; screening mammography (never, within the last year, not within the last year, unknown); and energy (in quintiles). Screening mammography was modeled as a time-varying covariate using information obtained in 1992, 1997, and 1999. We also examined energy-adjusted total fat as a potential confounder in the analysis. *In situ* breast cancers were included in the main analysis as cases; we also conducted sensitivity analyses excluding *in situ* tumors and those without information on tumor staging.

Potential effect modification of findings by potential for skin synthesis of vitamin D was examined by stratifying on UV index by state of residence for the subgroup of women (95.5%) who reported the same state of residence in 1982 and 2001. UV index levels were estimated using the 1997 monthly mean UV indices for major forecast cities in 50 states (40). We also stratified by ER status of the tumor, multivitamin use (current versus not current use), dietary fat intake (tertiles), HRT (current, past, never use), body mass index (<25, 25-30, >30), and family history (yes/no). The likelihood ratio test was used to test for significance in interaction models. Two-sided tests were statistically significant if $P \leq 0.05$.

Results

Dairy products and nutrients derived largely from dairy products (e.g., dietary calcium and dietary vitamin D) were highly correlated in the analytic cohort as expected (Table 1). Total calcium and total vitamin D included supplemental nutrients and, therefore, were not as strongly correlated with

Table 1. Correlation coefficients (*r*) between dairy, dietary calcium, and vitamin D intakes in CPS II Nutrition Cohort women

	Dietary calcium	Total calcium	Diet vitamin D	Total vitamin D	Total dairy	High-fat dairy	Low-fat dairy	Total milk	Low-fat milk
Diet calcium	1.00	0.56	0.89	0.39	0.78	0.02	0.82	0.80	0.80
Total calcium		1.00	0.48	0.50	0.40	−0.05	0.45	0.41	0.42
Diet vitamin D			1.00	0.41	0.70	0.06	0.77	0.83	0.81
Total vitamin D				1.00	0.26	−0.08	0.31	0.31	0.31
Total dairy					1.00	0.33	0.91	0.89	0.86
High-fat dairy						1.00	−0.09	0.12	−0.05
Low-fat dairy							1.00	0.89	0.93
Total milk								1.00	0.96
Low-fat milk									1.00

NOTE: Variables are in continuous form: dairy (g), calcium (mg).

other dietary variables. The largest contributors to dietary calcium included skim milk (38%), low-fat milk (32%), regular and low-fat cheese (15%), and regular and low-fat yogurt (7%). Top contributors to dietary vitamin D were similar, as follows: skim milk (42%), low-fat milk (38%), whole milk (5%), fish (5%), cereal (4%), and eggs (3%). Similarly, total dairy products were composed of skim milk (42%), low-fat milk (22%), whole milk (4%), regular and low-fat yogurt (11%), cheese and low-fat cheese (12%), and ice cream and low-fat ice cream (10%). Approximately 80% of low-fat dairy products consumed were from skim milk and low-fat milk, and the majority of milk consumed was in low fat or skim form (95%).

Table 2 provides a description of baseline characteristics and breast cancer risk factors according to intake of low-fat dairy products, dietary calcium, and total calcium, which were determined using predefined cut points. Ranges of intake were ~3- to 6-fold from the lowest to highest intake levels. Women in the highest categories of each exposure smoked less and were more physically active. Use of HRT and calcium and multivitamin supplement was more common among women in the highest exposure groups; differences were especially pronounced among women in extreme categories of total calcium intake. Women with higher total calcium intakes also reported a somewhat greater history of breast cysts, were leaner, and gained less weight since age 18 compared with women with low total calcium intake. Reproductive history did not vary by dietary exposure category. Total fat and alcohol intakes were lower with higher exposure levels, and dairy nutrients were tracked in the expected direction.

Table 2. Baseline characteristics according to consumption of low-fat dairy products and dietary and total calcium, CPS II Nutrition Cohort women (1992-2001)

Characteristic	Low-fat dairy (servings/d)			Dietary calcium (mg/d)			Total calcium (mg/d)		
	Low	Medium	High	Low	Medium	High	Low	Medium	High
	<0.5 (*n* = 16,064)	>1-2 (*n* = 22,360)	>3 (*n* = 7,681)	≤500 (*n* = 17,194)	>750-1,000 (*n* = 15,067)	>1,250 (*n* = 4,355)	≤500 (*n* = 10,620)	>1,000-1,250 (*n* = 9,120)	>1,750 (*n* = 6,645)
Mean age (y)	62.2	62.6	63.0	62.0	62.7	63.6	62.0	62.7	63.2
History of breast cyst (%)	27.4	29.1	30.9	27.9	29.4	30.1	25.8	29.7	34.6
Mean body mass index in 1992	25.6	25.6	25.7	25.8	25.5	25.2	26.1	25.4	24.3
Mean weight gain since age 18 y	29.6	29.0	30.0	30.8	28.0	26.3	32.4	28.1	22.5
Mean height (in)	64.4	64.6	64.8	64.4	64.6	64.6	64.4	64.7	64.6
Calcium supplement use (%)	30.2	34.3	34.2	30.3	35.3	35.9	1.6	48.4	93.7
Multivitamin supplement use (%)	35.3	44.6	46.6	35.7	45.8	48.9	13.6	53.5	67.3
Mean metabolic equivalents	10.9	12.4	13.2	10.6	12.8	13.6	9.9	12.9	13.9
Current smoker (%)	12.8	7.1	5.9	11.5	6.8	6.0	13.3	6.7	5.2
Nulliparous (%)	7.6	7.5	7.2	7.6	7.5	7.1	7.3	7.4	7.6
First birth after age 30 y (%)	7.2	7.8	8.5	7.1	7.6	8.1	7.0	7.7	7.4
Current HRT use (%)	31.1	35.8	37.1	31.9	36.3	36.3	26.2	36.6	48.4
Mean intakes of:									
Total dairy (servings/d)	0.74	1.79	4.24	0.70	2.12	4.15	0.64	2.26	2.42
Low-fat dairy (servings/d)	0.22	1.45	3.90	0.39	1.71	3.83	0.34	1.84	2.15
Total milk (servings/d)	0.23	1.11	3.51	0.19	1.38	3.54	0.17	1.53	1.80
Total calcium (mg/d)	706	1,043	1,544	648	1,146	1,787	395	1,120	2,293
Dietary calcium (mg/d)	442	772	1,261	396	858	1,490	390	848	1,002
Total vitamin D (IU/d)	220	337	488	209	360	574	113	387	560
Dietary vitamin D (IU/d)	80	176	327	78	194	388	78	195	239
Calories (kcal/d)	1,218	1,372	1,693	1,355	1,334	1,329	1,282	1,437	1,215
Total fat (g/d)	57	46	39	57	45	35	58	45	41
Alcohol (g/d)	5.8	4.4	3.3	7.0	3.4	2.3	6.6	4.4	3.3

Analyses are based on 2,855 cases of postmenopausal breast cancer identified over 536,815 person-years of follow-up from 1992 to 2001. The results with respect to nutrients are shown in Table 3. Women who consumed the most dietary calcium (>1,250 mg/d) had a 20% lower risk of postmenopausal breast cancer compared with those in the lowest category of intake (<500 mg/d; RR, 0.80; 95% CI, 0.67-0.95; P_{trend} = 0.02). Supplemental calcium (including both calcium supplements and multivitamins) was not associated with risk of breast cancer, with or without inclusion of dietary calcium in the model, whereas calcium supplements alone (additionally controlling for multivitamins) were marginally associated with reduced risk (RR, 0.90; 95% CI, 0.82-0.99, for ≥500 mg versus no use; not shown). Likewise, total calcium (diet plus all supplements) did not strengthen the association (for >1,750 mg total calcium/d; RR, 0.91; 95% CI, 0.79-1.06; P_{trend} = 0.07). However, there was a nonlinear lower risk at levels between 1,250 and 1,750 mg total calcium/d. Neither dietary nor total vitamin D intake (diet plus multivitamin sources of vitamin D) was significantly related to risk of breast cancer.

In analyses by foods (Table 4), consumption of at least two servings of dairy products daily (versus ≤0.5 servings/d) was inversely associated with breast cancer risk (RR, 0.81; 95% CI, 0.69-0.95; P_{trend} = 0.002). When we examined low-fat dairy products separately from high-fat dairy, associations were slightly stronger for low-fat dairy (controlled for other types). Total milk consumption (3 servings/d versus <1/2 cup/d) was nonsignificantly, inversely related to risk. When milk types were examined separately (compared with non–milk drinkers), consumption of low-fat and skim milk ≥3 servings/d (combined as ''low fat'') was marginally associated with lower risk. In these analyses, we considered individuals who reported cereal use but no milk consumption as ''non–milk drinkers.'' In analyses including these individuals as milk drinkers (with reference group including very small intake levels), results were not materially different.

In analyses that stratified by ER status of the tumor (Table 5), women in the highest dietary calcium category had 33% lower risk of ER+ tumors compared with women in the lowest category (RR, 0.67; 95% CI, 0.51-0.88; P_{trend} = 0.004). Dietary vitamin D was also significantly related to lower risk of ER+ (RR, 0.74; 95% CI, 0.59-0.93; P_{trend} = 0.006) but not ER− tumors (RR, 1.03; 95% CI, 0.61-1.73; P_{trend} = 0.84). Consumption of ≥2 servings/d of dairy products was associated with 29% lower risk of ER+ tumors than consumption of <0.5 cups/d (RR, 0.71; 95% CI, 0.56-0.91; P_{trend} <0.0003). Results were similar when we examined ER/PR status combined (e.g., ER+/PR+ was similar to ER+ alone and ER−/PR− was similar to ER− alone; there were too few cases to examine other combinations).

There was no significant interaction between the dietary exposures and multivitamin use, body mass index, HRT, family history of breast cancer, or total fat intake. Sensitivity analyses excluding women with *in situ* tumors were not materially different. However, women from states with lower UV exposure (UV index ≤ median of 4.2) had a lower risk of breast cancer with greater dietary vitamin D consumption >300 IU/d (RR, 0.81; 95% CI, 0.67-0.97) compared with women from these states who consumed ≤100 IU/d. Conversely, women from states with UV index >4.3 did not have lower risk with vitamin D consumption >300 versus ≤100 IU/d

Table 3. Age- and multivariate-adjusted RRs and 95% CIs of breast cancer according to calcium and vitamin D intake, CPS II Nutrition Cohort women (1992-2001)

Nutrient	*n*	Cases	RR* (95% CI)	RR† (95% CI)	P_{trend}
Dietary calcium (mg/d)‡					
≤500	17,194	739	1.00	1.00	
>500-≤750	25,435	1,065	0.96 (0.88-1.06)	0.95 (0.86-1.04)	
>750-≤1,000	15,067	634	0.96 (0.87-1.07)	0.96 (0.86-1.06)	
>1,000-≤1,250	6,516	265	0.93 (0.81-1.07)	0.91 (0.79-1.05)	
>1,250	4,355	152	0.79 (0.66-0.94)	0.80 (0.67-0.95)	0.02
Supplemental calcium‡ (mg/d)					
None	32,855	1,353	1.00	1.00	
<250	15,221	642	1.02 (0.93-1.12)	1.01 (0.92-1.11)	
250-<500	4,464	190	1.03 (0.88-1.20)	0.99 (0.85-1.16)	
500-<1,000	9,844	395	0.96 (0.86-1.07)	0.90 (0.81-1.01)	
1,000+	6,183	275	1.07 (0.94-1.22)	0.98 (0.86-1.12)	0.23
Total calcium (mg/d)					
≤500	10,620	457	1.00	1.00	
>500-≤750	17,880	729	0.94 (0.84-1.06)	0.91 (0.81-1.02)	
>750-≤1,000	14,023	581	0.95 (0.84-1.07)	0.92 (0.81-1.04)	
>1,000-≤1,250	9,120	407	1.02 (0.89-1.17)	0.97 (0.85-1.11)	
>1,250-≤1,500	6,296	248	0.90 (0.77-1.04)	0.84 (0.72-0.98)	
>1,500-≤1,750	3,983	144	0.81 (0.67-0.98)	0.76 (0.63-0.92)	
>1,750	6,645	289	0.99 (0.85-1.14)	0.91 (0.79-1.06)	0.07
Dietary vitamin D (IU/d)					
≤100	19,547	830	1.00	1.00	
>100-≤200	30,302	1,257	0.96 (0.88-1.05)	0.96 (0.88-1.05)	
>200-≤300	12,559	534	0.98 (0.88-1.09)	0.98 (0.88-1.10)	
>300	6,159	234	0.87 (0.75-1.00)	0.89 (0.76-1.03)	0.21
Total vitamin D (IU/d)					
≤100	12,707	529	1.00	1.00	
>100-≤200	19,575	826	1.00 (0.90-1.11)	0.99 (0.88-1.10)	
>200-≤300	8,494	326	0.91 (0.79-1.04)	0.90 (0.78-1.03)	
>300-≤400	5,339	220	0.98 (0.84-1.14)	0.95 (0.81-1.11)	
>400-≤500	6,304	241	0.91 (0.78-1.06)	0.86 (0.73-1.00)	
>500-≤600	6,425	304	1.12 (0.97-1.29)	1.08 (0.93-1.24)	
>600-≤700	4,609	206	1.05 (0.89-1.23)	1.03 (0.87-1.21)	
>700	5,114	203	0.94 (0.80-1.10)	0.95 (0.81-1.13)	0.98

*Age-adjusted model.
†Multivariate-adjusted model controlling for age, energy, history of breast cyst, family history of breast cancer, height, weight gain since age 18, alcohol use, race, age at menopause, age at first birth and number of live births, education, mammography history, and HRT.
‡Dietary and supplemental calcium are controlled for each other.

Table 4. Age- and multivariate-adjusted RRs and 95% CIs of breast cancer according to dairy product intake, CPS II Nutrition Cohort women (1992-2001)

Food	*n*	Cases	RR* (95% CI)	RR† (95% CI)	P_{trend}
Total dairy (servings/d)‡					
<0.5	7,034	300	1.00	1.00	
0.5-1	14,840	630	0.99 (0.86-1.13)	0.96 (0.83-1.10)	
>1-2	26,138	1,121	0.99 (0.88-1.13)	0.93 (0.82-1.06)	
>2-3	10,533	407	0.90 (0.77-1.04)	0.81 (0.69-0.95)	
>3	10,022	397	0.91 (0.78-1.06)	0.81 (0.69-0.96)	0.002
Low-fat dairy (servings/d)§					
<0.5	16,064	667	1.00	1.00	
0.5-1	16,778	707	1.00 (0.90-1.11)	0.96 (0.86-1.07)	
>1-2	22,360	947	1.00 (0.91-1.11)	0.94 (0.85-1.04)	
>2-3	5,684	223	0.94 (0.81-1.09)	0.85 (0.73-1.00)	
>3	7,681	311	0.95 (0.83-1.08)	0.86 (0.74-0.99)	0.016
High-fat dairy (servings/wk)§					
<0.3	16,544	678	1.00	1.00	
0.3-1	13,153	558	1.05 (0.94-1.17)	1.03 (0.92-1.16)	
>1-2	10,740	478	1.09 (0.97-1.23)	1.06 (0.94-1.19)	
>2-4	13,122	568	1.07 (0.96-1.19)	1.02 (0.91-1.15)	
>4	15,008	573	0.95 (0.85-1.06)	0.89 (0.79-1.00)	0.12
Total milk (servings/d)∥					
Non−milk drinker	18,923	810	1.00	1.00	
<0.5	7,209	279	0.91 (0.79-1.04)	0.90 (0.78-1.03)	
0.5-1	13,269	556	0.97 (0.87-1.08)	0.96 (0.86-1.06)	
>1-2	19,261	805	0.97 (0.88-1.07)	0.94 (0.85-1.04)	
>2-3	3,014	129	1.00 (0.83-1.20)	0.97 (0.80-1.17)	
>3	6,891	276	0.91 (0.80-1.05)	0.88 (0.76-1.02)	0.13
Milk type (servings/d)					
Non−milk drinker	18,923	810	1.00	1.00	
Whole milk only	2,183	84	0.92 (0.74-1.16)	0.97 (0.78-1.22)	
Low-fat milk only <0.5	6,062	238	0.92 (0.80-1.06)	0.90 (0.78-1.04)	
Low-fat milk only 0.5-1	11,561	493	0.99 (0.88-1.10)	0.96 (0.86-1.08)	
Low-fat milk only >1-2	17,225	722	0.97 (0.88-1.07)	0.94 (0.85-1.04)	
Low fat milk only >2-3	2,418	104	1.00 (0.81-1.22)	0.95 (0.77-1.17)	
Low-fat milk only >3	6,318	255	0.92 (0.80-1.06)	0.88 (0.76-1.02)	
Combination of milks	3,877	149	0.90 (0.76-1.07)	0.90 (0.75-1.07)	—

*Age-adjusted.
†Additionally controlled for energy, history of breast cyst, family history of breast cancer, height, weight gain since age 18, alcohol use, race, age at menopause, age at first birth and number of live births, education, mammography history, and HRT use.
‡Dairy products include low fat cheese, yogurt, low fat yogurt, ice cream, whole milk, low fat milk, skim milk, and cheese from pizza.
§Low-fat dairy and high-fat dairy are additionally controlled for each other.
∥Milk products include whole milk, low fat milk, and skim milk.

(RR, 1.05; 95% CI, 0.82-1.35; $P_{interaction}$ = 0.05). Similar findings were observed by UV index for >2 versus <0.5 servings/d of low-fat dairy (RR, 0.77; 95% CI, 0.66-0.90 within low UV index states and RR, 1.05; 95% CI, 0.85-1.29 within high UV index states, $P_{interaction}$ = 0.03) and >2 versus <0.5 total dairy servings/d (RR, 0.76; 95% CI, 0.64-0.91 within low UV index states and RR, 0.93; 95% CI, 0.73-1.17 within high UV index states, $P_{interaction}$ = 0.02).

Discussion

In this large prospective U.S. cohort, high intake of dietary calcium and low-fat dairy products was associated with a moderately lower risk of developing postmenopausal breast cancer compared with women with lowest intake levels. Supplemental calcium or higher levels of total calcium (diet plus supplements) and vitamin D were not related to overall breast cancer risk. However, vitamin D, calcium, and dairy products were inversely associated with risk of ER+ breast tumors, but not ER− tumors.

Our overall findings suggest that dairy products, composed mainly of low-fat sources or some component within these foods, are associated with a small but significantly lower risk of breast cancer in postmenopausal women. Three prospective studies of low-fat dairy products or dietary calcium in relation with postmenopausal breast cancer risk had mixed results (18, 29, 41). In the Netherlands Cohort Study, consumption of skim milk and skim milk products was unrelated to postmenopausal breast cancer risk (RR, 1.04, for median intake of 203 g/d or 1 cup/d versus none; ref. 41). In our study, risk was not lower until intake reached ≥480 g/d (2 cups/d), implying that greater intakes may be necessary to lower risk. In a large pooled analysis of U.S. and European cohorts (18), dairy products were not related to breast cancer risk but milk fortification policies may be different in European countries. Shin et al. (29) observed a significant inverse association between low-fat dairy products, dietary calcium, and premenopausal breast cancer risk in the Nurses' Health Study using repeated FFQs to cumulatively update diet over a 16-year follow-up period. However, these dietary factors were not related to postmenopausal breast cancer, except in analyses limited to baseline exposure when dietary calcium was associated with a 15% statistically significantly lower risk (29).

The high correlations of nutrients in dairy products make it challenging to identify the single or combined variables that may contribute to lower breast cancer risk. Dietary calcium was more strongly inversely associated with risk than other factors we examined, but the hypothesized mechanisms are not as compelling as those for vitamin D. One hypothesis for calcium centers on modification of fat-stimulated proliferation (3) but dietary fat is not consistently related to breast cancer risk (42, 43) and was not a modifier in this analysis. If calcium is truly a causal factor, a possible explanation for the weaker association with supplemental calcium is that lactose present in dairy products enhances calcium absorption. Lactose has also been hypothesized to reduce breast cancer risk by blocking gonadotropin release, which would down-regulate estrogen synthesis (29). Use of calcium supplements was also more common among women taking HRT in our cohort; thus, residual confounding by HRT use among calcium supplement users may also account for the weaker associations with supplemental calcium. Low-fat dairy products were associated with a slightly lower risk of breast cancer compared with high-fat dairy products, suggesting that conjugated linoleic acid may not be the responsible factor, although, in this study, we were not able to measure total conjugated linoleic acid intake.

Although our findings for vitamin D were not as strong as for other dietary variables, the biological plausibility for a role for vitamin D in breast carcinogenesis is strong. Both normal and neoplastic breast tissue express the nuclear receptor for $1,25(OH)_2D$. Several studies report tissue-specific expression of 1,α-hydroxylase and autocrine/paracrine synthesis of $1,25(OH)_2D$ (6, 44). Little is known about factors that may influence $1,25(OH)_2D$ synthesis and degradation in breast tissue but greater substrate availability should be important (7). Others have observed inverse associations between higher 25-hydroxyvitamin D levels or sun exposure and breast cancer risk (7, 27). Vitamin D intake may be more strongly associated with risk than we were able to ascertain had we assessed sun exposure or 25-hydroxyvitamin D blood levels. Among women with expected lower UV synthesis of vitamin D (states with lower UV index), we observed stronger inverse associations with dietary vitamin D compared with women who lived in states with high UV index.

In our study, inverse associations between dietary factors and postmenopausal breast cancer risk were somewhat stronger in women with ER+ tumors. This is consistent with recent studies showing that risk factors for breast cancer vary by ER and PR status (45). Previous studies suggest that ER+ breast cancer cell lines are more sensitive to $1,25(OH)_2D$-mediated growth regulation compared with ER− cell lines (4, 5, 46). Although total and dietary vitamin D was not related to overall breast cancer risk in our study, vitamin D was inversely related to risk of ER+ tumors. This suggests a role of

Table 5. Relation of dietary variables to risk of breast cancer by ER status

Variable	ER+ cases			ER− cases		
	Cases	RR	P_{trend}	Cases	RR	P_{trend}
Dietary calcium (mg/d)*						
≤500	351	1.00		62	1.00	
>500-≤750	471	0.87 (0.76-1.00)		87	0.97 (0.69-1.34)	
>750-≤1,000	282	0.88 (0.75-1.03)		44	0.84 (0.57-1.25)	
>1,000-≤1,250	116	0.82 (0.66-1.01)		23	1.03 (0.64-1.69)	
>1,250	63	0.67 (0.51-0.88)	0.004	11	0.77 (0.40-1.47)	0.49
Supplemental calcium (mg/d)*						
None	596	1.00		113	1.00	
<250	277	0.99 (0.85-1.14)		44	0.84 (0.59-1.19)	
250-<500	84	0.99 (0.79-1.25)		19	1.24 (0.76-2.02)	
500-<1,000	193	0.98 (0.83-1.15)		32	0.92 (0.62-1.37)	
1,000+	133	1.06 (0.87-1.28)	0.82	19	0.85 (0.52-1.39)	0.63
Total calcium (mg/d)						
≤500	212	1.00		36	1.00	
>500-≤750	313	0.83 (0.69-0.98)		73	1.20 (0.81-1.80)	
>750-≤1,000	262	0.87 (0.72-1.04)		32	0.69 (0.43-1.12)	
>1,000-≤1,250	194	0.96 (0.79-1.18)		29	0.97 (0.59-1.59)	
>1,250-≤1,500	104	0.72 (0.57-0.92)		20	0.95 (0.55-1.66)	
>1,500-≤1,750	65	0.71 (0.53-0.94)		11	0.84 (0.42-1.66)	
>1,750	133	0.87 (0.70-1.09)	0.13	26	1.14 (0.68-1.92)	0.78
Dietary vitamin D (IU/d)						
≤100	404	1.00		64	1.00	
>100-≤200	551	0.85 (0.75-0.97)		102	1.05 (0.77-1.44)	
>200-≤300	231	0.86 (0.73-1.02)		42	1.06 (0.71-1.57)	
>300	97	0.74 (0.59-0.93)	0.006	19	1.03 (0.61-1.73)	0.84
Total vitamin D (IU/d)						
≤100	243	1.00		41	1.00	
>100-≤200	356	0.92 (0.78-1.08)		73	1.18 (0.81-1.74)	
>200-≤300	149	0.88 (0.72-1.08)		26	0.99 (0.60-1.62)	
>300-≤400	109	1.00 (0.80-1.26)		12	0.74 (0.38-1.41)	
>400-≤500	126	0.95 (0.76-1.18)		15	0.75 (0.41-1.37)	
>500-≤600	129	0.98 (0.79-1.21)		22	1.08 (0.64-1.83)	
>600-≤700	87	0.93 (0.73-1.19)		17	1.15 (0.65-2.03)	
>700	84	0.84 (0.65-1.09)	0.57	21	1.35 (0.79-2.33)	0.82
Low-fat dairy (servings/d)†						
<0.5	307	1.00		50	1.00	
0.5-1	328	0.95 (0.81-1.11)		55	1.03 (0.70-1.52)	
>1-2	422	0.88 (0.76-1.03)		80	1.13 (0.79-1.64)	
>2-3	91	0.72 (0.56-0.92)		14	0.82 (0.44-1.52)	
>3	135	0.76 (0.61-0.94)	0.002	28	1.22 (0.74-2.03)	0.59
Total dairy (servings/d)						
<0.5	136	1.00		25	1.00	
0.5-1	299	0.98 (0.80-1.20)		52	1.01 (0.62-1.63)	
>1-2	497	0.88 (0.72-1.07)		87	0.98 (0.62-1.56)	
>2-3	175	0.71 (0.56-0.91)		25	0.74 (0.41-1.34)	
>3	176	0.73 (0.57-0.93)	0.0003	38	1.23 (0.70-2.15)	0.77

NOTE: Models are adjusted for age, energy, history of breast cyst, family history of breast cancer, height, weight change from age 18 to 1992, alcohol use, parity and age at first birth, education, HRT use, and mammography history.
*Dietary and supplemental calcium are controlled for each other.
†Additionally controlled for high-fat dairy intake.

improved vitamin D status on reducing ER+ breast cancer, which needs confirmation in other studies.

Limitations of this study include the inability to quantify vitamin D status precisely because we did not inquire about sun exposure and were not able to measure plasma 25-hydroxyvitamin D levels directly. However, our dietary instrument was validated for calcium and dairy intakes (38) and we were able to examine findings by UV index for the 21 enrollment states of our participants. Our findings are generalizable to the adult diet of mostly White, middle class postmenopausal women. We were unable to determine whether dietary exposures earlier in life influence postmenopausal breast cancer risk. We were missing data on ER status for 47% of the population. Examination of demographic and risk factor characteristics among women with and without ER tumor status revealed no important differences. The number of ER− tumors in our sample was limited (n = 227). Thus, we cannot rule out that we missed associations in this subset due to lack of statistical power.

In summary, postmenopausal women who consumed greater intakes of dietary calcium and dairy products, primarily from low-fat sources, were at lower risk of breast cancer in our cohort. The lower risk was more pronounced in ER+ breast cancer. Supplemental sources of these nutrients were not as strongly related to risk. Future studies should clarify specific components responsible and the relation between these factors and ER status.

References

1. Lipkin M, Newmark HL. Vitamin D, calcium, and prevention of breast cancer: a review. J Am Coll Nutrition 1999;18:392–7S.
2. Martinez ME, Willett WC. Calcium, vitamin D and colorectal cancer: a review of the epidemiologic evidence. Cancer Epidemiol Biomarkers Prev 1998;7:163–8.
3. Jacobsen EA, James KA, Newmark HL, Carroll KK. Effects of dietary fat, calcium, and vitamin D on growth and mammary tumorigenesis induced by 7,12-dimethylbenz(*a*)anthracene in female Sprague-Dawley rats. Cancer Res 1989;49:6300–3.
4. Colston K, Welsh J. Vitamin D and breast cancer. In: Feldman D, editor. Vitamin D. Vol. II. Stanford (California): Elsevier Academic Press; 2005: p. 1663–77.
5. Colston KW, Berger U, Coombes RC. Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet 1989;1:188–91.
6. Townsend K, Banwell CM, Guy M, et al. Autocrine metabolism of vitamin D in normal and malignant breast tissue. Clin Cancer Res 2005;11:3579–86.
7. Lowe LC, Guy M, Mansi JL, et al. Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population. Eur J Cancer 2005;41:1164–9.
8. James SY, Mackay AG, Binderup L, Colston KW. Effects of a new synthetic vitamin D analog, EB1089, on the oestrogen-responsive growth of human breast cancer cells. J Endocrinology 1994;141:555–63.
9. Swami S, Krishnan AV, Feldman D. 1,25-dihydroxyvitamin D3 down-regulates estrogen receptor abundance and suppresses estrogen actions in MCF-7 human breast cancer cells. Clin Cancer Res 2000;6:3371–9.
10. Vink-van Wijngaarden T, Pols HAP, Buurman CJ, Birkenhager JC, van Leeuwen JPTM. Inhibition of insulin- and insulin-like growth factor-I-stimulated growth of human breast cancer cells by 1,25-dihydroxyvitamin D3 and the vitamin D3 analogue EB1089. Eur J Cancer 1996;32A:842–8.
11. Campbell MJ, Gombart AF, Kwok SH, Park S, Koeffler HP. The anti-proliferative effects of 1,25(OH)2D3 on breast and prostate cancer cells are associated with induction of BRCA1 gene expression. Oncogene 2000;19:5091–7.
12. Ip C, Chin SF, Scimeca JA, Pariza MW. Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. Cancer Res 1991;51:6118–24.
13. Grosvenor CE, Picciano MF, Baumrucker CR. Hormones and growth factors in milk. Endocr Rev 1993;14:710–28.
14. Outwater JL, Nicholson A, Barnard N. Dairy products and breast cancer: the IGF-I, estrogen, and bGH hypothesis. Med Hypotheses 1997;48:453–61.
15. Giovannucci E, Pollak M, Liu Y, et al. Nutritional predictors of insulin-like growth factor I and their relationships to cancer in men. Cancer Epidemiol Biomarkers Prev 2003;12:84–9.
16. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. Cancer Epidemiol Biomarkers Prev 2002;11:852–61.
17. Moorman PG, Terry PD. Consumption of dairy products and the risk of breast cancer: a review of the literature. Am J Clin Nutr 2004;80:5–14.
18. Missmer SA, Smith-Warner SA, Spiegelman D, et al. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. Int J Epidemiol 2002;31:78–85.
19. Katsouyanni K, Willett W, Trichopoulos D, et al. Risk of breast cancer among Greek women in relation to nutrient intake. Cancer 1988;61:181–5.
20. Negri E, La Vecchia C, Franceschi S, et al. Intake of selected micronutrients and the risk of breast cancer. Int J Cancer 1996;65:140–4.
21. Braga C, La Vecchia C, Negri E, Faranceschi S, Parpinel M. Intake of selected foods and nutrients and breast cancer risk: an age- and menopause-specific analysis. Nutr Cancer 1997;28:258–63.
22. Witte JS, Ursin G, Siemiatycki J, Thompson WD, Paganini-Hill A, Haile RW. Diet and premenopausal bilateral breast cancer: a case-control study. Breast Cancer Res Treat 1997;42:243–51.
23. Potischman N, Swanson CA, Coates RJ, et al. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. Int J Cancer 1999;82:315–21.
24. Levi F, Pasche C, Lucchini F, La Vecchia C. Dietary intake of selected micronutrients and breast-cancer risk. Int J Cancer 2001;91:260–3.
25. Boyapati SM, Shu XO, Jin F, et al. Dietary calcium intake and breast cancer risk among Chinese women in Shanghai. Nutr Cancer 2003;46:38–43.
26. Adzersen KH, Jess P, Freivogel KW, Gerhard I, Bastert G. Raw and cooked vegetables, fruits, selected micronutrients, and breast cancer risk: a case-control study in Germany. Nutr Cancer 2003;46:131–7.
27. John EM, Schwartz GG, Dreon DM, Koo J. Vitamin D and breast cancer risk: the NHANES I Epidemiologic Follow-up Study, 1971-1975 to 1992. Cancer Epidemiol Biomarkers Prev 1999;8:399–406.
28. Knekt P, Jarvinen R, Seppanen R, Pukkala E, Aromaa A. Intake of dairy products and the risk of breast cancer. Br J Cancer 1996;73:687–91.
29. Shin M, Holmes MD, Hankinson SE, Wu K, Colditz GA, Willett WC. Intake of dairy products, calcium, and vitamin D and risk of breast cancer. J Natl Cancer Inst 2002;94:1301–11.
30. Calle EE, Rodriguez C, Jacobs EJ, et al. The American Cancer Society Cancer Prevention Study II Nutrition Cohort—Rationale, study design, and baseline characteristics. Cancer 2002;94:2490–501.
31. Bergmann MM, Calle EE, Mervis CA, Miracle-McMahill HL, Thun MJ, Heath CW. Validity of self-reported cancers in a prospective cohort study in comparison with data from state cancer registries. Am J Epidemiol 1998;147:556–62.
32. Calle EE, Terrell DD. Utility of the National Death Index for ascertainment of mortality among Cancer Prevention Study II participants. Am J Epidemiol 1993;137:235–41.
33. Block G, Hartman AM, Naughton D. A reduced dietary questionnaire: development and validation. Epidemiology 1990;1:58–64.
34. Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. Recent patterns of medication use in the ambulatory adult population of the United States. The Slone Survey. JAMA 2002;287:337–44.
35. Block G, Coyl L, Smucker R, Harlan LC. Health Habits and History Questionnaire: diet history and other risk factors. Personal computer system documentation. Bethesda (Maryland): National Cancer Institute Division of Cancer Prevention and Control, NIH 1989.
36. Agricultural Research Service. Provisional table on vitamin D content of foods. Beltsville (Maryland): U.S. Department of Agriculture; 1991.
37. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
38. Flagg EW, Coates RJ, Calle EE, Potischman N, Thun MJ. Validation of the American Cancer Society Cancer Prevention Study II Nutrition Survey Cohort Food Frequency Questionnaire. Epidemiology 2000;11:462–8.
39. Cox DR. Regression models and life-tables. J R Stat Soc [B] 1972;34:187–220.
40. U.S. Environmental Protection Agency. How is the UV index calculated? Vol. 2005. http://www.epa.gov/sunwise/uvcalc.html.
41. Voorips LE, Brants HAM, Kardinaal AFM, Hiddink GJ, van den Brandt PA, Goldbohm RA. Intake of conjugated linoleic acid, fat, and other fatty acids in relation to postmenopausal breast cancer: the Netherlands Cohort Study on Diet and Cancer. Am J Clin Nutr 2002;76:873–82.
42. Smith-Warner SA, Spiegelman D, Adami HO, et al. Types of dietary fat and breast cancer: a pooled analysis of cohort studies. Int J Cancer 2001;92:767–74.
43. Bingham SA, Luben R, Welch A, Wareham N, Khaw KT, Day N. Are imprecise methods obscuring a relation between fat and breast cancer? Lancet 2003;362:212–4.
44. Cross HS, Peterlik M, Reddy GS, Schuster I. Vitamin D metabolism in human colon adenocarcinoma-derived Caco-2 cells: expression of 24-hydroxyvitamin D3-1a-hydroxylase activity and regulation of side-chain metabolism. J Steroid Biochem Mol Biol 1997;62:21–8.
45. Colditz GA, Rosner B, Chen WY, Holmes MD, Hankinson SE. Risk factors for breast cancer according to estrogen and progesterone receptor status. J Natl Cancer Inst 2004;96:218–28.
46. Welsh J, Wietzke JA, Zinser GM, et al. Impact of the vitamin D3 receptor on growth-regulatory pathways in mammary gland and breast cancer. J Steroid Biochem Mol Biol 2003;83:85–92.