

Review

Vitamin D, Calcium, and Breast Cancer Risk: A Review

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Abstract

Vitamin D and calcium are metabolically interrelated and highly correlated dietary factors. Experimental studies have shown their anticarcinogenic effects due to their participation in regulating cell proliferation, differentiation, and apoptosis in normal and malignant breast cells. Given the emerging interest in their potential roles in the etiology of breast cancer, we review the current epidemiologic literature on dietary and/or supplemental intakes of vitamin D, endogenous circulating levels of vitamin D, and dietary and/or supplemental intakes of calcium in relation to breast cancer risk. To place these studies in context, we also provide a brief review of other supporting epidemiologic evidence. Despite inconsistent results from the epidemiologic studies, several lines of evidence suggest that vitamin D and calcium may be involved in the development of breast cancer. Specifically, (a) there is some epidemiologic evidence for inverse associations

between vitamin D and calcium intakes and breast cancer; (b) serum, plasma, and/or blood levels of vitamin D metabolites have been inversely associated with breast cancer risk in some studies; (c) high sunlight exposure, presumably reflecting vitamin D synthesis in the skin, has been associated with a reduced risk of breast cancer; (d) vitamin D and calcium intakes have been inversely related to breast density, an intermediate end point for breast cancer; (e) calcium has been associated with a reduced risk of benign proliferative epithelial disorders of the breast, putative precursors of breast cancer; and (f) certain polymorphisms of the vitamin D receptor might modify breast cancer susceptibility. To further confirm the potential protective effects of calcium and vitamin D on breast cancer, well-designed cohort studies and clinical trials are warranted. (Cancer Epidemiol Biomarkers Prev 2006;15(8):1427–37)

Introduction

Breast cancer is the most commonly diagnosed cancer among U.S. women (1). An estimated 211,240 new cases of invasive breast cancer and 58,490 new cases of *in situ* breast cancer were predicted among U.S. women for 2005 (1). In terms of mortality, breast cancer ranks second only to lung cancer as a cause of death from cancer among U.S. women, with 40,410 breast cancer deaths predicted for 2005 (1). Given the magnitude of the problem, considerable effort has been devoted to elucidation of the etiology of breast cancer. Indeed, many factors have been related to altered breast cancer risk, including certain menstrual (age at menarche and age at menopause), reproductive (childbearing and lactation), and anthropometric [body mass index (BMI) and weight gain] factors as well as exogenous estrogen use, endogenous hormone levels, family history of breast cancer, history of benign breast disease (BBD), ionizing radiation, and alcohol consumption (2, 3). However, because these factors do not fully explain the epidemiology of breast cancer, identification of additional avenues of etiologic investigation is warranted.

Ecological studies have associated high levels of sunlight exposure with low breast cancer incidence and mortality rates (4–7). These observations, together with experimental evidence showing anticarcinogenic properties of vitamin D, have led to the hypothesis that high levels of vitamin D might reduce the risk of breast cancer (8–11). In the United States, the main sources of dietary vitamin D intake are vitamin D–fortified dairy products, which are also rich sources of dietary calcium.

In rats, calcium has been shown to reduce fat-induced mammary cell proliferation by maintaining the intracellular calcium concentration (12). Moreover, vitamin D and calcium are metabolically interrelated and highly correlated dietary factors that may influence breast cancer risk through a variety of common or different mechanisms (13).

Given the emerging interest in the potential roles of vitamin D and calcium in the etiology of breast cancer, we review here the current epidemiologic literature on dietary and/or supplemental intakes of vitamin D, endogenous circulating levels of vitamin D, and dietary and/or supplemental intakes of calcium in relation to breast cancer risk. To place these studies in context, we also provide a brief review of the sources, metabolism, and anticarcinogenic properties of vitamin D and calcium as well as other supporting epidemiologic evidence.

Sources and Metabolism

Vitamin D. Humans ingest vitamin D from foods, such as fish, eggs, and fortified dairy products, and from vitamin D–containing multivitamins and supplements (14). The two naturally occurring forms of vitamin D are cholecalciferol (vitamin D₃) from animal sources and ergocalciferol (vitamin D₂) from plant sources (15). Recent studies in humans have provided evidence that vitamin D₃ is more efficient than vitamin D₂ in increasing serum 25-hydroxyvitamin D [25(OH)D], the precursor of the biologically active form of vitamin D, 1,25(OH)D (16). In the United States, the recommended daily vitamin D intake is 200, 400, and 600 IU for adults <50, 50 to 70, and >70 years old, respectively (14). An additional source of vitamin D is sunlight exposure, which can convert 7-dehydrocholesterol, a cholesterol-like precursor, into vitamin D₃ in the skin (17).

The pro–hormone vitamin D, in the form of vitamin D₂ or D₃, is first metabolized to 25(OH)D in the liver and then

Received 1/30/06; revised 5/15/06; accepted 6/1/06.

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doi:10.1158/1055-9965.EPI-06-0075

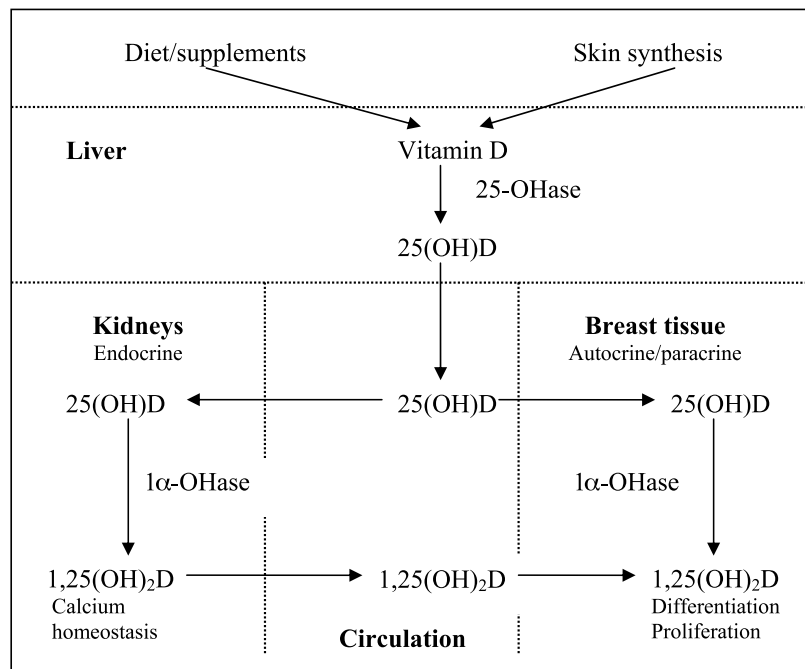


Figure 1. A simplified diagram of vitamin D metabolism. Vitamin D (from diet and supplements or from synthesis in the skin) is first metabolized to 25(OH)D in the liver and then further metabolized to 1,25(OH)₂D by 1- α -hydroxylase in the kidneys (endocrine pathway) and breast tissue (autocrine/paracrine pathway).

further metabolized to 1,25-dihydroxyvitamin D [1,25(OH)₂D] by 1- α -hydroxylase in the kidneys and other target tissues (see Fig. 1; ref. 18). Both 25(OH)D and 1,25(OH)₂D can be degraded through the catalysis of vitamin D 24-hydroxylase in various tissues, including the breast (19). Therefore, vitamin D status in the circulation depends on exogenous vitamin D sources (from dietary and supplemental intake), endogenous production (through synthesis in the skin), and activities of vitamin D metabolic enzymes. In human plasma, the concentration of 25(OH)D (>20 ng/mL) is ~1,000 times higher than that of 1,25(OH)₂D (20-60 pg/mL; ref. 20). Circulating 25(OH)D concentration varies with dietary intake and exposure to sunlight and is considered to be the best indicator of vitamin D status (14). In contrast, the circulating concentration of 1,25(OH)₂D is maintained in a relatively narrow range due to tight regulation by renal 1- α -hydroxylase. Various epithelial cells, such as those in the prostate, breast, and colon, have been shown to express vitamin D 1- α -hydroxylase (21). However, circulating 1,25(OH)₂D produced by these extrarenal tissues is undetectable in anephric conditions (15).

Calcium. Calcium is required for all living cells to maintain their structure and functions (22, 23). Humans ingest calcium from calcium-rich diets, such as dairy products and supplements. In the adult human body, 99% of calcium is found in mineralized tissues (bones and teeth), in which it is present as calcium phosphate or calcium carbonate (24). The remaining 1% is found in the blood, extracellular fluid, and various tissues. The concentration of calcium in the plasma, in which it is present as ionized calcium, is maintained dynamically within a tightly regulated range through intestinal calcium absorption, renal calcium excretion and reabsorption, and skeletal calcium storage and resorption (24).

Interrelationship between Vitamin D and Calcium. Circulating 1,25(OH)₂D plays an important role in calcium homeostasis by participating in a feedback loop that maintains the level of calcium within its regulated range (25). The level of circulating 1,25(OH)₂D varies inversely with that of calcium intake (26). In response to inadequate intake of calcium, increased production of 1,25(OH)₂D leads to increased calcium absorption. Furthermore, 1,25(OH)₂D facilitates the cellular uptake of calcium from circulating blood (25). It has been shown that the addition of 1,25(OH)₂D to mammary gland

explants enhances calcium uptake into its functionally differentiated epithelial cells (27). On the other hand, circulating levels of calcium influence the activity of renal 1- α -hydroxylase and thus the circulating concentration of 1,25(OH)₂D (26). Therefore, in normal physiologic states, vitamin D and calcium are metabolically interrelated (13) and blood levels of both calcium and 1,25(OH)₂D are maintained in relatively narrow ranges.

Anticarcinogenic Properties

Vitamin D. 1,25(OH)₂D, the biologically active form of vitamin D, exerts its effects mainly through binding to nuclear vitamin D receptor (VDR) and further binding to specific DNA sequences, namely vitamin D response elements (28). Through this genomic pathway, 1,25(OH)₂D modulates expression of specific genes in a tissue-specific manner (29). Experimental studies have shown that 1,25(OH)₂D can inhibit cellular proliferation, induce differentiation and apoptosis, and inhibit angiogenesis in normal and malignant breast cells (8-11). In rodent models, high intake of vitamin D has been shown to suppress high-fat diet-induced epithelial hyperproliferation and tumorigenesis of the mammary gland (12, 30). In addition, a nongenomic pathway of 1,25(OH)₂D has been shown, in which 1,25(OH)₂D interacts largely with membrane VDR to exert its biological effects by altering intracellular calcium channels (20). However, the involvement of this pathway in carcinogenesis and cancer prevention is not clearly defined.

Two distinct pathways of vitamin D biosynthesis and action have been proposed in mammary carcinogenesis, one involving 1,25(OH)₂D and the other involving 25(OH)D (8, 15). In the endocrine pathway, circulating 1,25(OH)₂D reaches the breast tissue to exert its anticarcinogenic effect. The other pathway is the autocrine/paracrine pathway, in which circulating 25(OH)D reaches the breast tissue and is further catalyzed to 1,25(OH)₂D by the 1- α -hydroxylase in the breasts. The locally produced 1,25(OH)₂D may bind to VDR and therefore regulate cell proliferation, differentiation, and apoptosis (15).

Calcium. The importance of calcium in carcinogenesis derives from its participation in regulating cell proliferation, differentiation, and apoptosis (31-33). Increasing the concentration of calcium decreases cell proliferation and induces

differentiation of mammary cells in experimental studies (30, 34, 35). In rodent models, high intake of calcium has been shown to suppress high-fat diet-induced epithelial hyperproliferation of the mammary gland and mammary tumorigenesis induced by 7,12-dimethylbenz(a)anthracene (12, 30). Evidence is available that calcium at least partially exerts its anticarcinogenic effects through vitamin D. For example, calcium is one of the key mediators of apoptosis induced by vitamin D compounds in breast cancer cells (32).

Epidemiologic Studies of Vitamin D, Calcium, and Breast Cancer Risk

Identification of Relevant Studies. We conducted MEDLINE searches to identify possible epidemiologic studies for inclusion in the review. To identify the studies of breast cancer risk in relation to vitamin D and calcium, we searched using the term "breast cancer" in combination with the following terms: vitamin D, calcium, micronutrient(s), dietary, diet(s), nutrition, nutritional, nutrient(s), dairy, 1,25-dihydroxyvitamin D, and 25-dihydroxyvitamin D. The MEDLINE searches were supplemented by searching for related articles referenced in relevant published studies and reviews. To be included in the review, a study must have been published in English, with a case-control, cohort, or cross-sectional study design, and with the primary outcome of breast cancer.

Dietary and Supplemental Vitamin D Intake. To date, there have been several epidemiologic studies of the association between vitamin D and breast cancer risk. However, their results have not been consistent. In the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study (36), frequent recreational and occupational sunlight exposure was inversely associated with breast cancer risk. Consistent with previous ecological studies, this study suggested that women residing in the northeast of the United States might experience a higher risk of developing breast cancer than women residing in other regions of the United States. These results raised the possibility that levels of vitamin D synthesis in the skin due to sunlight exposure might be inversely associated with breast cancer risk. However, this study showed no associations between dietary and supplemental vitamin D intakes and breast cancer (Table 1). The null results for dietary vitamin D were consistent with those reported by three case-control studies (37-39). However, the interpretation of the results from those case-control studies is potentially compromised due to their small sample sizes and the possibility of selection bias. In contrast, in the Nurses' Health Study, there was an inverse association between vitamin D intake and breast cancer risk among premenopausal women but no association among postmenopausal women (40). Consistent with this observation, a recently published study based on the Cancer Prevention Study II Nutrition Cohort observed no associations of breast cancer with total and dietary vitamin D intakes among postmenopausal women (13).

It has been suggested that adolescent diet may be an important predisposing factor for breast cancer risk later in life (25, 41). However, two recently published studies failed to establish any association between dietary vitamin D intake during high school and risk of breast cancer in adulthood (Table 1; refs. 42, 43). Notably, dietary (or total) intake of vitamin D is not a complete measure of vitamin D status. Furthermore, measurement error may limit studies of dietary intake of this nutrient in relation to breast cancer risk.

Endogenous Circulating Vitamin D Levels. Several studies have examined the association between endogenous vitamin D levels and breast cancer risk (Table 2). In a hospital-based

case-control study, an inverse association was observed between 1,25(OH)₂D levels measured in whole blood collected at the time of diagnosis and breast cancer risk (44). Given that there were similar 1,25(OH)₂D levels in women with ductal carcinoma *in situ* and in women with invasive ductal carcinoma, the observed inverse association with breast cancer might not have resulted from an effect of the invasive disease on blood 1,25(OH)₂D levels. In contrast, a nested case-control study with 96 breast cancer cases and 96 controls found no association between prediagnostic 1,25(OH)₂D levels and breast cancer risk among postmenopausal women (45) possibly due to the relatively small sample size. Although statistically insignificant, plasma levels of 1,25(OH)₂D were associated with reduced risk of breast cancer in a case-control study nested in the Nurses' Health Study (46).

The circulating concentration of 25(OH)D is considered to be an excellent measure of the availability of vitamin D from the diet and supplements and from synthesis in the skin (47). Its potential importance in breast carcinogenesis is due to the fact that 25(OH)D can be metabolized to 1,25(OH)₂D by 1- α -hydroxylase in breast tissue and therefore may be more representative of intracellular levels of 1,25(OH)₂D than circulating levels of 1,25(OH)₂D (48). The early case-control study of breast cancer by Janowsky et al. (44) found similar blood levels of 25(OH)D among study subjects regardless of disease status. Although statistically insignificant, an inverse association between plasma levels of 25(OH)D and risk of breast cancer were observed in the case-control study nested in the Nurses' Health Study (46). Furthermore, a recent case-control study observed that women with plasma 25(OH)D concentration <50 nmol/L had >5 times higher risk of breast cancer than those with plasma concentration exceeding 150 nmol/L (49). To date, no studies have been published investigating intracellular or tissue levels of 1,25(OH)₂D and 25(OH)D in association with breast cancer risk.

Dietary and Supplemental Calcium Intake. The majority of studies on calcium intake and breast cancer risk published to date have been case-control studies (Table 3). Among six hospital-based case-control studies, four reported an inverse association between dietary calcium intake and breast cancer risk (50-53), whereas the remainder reported a nonsignificantly reduced risk of breast cancer among women with high dietary consumption of calcium (37, 54). However, the interpretation of these results is potentially compromised by the fact that hospital-based case-control studies are subject to selection bias. Furthermore, with the exception of the study by Negri et al. (51), these studies were relatively small, with sample sizes <350 for the case groups and <450 for the control groups. Moreover, apart from the study by Adzersen et al. (50), these studies failed to control for some of the well-documented breast cancer risk factors in multivariate analyses.

There have been three population-based case-control studies of dietary calcium intake and breast cancer risk. An early population-based case-control study in the Netherlands suggested that women who consumed relatively high levels of dietary calcium and fiber might have a decreased risk of breast cancer (55). However, this study was relatively small and failed to control for potential confounding factors other than age and fat intake. A more recent population-based case-control study among Chinese women observed that dietary calcium intake had a statistically nonsignificant inverse association with risk of breast cancer among premenopausal and postmenopausal women (56). In contrast, a small familial matched case-control study found no association between dietary calcium intake and breast cancer risk (39). Notably, none of these published case-control studies have taken supplemental calcium intake into consideration.

Table 1. Dietary and/or supplemental intake of vitamin D and breast cancer risk

First author (ref.), year, study place	Years of data collection	Study design	No. cases/controls (cohort)	Comparison*	RR (95% CL)	Variables adjusted for
Simard (38) [†] , 1991, Canada	1981-1983	Case-control study	108/322	Not stated	No association	Not stated
Witte (39), 1997, United States [‡] and Canada	1989	Familial matched case-control study	140/222	Not stated	No association	Not stated
John (36), 1999, [‡] United States	1971-1992	Cohort study	179/4,747	Dietary vitamin D (IU), ≥ 200 vs < 100 IU Supplemental vitamin D, daily vs never	0.85 (0.59, 1.24) 0.89 (0.60, 1.32)	Age, education, age at menarche, BMI, alcohol, physical activity, and calcium intake
Levi (37), 2001, Switzerland	1993-1999	Hospital-based case-control study	289/442	Dietary vitamin D (tertiles), T3 vs T1	1.43 (0.90, 2.26)	Age, education, parity, menopausal status, BMI, energy, alcohol drinking
Shin (40), 2002, United States [§]	1980-1996	Cohort study	3,172/88,691	Total vitamin D (IU/d) Premenopausal, > 500 vs ≤ 150 Postmenopausal, > 500 vs ≤ 150	0.72 (0.55, 0.94) 0.94 (0.80, 1.10)	Age, period, physical activity, BBD, family history of breast cancer, height, weight change since age 18, BMI at age 18, age at menarche, parity, age at first birth, alcohol intake, energy, glycemic index, β -carotene, and vitamin E
Frazier (42), 2003, United States	1980-1986	Nested case-control study	843/8,430	Dietary vitamin D during adolescence (quintiles), Q5 vs Q1	0.96 (95% CL unknown)	Age, age at menarche, menopausal status, family history, BBD, adult height, parity/age at first birth, HRT, BMI at 18, alcohol intake in 1980, and vitamin A intake
Frazier (43), 2004, United States [¶]	1989-1998	Cohort study	361/47,355	Dietary vitamin D during adolescence (quintiles), Q5 vs Q1	0.92 (0.66, 1.27)	Age, period, height, parity and age at first birth, BMI at 18, age at menarche, family history of breast cancer, history of BBD, menopausal status, alcohol, weight gain since age 18
McCullough (13), 2005, United States ^{**}	1992-2001	Cohort study	2,855/68,567	Total vitamin D (IU/d), > 700 vs ≤ 100 Dietary vitamin D (IU/d), > 300 vs ≤ 100	0.95 (0.81, 1.13) 0.89 (0.76, 1.03)	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 no. live births, education, mammography history, and HRT

Abbreviations: RR, relative risk; 95% CL, 95% confidence limit; HRT, hormone replacement therapy.

*The associations are presented by menopausal status whenever the studies reported them separately or the studies were restricted to either premenopausal or postmenopausal women. Otherwise, the associations for a combination of premenopausal and postmenopausal women are presented.

[†]No RR were presented.

[‡]This study was based on First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study. Only White women were included.

[§]This study was based on the Nurses' Health Study. Cumulative vitamin D intakes were used in the analyses.

^{||}The study was nested in the Nurses' Health Study. Confidence intervals were not presented.

[¶]This cohort study was based on Nurses' Health Study II.

^{**}This study, an analysis of postmenopausal women, was conducted in the Cancer Prevention Study II Nutrition Cohort.

To date, three cohort studies have been published investigating the association between calcium intake and breast cancer risk. An early study associated dietary calcium intake with a reduced risk of breast cancer after following a cohort of 4,697 Finnish women for 25 years (57). However, the study was relatively small (only 88 breast cancer cases), and the well-established risk factors for breast cancer were not adjusted for. The second study was conducted among the Nurses' Health Study cohort with repeated measurements of

calcium intake (40). This large cohort study found that breast cancer risk was inversely associated with total calcium intake (dietary plus supplemental intake) and calcium intake from diet. However, the inverse association was present only among premenopausal women. In contrast, a recently published study based on the Cancer Prevention Study II observed inverse associations between breast cancer risk and total and dietary calcium intakes among a cohort of postmenopausal women (13). The associations between

calcium intake and breast cancer risk by menopausal status warrant further investigation.

Other Epidemiologic Evidence

Vitamin D and Calcium Intake in Relation to Breast Density. Mammographic breast density is strongly related to breast cancer risk (58, 59). Women with density in >75% of the breast have four to five times the risk of breast cancer of women with little or no breast density (60). It has been suggested that mammographic breast density may serve as an intermediate end point for breast cancer risk in the investigation of potential approaches for prevention of the disease (61). Four cross-sectional studies and one longitudinal study have investigated calcium and/or vitamin D intakes in relation to breast density (Table 4). Among the early three cross-sectional studies (61-63), two studies (61, 63) linked relatively high dietary intakes of vitamin D and calcium to lower breast density among premenopausal and postmenopausal women. In a recent study, data were stratified by menopausal status and vitamin D and calcium intakes from diet and supplements were evaluated separately (64). This study revealed that breast density was inversely associated with dietary calcium and vitamin D intakes but not with supplemental calcium and vitamin D intakes among premenopausal women. Furthermore, no associations were observed between dietary and supplemental calcium and vitamin D intakes and breast density among postmenopausal women. In the only longitudinal study to date, high dietary calcium intake was associated with low breast density among a cohort of 1,668 premenopausal and postmenopausal women (65). Given the strong association between breast density and breast cancer risk, the lower breast density associated with higher levels of calcium

and vitamin D supports the notion that high intakes of vitamin D and calcium may be associated with a reduction of breast cancer risk.

Vitamin D and Calcium Intake in Relation to BBD. BBD consists of many histologic entities, which can be categorized broadly into two major groups: nonproliferative BBD and benign proliferative epithelial disorders of the breast with or without atypia (66). Women with benign proliferative epithelial disorder of the breast are at increased risk of developing subsequent breast cancer, whereas those with nonproliferative BBD are not (67). Along with epidemiologic studies, experimental studies suggest that nonatypical proliferative changes and atypical hyperplasia represent successive steps preceding the development of *in situ* cancer and then invasive carcinoma of the breast (68). Given the roles of calcium and vitamin D in cell proliferation and differentiation, it is conceivable that they might be related to risk of benign proliferative epithelial disorder. To date, only two epidemiologic studies have explored the association between calcium intake and BBD. A case-cohort study conducted in the Canadian National Breast Screening Study observed a reduced risk of benign proliferative epithelial disorder among women who had relatively high dietary calcium intake albeit not in a dose-dependent pattern (69). Similarly, a small hospital-based case-control study suggested an inverse association between dietary calcium intake and BBD (70). However, the case group in this study consisted of all types of BBD combined, thereby limiting the conclusions that can be drawn. To date, there have been no published studies that have evaluated the relation between vitamin D intake and risk of BBD.

VDR Polymorphisms and Breast Cancer Risk. The VDR is a key mediator of the vitamin D pathway. It is expressed in normal and malignant breast cells (71). It has been

Table 2. Endogenous vitamin D levels and breast cancer risk

First author (ref.), year, study place	Years of data collection	Study design	No. cases/controls	Comparison*	OR (95% CL)	Variables adjusted for
Hiatt (45), 1998, United States [†]	1964/1972-1991	Nested case-control study	96/96	Serum 1,25(OH) ₂ D (pg/mL), ≥51 vs <32	1.0 (0.2, 3.4)	Education, parity, history of breast biopsy, alcohol, breast cancer in mother and sister
Janowsky (44), 1999, United States [‡]	1990-1991	Hospital-based case-control study	131/149	Blood 1,25(OH) ₂ D (pmol/mL), ≤33.61 vs >62.94	5.3 (2.1, 13.4)	Age, assay batch, month of blood draw, clinic, and sample storage time
Lowe (49), 2005, United Kingdom	1998-2003	Hospital-based case-control study	179/179	Blood 25(OH)D Plasma 25(OH)D (nmol/L), <50 vs >150	No association [§] 5.83 (2.31, 14.7)	Matching variables, time of year, age at sampling, and menopausal status
Bertone-Johnson (46), 2005, United States	1989/1990-1996	Nested case-control study	701/724	Plasma 25(OH)D (quintile), Q5 vs Q1	0.73 (0.49, 1.07)	Matching variables, BMI at age 18, parity/age as first birth, family history of breast cancer, history of BBD, HRT, age at menarche, age at menopause, alcohol intake, and plasma α-carotene
				Plasma 1,25(OH) ₂ D (quintile), Q5 vs Q1	0.76 (0.52, 1.11)	

Abbreviation: OR, odds ratio.

*The associations shown are for all subjects combined. No associations by menopausal status were reported by these studies.

[†]This study was nested in a cohort of 95,000 women. During an average 15.4-year follow-up, 2,131 new breast cancer patients were identified from the cohort. Ninety-six White cases and 96 White controls were randomly selected from study subjects with serum collected in 1964 to 1972.

[‡]Blood samples were collected after diagnosis. Results presented here are for White women only because sample sizes for other ethnic groups were too small.

[§]The article showed that the blood 25(OH)D level did not differ between cases and controls. However, the article did not present adjusted OR and 95% CL for the association between blood 25(OH)D level and breast cancer risk.

^{||}This study was nested in the Nurses' Health Study.

Table 3. Dietary and/or supplemental intake of calcium and breast cancer risk

First author (ref.), year, study place	Years of data collection	Study design	No. cases/controls (cohort)	Comparison*	RR (95% CL)	Variables adjusted for
Katsouyanni (54), 1988, Greece	1983-1984	Hospital-based case-control study	120/120	Dietary calcium (centiles), C90 vs C10 [†]	0.56 (0.27, 1.16)	Age, education, and interviewer
Zaridze (52), 1991, Russia	1987-1989	Hospital-based case-control study	139/139	Dietary calcium (quartiles) Premenopausal Postmenopausal, Q4 vs Q1	No association [‡] 0.20 (0.05, 0.84)	Energy intake, age at menarche, and education
Van't Veer (55), 1991, Netherlands [§]	1985-1987	Population-based case-control study	133/289	Dairy calcium/fiber ≤464.4/≤26.2 >464.4/>26.2	1.0 (ref.) 0.58 (0.32, 1.05)	Age and fat intake
Landa (53), 1994, Spain	1987-1988	Hospital-based case-control study	100/100	Dietary calcium (tertiles), Q3 vs Q1	0.4 (0.2, 0.9)	Energy intake
Negri (51), 1996, Italy	1991-1994	Hospital-based case-control study	2,569/2,588	Dietary calcium (quintiles), Q5 vs Q1	0.80 (0.7, 1.0)	Age, center, education, parity, energy, and alcohol intake
Knekt (57), 1996, Finland	1966/1972-	Cohort study	88/4,697	Dietary calcium (tertiles), T3 vs T1	0.44 (0.24, 0.80)	Not stated
Witte (39), 1997, United States and Canada	1989	Familial matched case-control study	140/222	Not stated	No association [‡]	Not stated
Levi (37), 2001, Swiss	1993-1999	Hospital-based case-control study	289/442	Dietary calcium (tertiles), T3 vs T1	0.73 (0.44, 1.22)	Age, education, parity, menopausal status, BMI, total energy intake, and alcohol drinking
Shin (40), 2002, United States [¶]	1980-1996	Cohort study	3,172/88,691	Total calcium (mg/d) Premenopausal, >1,000-1250 vs ≤500 Postmenopausal, >1,000-1250 vs ≤500	0.75 (0.57, 0.99) 0.90 (0.76, 1.07)	Age, period, physical activity, BBD, family history of breast cancer, height, weight change since age 18, BMI at age 18, age at menarche, parity, age at first birth, alcohol intake, energy, glycemic index, β-carotene, and vitamin E
Adzersen (50), 2003, Germany	1998-2000	Hospital-based case-control study	310/353	Dietary calcium (mg/d), 870-1,180 vs <558	0.42 (0.23, 0.75)	Age, energy, age at menarche, age at first birth, age at menopause, mother/sister with breast cancer, current smoking, history of BBD or operation, BMI, alcohol, and HRT
Boyapati (56), 2003, China	1996-1998	Population-based case-control study	1,459/1,556	Dietary calcium (deciles) Postmenopausal, D10 vs D1 Premenopausal, D10 vs D1	0.72 (0.38, 1.37) 0.72 (0.38, 1.37)	Age, energy, menopausal status (for all subjects only), family history among first-degree relatives, history of fibroadenoma, age at first live birth, BMI, education, income, protein, fruits, and vegetables
McCullough (13), 2005, United States**	1992-2001	Cohort study	2,855/68,567	Postmenopausal Dietary and supplemental (mg/d), >1,500 to ≤1,750 vs ≤500 Dietary calcium (mg/d), >1,250 vs ≤500 Supplemental calcium (mg/d), >1,000 vs none	0.76 (0.63, 0.92) 0.80 (0.67, 0.95) 0.98 (0.86, 1.12)	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 no. live births, education, mammography history, and HRT

*The associations are presented by menopausal status whenever the studies reported them separately or the studies were restricted to either premenopausal or postmenopausal women. Otherwise, the associations for a combination of premenopausal and postmenopausal women are presented.

[†]RR and 95% CL were estimated for consumption of calcium equal to the value of the 90th centile versus consumption equal to the value of the 10th centile.

[‡]Detailed estimates were not presented.

[§]Joint effects of calcium and fiber were presented.

^{||}The cohort was recruited between 1966 and 1972 and followed up for 25 years.

[¶]The study was based on the Nurses' Health Study. Cumulative calcium intake was used in analyses.

**This study, an analysis of postmenopausal women, was conducted in the Cancer Prevention Study II Nutrition Cohort.

Table 4. Calcium and vitamin D intake and breast density

First author (ref.), year, study place	Years of data collection	Study design	Sample size	Comparison*	Estimates	Variables adjusted for	
Vachon (62), 2000, United States	1990-	Cross-sectional study	1,508	Total vitamin D (IU/d)	Mean (95% CL) % breast density [†]	Age, BMI, WHR, physical activity, age at menarche, age at first birth and no. births combined, alcohol, smoking, family history of breast cancer, HRT, and oral contraceptive use (premenopausal women only)	
				Premenopausal, ≤188.7	40 (34, 45)		
				Premenopausal, >562.8	42 (35, 48)		
				Postmenopausal, ≤188.7	32 (30, 35)		
				Postmenopausal, >562.8	32 (30, 34)		
Holmes (63), 2001, United States	1986-1990	Cross-sectional study	885	Premenopausal	Mean % density across quintiles (P) [‡]	Age and BMI	
				Dietary vitamin D intake	45, 41, 38, 42, 33 (0.02)		
				Dietary calcium intake	44, 47, 37, 37, 37 (0.01)		
Bérubé (61), 2004, United States	1988-1990	Cross-sectional study	543	Dietary vitamin D (IU/d), 200+ vs <50	OR (95% CL) [§] : 0.24 (0.11, 0.53)	Age, BMI, age at menarche, no. birth/age at first birth, oral contraceptive use, menopausal status, HRT, family history of breast cancer, education, alcohol, caloric intake, and smoking	
				Dietary calcium (mg/d), 1,000+ vs <499	OR (95% CL) [§] : 0.24 (0.10, 0.57)		
Bérubé (64), 2005, Canada	2001-2002	Cross-sectional study	1,560	Vitamin D (100 IU increase)	β (P)	Age, BMI, age at menarche, no. birth, age at first birth, duration of oral contraceptive and HRT use, history of breast biopsies, family history of breast cancer, education, alcohol, caloric intake, physical activity, and smoking	
				Premenopausal			
				Food	-1.8 (0.008)		
				Supplements	-1.0 (0.16)		
				Total	-1.4 (0.004)		
				Postmenopausal			
				Food	-0.4 (0.40)		
				Supplements	0.4 (0.29)		
				Total	0.1 (0.76)		
				Calcium (100 mg increase)			
				Premenopausal			
				Food	-0.7 (0.005)		
				Supplements	-0.7 (0.06)		
				Total	-0.8 (0.0004)		
				Postmenopausal			
Food	0.1 (0.72)						
Supplements	0.2 (0.46)						
Total	0.1 (0.49)						
Masala (65), 2005, Italy	1993-2000	Longitudinal study	1,668	Dietary calcium (tertiles), T3 vs T1	OR (95% CL) [¶] : 0.67 (0.47, 0.94)	Age, education, BMI, menopausal status, and total caloric intake	

*The associations are presented by menopausal status whenever the studies reported them separately or the studies were restricted to either premenopausal or postmenopausal women. Otherwise, the associations for a combination of premenopausal and postmenopausal women are presented.

[†]Assessment was based on mean (95% CL) of % breast density.

[‡]The study was published as an abstract. No detailed information on the 95% CL was presented.

[§]This study compared women with mammographic density of ≥70% with those with mammographic density of ≤30%.

^{||}β, estimated from linear regression analyses, represented absolute mean decrease or increase in breast density (%) for increments of 100 IU vitamin D or 100 mg calcium, respectively.

[¶]The study compared women with high mammographic breast density with those with low mammographic breast density.

hypothesized that genetic polymorphisms in the VDR may influence breast cancer risk due to their potential effects on VDR gene expression and protein function. Many polymorphisms in the VDR gene have been identified, among which FOK1, BSM1, APA1, TAQ1, and Poly(A) have been studied the most frequently (72). The FOK1 polymorphism in exon 2 causes a T-to-C transition, resulting in a protein (encoded by C allele, also known as F allele) that is three amino acids shorter than the protein encoded by T allele (also known as f allele). The longer protein is less transcriptionally active than the shorter protein (73). Moreover, the ff genotype has been associated with decreased bone mineral density in multiple ethnic groups (74-76). BSM1, APA1, TAQ1, and Poly(A), located at the 3' end of the VDR gene, are in strong linkage disequilibrium. Their functional significance is not well understood, although limited data have shown that the length polymorphism Poly(A) may influence the transcriptional efficiency and stability of the VDR mRNA (72, 77).

To date, 13 epidemiologic studies have investigated various VDR gene polymorphisms in relation to breast cancer risk (Table 5). A large nested case-control study found a positive association between the ff genotype of FOK1 and breast cancer risk, whereas four earlier studies found no association with this genotype (78-82). Of the eight epidemiologic studies that have assessed the associations between the TAQ1 genotype and breast cancer risk, seven observed no association and one observed a positive association between T allele and breast cancer risk (82-88). As for the length polymorphism Poly(A), its long sequences were linked to a reduced risk of breast cancer in one study but were associated with an increased risk of breast cancer in two other studies (79-81). Studies on BSM1 and APA1 have yielded even more confusing information with negative, positive, or no associations reported by different studies (49, 78-84, 86, 89). These discrepancies might have resulted from study design issues and might also have resulted from

intrinsic population differences. Most of these studies were limited by their small sample sizes, potential selection bias due to case-control study designs, and failure to control for potential confounding factors. Moreover, linkage disequilibrium might differ among different populations. Nevertheless, modification of breast cancer susceptibility by certain VDR polymorphisms supports the notion that the VDR pathway and therefore vitamin D might modify breast cancer risk.

Calcium-Sensing Receptor and Breast Cancer Risk. The calcium-sensing receptor is expressed in human parathyroid gland, kidneys, and other tissues, such as colon and breasts (90). By influencing parathyroid hormone secretion and renal calcium reabsorption, the calcium-sensing receptor plays an

important role in maintaining extracellular calcium concentration (91). The calcium-sensing receptor is involved in regulating several cellular processes, including proliferation, differentiation, and apoptosis (92). Its presence in normal and malignant breast tissue suggests its potential role in mammary carcinogenesis (90). Three nonsynonymous polymorphisms (Ala⁹⁸⁶Ser, Arg⁹⁹⁰Gly, and Gln¹⁰¹¹Glu) of the calcium-sensing receptor gene have been identified. The Ala⁹⁸⁶Ser polymorphism has been found to influence extracellular calcium concentration (93, 94), whereas the functional significance of the other two polymorphisms is not well defined. To date, there have been no studies investigating calcium-sensing receptor polymorphisms and breast cancer risk.

Table 5. VDR polymorphisms and breast cancer risk

First author (ref.), year, study place	Years of data collection	Ethnicity	Study design	No. cases/controls	Comparison*	OR (95% CL)	Variables adjusted for
Chen (78), 2005, United states	1989/1990-2000	Mainly Caucasian	Nested case-control study	1,234/1,676	FokI: ff vs FF Premenopausal Postmenopausal BsmI: BB vs bb Premenopausal Postmenopausal	2.18 (1.18, 4.00) 1.25 (0.94, 1.66) 0.92 (0.46, 1.85) 0.94 (0.69, 1.26)	BMI, parity/age at first birth, family history of breast cancer in first-degree relative, BBD, alcohol, age at menarche, and age at menopause
Lowe (49), 2005, United Kingdom	1998-2003	Caucasian	Hospital-based case-control study †	179/179	BsmI: bb vs BB BsmI: Bb vs BB	2.02 (1.03, 3.97) 0.71 (0.37, 1.36)	Matching variables, time of year, age at sampling, and menopausal status
Guy (79), 2004, United Kingdom ‡	1998-2002	Caucasian	Hospital-based case-control study †	398/427	BsmI: bb vs BB Poly(A): LL vs SS FokI: FF vs ff ApaI: aa vs AA TaqI: TT vs tt	1.88 (1.19, 2.95) 1.90 (1.20, 3.01) 1.31 (0.84, 2.06) 0.80 (0.54, 1.19) 0.71 (0.42, 1.19)	Age, HRT usage, and menopausal status
Sillanpaa (83), 2004, Finland	1990-1995	Caucasian	Population-based case-control study	483/482	BsmI: BB vs bb TaqI: TT vs tt	0.76 (0.25, 2.29) 1.04 (0.27, 4.49)	Age, age at menarche, age at first full-term pregnancy, no. pregnancies, family history of breast cancer, and history of BBD
Buyru (84), 2003, Turkey	Not stated	Turkish	Case-control study	78/27	BsmI: BB vs bb TaqI: TT vs tt	0.76 (0.25, 2.29) 1.04 (0.27, 4.49)	Not stated
Newcomb (85), 2002, United states	1998	Not stated	Population-based case-control study	403/383	TaqI: tt vs TT Premenopausal Postmenopausal	1.15 (0.67, 3.41) 0.85 (0.47, 1.54)	Age, family history of breast cancer, BMI, age at first birth, HRT, and menopausal status
Hou (86), 2002, Taiwan	Not stated	Chinese	Hospital-based case-control study	34/169	BsmI: Bb vs bb TaqI: Tt vs TT ApaI: aa vs AA	1.81 (0.72, 4.55) 1.87 (0.64, 5.42) 0.52 (0.19, 1.40)	Not stated
Bretherton-Watt (80), 2001, United Kingdom ‡	Not stated	Caucasian	Hospital-based case-control study †	181/241	BsmI: bb vs BB Poly(A): LL vs SS FokI: FF vs ff	2.32 (1.23, 4.39) 2.46 (1.29, 4.70) 1.17 (0.65, 2.08)	Not stated
Ingles (81), 2000, United States	Not stated	Latinas	Nested case-control study	143/300	BsmI: BB vs bb Poly(A): SS vs LL FokI: ff vs FF	2.2 (1.0, 4.7) 3.2 (1.5, 6.9) 1.1 (0.6, 2.9)	Age
Curran (82), 1999, Australia	Not stated	Australian	Hospital-based case-control study	135/110	ApaI: a vs A TaqI: T vs t FokI: F vs f TaqI: tt vs TT	1.56 (1.09, 2.24) 1.45 (1.00, 2.00) 0.99 (0.69, 1.43) 0.79 (0.45, 1.39)	Not stated
Dunning (87), 1999, United Kingdom§	1992-1996	Caucasian	Case-control study	211/268	TaqI: tt vs TT	1.05 (0.72, 1.53)	Not stated
Dunning (87), 1999, United Kingdom§	1991-1996	Caucasian	Case-control study	740/359	TaqI: tt vs TT	1.05 (0.72, 1.53)	Not stated
Lundin (88), 1999, Sweden	1980-1993	Swedish	Hospital-based case-control study	111/130	TaqI	No association	Not stated
Ruggiero (89), 1998, Italy	Not stated	Italian	Hospital-based case-control study	88/167	BsmI: bb vs BB	0.89 (0.44, 1.81)	Not stated

*The associations are presented by menopausal status whenever the studies reported them separately or the studies were restricted to either premenopausal or postmenopausal women. Otherwise, the associations for a combination of premenopausal and postmenopausal women are presented.

†Prevalent breast cancer cases were included.

‡The study by Bretherton-Watt is the pilot study for the study by Guy.

§The same article presented two separate studies.

||No OR and 95% CL were presented. Genotype distributions were similar for cases and controls.

Discussion and Conclusion

Despite inconsistent results from the epidemiologic studies, several lines of evidence suggest that vitamin D and calcium might be involved in the development of breast cancer. Specifically, (a) vitamin D and calcium have shown anticarcinogenic properties in experimental studies; (b) some epidemiologic studies have suggested inverse associations between vitamin D and calcium intakes and breast cancer; (c) serum, plasma, and/or blood levels of vitamin D metabolites have been inversely associated with breast cancer risk in some studies; (d) high sunlight exposure, presumably reflecting vitamin D synthesis in the skin, has been associated with a reduced risk of breast cancer; (e) vitamin D and calcium intakes have been inversely related to breast density, an intermediate end point for breast cancer; (f) calcium has been associated with a reduced risk of benign proliferative epithelial disorder of the breast, putative precursors of breast cancer; and (g) certain polymorphisms of the VDR might modify breast cancer susceptibility.

Experimental evidence supports the hypothesis that the association between vitamin D intake and breast cancer may be stronger for premenopausal women than for postmenopausal women. The evidence is based on the biological interactions among vitamin D, the VDR, estrogen, and insulin-like growth factor-I (IGF-I). First, vitamin D has been shown to suppress the proliferative activity of both 17 β -estradiol and IGF-I, inhibit the antiapoptotic effect of IGF-I, and down-regulate the levels of estrogen receptors and IGF-I receptors (18, 95-97). Second, estrogen and IGF-I have been found to up-regulate VDR expression in breast cancer cells (15, 97). The potentially stronger effect of vitamin D on breast cancer among premenopausal women than postmenopausal women may be explained by the fact that the former have higher circulating levels of estrogen and IGF-I than the latter (98, 99). Two cohort studies (13, 40) showed no reduction in breast cancer risk in association with vitamin D intake in postmenopausal women, whereas one of them (40) found a reduced breast cancer risk in premenopausal women.

Vitamin D and calcium are strongly correlated and share similar anticarcinogenic effects on mammary gland. Hence, any apparent effect of vitamin D on breast cancer risk might be due in part to an effect of calcium and vice versa. However, few epidemiologic studies have investigated the joint and independent effects of vitamin D and calcium on breast cancer risk. To date, there have been three epidemiologic studies (13, 37, 40) that assessed the associations of breast cancer with calcium and vitamin D intakes within the same study populations. Among them, the case-control study by Levi et al. (37) and the cohort study by McCullough et al. (13) did not evaluate the joint and independent effects of calcium and vitamin D on breast cancer. Although mainly reporting separate associations of breast cancer with calcium and vitamin D, the cohort study by Levi et al. (37) also reported that calcium intake from dairy foods was inversely associated with premenopausal breast cancer within strata of vitamin D intake. Distinguishing the independent associations of vitamin D and calcium with breast cancer will be difficult due to their high correlation. To address this issue, observational studies with very large sample sizes or clinical trials with appropriate study designs are required for future investigation. An interaction between calcium and vitamin D has been shown in the prevention of colorectal neoplasia in some studies (100-103). A study by Grau et al. showed that calcium supplementation lowered the risk of colorectal adenoma only among subjects with a high level of 25(OH)D and that 25(OH)D was inversely associated with the risk only among subjects who received calcium supplements (100). Furthermore, Grau et al. (100) hypothesized that the interaction might have occurred due to the fact that vitamin D controls

intracellular calcium gradients (104) and increases expression of the calcium-sensing receptor (91). To date, the only study that has investigated the interaction between calcium and vitamin D in breast cancer yielded a null result (37). Further investigation of this interaction in the development of breast cancer is warranted.

To further confirm the potential protective effects of calcium and vitamin D on breast cancer, well-designed cohort studies and clinical trials are warranted. Although not designed to assess breast cancer as a primary end point, the Women's Health Initiative clinical trial of calcium and vitamin D supplementation may provide us with valuable information on this topic (105).

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