

Vitamin D and Breast Cancer Risk

Introduction

Human beings are photosynthetic organisms. In the presence of sunlight, a form of cholesterol stored in the skin is transformed into vitamin D. More specifically, the energy from solar ultraviolet B radiation converts 7-dehydrocholesterol into vitamin D₃ (cholecalciferol), an inert form of vitamin D that becomes biologically activated through a two-step metabolic process involving both the liver and the kidney (Figure 1). From here, fully functional 1,25-dihydroxyvitamin D travels through the blood on carrier proteins to various target tissues. Most famously, vitamin D stimulates the expression of proteins involved in transporting calcium and phosphorus across the gut wall and thus facilitates the mineralization of bone and electrochemical signaling.^{1,2} But many tissues in the body – including lymphocytes and the breast – also display vitamin D receptors or synthesize 1,25-dihydroxyvitamin D outright from its inactive precursor form.³ As such, vitamin D is not actually a vitamin at all (that is, an essential micronutrient that catalyzes chemical reactions) but is more properly regarded as a hormone (that is, a chemical messenger involved in signaling and regulatory pathways). Through mediation by nuclear hormone receptors, the misnamed hormone called vitamin D acts as a transcription factor that alters the expression of many genes.³ In so doing, it modulates inflammatory responses and participates in regulating cell growth and differentiation. In the breast, vitamin D exerts anti-proliferative, pro-differentiating, and apoptotic effects.²

In the last 10 to 15 years, vitamin D has become the subject of growing interest as an environmental factor that may be associated with the reduction of risk of a spectrum of cancers, including breast cancer.⁴⁻⁶ In 1990, an ecological study conducted by Garland and coworkers reported an inverse correlation between U.S. breast cancer mortality rates and exposure to solar radiation and hypothesized that vitamin D produced by sunlight exposure might have more than a correlative role in the regional differences in breast cancer mortality.⁷ Since then, a substantial body of experimental evidence has accumulated that 1,25-dihydroxyvitamin D has anti-cancer effects both *in vitro* and *in vivo*. Human epidemiologic studies are conflicting but mostly provide support for cell culture and animal studies. All together, prospective and retrospective studies suggest that vitamin D deficiency is associated with a 30–50 percent increase in breast cancer risk and with poorer survival among those so diagnosed.⁸ These studies also suggest that the apparent cancer-preventive effect of vitamin D can involve various pathways, including calcium-dependent processes. In addition, there is much ongoing interest in the possibility that vitamin D could be used as a treatment for cancer.⁹

This chapter focuses narrowly on the influence of vitamin D on breast cancer risk and mortality. It does not review the evidence for its effects on the development of other cancers – including colon, prostate, and lung – where it is also apparently involved in risk reduction¹⁰ – or on other disorders, such as cardiovascular disease and multiple sclerosis (see Holick⁸ for a review). Nor does it comment on the ongoing controversy regarding the opposing public health goals of sun

protection to prevent skin cancer and sun exposure to promote vitamin D₃ synthesis. (See Gilcrest,¹¹ who argues that the controversy is being fueled, in part, by the indoor tanning industry. See also chapter I.C., which examines compounds in personal care products, including sunscreens, as a source of exposure to endocrine-disrupting chemicals.)

Perhaps more than any other single factor, vitamin D has profound connections with all three sectors of this report. As a photosynthetically produced hormone, vitamin D is deeply involved with physical environment. Air pollution, for example, is known to interfere with sunlight-induced vitamin D synthesis. Vitamin D synthesis is also influenced by season, latitude, altitude, time of day, and cloud cover.¹²

Because the skin pigment melanin competes with 7-dehydrocholesterol for solar UV B radiation, darker-skinned people require more time in the sun to generate adequate stores of vitamin D than do lighter-skinned individuals. Thus, vitamin D may potentially be involved with racial and ethnic disparities in breast cancer progression and survival. African American women, for example, are ten times more likely than white women to suffer from hypovitaminosis D, according to data collected as part of the National Health and Nutrition Examination Survey.¹³ Individuals confined to nursing homes are often sunlight and vitamin D-deprived, raising questions about vitamin D's role in breast cancer among disabled women who are homebound or confined to institutions.

Because, the structures of workplaces and neighborhoods mediate sunlight exposure, vitamin

D is also a dimension of the built environment. Indeed, vitamin D was first identified – and misclassified as a vitamin – during attempts to prevent and cure the scourge of rickets among urban children in 19th century Europe. The rise of rickets – a bone-deforming disease that is the result of acute vitamin D deficiency – corresponded to a dramatic change in the built environment that was ushered in with the advent of the Industrial Revolution when indoor factory work, tenement living, and smoggy air of industrial cities replaced outdoor farm life. As a consequence, sunlight exposure for a large sector of the European and British population markedly decreased.¹

Vitamin D's involvement with the physical environment, race, and the built environment make it an intriguing topic for California-based breast cancer research. With the longest latitudinal gradient of any state, areas of high air pollution, a diversity of built environments ranging from urban to rural, and a population containing many different skin colors, California is a good laboratory for a study of vitamin D's influence on breast cancer incidence and outcome.

Concept/Exposure Definition

During the race to find a cause for rickets, two independent discoveries were made: sunbathing could cure rickets in children, and cod-liver oil could cure rickets in dogs that had been confined indoors away from sunlight. By 1924, U.S. children began consuming milk and bread that had been irradiated with ultraviolet light, and the epidemic of rickets quickly dwindled.¹ Because of this unusual history, the term vitamin D is used in reference to two fat-soluble compounds,

cholecalciferol (vitamin D₃), which is manufactured by irradiated skin, and ergocalciferol (vitamin D₂), which is created during the irradiation of foods. Both were originally named and defined by their ability to both prevent and cure rickets. Both of these compounds require a two-step metabolism, as described above, for conversion to their active form, 1,25(OH)₂ D.

Sources of Exposure

(The following sections are summarized from several comprehensive reviews on various aspects of vitamin D and health¹⁴⁻²².)

There are four contemporary sources of vitamin D. The leading source by far is sunlight exposure. Another source is dietary and includes a limited number of foods contain naturally occurring vitamin D₃. These are egg yolk, liver, and oily

saltwater fish such as herring, salmon, and sardines. Certain mushrooms (for example, shitake) are a natural source of vitamin D₂. A third source is foods that are naturally low in vitamin D but which have been fortified with synthetic vitamin D₃ or D₂. These include liquid milk (but not cheese or ice cream, which are not fortified) and certain brands of cereals, bread, orange juice, and margarine. Last are supplements in the form of multivitamins containing vitamin D, vitamin D tablets, or cod liver oil. These may contain either vitamin D₃ or D₂. While excessive exposure to sunlight does not lead to overproduction of vitamin D₃, overdosing on vitamin D supplements can produce intoxication. Indeed, baits laced with vitamin D are sometimes used as rat poison.³

Whatever the source, vitamin D must undergo metabolic activation as shown in figure 1 and discussed below.

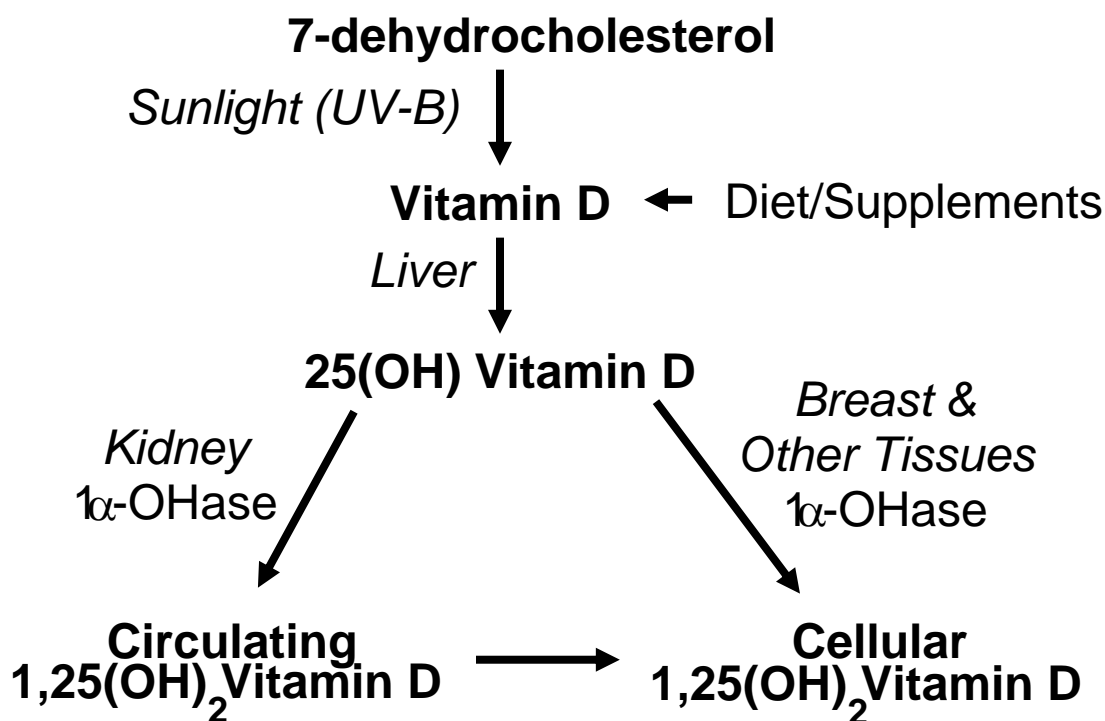


Figure 1. Synthesis and Metabolism of Vitamin D

Vitamin D from Sunlight

In the United States, sun exposure accounts for up to 90% of the circulating 1,25(OH)₂ D precursor, 25-dihydroxyvitamin D [25(OH)D, calcidiol] levels.

Cutaneous vitamin D production is modified by both *personal, social, and environmental* factors.¹⁸

Personal factors include constitutive skin pigmentation, age, body type, use of sunscreen, and wearing of protective clothing. As described above, people of different races diverge in the level of expression of melanin within melanocytes. Melanin contained within the melanocytes competes with 7-dehydrocholesterol for UV-B radiation. Humans differ in the levels of melanin expressed in epidermal melanocytes and higher levels of melanin in the epidermis decrease vitamin D production.

In fact, given an equal amount of sun exposure, light-pigmented individuals are 5–10 fold more efficient in converting 7-dehydrocholesterol to previtamin D.²³

Age is also an important variable. Levels of 7-dehydrocholesterol decrease linearly with age such that the epidermis of an 80-year-old has levels that are about half that of a 20-year-old. Thus, at any given sun exposure, a college student can produce twice the vitamin D₃ as her grandmother can.

Body type may also play an important role in the bioavailability of vitamin D. Vitamin D₃ is fat-soluble compound that is stored in body fat compartments. In this way, vitamin D produced

during the summer months can be used in the winter when ambient light may be insufficient to create more. Accordingly, individuals with different BMI values may differ both in their bioavailability and reserves of vitamin D.

Sunscreens are very effective inhibitors of cutaneous vitamin D photosynthesis. A sun protection factor of 15 effectively halts vitamin D production.

Environmental factors also influence cutaneous vitamin D₃ photosynthesis. These factors include geographic latitude, altitude, season, time of day, cloud cover, and air pollution. The solar zenith angle – which varies with season, latitude and time of day – is a major determinant of the amount of solar radiation that reaches the earth's surface. The more oblique the sun's zenith angle is in the winter, the fewer UV-B photons per unit area strike the earth's surface. Consequently, at northern (or southern) latitudes, no vitamin D is produced by photosynthesis during the winter. The length of the season that is prohibitive of vitamin D production varies with latitude. For example, in Boston, which is situated at 42°N, vitamin D synthesis is not possible from November through February. California's northern border is also located at 42°N. Below 37°N, which is the latitude of San Francisco, winter UV-B irradiation is sufficient, and vitamin D₃ photosynthesis takes place year-round. Vitamin D production is also affected by changes in the angle of the sun throughout the day; the photosynthetic period is thus limited to the hours of 10 am to 3 pm. Complete cloud cover reduces UV irradiance by half, and complete shade reduces it by 60 percent. Industrial pollution increases shade and decreases

sun exposure and can even contribute to the development of rickets.²⁴

Vitamin D metabolism

Vitamin D made in the skin or absorbed from the diet or supplements does not have biologic activity and must undergo a two-step metabolic process to its biologically active form. The steps in this process are illustrated in Figure 1.

Whatever its source, vitamin D that enters the circulatory system is bound largely to vitamin D-binding protein. This protein also binds metabolically modified forms.

Upon circulatory delivery to the liver, vitamin D is hydroxylated at the 25 position by a cytochrome P450-like enzyme. This reaction produces 25(OH) vitamin D [25(OH)D or calcidiol], the major circulating vitamin D metabolite. The production of 25(OH)D is not tightly regulated and its serum levels reflect the degree of vitamin D photosynthesis in the skin and that absorbed from the diet. Because of its longer half-life, 25(OH)D is thought to provide the best estimate of vitamin D exposure.

25(OH)D is converted to the active hormone 1,25(OH)₂D by the enzyme 25-hydroxyvitamin D 1 α -hydroxylase (1 α -hydroxylase). This enzyme is also cytochrome P450-like and is localized in the kidneys, as well as other tissues including mammary tissue. The renal enzyme is best studied and has been found to have tight regulation. Parathyroid hormone and low level of serum phosphate have been found to induce the activity of this enzyme whereas calcium and the end product 1,25(OH)₂D repress its activity.

1 α -hydroxylase is expressed in both normal and malignant tissues including that of the breast. While the non-renal enzyme is thought to be identical to the renal enzyme, it has been found to be unaffected by the regulators of renal activity.

1,25(OH)₂D has both endocrine and paracrine activities. Following its formation in the kidney, it can function as an endocrine hormone to act as a positive regulator of calcium (and phosphate) homeostasis. 1,25(OH)₂D accomplishes this in three ways: a) by increasing calcium and phosphate absorption in the small intestine; b) by interacting with parathyroid hormone to enhance calcium and phosphate mobilization from bone; and c) by decreasing renal excretion of these ions. At the molecular level these processes occur following the binding of 1,25(OH)₂D to the vitamin D receptor (VDR). The VDR is a member of the steroid and thyroid hormone receptor supergene family and is present in most tissues including normal and malignant breast tissue. The VDR bound to 1,25(OH)₂D acts by forming a heterodimer with the retinoid-X receptor and inducing transcription of target genes. 1,25(OH)₂D also has biologic effects which occur too rapidly to involve transcription induction and may possibly involve a recently identified membrane receptor the 1,25, D₃-membrane-associated rapid response, steroid-binding protein. The functioning of this protein is at this time not fully understood.²⁵

The affinity of the VDR for 1,25(OH)₂D is about 1000 times that of any of the other circulating vitamin D metabolites. Nonetheless, the total serum concentration of 25(OH) D is about 1000 times higher than 1,25(OH)₂D. Because of the

high total serum 25(OH)D concentrations, this compound could potentially compete for VDR binding and affect the biological activity of 1,25(OH)₂D. However, differential binding affinities of 25(OH)D and 1,25(OH)₂D to the vitamin D binding protein has a modulating effect such that the free concentration of 25(OH)D is only twice that of 1,25(OH)₂D. This effect eliminates any potential VDR binding competition between these two compounds. Knock-out mice of the vitamin D binding protein display mild dysfunction and are susceptible to vitamin D deficiency.²⁶

25(OH)D, the product of hepatic metabolism may also have paracrine activity. It is now known that 1 α -hydroxylase is expressed in many non-renal tissues, including breast cells^{27,28} and the delivery of this compound via the circulation may allow for synthesis of²⁸ 1,25(OH)₂D synthesis in the breast and other tissues containing this enzyme. However, this effect remains theoretical as formation of 1,25(OH)₂D has not been measured in any of these tissues.

Both 25(OH)D and 1,25(OH)₂D are hydroxylated at the 24 position by another cytochrome P450 enzyme 24-hydroxylase. It is considered a key enzyme in vitamin D catalysis. Dysregulation of 24-hydroxylase expression has been demonstrated to occur in breast cancer cell lines and breast tumors. This enzyme may play an important role in the functioning of vitamin D during the carcinogenesis process.

Daily vitamin D requirements

In 1997, the Institute of Medicine of the National Academy of Sciences made age-dependent daily

recommendations for vitamin D intake. These were: 1) 200 IU for children and adults up to age 50; 2) 400 IU for adults aged 50 to 70 years; and 3) 600 IU for adults older than 70 years. Based on clinical trials examining reduction of bone fractures, a group of prominent nutritionists have recently suggested that these recommendations are far too low. Potential for cancer risk reduction were also mentioned in this statement of concern. The body's daily needs for vitamin D have been estimated at an average of 3,000–5,000 IU.²¹ Such needs are clearly not being met. For example, typical multivitamins contain 400 IU of vitamin D (in the form of D₃ or D₂). Casual sun exposure (on face, arms and hands) of 5–15 minutes/day 2–3 times a week (depending on latitude, season, and skin pigmentation) supplies the equivalent of 1000 IU of vitamin D₃. Garland et al.²⁹ found that individuals with a serum level of 52 ng/ml of 25(OH)D had a 50 percent lower risk of breast cancer compared to individuals with less than 13 ng/ml. A serum level of 52 corresponds to an intake of 4000 IU/day.

Vitamin D Deficiency

Vitamin D deficiency has been reported in all races as well as in areas with potentially adequate solar exposure. This deficiency is especially prominent in the black population. A recent NHANES based study found that 42% of black women of reproductive age exhibited hypovitaminosis D. By contrast, only 4 percent of white women did.¹³ Hypovitaminosis D is also common in sunny countries in which cultural practices prescribe that women be heavily veiled in public.³

Biologic Plausibility

The biological activity of vitamin D and calcium support the idea that these entities may play a role in breast cancer etiology. *In vitro* and *in vivo* studies in normal and malignant breast cells have shown that 1,25(OH)₂D can inhibit proliferation, induce differentiation, stimulate apoptosis, and inhibit angiogenesis.^{9, 30-34} High intake of vitamin D has also been demonstrated to inhibit proliferation and tumor formation in a rodent model of mammary carcinogenesis.

As vitamin D plays an important role in the regulation of calcium, it is possible that any activity associated with vitamin D may involve calcium, which itself has anticancer activity. Like vitamin D, calcium is involved in the regulation of proliferation, differentiation and apoptosis in mammary cells *in vitro*. In addition, some studies suggest interplay between vitamin D and calcium. Studies have also demonstrated activity for calcium in rodent models of mammary carcinogenesis. At high levels of intake, calcium is able to inhibit carcinogenesis in two rodent models of mammary carcinogenesis, one involving tumor induction by 7,12-dimethylbenzanthracene and the other by a high-fat diet.

Review of the Epidemiologic Literature

The epidemiologic evidence on vitamin D and breast cancer is more limited than the *in vitro* and *in vivo* data but certainly suggests a protective role for vitamin D in the pathogenesis of breast cancer, especially in halting progression. Low levels of vitamin D are associated with more advanced cancers.³⁵

A wide range of ecological studies has linked low levels of sunlight with high breast cancer rates.²⁹ However, until recently, it was not possible to estimate the dose-response relationship. A pooled analysis conducted in 2007 to assess the association between vitamin D metabolite 25(OH)D in the serum and breast cancer risk found that individuals with the highest circulating vitamin D had a 50 percent lower risk of breast cancer than those with the lowest levels. Pooled odds ratios for breast cancer from lowest to highest quintile were 1.00, 0.90, 0.70, 0.70, 0.50.²⁹ Human studies show that vitamin D levels are higher in controls than in cases and decrease further in patients with bone metastases. Moreover, circulating vitamin D levels are lower in patients with advanced breast cancer than in those with early breast cancer.³⁵

The ecological studies that focus on latitudinal gradient find stronger associations with mortality than with incidence. Garland et al. noted in 1990⁷ that U.S. breast cancer mortality rates in white women were highest in the Northeast and lowest in the South, and based on correlation with solar radiation levels they proposed the hypothesis that vitamin D from sun exposure and diet may lower breast cancer mortality. A geographic gradient for breast cancer incidence has been evident for white women in all 5-year periods between 1950 and 1994,³⁶ and inverse correlations with solar radiation have been reported for both white and black women.^{37, 38} However, a recent analysis of more detailed mortality data showed that in white women aged 20–49 years, the breast cancer mortality gradient had disappeared by 1990–1999, largely due to a greater decline in mortality rates in the Northeast than in the South.³⁹ A similar

trend was seen in women aged ≥ 50 years: the Northeast/South gradient diminished over the last three decades as breast cancer mortality rates increased in the South.

Higher mortality rates may reflect exposure to factors (e.g., vitamin D deficiency) that increase tumor progression and metastasis, thereby shortening survival and increasing mortality. Recent epidemiologic data by John et al.⁴⁰ provide some support for this hypothesis.

In contrast to the results for breast cancer mortality, the results of studies examining breast cancer incidence over geographic gradients are not supportive of the importance of solar exposure in breast cancer etiology. SEER incidence data for breast cancer do not show a Northeast-South gradient,⁴¹ though a recent analysis of the incidence data for the year 1999 from additional high-quality cancer registries shows lower breast cancer incidence rates in the South than in the Northeast (cited in Sturgeon 2004³⁹). In the Nurses' Health Study, which utilizes a large, national U.S. cohort, incidence rates in both premenopausal and postmenopausal women were similar across four major U.S. regions, after adjustment for individual risk factors of the participants.⁴² In California, where latitude spans from 32–42°N, breast cancer incidence rates among participants of the California Teachers Study did not display a north-south gradient. Following adjustment for personal and ecologic risk factors, incidence rates were 33% higher in the urban areas of San Francisco and 29% higher in Los Angeles than in the remainder of the state.⁴³ These findings emphasize the importance of other factors in explaining the results of studies

examining regional differences in incidence rates. The true importance of sun exposure in these studies cannot be assessed, as it was not examined in this occupational cohort of school employees that works largely indoors.

Three ecological studies have examined the relationship of place of residence, with and without consideration of average sunlight exposure, and breast cancer risk. Residential solar radiation levels was inversely associated with breast cancer mortality in a nationwide death-certificate based case-control study.⁴⁴ A statistically significant reduction of 18% was reported but solar radiation levels were based on state averages and risk was only adjusted for age. A case-control study examined breast cancer risk in Marin County California, viewing this as a high incidence population. This study found increased risks of premenopausal breast cancer in women under age 50 years who were born in the Northeast (OR = 6.2; 95% CI = 1.0–7.9) or who had ever lived in the Northeast before age 21 years (OR = 6.2; 95% CI = 2.2–17.8).⁴⁵ Small and non-significant effects were reported for postmenopausal breast cancer risk. In addition, no correlation was made to sunlight exposure. A better-designed and more complete nationwide cohort study was conducted using NHANES data. Data on sunlight levels in the state of longest residence were combined with a physician-conducted sun-exposure evaluation.⁴⁶ A statistically non-significant but substantial (42%) decrease in breast cancer risk was reported for women with moderate-to-considerable sun exposure who lived in a state with high solar radiation.

Four studies have assessed breast cancer risk in relation to personal sun exposure histories. A Canadian population-based case-control study found reduced breast cancer risk associated with increasing sun exposure from ages 10–19.⁴⁷ The NHANES based study by John et al. discussed above reported moderate decreases in risk among women who self-reported frequent (vs. rare or never) recreational activity (OR = 0.66; 95% CI = 0.44–0.99) and occupational sun exposure (OR = 0.64; 95% CI = 0.41–0.98).⁴⁶ A second study utilizing national death certificates and sun exposure based job titles reported weak statistically non-significant decreases in the risk of breast cancer mortality.⁴⁴ Decreases were reported for women living in areas of high sunlight and working as farmers (OR = 0.90) or outside (OR = 0.75). In contrast, in the Nurses Health Study, the decrease in cancer risk associated with total or dietary vitamin D (discussed below) was not affected by adjustment for outdoor activity (or place of residence).⁴⁸

A novel measure of sun exposure based on skin pigmentation measurements has been developed to serve as an index of past sun exposure.^{40, 49, 50} The index is calculated as the difference between skin color on the upper inner arm (a site with very little to no exposed to sunlight) and the central forehead (a site with high sunlight exposure). These studies, which are as yet unpublished, reported an inverse association between the sun exposure index and advanced breast cancer. However, no association was found with localized breast cancer.⁴⁰

Serum measures of vitamin D concentration have the distinct advantage that they provide direct measures of exposure and the disadvantage that

they may represent exposures over a relatively short time period in the life span. Such studies have used two measures – that of the active metabolite of vitamin D, 1,25(OH)₂D and that of its precursor, 25(OH)D. Some scientists have justifiably argued that 25(OH)D is the more relevant measure for several reasons. First, the circulating concentration of 1,25(OH)₂D provides a poor measure of vitamin D status as its plasma concentrations are tightly regulated and change only in cases of extreme deficiency. Second, 25(OH)D levels provide a measure of the availability of vitamin D from diet, supplements, and skin photosynthesis and are accordingly more valid. In addition, they may also be a more relevant measure for breast cancer risk assessment since 25(OH)D may be converted to its active metabolite, 1,25(OH)₂D, by 1 α -hydroxylase in breast tissue.

Three studies assessed breast cancer risk in relation to circulating 25(OH)D. A hospital-based study found no differences in cases and controls.⁵¹ In contrast, a case-control study nested within the Nurses Health study reported an inverse relationship between high 25(OH)D levels and breast cancer risk (OR = 0.73 for highest compared with lowest quintile).⁵² The results were suggestive, but not statistically significant (P trend = 0.06). A hospital based case-control study in Britain found a strong statistically significant association with breast cancer risk (OR = 5.83) for women with low compared with high circulating levels of 25(OH)D.⁵³

Three studies have assessed serum levels of 1,25(OH)₂D for an association with breast cancer risk. The first of these studies is a small nested

case-control study which reported no association between pre-diagnostic 1,25(OH)₂D levels and postmenopausal breast cancer risk.⁵⁴ A second small hospital-based case-control study reported a strong statistically significant association (OR = 5.3) comparing low and high 1,25(OH)₂D levels.⁵¹ The third study was a large case-control study nested within the Nurses Health Study. This study reported a modest (OR = 0.76), statistically insignificant association between high versus low 1,25(OH)₂D levels and breast cancer risk.^{51, 52, 54}

A number of studies have examined dietary vitamin D intake and breast cancer risk. Interpretation of these studies relative to vitamin D is complicated as vitamin D and calcium intake are highly correlated. This is especially important as they have similar activities against breast cancer cells, share regulatory pathways, and may act in conjunction with each other. According to a prospective investigation of 30,000 women in the Women's Health Study, higher dietary intakes of calcium and vitamin D were associated with lower breast cancer risk among premenopausal, but not postmenopausal, women. Moreover, premenopausal women with low calcium and vitamin D intake were more likely to have large and poorly differentiated tumors.⁵⁵

Six other studies have also explored the association of dietary vitamin D intake and breast cancer risk.³⁴ Consistent with the Women's Health Study, a statistically significant reduction in breast cancer risk was found in the large cohort Nurses' Health Study for premenopausal women (RR = 0.72) for women whose total daily intake was more than 500 IU compared to women whose intake was less than 150 IU [Shin 2002]. Dietary

vitamin D intake was associated with a similar but marginally statistically significant risk reduction. Further adjustment for residential area and history of sunlight exposure did not change these relationships. As in the Lin study, this investigation found no association with breast cancer risk for postmenopausal women.⁴⁸

Similarly, a national U.S. cohort of 68,567 women found no association between total and dietary vitamin D and breast cancer risk among postmenopausal women as a whole.⁵⁶ However when estrogen-receptor positive (ER+) and negative (ER-) cases were examined separately, a weak, statistically significant reduction in risk was found for postmenopausal women with ER+ tumors (RR = 0.74). The comparison groups in this examination were women with dietary intakes greater than 300 IU/day relative to women with intakes less than 100 IU/day. There was also a significant dose-response relationship.

Cellular studies support such an ER-related effect. Experimental studies, using mammary cell lines, have reported that ER+ cell lines are more sensitive to 1,25(OH)₂D than ER- cell lines.³¹ Surprisingly, examination of total (rather than merely dietary) vitamin D intake, was not associated with risk. These authors also reported a potentially important modifying effect of UV exposure for women from cloudy localities. Among women from states with little sunlight, vitamin D intake had a significant effect on breast cancer risk. Risk was statistically significantly reduced (RR = 0.81) for daily intakes of > 300 IU/day vs. ≤ 100 IU/day. On the other hand, for women from states with higher sunlight exposure levels, vitamin D intake (> 300 IU/day vs. ≤ 100

IU/day) was not associated with breast cancer risk (RR = 1.05).

Three other small, case-control studies have examined women of all ages and reported no association between dietary vitamin D and breast cancer risk.⁵⁷⁻⁵⁹ No statistically significant association with vitamin D intake was reported by an NHANES based cohort study.⁴⁶

Animal studies and some human epidemiologic studies have suggested that adolescence may be a critical period affecting future breast cancer risk. Frazer and coworkers have conducted two studies that retrospectively examined vitamin D intake during adolescence. In both studies they found no association with breast cancer risk.^{60, 61}

Preliminary data (published abstract) from the Women Health Initiative (WHI), a randomized clinical trial, suggest that daily intake of 400 IU of vitamin D₃ and 1000 mg of elemental calcium did not reduce the incidence of invasive breast cancer among postmenopausal women after 7 years of follow-up (HR = 0.96, 95% CI = 0.85–1.09).⁶² However, in the placebo group, total vitamin D intake at baseline was associated with reduced risk.

The studies conducted to date do not provide a completely clear picture on the effect of dietary and supplemental vitamin D on breast cancer risk. However, examination of the results as a whole may add some potentially encouraging clarity. Beneficial effects have been reported for some subgroups of the women studied, including premenopausal women, postmenopausal women with ER+ tumors, and women living in sunless areas. Accordingly, inclusion of these three

subgroups along with others in the analyses could potentially obscure the results.

The finding of reduced risk in regions of low UV is consistent with lower circulating levels of 25(OH)D due to lack of vitamin D synthesis during the winter months and higher prevalence of vitamin D insufficiency/deficiency. Thus, high dietary vitamin D intake is a more important contributor to circulating levels of 25(OH)D in individuals who live in low-UV regions without year-round vitamin D synthesis, than in individuals who live in high-UV regions. Thus, the effect of dietary vitamin D on breast cancer risk may be masked in high-UV regions. An alternative interpretation is that a daily intake of 400 IU may be too small to influence breast cancer risk. Among fair-skinned Caucasians, casual sun exposure for 10–15 minutes corresponds to an oral intake of 1000 IU. A serum 25(OH)D level of 52 ng/mL, which corresponds to oral intake of 4000 IU/day, has been associated with a 50% reduction in breast cancer risk.²⁹ Thus, oral intake much higher than 400 IU may be necessary to reduce breast cancer risk.

A number of studies have assessed associations of common polymorphisms in several regions of the vitamin D receptor (VDR) gene and breast cancer risk.^{34, 63} The most commonly studied SNPs include a polymorphic site in exon 2 near the 5' end of the gene that is identified by *FokI* restriction enzyme, which has two potential translation initiation sites and results in VDR proteins that differ in length by three amino acids and differ in transcriptional activity. At the 3' end of the gene, two polymorphic sites in intron 8 are identified with *BsmI* and *ApaI* restriction enzymes,

and a third in exon 9 is identified by *TaqI* restriction enzyme. The *BsmI*, *Apa*, *I* and *TaqI* SNPs are not functional, but are strongly linked with the *poly(A)* microsatellite repeat located in the 3' untranslated region (UTR) which may influence VDR mRNA stability.

Studies of associations between breast cancer risk and VDR variants, while inconsistent, are largely null. Three relatively large case-control studies in white women from the US,⁶⁴ the UK,⁶⁵ and Sweden⁶⁶ found no association with *TaqI*. Other smaller studies found no association in white, Hispanic and African-American women from California⁴⁰ or increased risks in relation to the *TaqI* T allele in women from Australia,⁶⁷ China⁶⁸ or Taiwan.⁶⁹ Several studies found no association with *FokI*.^{40, 64, 65, 70} In the largest study conducted to date, *FokI* ff (vs FF) was associated with a significantly increased risk (OR = 1.34, 95% CI = 1.06–1.69),⁷¹ as previously reported for Hispanics.⁷² Most of these studies had design limitations such as small size or limited adjustment for confounding factors. More study will be required to clarify this potentially complex area of research.

Since any potential effect of the VDR protein on cancer development is dependent on the availability of its transactivation ligand, 1,25(OH)₂D, it may be critical to consider the association between VDR variants and breast cancer in the context of substrate availability. A nested case-control study within the Nurses Health Study reported no effect of serum levels of 25(OH)D or 1,25(OH)₂D on the association of the *FokI* genotype with breast cancer risk.⁷¹ In agreement with these results, an unpublished case-

control study found that the inverse association of the sun exposure index with breast cancer risk was not modified by VDR genotype, though stratification produced relatively small groups for analysis.⁴⁰ In contrast, a small, British case-control study reported a strong, statistically significant increase in breast cancer risk (OR = 6.82) associated with the *BsmI* bb VDR genotype and low levels of 25(OH)D,⁵³ It should be noted that there was also a statistically significant trend for this relationship but that the analysis was limited as there was only adjustment for age and menopausal status.

Breast density – the relative proportion of glandular tissue to fat tissue in the breast – is also affected by vitamin D. High breast density (lots of ductal tissue and little fat) is strongly related to breast cancer risk and has been proposed as an intermediate marker in breast cancer prevention efforts. Emerging evidence shows that vitamin D is associated with lower breast density, especially among premenopausal women,^{73, 74} perhaps reflecting vitamin D's antiproliferative effects, as seen in cell culture experiments. Moreover, a Canadian study has found that breast density changes seasonally, reaching a peak in early spring when circulating blood levels of vitamin D are lowest. Changes in blood vitamin D were inversely related to breast density and were seasonally synchronized.⁷⁵

As 1,25(OH)₂D has been shown to decrease proliferation, increase differentiation and inhibit metastasis, it is plausible that various aspects of vitamin D availability and functioning and availability might play a role in survival from breast cancer. As early as 1989, Colston and

coworkers reported significantly longer survival for breast cancer patients with *VDR* positive tumors.⁷⁶ Two studies of women from Norway have also supported this idea. Both studies examined how seasonal variation in UV radiation at the time of diagnosis may affect survival from breast and other cancers. Norway is well suited to such a study as it has a spread in latitude of more than 10 degrees (as does California); long summer days and long winter nights provide considerable seasonal variation. In Norwegian women, survival was highest when the season of diagnosis occurred during the summer or fall months when vitamin D photosynthesis was potentially highest.⁷⁷ The second study also found a summer effect. Recently, Lim and coworkers⁷⁸ published similar findings for breast cancer mortality rates among women in the United Kingdom.

Very few studies have examined diet after breast cancer diagnosis and survival. Holmes and coworkers conducted such a study of the Nurses' Health Study Cohort.⁷⁹ This study reported a 27% decrease in breast cancer risk for women with the highest dietary vitamin D intake (not including supplements) relative to those with the lowest levels of intake. This decrease bordered on statistical significance, and there was a statistically significant trend across the quantiles of intake (P value, trend = 0.05). A decrease in risk was also reported when supplements were included in intake values (RR = 0.86) but these results were not statistically significant.

Demonstration of anti-proliferative effects of 1,25(OH)₂D has led to the active investigation of 1,25(OH)₂D as an agent in cancer therapy.^{4, 30} Administration of 1,25(OH)₂D can lead to life-

threatening hypercalcemia. This effect is the major challenge in the use of 1,25(OH)₂D chemotherapeutically and in the design synthetic vitamin D analogues. Hundreds of analogues have been developed, many have been assessed for their effects on breast cancer cell growth versus their hypercalcemic activity. Some of these analogs are currently being tested in clinical trials. Vitamin D analogues may have potential as cancer chemopreventive agents but this remains an unexplored area.

Discussion and Future Directions

All together, the experimental and human studies provide evidence for a protective effect of vitamin D on the breast. In vivo and in vitro studies show clear anticarcinogenic effects that manifest as a result of vitamin D's hormonal regulation of cell proliferation, differentiation, and apoptosis in both normal and malignant breast cells.³⁴ In some, but not all, human studies, sunlight exposure and serum levels of vitamin D are inversely related to breast cancer risk among subgroups of women. The benefits of vitamin D for the breast appear strongest for premenopausal women. Possibly because of its anti-proliferative effects, vitamin D is also inversely associated with breast density, which is itself a risk factor for breast cancer. The finding that 4 of every 10 young African American women exhibit hypovitaminosis D – a rate 10-fold that of their white counterparts – raises public health concerns, especially in light of the fact that low vitamin D levels are associated with more advanced breast cancers.

Outstanding questions include:

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- 1) What are the racial/ethnic differences in sunlight exposure and circulating vitamin D levels among women in California?
- 2) Can differences in blood serum levels of vitamin D explain racial disparities in breast cancer progression, mortality or aggressiveness? Palmieri³⁵ notes that darker skin pigmentation has been associated with larger-sized tumors and increased frequency of lymph node involvement. Are racial differences in vitamin D status – and perhaps access to sunlight--playing a role here?
- 3) How does air pollution interfere with UV B irradiance and thereby vitamin D photosynthesis among women in California?
- 4) Is timing of exposure important for the anti-carcinogenic benefits of vitamin D?
- 5) What aspects of the built environment – housing density, sidewalks, safety, playgrounds, community gardens, workplace policies, distance to shopping and schools – influence vitamin D levels among inhabitants and thereby the pathogenesis of breast cancer?
- 6) Recommendations for beneficial sunlight exposure presume light-colored skin. Among fair-skinned Caucasians, for example, casual sun exposure for 10–15 minutes is said to correspond to an oral intake of 1000 IU. What are the correspondences and recommendations for black, Hispanic, and Asian women? And how do these recommendations vary by latitude within California?
- 7) Vitamin D is fat-soluble. How does body mass index affect bioavailable vitamin D metabolites?
- 8) What are the vitamin D profiles for teachers, nurses, and other occupational groups known to have high rates of breast cancer, and how do these compare to the general population?

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