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Vitamin D Acquisition and Breast Cancer Risk

Faustino R. Pérez-López, MD, Peter Chedraui, MD, MSc, and
Javier Haya, MD

Objective: The aim of the study was to focus on the association of vitamin D and breast cancer. *Methods:* The study of evidence concerning vitamin D's influence on the origin and development of breast cancer from a PubMed and individual searches. *Results:* Body sunlight exposure may reduce the prevalence of breast cancer. However, these studies correspond to global populations of different countries and regions without considering other geographic factors and individual, ethnic, and cultural factors that may affect sunlight exposure. *Epidemiological analyses* show that low vitamin D ingestion is associated with increased risk of breast cancer. *Studies measuring serum vitamin D metabolites* in women who were followed many years suggest that low circulating 25-hydroxyvitamin D₃ levels are associated with increased breast cancer risk. *Conclusions:* Although there are controversial results, it seems plausible that sufficient endogenous vitamin D levels may have a protective function on mammary cells, reducing breast cancer risk.

KEY WORDS: 1,25-Dihydroxyvitamin D₃, 25-hydroxyvitamin D₃, breast cancer, vitamin D.

INTRODUCTION

Breast cancer is one of the most common malignancies in women. It is a multifactorial disease in which changes in cellular biology are affected by a large number of variables. It seems that breast cancer results from the accumulation of a large number of genetic mutations. The immune and apoptotic systems may eliminate abnormal cells without any further health consequences. However, when the defence system is altered, the accumulation of abnormal cells may result in tumor growth and general dissemination.¹ Breast cells are under hormone regulation as estrogen and progesterone control mitosis rate. Estrogens also interfere with the immune surveillance in breast cancer.² However, there are many other hormonal

signals and growth factors involved in mammary carcinogenesis.³⁻⁵ On the other hand, epidemiologic and biochemical studies are searching new additional breast cancer risk factors as well as new targets for preventive therapies.^{6,7} Breast cancer incidence patterns reflect to a certain extent some basic societal characteristics, such as lifestyle factors. One important lifestyle factors is sun exposure and tanning habits as an indirect measure of vitamin D synthesis; a second factor is related to food and micronutrients.

Vitamin D is an essential hormone produced mainly from skin exposure to sunlight ultraviolet (UV) B radiation, with a small quantity provided by diet. Vitamin D deficiency is presently a common problem that can lead to a number of serious health conditions beyond the musculoskeletal system. Thus, vitamin D deficiency can lead to or exacerbate osteopenia, osteoporosis, muscle weakness, fractures, common cancers and autoimmune, infectious, and cardiovascular diseases.⁸⁻¹⁰ In addition, Dobnig et al¹¹ have found that normal vitamin D levels can also have an impact on death rates regardless of the primary cause of death, and participants are more likely to live longer than those with a deficiency. However, it is not clear why vitamin D could have such a beneficial effect on mortality. Studies over the past years indicate an important potential role for vitamin D in cancer

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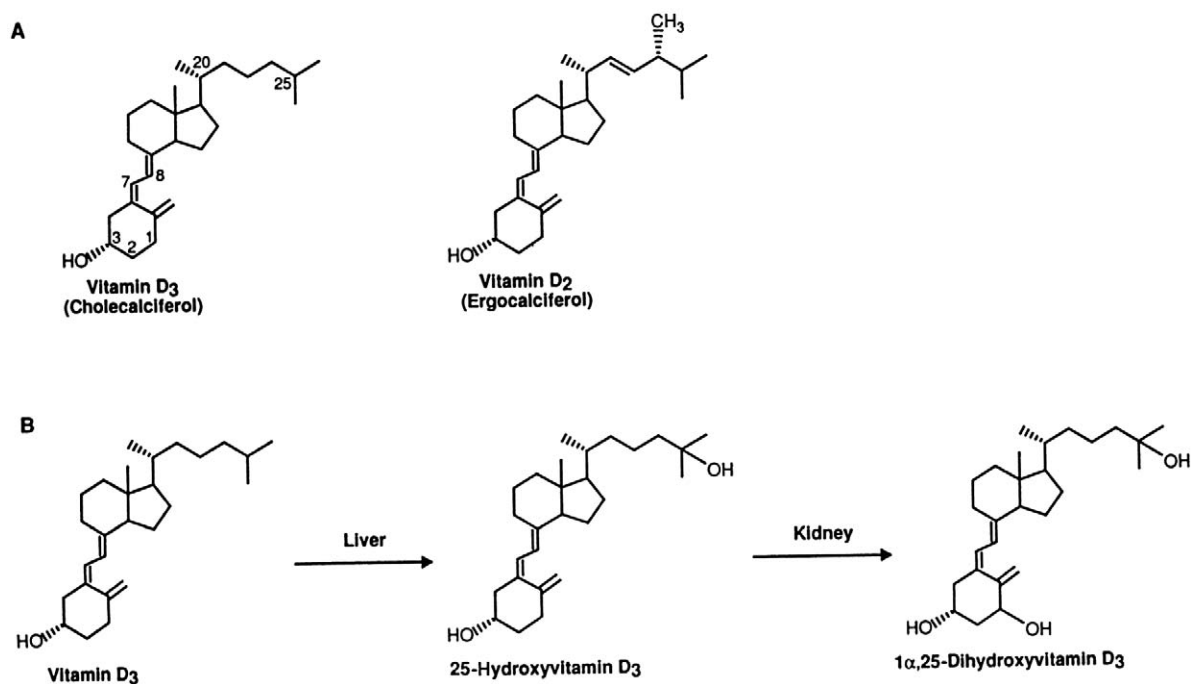


Figure 1. General vitamin D biosynthesis. A, vitamin D₃ and vitamin D₂. B, 2-step hydroxylation.

prevention, survival, and treatment. Scientific evidence regarding the pathophysiology of vitamin D on the mammary gland has increased. Thus, mammary cells have vitamin D receptors (VDRs) as well as hydroxylases for the regulation of local hormone synthesis. The following review, based on PubMed and personal queries, will address and discuss published information regarding vitamin D's influence on the different aspects of breast cancer.

PATHOPHYSIOLOGY OF VITAMIN D

Vitamin D is a group of fat-soluble prohormones, the 2 major forms of which are ergocalciferol (vitamin D₂) and cholecalciferol (vitamin D₃). Ultraviolet B sunlight (290–315 nm) is absorbed by the skin and converts the natural precursor 7-dehydrocholesterol into vitamin D₃ that is bound by vitamin D binding protein (DBP) into the capillary bed.^{8,12} Geography, climate, and air pollution determines how much vitamin D people are able to manufacture from sun exposure. Vitamin D₂ and vitamin D₃ are also ingested through diet. After ingestion, they are incorporated into chylomicrons, transported through the lymphatic system, and subsequently released into the venous circulation, where vitamin D is bound to lipoproteins and DBP.¹³ Both vitamin D from the skin and diet enter the liver in which they are converted to

25 hydroxyvitamin D (25(OH)D) or calcidiol, the primary circulating form of vitamin D. The conversion is carried out by 25-hydroxylases (CYP27A1, CYP2R1, CYP3A4, and CYP2J3).¹³ A second hydroxylation, mainly taking place in the kidney, is performed by 25(OH)D-1α-hydroxylase (1α-hydroxylase or CYP27B1) and gives rise to the active hormone 1α,25-dihydroxyvitamin D (1,25(OH)₂D), dihydrocholecalciferol, or calcitriol (Figure 1). Renal 1α-hydroxylase is under the endocrine control of 1,25(OH)₂D, parathyroid hormone (PTH), and calcitonin that stimulate 1α-hydroxylase gene expression, while the 1,25(OH)₂D action is exerted by the VDR.¹⁴

Several factors have been found to alter vitamin D circulating levels (Table 1). There has been a debate in recent years regarding 25(OH)D serum concentration reference values used to define vitamin D deficiency and insufficiency. Currently, the traditional 10 ng/mL cutoff serum value for 25(OH)D deficiency is too low to indicate vitamin D adequacy. A low serum 25(OH)D concentration is associated with physiologic, pathologic, and clinical evidence of vitamin D deficiency, including increased PTH secretion, increased bone turnover, osteoporosis, mild osteomalacia, and an increased risk of fractures. However, to prevent more subtle or long-term effects of vitamin D insufficiency, circulating concentrations of at least 30 ng/mL 25(OH)D may be warranted.¹⁵ The physiologically active hormone

Table 1. Risk Factors for Vitamin D Deficiency

Reduced skin synthesis
Season, latitude, time of day
Skin pigment
Sunscreen use
Aging
Decreased bioavailability
Malabsorption
Obesity
Increased catabolism
Anticonvulsants, glucocorticoids, highly active antiretroviral therapy
Pregnancy and breast feeding
Decreased synthesis of 25-OH(D)
Liver failure
Decreased synthesis of 1,25(OH) ₂ D
Chronic kidney disease

Abbreviations: 1,25(OH)₂D, 1 α ,25-dihydroxyvitamin D; 25(OH)D, 25 hydroxyvitamin D.

1,25(OH)₂D has at least 4 major biological functions: (1) calcium and phosphorus homeostasis regulation and metabolism, (2) regulation of other hormones, (3) cell differentiation induction and growth modulation, and (4) immune system regulation and a possible role in the prevention of cancer and immune-mediated diseases. Recent evidences suggest that vitamin D plays additional roles in cellular differentiation and proliferation control in a variety of cell types, including mammary cells.

Vitamin D binding protein is a multifunctional plasma glycoprotein present in most vertebrates. In humans, it has a molecular weight of about 52 to 58 kd. It is a highly polymorphic protein that acts as the main carrier for vitamin D and its hydroxylated metabolites, showing the highest affinity for 25(OH)D. Vitamin D binding protein is synthesized in significant amounts only by the liver and secreted into the blood. Other vitamin D carrier proteins include albumin and α -fetoprotein. Changes in 25(OH)D levels related to age, gender, or fat mass do not seem to be due to transporting protein alterations.^{16,17}

The biologically active hormone 1,25(OH)₂D interacts with the VDR to regulate proliferation and apoptosis in a variety of tissues, including the mammary gland. 25 Hydroxyvitamin D is almost devoid of a direct effect on the VDR because its binding is only 2% of that of the active hormone and due to the fact that 25(OH)D is about 1000 times more tightly bound to serum DBP.¹⁸ In each tissue, VDRs are regulated to a different set point, according to physiologic inputs such as calcium and 1,25(OH)₂D. In vitro studies show that 1,25(OH)₂D

upregulates the VDR at least partially through the activation of gene expression, often increasing VDR number, and in other circumstances obtaining VDR stabilization.¹⁹ However, VDR upregulation is not uniform in all tissues. Calcium and 1,25(OH)₂D have a mild effect on duodenal VDRs, while the same agents regulate VDR expression at the parathyroid gland and kidney.²⁰

The 25(OH)D-24-hydroxylase (24-hydroxylase, CYP24A1) plays an important role in regulating concentrations of both the 25(OH)D precursor and the 1,25(OH)₂D hormone (Figure 1). It converts 25(OH)D and 1,25(OH)₂D to 24,25-dihydroxyvitamin D₃ and 1,24,25-trihydroxyvitamin D₃, respectively. The 24-hydroxylase depends on VDR activity, and is likely controlled by a short-lived repressor protein.²¹

Vitamin D's primary excretion route is via the bile into the feces, although it is also metabolized to water-soluble metabolites, such as calcitroic acid, and excreted in the urine.

VITAMIN D AS A CELL GROWTH AND DIFFERENTIATION REGULATOR

The combined presence of the 1 α -hydroxylase enzyme as well as the specific receptor in several tissues may explain the new roles for 1,25(OH)₂D unrelated to calcium homeostasis.²²⁻²⁴ The 1 α -hydroxylase activity has been demonstrated in inflammatory cells including the monocyte/macrophage group. Parathyroid hormone and calcitonin do not change 1 α -hydroxylase expression in the human monocytic cell line THP-1, which can be differentiated into macrophage-like cells, whereas interferon- γ treatment augments macrophage 1 α -hydroxylase concentration.²⁵ On the other hand, macrophages and dendritic cells are capable of performing both hydroxylation steps of vitamin D₃ metabolism suggesting a possible role for local 1,25(OH)₂D synthesis.²⁶ Mammary cells also express 1 α -hydroxylase suggesting that this enzyme converts precursor 25(OH)D metabolite into 1,25(OH)₂D, while no synthesis by 25(OH)D was obtained in mammary cells derived from 1 α -hydroxylase in null mice.²⁷

Malignant cells have high basal 25(OH)D-24-hydroxylase (24-hydroxylase or CYP24) levels and have the potential of inducing CYP24 in response to 1,25(OH)₂D. This 24-hydroxylase would rapidly degrade the active steroid hormone and 25(OH)D (Figure 2) to less potent metabolites.²⁸

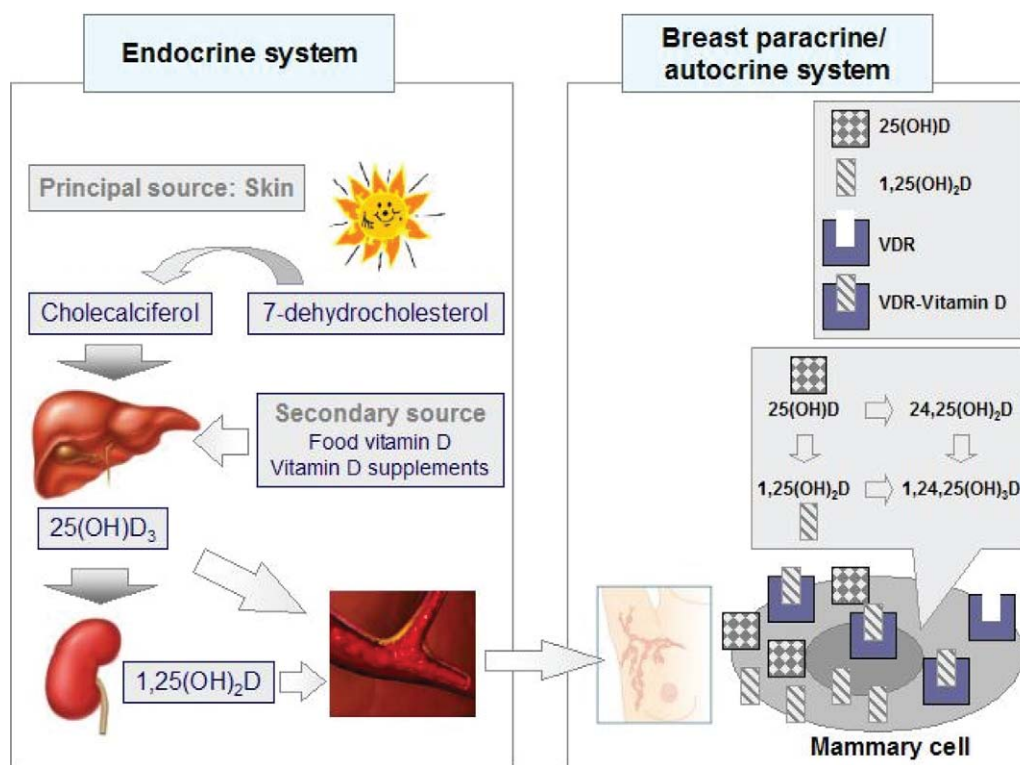


Figure 2. Vitamin D acquisition (left) through sunlight effect on the exposed skin, and food and supplement ingestion. Liver 24-hydroxylation of vitamin D, and kidneys 1α -hydroxylation complete the hormone synthesis. Mammary synthesis (right) of $1,25(\text{OH})_2\text{D}$ from circulating $25(\text{OH})\text{D}$ is produced by 1α -hydroxylase, while 24 hydroxylation inactivates $25(\text{OH})\text{D}$ and $1,25(\text{OH})_2\text{D}$ to $24,25(\text{OH})_2\text{D}$ and $1,24,25(\text{OH})_3\text{D}$, respectively. $1,25(\text{OH})_2\text{D} = 1\alpha,25$ -dihydroxyvitamin D; $25(\text{OH})\text{D} = 25$ hydroxyvitamin D; $24,25(\text{OH})_2\text{D} = 24,25$ dihydroxyvitamin D; $1,24,25(\text{OH})_3\text{D} = 1,24,25$ trihydroxyvitamin D.

Steroid hormones and the secosteroid vitamin D_3 modulate development, growth, and differentiation in various cell types. These actions are mediated by specific hormonal intracellular receptors, which in turn will define genetic sequencing. These hormones also influence growth factors and cytokines, requiring complex control mechanisms. The VDR is expressed by almost all cells, and about 3% of the mouse or human genome is regulated, directly and/or indirectly, by the vitamin D endocrine system. The immune system of vitamin D-deficient mice is grossly normal but after exposure to predisposing factors displays increased sensitivity to autoimmune diseases such as inflammatory bowel disease or type 1 diabetes.²⁹ Extensive investigation has shown that cancer cells express VDRs that may regulate more than 60 genes involved in cell differentiation, antiproliferative and antimetastatic effects.³⁰ However, many breast cancer cell lines are less sensitive to $1,25(\text{OH})_2\text{D}$ than normal mammary epithelial cells. Reduced sensitivity to $1,25(\text{OH})_2\text{D}$ has been linked to vitamin D metabolizing enzyme alterations as well as VDR expression or function

downregulation. It has been demonstrated in VDR knockout mice that vitamin D may also act through other physiological mechanisms that are VDR independent.³¹

There is abundant evidence that $1,25(\text{OH})_2\text{D}$ inhibits growth in several cancer cell types (including breast cancer) and angiogenesis which are responsible for its antitumor effects in different experimental models.³²⁻³⁵ There is also in vitro evidence concerning vitamin D effects on normal and malignant breast tissue, suggesting a relevant role in tumorigenesis.^{27,36,37}

SUNLIGHT AND BREAST CANCER

Epidemiological studies have suggested the hypothesis that sunlight deprivation and the associated reduction in the circulating levels of vitamin D_3 derivatives may lead to an increased incidence of solid carcinomas. Peller et al³⁸ were the first to propose that sunlight exposure decreases cancer risk. Apperly et al³⁹ showed an association between latitude and cancer mortality reviewing

cancer death statistics across North America and Canada. Compared to cities between latitudes 10° and 30°, those at latitudes 30° to 40° had an 85% higher cancer death rate, rate which was 118% and 150% higher for cities at latitudes 40° to 50° and 50° to 60°, respectively. Garland et al⁴⁰ and Schwartz et al⁴¹ have proposed that low sun exposure could be related to the development of several internal solid cancers. Thus, the incidence for many cancers would be higher as the distance from the equator increases. Garland et al⁴⁰ reported geographical variation in breast cancer mortality in the United States associated with geographic UV measurements for more than 9 years. Thus, breast cancer deaths varied 1.8-fold from low rates in the sunny areas of the south and southwest to high rates in lower sunlight areas such as New York. The strong latitude gradient in cancer mortality does not appear to be associated with socioeconomic factors, although this would not be the only factor to be accounted for because others might possibly explain an ecologic association.⁴²

In a case-control study based on US death certificates, breast cancer risk was significantly reduced among participants with high residential sunlight exposure and with high occupational exposure in nonfarming outdoor jobs.⁴³ In addition, there is an inverse association between regional environment UVB radiation and mortality rates in 3953 United States counties for at least 11 cancer types, including that of the breast.⁴⁴ Inverse associations between environment UVB radiation and mortality due to breast, endometrium, ovary, prostate, and kidney cancers and multiple myeloma and non-Hodgkin lymphomas have been reported across European countries.⁴⁵ The US studies suggest that the addition of population level information on variables related to diet and socioeconomic factors did not alter the correlations between UV exposure and mortality rates. However, this may not be true in Europe, where potential confounding factors may differ from those in the United States. This inverse relationship has been confirmed across a broad range of cancers that have variable risk factors.⁴⁶ Nevertheless, the Nurses' Health Study population did not show a geographic gradient for breast cancer incidence (diagnosed cases) when adjusted for age and established risk factors, because breast cancer incidence was similar among premenopausal and postmenopausal women of 4 studied North American regions.⁴⁷

In the first National Health and Nutrition Examination Survey (NHANES) cohort that includes 5009 women with baseline dermatological examination, it was found that increased sunlight exposure assessed by different end

points were all associated with a small breast cancer risk reduction, the highest reductions being in women living in high sunlight regions with no risk reduction in low sunlight regions.⁴⁸ In a population-based case-control study, the relationship between sources of vitamin D and breast cancer risk was assessed among 972 breast cancer patients from the Canadian Ontario Cancer Registry and 1135 controls.⁴⁹ When outdoor activities were classified in quartiles, reduced breast cancer risk was significantly associated with the highest sun exposure quartile versus the lowest from ages 10 to 19. There were also breast cancer risk inverse associations with cod liver oil use and high milk consumption. The association was weaker from ages 20 to 29 and there was no association for ages 45 to 54. These results suggest that outdoor activities—a surrogate index of sun exposure and skin vitamin D synthesis—might be crucial at the time breasts are developing, and vitamin D-enriched diet or supplements—such as cod liver oil or milk—could also give a significant protection against breast cancer risk.

Tuohimaa et al⁵⁰ followed more than 400 000 skin cancer patients with record linkage to cancer registries, studying the pattern of second primary cancers. They reported that vitamin D production in the skin might decrease the risk of several solid cancers, including that of the breast. However, their results and hypotheses have been criticized because individuals' behavior on sunny days may be associated with climate, lifestyle, fertility pattern, diet, physical activity, and other variable factors dependent on nationality and cultural background.⁵¹

The vitamin D hormone system seems to be the molecular link between sunlight and the most prevalent cancers, with incidence rates increasing with age.⁵²⁻⁵⁴ Mohr et al⁵⁵ have studied the inverse relationship between age-standardized breast cancer incidence rates in 107 countries, modeling and measuring serum 25(OH)D to determine whether breast cancer incidence is associated with sunlight exposure. The authors found a protective effect of UVB irradiance on breast cancer risk that seemed independent of fertility rate, overweight, alcohol intake, energy intake, and other factors. However, the study refers to countries rather than people and findings that apply to aggregates may not apply to individuals. Despite these results, increasing sun exposure may not be the way to decrease breast cancer risk because prolonged and cumulative sunlight doses may increase skin cancer and ageing, cataracts and other complications. Nevertheless, a regular small-dose sunlight exposure may have benefits for both breast development and general health.

Table 2. Clinical Studies on Diet, Vitamin D Supplements and Breast Cancer Risk

Authors	Population	Outcome
Simard et al ⁵⁶	Case-control (108/322) study of diet intake and breast cancer risk	Twice as many breast cancer patients than controls had a higher consumption of vitamin D
John et al ⁴⁸	5509 women from the First National Health and Nutrition Examination Survey cohort followed (190 developed incident breast cancer)	Sunlight and dietary vitamin D reduce the risk of breast cancer
Shin et al ⁵⁷	88 691 women from the Nurses' Health Study cohort (3482 women developed incident invasive breast cancer)	High intake of low-fat dairy foods was associated with reduced risk of breast cancer in premenopausal women
McCullough et al ⁵⁸	34 321 postmenopausal women from the Iowa Women's Health Study cohort (2855 women developed incident breast cancer)	Vitamin D intake of >800 IU/d appears to be associated with a small decrease in risk of breast cancer among postmenopausal women
Robien et al ⁵⁹	34 321 postmenopausal women followed for breast cancer incidence from 1986 to 2004	Vitamin D intake of >800 IU/d is associated with a small decrease in risk of breast cancer among postmenopausal women
Lin et al ⁶⁰	10 578 premenopausal and 20 909 postmenopausal women 45 years or older in the Women's Health Study followed for 10 years (276 premenopausal and 743 postmenopausal women developed incident breast cancer)	High intakes of calcium and vitamin D is associated with a lower risk of developing premenopausal breast cancer
Abbas et al ⁶¹	Case-control (278/666) study of German premenopausal women to determine breast cancer risk	Breast cancer risk was significantly inversely associated with vitamin D intake, and its protective effect was independent of dietary calcium intake
Knight et al ⁴⁹	Case-control (972/1135) study of the Ontario Cancer Registry	Low breast cancer risk was associated with increasing sun exposure from ages 10 to 19. Breast cancer risk was inversely associated with cod liver use and high milk consumption. The associations were weaker from ages 20 to 29.
Lappe et al ⁶²	1179 community-dwelling healthy postmenopausal women aged 55 years or older were randomized to receive calcium, vitamin D + calcium, or placebo for 4 years	Vitamin D significantly reduced the risk of breast cancer while calcium had no significant effect

VITAMIN D INTAKE AND BREAST CANCER RISK

Vitamin D intake has been associated with breast cancer risk (Table 2). The first publication concerning the influence of food and its vitamin D content on breast cancer risk was a case-control study showing that mean daily intake of vitamin D was higher in breast cancer patients as compared to the controls,⁵⁶ data which are contrary to current findings indicating that higher intake may decrease breast cancer risk. The effects of sunlight exposure, diet, and supplements on the risk of breast cancer have been studied in the first NHANES cohort, which included 5009 white women who completed dietary surveys from 1971 to 1974 and were followed up to 1992.⁴⁸ The adjusted relative risk was calculated based on 190 cases that developed incident breast cancer. The average vitamin D intake provided by food was slightly lower, although not significantly, among breast cancer cases than in controls. The proportion of women with an intake of at least 200 IU was 22% among breast cancer cases as compared to 26% in controls. The values were not

significantly different probably due to the very low vitamin D intake. However, the authors found a cancer risk reduction in women living in high sunlight exposure.

The 16-year follow-up of 88 691 women in the Nurses' Health Study cohort from the date of return of their food-frequency questionnaire provided interesting information about intake of dairy products, calcium, and vitamin D and the risk of breast cancer.⁵⁷ At follow-up, incident breast cancer appeared in 3482 of these women (premenopausal = 827, postmenopausal = 2345, and uncertain menopausal status = 310). High intake of low-fat dairy foods was associated with reduced breast cancer risk in premenopausal women. Similar inverse associations were detected between breast cancer and calcium/vitamin D intake.

McCullough et al⁵⁸ evaluated 68 567 postmenopausal women from the Cancer Prevention Study II Nutrition Cohort who completed a detailed questionnaire. During follow-up, 2855 cases of incident breast cancer were identified. Consumption starting at 2 or more servings of dairy products per day was inversely associated with a

lower breast cancer risk as compared with <0.5 servings/d. The associations were slightly stronger in women with estrogen receptor (ER)-positive tumors comparing highest to lowest dietary intake. The authors concluded that dietary calcium and/or some other dairy components may slightly reduce breast cancer risk in postmenopausal women. A similar type of study by Robien et al⁵⁹ evaluated vitamin D intake and breast cancer risk in 34 321 postmenopausal women from the Iowa Women's Health Study cohort. The relative risk for breast cancer was significantly lower in women who consumed >800 IU/d versus <400 IU/d total vitamin D intake, this association being the strongest in the first 5 years after basal diet assessment. These results suggest that a vitamin D intake >800 IU/d is associated with a small decrease in breast cancer risk.

Lin et al⁶⁰ evaluated more than 30 000 women aged 45 years and older from the Women's Health Study cohort who were healthy at baseline and were followed 10 years in average. High calcium and vitamin D intake through food sources and nutritional supplements reduced breast cancer risk in premenopausal women nearly by one third. The association was strongest for the most aggressive tumors and not found in postmenopausal women. Women who had high vitamin D and calcium intake were leaner, more physically active, consumed less fat and alcohol, and engaged in a healthy lifestyle.

Abbas et al⁶¹ reported the results from a population-based case-control study that examined the independent and joint effects of dietary vitamin D and calcium on breast cancer risk in a German cohort. Dietary information was assessed in 278 premenopausal cases and 666 matched controls. Breast cancer risk was significantly inversely associated with vitamin D intake; contrary to this, dietary calcium was not. Therefore, dietary vitamin D seems to have a protective role on breast cancer risk in premenopausal women, independent of calcium intake. Furthermore, data obtained from the Ontario Cancer Registry, as discussed above, suggest that cod liver oil and high milk intake might be relevant during breast development, many years before breast cancer diagnosis.⁴⁹

In 1 study, postmenopausal women aged 55 years and older were randomly assigned to 1 of 3 groups and followed up for 4 years to determine fracture and cancer incidence.⁶² One group took 1400 to 1500 mg/d of calcium supplement, a second the same amount of calcium plus 1100 IU/d of vitamin D, and the third group daily placebo pills. After 4 years, women in the combined treatment had a 60% lower breast cancer risk as compared to the placebo group, while women who received

calcium alone had a 47% lower risk than women on placebo. The effect of the combined treatment was more significant when the results of the first year were eliminated to reduce bias due to possible cancer cases that had not yet been diagnosed. With this correction, a significant effect for calcium alone when compared to placebo was not evidenced. Although the results are interesting, the small studied population must be mentioned. Despite this, if results are confirmed, it might be possible to obtain some preventive effect on breast cancer using vitamin D at a dose higher than that considered in other previous studies already discussed.

SERUM VITAMIN D AND BREAST CANCER RISK

Vitamin D from both skin synthesis and digestive absorption are converted in the liver within 48 hours into 25(OH)D. This has a biological half-life of at least 2 months, and its serum level is the accepted measure of endogenous vitamin D status (skin biosynthesis and food or supplement intake). However, serum 25(OH)D levels do not indicate the amount stored in other tissues. Blood 1,25(OH)₂D is not a good indicator of vitamin D status because it has a short half-life of 15 hours and serum levels are under PTH, calcium, and phosphate feedback influences. 1 α ,25-Dihydroxyvitamin D breast tissue synthesis may contribute to maintain normal cellular function at the breast, and when circulating levels of 25(OH)D precursors decrease, breast tissue processes would be altered, increasing breast cancer risk. The protective effects of vitamin D result from its role as a nuclear transcription factor that regulates cell growth, differentiation, apoptosis, and a wide range of cellular mechanisms pivotal to the development of cancer. 1 α ,25-Dihydroxyvitamin D has significant effects on cell growth and normal and tumoral mammary gland differentiation, antiproliferative effects and apoptosis induction being the most dominant. In the MCF-7 human breast carcinoma cell line, the effects of 1,25(OH)₂D are mediated through different hormone factors, including growth hormone, prolactin, and a pituitary transcription factor-1.⁶³ Different studies have explored the possible association between low blood vitamin D metabolites and breast cancer risk (Table 3).

Breast Cancer Risk and Serum Vitamin D Metabolites

A prospective case-control study nested within the Nurses' Health Study cohort had evaluated the relation

Table 3. Serum 25(OH)D and/or 1,25(OH)₂D Levels and Breast Cancer Risk

Authors	Population	Outcome
Garland et al ⁶⁴	Pooled analysis of 4 observational studies	There is an inverse association between 25(OH)D considering the extreme quintiles
Abbas et al ⁶⁵	Case-control study of 25(OH)D and risk of postmenopausal breast cancer (1394 cases and 1365 controls)	Serum 25(OH)D concentration was significantly inversely associated with postmenopausal breast cancer risk. The association was stronger in women never using menopausal HT compared with past and current users
Freedman et al ⁴³	Case-control study 25(OH)D and 1,25(OH) ₂ D levels in postmenopausal women (1005 breast cancer and 1005 noncases)	The relative risk of breast cancer for the highest quintile 25(OH)D and 1,25(OH) ₂ D versus the lowest were not significantly different
Pilz et al ⁶⁶	25(OH)D and 1,25(OH) ₂ D in 3299 patients from the Ludwigshafen Risk and Cardiovascular Health study were followed for the breast cancer incidence and evolution	Low levels of 25(OH)D are associated with increased risk of fatal breast cancer in patients referred to coronary angiography
Mawer et al ⁶⁷	Cross-sectional study of patients with early and advanced breast cancer	1,25(OH) ₂ D fell in breast cancer patients whose disease progressed in 6 months
Palmieri et al ⁶⁸	204 women with early breast cancer and 75 patients with locally advanced or metastatic disease	Patients with early-stage disease had significantly higher serum 25(OH)D and significantly lower PTH than those with advanced disease
Goodwin et al ⁶⁹	Breast cancer cohort from 3 Toronto Hospital	Vitamin D deficiency is associated with poor prognosis
de Lyra et al ⁷⁰	21 postmenopausal women with breast cancer submitted to 1-month vitamin D treatment	Reduction of Ki67 expression after 1 month treatment with 1,25(OH) ₂ D 0.50 µg/d

Abbreviations: HT, hormone therapy; PTH, parathyroid hormone; 1,25(OH)₂D, 1 α ,25-dihydroxyvitamin D; 25(OH)D, 25 hydroxyvitamin D.

between vitamin D metabolites and breast cancer risk.⁷¹ Thus, 25(OH)D and 1,25(OH)₂D were measured and women were followed up for 6 years. Hormone values from women who developed breast cancer were matched to cancer-free controls classified according to age, menopausal status, and other factors. Compared to control, patients with breast cancer had significantly lower mean serum levels of 25(OH)D. The 25(OH)D serum level was inversely associated with the risk of ER-/ α -progesterone receptor (PR) - tumors, but not with ER+/ α -PR+ and ER+/ α -PR- tumors. Although serum 1,25(OH)₂D levels were also lower among cases, this was not significant when compared to controls. The inverse associations were stronger for both metabolites in women aged 60 years and older, although not statistically significant.

Garland et al⁶⁴ reported a pooled analysis of various publications^{21,64,71-73} that included breast cancer risk and serum 25(OH)D. Patients were grouped by quintile values according to the following serum 25(OH)D values: 6, 18, 29, 37, and 48 ng/mL. Pooled odds ratio for breast cancer from lowest to highest quintile were 1.0, 0.90, 0.70, 0.70, and 0.50. Although the analysis has some limitations, it seems that there is an inverse association between vitamin D and the risk of breast cancer considering the extreme 25(OH)D values. A German case-control

study also assessed the association of 25(OH)D serum concentrations and breast cancer risk in postmenopausal women.⁶⁵ This study included 1394 cases and 1365 controls matched for the year of birth and the time of blood collection and grouped by 25(OH)D levels: <30, 30 to 45, 45 to 60, 60 to 75, and \geq 75 nM. Odds ratio were calculated against the lowest vitamin D concentration (<30 nM), results being 0.57, 0.49, 0.43, and 0.31, respectively. These results suggest a strong protective effect for postmenopausal breast cancer through a better vitamin D acquisition. The association was stronger in women never using hormone replacement therapy compared with past and current users.

In a prospective study regarding serum 25(OH)D levels and cancer mortality, Freedman et al⁷⁴ followed 16 818 participants from the third NHANES cohort. After nearly a decade of follow-up, 536 participants had died of cancer. Cancer mortality was not related to the circulating vitamin D levels for the overall group nor was it related after stratifying for ethnicity, sex, and age. However, there was an inverse association between 25(OH)D serum levels and colorectal mortality. Thus, vitamin D levels of 80 nmol/L or more were associated with a 72% reduction in colorectal cancer risk mortality as compared with patients with levels <50 nmol/L. The authors'

conclusion have been criticized and results interpreted contrarily—adequate serum 25(OH)D levels have a substantial protective association on breast cancer mortality rates.⁷⁵ Thus, mortality was significantly lower in women whose serum 25(OH)D levels were at or above the median, 62.5 nmol/L, compared to those below it. More recently, Freedman et al⁷⁶ reported the serum levels of 2 vitamin D metabolites in a postmenopausal women cohort. Serum 25(OH)D and 1,25(OH)₂D levels were measured between 1993 and 2001 in postmenopausal women aged 55 to 74 years from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial cohort. After a 3.9-year median follow-up, the authors identified 1005 incident breast cancers, which were matched to 1005 noncases. Breast cancer relative risks were calculated by quintile for each metabolite. Obtained relative risks for the highest versus the lowest quintile were not statistically significant for either 25(OH)D or 1,25(OH)₂D. Results did not change even when the first 2 years of follow-up were excluded. Although the authors do not rule out an association for younger women, longer follow-up, or earlier assessed vitamin D metabolite values.

Pilz et al⁶⁶ measured both 25(OH)D and 1,25(OH)₂D in 3299 patients from the Ludwigshafen Risk and Cardiovascular Health cohort. Blood sampling was performed before coronary angiography and mortality were recorded during a median follow-up of nearly 8 years. After adjustment for confounders, there was no association between 1,25(OH)₂D levels and increased fatal cancer risk in these patients. On the contrary, 25(OH)D levels showed an inverse association with fatal cancer. This study supports an inverse association between vitamin D levels and breast cancer as postulated by Freedman et al⁷⁶ (interpreting the results of Garland et al⁷⁵). Although the populations were quite different, it may be likely that long-term low vitamin D levels are deleterious for mammary cells.

Breast Cancer Progression

The role of endogenous 1,25-(OH)₂D at different clinical breast stages has been previously studied in a cross-sectional study.⁶⁷ Hormone levels were highest in early disease, fell in normocalcemic patients with bone metastases, and were lowest in hypercalcemic patients. In normocalcemic patients with bone metastases submitted to hormone therapy, serum 1,25(OH)₂D fell in patients whose disease progressed in a 6-month period, whereas it remained stable in those who responded to treatment. Another publication addressed to clarify a possible role

of vitamin D in cancer progression measured serum 25(OH)D levels in early and advanced breast cancer.⁶⁸ Levels were significantly higher in early-stage breast cancer as compared to cases with advanced disease. In addition, PTH levels were significantly lower in early cases compared to advanced ones. Thus, it could be speculated that the active hormone may control secondary tumor growth, or its synthesis decreases as tumor growth progresses.

Goodwin et al⁶⁹ recently reported on 25(OH)D serum levels and the risk for distant recurrence and death in a prospective early breast cancer cohort of 512 women enrolled from 3 Toronto Hospitals followed up for a median of 11.6 years. Only 24% of women had the recommended vitamin D level, and low vitamin D levels were associated with higher risk for distant metastases and worse survival: 116 women (22.7%) had distant recurrences and 106 (20.7%) died during the 11.6-year median follow-up. Low vitamin D levels were significantly associated with premenopausal status, high body mass index (BMI), high insulin levels, and high tumor grade. Distant disease-free survival was significantly worse in women with deficient vitamin D levels as compared to patients with adequate levels. These associations were independent of age, BMI, ER status, and T and N stage and tumor grade. The authors concluded that vitamin D deficiency is associated with poor prognosis, although chance was not ruled out and few women with very high vitamin D levels seemed to have worse survival.

The antiproliferative effects of supplementing 1,25(OH)₂D over a 30-day period have been reported in 21 postmenopausal women with breast cancer being evaluated for tumor dimension (ultrasound), cell proliferation rate (Ki67 expression), and serum 1,25(OH)₂D levels.⁷⁰ The first 10 (group GI) and the next 11 patients (group GII), respectively received oral 1,25(OH)₂D at 0.25 and 0.50 µg/d. One third of women presented 25(OH)D insufficiency (7–20 ng/mL) and no patients presented 1,25(OH)₂D below the reference level (15.9 pg/mL). There were not differences in 1,25(OH)₂D serum levels and tumor dimensions evaluated by ultrasound before and after supplementation. There was a small reduction in Ki67 expression in GI and a significant reduction in GII after treatment, suggesting that a short period of supplementation may reduce Ki67 expression, an index of antiproliferative effects. These results correlate well with *in vitro* studies showing that 1,25(OH)₂D profoundly affects breast cancer cell phenotype and reverts myoepithelial features associated with more aggressive forms and poor prognosis in human breast cancer.⁷⁷

On the other hand, a 15% to 25% better survival has been reported for patients diagnosed during summer, and a slight beneficial effect for residents of the high UV region for some cancer forms.⁷⁸ Breast cancer prognosis was best for women diagnosed during the summer season. All these retrospective studies have diagnosis-related factors such as chemotherapy or lack of sunlight after prolonged stays that might have contributed to low vitamin D levels among breast cancer patients.

FINAL REMARKS

Although accumulated evidence regarding the association between different vitamin D metabolite end points and breast cancer risk is inconclusive, it seems plausible that mammary cells are under vitamin D regulation. Results from different studies do not always correlate due to many factors, including population heterogeneity, difficulties in determining the precise and continuous vitamin D status, lack of vitamin D determination at young ages and many years before breast cancer is expressed, other lifestyle variables that influence breast cancer risk, used end points, and so on. It seems that there is not a simple explanation on how vitamin D participates in breast cancer development and progression, although it is plausible that low levels have deleterious effects on mammary cells.

A 25(OH)D level of less than 30 ng/mL expresses a suboptimal vitamin D status capable of suppressing PTH hormone secretion.^{8,9,79,80} When low vitamin D levels are reduced for a long period, it seems likely that mammary cell physiology could be altered including 1,25(OH)₂D local synthesis, and the regulation of VDR, growth factors, and other hormones. The active hormone 1,25(OH)₂D regulates calcium transport during lactation, maintenance of mammary cell differentiation, and milk protein production along with other hormones.^{81,82} In addition, 1,25(OH)₂D inhibits carcinogen-induced pre-neoplastic lesions,⁸³ and vitamin D analogs inhibit cell proliferation in a number of breast cancer cell lines expressing positive ER+ as well as ER- ones, although the antiproliferative effects seem to be less consistent in ER- tumors.^{84,85} These results suggest an association between estrogens and vitamin D and the breast carcinogenesis process. In vitro studies have suggested that vitamin D exerts anticarcinogenic effects on breast cancer cells expressing high levels of insulin growth factor I (IGF-I) and IGF binding protein 3.⁸⁶⁻⁸⁸ Because serum levels of IGF-I and/or IGF binding protein 3 decline with increasing age, the interaction between IGF pathways and

vitamin D in breast cancer would be more important for premenopausal women than postmenopausal women. Therefore, maintaining adequate vitamin D levels at younger ages would be more effective in preventing breast cancer.

The anticancer effect of vitamin D has been explained by the presence of 1 α -hydroxylase in a number of cancer types which converts 25(OH)D to the active hormone 1,25(OH)₂D which has strong antiproliferative and differentiating effects on various cell types including breast cancer cells.^{89,90} Thus, low intracellular 1,25(OH)₂D might increase the risk of cancer transformation. The antiproliferative actions of 1,25(OH)₂D have been related to biochemical changes in cell cycle regulators, by counteracting the growth-promoting effects of estrogens and induction of apoptosis in breast cancer cells.^{91,92} Furthermore, 1,25(OH)₂D has been shown to inhibit the proliferation and metastasis of breast cancer.⁹³

Accepted ways to decrease woman's risk of breast cancer include avoidance of weight gain and hormone treatments, moderation in alcohol consumption if any, regular physical activity, and a healthy and balanced diet. Adequate continuous high 25(OH)D levels, either by moderate sun exposure or by high vitamin D intake, might also be a protective measure against breast cancer as well as to maintain bone health and prevent other chronic low vitamin D level-related conditions. A 15-minute sun bath around noon may produce 20 000 IUs in whites. Darker skinned individuals require a longer exposure to produce the equal amount. Individuals who live in areas with high air pollution and those at higher latitudes—where sun ray angle is not sufficient to produce adequate amounts of vitamin D at the skin—have significant risks of exhibiting low vitamin D levels. Seasonal variations and latitudinal gradients of 25(OH)D have been reported, and skin's ability to produce vitamin D decreases with age. Thus, the individual response to sun exposure is variable in terms of vitamin D circulating levels. In individuals reluctant to expose skin to sunlight or those living at latitudes far from the equator, supplementation with 1000 IU of vitamin D per day would normalize endogenous vitamin D and improve general health. This dose is devoid of adverse health effects.^{80,94,95}

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