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Breast Cancer

Vitamin D and Breast Cancer

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ABSTRACT

In addition to its role in calcium homeostasis and bone health, vitamin D has also been reported to have anticancer activities against many cancer types, including breast cancer. The discovery that breast epithelial cells possess the same enzymatic system as the kidney, allowing local manufacture of active vitamin D from circulating precursors, makes the effect of vitamin D in breast cancer biologically plausible. Preclinical and ecologic studies have suggested a role for vitamin D in breast cancer prevention. Inverse associations have also been shown between serum 25-hydroxyvitamin D level (25(OH)D) and breast cancer development, risk for breast cancer recurrence, and mortality in women with early-stage breast cancer. Clinical trials of vitamin D supplementation, however, have yielded inconsistent results. Regardless of whether or not vitamin D helps prevent breast cancer or its recurrence, vitamin D deficiency in the U.S. population is very common, and the adverse impact on bone health, a particular concern for breast cancer survivors, makes it important to understand vitamin D physiology and to recognize and treat vitamin D deficiency. In this review, we discuss vitamin D metabolism and its mechanism of action. We summarize the current evidence of the relationship between vitamin D and breast cancer, highlight ongoing research in this area, and discuss optimal dosing of vitamin D for breast cancer prevention.
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INTRODUCTION

Vitamin D is critical for bone health, and sufficient levels can reduce the risk for hip fracture in women. There also is increasing evidence that vitamin D has effects on other body systems, and that adequate supplies of vitamin D are required for optimal health. In 1990, Garland et al. [1] first reported an inverse association between total average annual sunlight energy that strikes the ground and age-adjusted breast cancer mortality in the U.S. The most probable mechanism linking sunlight exposure to a lower risk for breast cancer is increased photosynthesis of vitamin D. Subsequently, inverse associations have been suggested between serum 25-hydroxyvitamin D level (25(OH)D) and breast cancer development, risk for breast cancer recurrence, and mortality in women with early-stage breast cancer. This review summarizes vitamin D metabolism and its mechanism of action, the current evidence on the relationship between vitamin D and breast cancer, and the optimal dosing of vitamin D for breast cancer prevention.

METHODS

A literature search was conducted to identify studies assessing the association between vitamin D and breast cancer risk. We searched MEDLINE, PubMed database, and the American Society of Clinical Oncology and San Antonio Breast cancer Symposium proceedings prior to February 28, 2011 for relevant reports. Search terms included "vitamin D," "25-hydroxyvitamin D," "1,25-dihydroxyvitamin D," and "breast cancer." Titles and abstracts were reviewed for relevance. Cross-referencing was used to identify any missing studies in the database search.

VITAMIN D METABOLISM AND MECHANISM OF ACTION

Vitamin D is obtained from both dietary sources and exposure to sunlight. Dietary sources include oily fish such as salmon, eggs, and fortified dairy products. The two naturally occurring forms of vitamin D are cholecalciferol (vi-

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tamin D_3) from animal sources and ergocalciferol (vitamin D_2) from plant sources. However, most vitamin D in circulation is produced naturally when 7-dehydrocholesterol in the skin is exposed to ultraviolet B (UVB) radiation to produce vitamin D_3 [2].

Vitamin D is first converted to 25(OH)D, the major circulating metabolite, by 25-hydroxylases in the liver [2]. 25(OH)D then undergoes a second hydroxylation in the kidney into 1,25 dihydroxyvitamin D ($1,25(OH)_2D$), by 1-α-hydroxylase (CYP27B) (Fig. 1) [2]. $1,25(OH)_2D$, also known as calcitriol, is the biologically active form of vitamin D and exerts its action by binding to an intracellular receptor, the vitamin D receptor (VDR) [3]. VDR, first identified in a breast cancer cell line in 1979, belongs to the superfamily of nuclear receptors for steroid hormones and regulates gene expression by acting as a ligand-activated transcription factor [3]. In addition to its main function of maintaining extracellular calcium levels, the activation of VDR influences up to 200 genes that mediate cellular growth, differentiation, and apoptosis [4].

The best indicator of total body vitamin D stores is 25(OH)D because its half-life is far greater than that of vitamin D or $1,25(OH)_2D$ [5]. The serum 25(OH)D concentration is determined mainly by sunlight exposure, because most foods have little vitamin D. In the absence of adequate sun exposure, vitamin D deficiency may occur rapidly. Risk factors for vitamin D deficiency include obesity, low dietary intake, dark skin pigmentation, lack of sun exposure, and older age [6, 7].

Vitamin D and Breast Cancer—Preclinical Studies

Several extrarenal tissues in the body, including the breast, contain the 1-α-hydroxylase enzyme required for generation of the active vitamin D metabolite, $1,25(OH)_2D$, from circulating 25(OH)D [8]. The concentration of circulating 25(OH)D appears to be the key factor regulating tissue-specific synthesis of the active form of vitamin D [8, 9]. The locally synthesized $1,25(OH)_2D$ can bind to VDRs present in the breast epithelium to regulate the expression of many genes [9]. In addition, breast cells also contain 24-hydroxylase enzyme (CYP24), which converts $1,25(OH)_2D$ into less active metabolites such as 24,25-dihydrohydroxyvitamin D_3 and 1,24,25-trihydroxyvitamin D_3 [9, 10]. Therefore, breast cells contain all the components of a vitamin D signaling axis that coordinates the local synthesis and metabolism of $1,25(OH)_2D$ and its signal transduction via VDRs.

Many studies have examined the effects of vitamin D on mammary carcinogenesis in vitro and in animal models, and the data support a protective role for vitamin D in breast cancer development [11–20]. In addition, mice rendered vitamin D deficient exhibit enhanced cancer development [21], as do *VDR* knockout mice [22]. Several mechanisms underlying the inhibitory effects of vitamin D on the growth of breast cancer cells have been proposed.

Growth Arrest and Apoptosis

$1,25(OH)_2D$ has been shown to induce cell-cycle arrest by increasing the expression of cyclin-dependent kinase inhibitors such as p21 and p27 in MCF-7 breast cancer cell lines [11, 12]. The active vitamin D metabolite also can regulate the expression of oncogenes such as c-*myc* and c-*fos* and the actions of several growth factors, including epidermal growth factor, transforming growth factor, and insulin-like growth factor (IGF)-1 [13].

$1,25(OH)_2D$ also can induce morphological changes associated with apoptosis in breast cancer cells [14]. These changes could be related to regulation of the Bcl-2 family of genes that leads to a relatively lower expression level of antiapoptotic proteins such as Bcl-2 and Bcl-XL versus proapoptotic proteins such as Bax and Bak [13, 23].

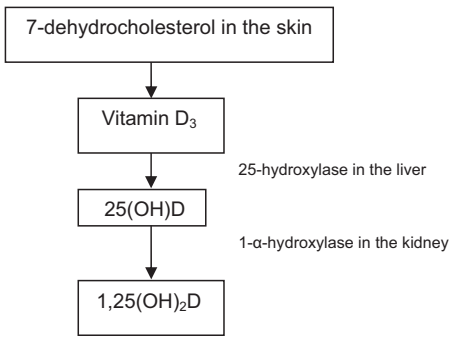

Figure 1. Vitamin D metabolism.

Inhibition of Invasion and Metastasis

Vitamin D is critical for bone health. Vitamin D deficiency results in elevated parathyroid hormone (PTH) secretion, which stimulates the osteoblastic PTH receptor to increase expression of receptor activator of nuclear factor κB ligand, a potent activator of osteoclast recruitment and bone resorption [24]. Vitamin D deficiency has been shown to promote the growth of human breast cancer cells in the bones of nude mice, suggesting that vitamin D might promote cancer growth by altering the bone microenvironment [15].

In some breast cancer cell lines, $1,25(OH)_2D$ increases the expression of E-cadherin, which prevents invasion and metastasis [16]. In addition, $1,25(OH)_2D$ has potent antiangiogenic activity that likely contributes to its inhibition of tumor invasion [13]. $1,25(OH)_2D$ also has been shown to decrease the activity of matrix metalloproteinases (MMPs), urokinase-type plasminogen activator, and tissue-type plasminogen activator and to increase the expression of plasminogen activator inhibitor and MMP inhibitor 1, which are all important mediators of invasion and metastasis [17].

Anti-Inflammation

$1,25(OH)_2D$ has been shown to downregulate the expression of cyclooxygenase-2 (COX-2), which plays a critical role in prostaglandin synthesis in several human breast cancer cell lines [18]. It also increases the expression of 15-hydroxyprostaglandin dehydrogenase, which catalyzes the conversion of prostaglandins to biologically inactive ketoderivatives [18]. Prostaglandins have been suggested to play a role in the development and progression of breast cancer [25]. Prostaglandins

released from breast cancer cells or surrounding tissues stimulate tumor progression by promoting cell proliferation and resistance to apoptosis and stimulating tumor cell invasion and angiogenesis [25]. A high expression level of COX-2 in breast cancer has been shown to correlate with high grade, large tumor size, and poor prognosis [26].

Estrogen Pathway Inhibition

Several studies have suggested that $1,25(OH)_2D$ can inhibit both the synthesis and the biological actions of estrogens [18–20]. $1,25(OH)_2D$ suppresses the estrogen pathway by reducing the expression of the gene coding aromatase, the enzyme that converts androgens to estrogens [18]. $1,25(OH)_2D$ also downregulates estrogen receptor (ER)-α, the nuclear receptor that mediates the actions of estrogen [19, 20]. The combined actions of $1,25(OH)_2D$ can decrease the levels of estrogens and the receptor that mediates their signaling.

Vitamin D and Breast Cancer—Epidemiological Studies

Sunlight Exposure

Early epidemiologic studies of breast cancer and vitamin D have shown strong inverse associations between sunlight exposure and breast cancer incidence and mortality [1, 27, 28]. In particular, a study by Garland et al. [1] of 87 U.S. counties reported strong correlations between lower sunlight exposure and age-adjusted breast cancer mortality rates, with the highest rates in the Northeast, compared with the Southwest. The link between sunlight and breast cancer also has been reported in other countries [29]. In addition, several studies have shown a better prognosis for breast cancer patients following diagnosis or treatment initiation in the summer or fall [30–32]. This seasonal effect was hypothesized to be the result of greater vitamin D during a period of higher sunlight exposure at the time of diagnosis [30–32].

In a retrospective cohort study from the first National Health and Nutrition Examination Survey (NHANES), 5,009 white women completed in-person interviews and dermatological examinations to assess their vitamin D exposure. Women who self-reported frequent sun exposure at baseline had a 33% lower risk for breast cancer than those who reported never or rare sun exposure over 17 years of follow-up [33]. A lower breast cancer risk was also seen for women who lived in the U.S. regions of high solar radiation [33]. One of the limitations of that study is the healthy patient bias, whereby women who reported frequent sun exposure were likely to be more physically active and in better general health than those who had less sun exposure.

Vitamin D Intake

Six case–control studies have examined the relationship between vitamin D intake and breast cancer risk (Table 1) [34–39]. The largest was an Italian study that included 2,569 cases and 2,588 controls in which a 78-item food frequency questionnaire was used to collect information on dietary sources of vitamin D. Women with the highest vitamin D intake (>190 IU) had a 34% lower risk for breast cancer than those with the lowest vitamin D intake (<60 IU) [38]. The odds ratios (ORs) were 0.80 (95% confidence interval [CI], 0.64–0.99) and 0.78 (95% CI, 0.66–0.92) among pre- or perimenopausal and postmenopausal women, respectively [38]. The strengths of the study are the large dataset and the use of a reproducible and valid food frequency questionnaire [40]. The study results were adjusted for many known risk factors for breast cancer. Limitations of the study include the absence of information on sun exposure or serum levels of vitamin D and the use of hospital-based controls.

Two other case–control studies also reported a relatively lower breast cancer incidence with greater vitamin D intake [34, 35]. However, three studies did not show an association between vitamin D intake and breast cancer risk [36, 37, 39]. Inconsistencies among studies may be a result of differences in methods for selecting cases and controls, dietary intake data collection tools, and referent time periods.

Among the six cohort studies that addressed the relationship between breast cancer risk and vitamin D intake (Table 1) [33, 41–45], the largest is the Nurse's Health Study, which included 88,691 women [45]. Vitamin D intake was assessed by a 61-item food frequency questionnaire every 4 years, and high vitamin D intake was associated with a statistically significant lower risk for premenopausal breast cancer (OR, 0.72; 95% CI, 0.55–0.94) [45]. The inverse association was not observed for postmenopausal breast cancer. The strengths of the study are the prospective nature of the study design, the large dataset, the long duration of dietary intake assessment, and the ability to adjust for known breast cancer risk factors and other potential confounders, such as total fat and B-carotene intake. Limitations of the study again are the healthy patient effect and the absence of information on sun exposure or serum levels of vitamin D.

A similar finding was reported in the Women's Health Study cohort that included 10,578 premenopausal women and 20,909 postmenopausal women [42]. Higher intake of vitamin D was associated with a lower risk for breast cancer in premenopausal women (OR, 0.65; 95% CI, 0.42–1.00) but not in postmenopausal women (OR, 1.30; 95% CI, 0.97–1.13) [42]. Other studies that included predominantly postmenopausal women either showed a trend toward a lower breast cancer risk with higher vitamin D intake [44, 45] or did not show a protective effect of higher vitamin D intake for breast cancer [33]. Only one study reported a protective effect of high dietary vitamin D for postmenopausal breast cancer, but only in ER^+ tumors [43]. In summary, most of the cohort studies that were similar in design did observe a lower risk for breast cancer with higher vitamin D intake, particularly in premenopausal women.

A possible explanation for the observed difference by menopausal status may be related to the relationship between vitamin D and IGFs. In vitro studies have suggested that vitamin D can inhibit IGF-1–stimulated growth of breast cancer cells [46]. In addition, vitamin D has been shown to increase production of IGF-binding protein 3, a member of the IGF-binding protein family that regulates the mitogenic effects of

Table 1. Studies of vitamin D intake and breast cancer risk

Study	Description	Study patients	Data collection	Comparison (vitamin D intake)	OR (95% CI)
Abbas et al. (2007) [34]	Population-based case–control study in Germany, 1992–1995	278 premenopausal cases and 666 age-matched controls	176-item FFQ	≥200 IU/day versus <80 IU/day	0.5 (0.26–0.96)
Knight et al. (2007) [35]	Population-based case–control study in Canada, 2003–2004	972 cases and 1,135 controls	Telephone interview	Cod liver oil use versus none	0.76 (0.62–0.92)
				≥10 glasses of milk/week versus none	0.62 (0.45–0.86)
Levi et al. (2001) [36]	Hospital-based case–control study in Switzerland, 1993–1999	568 cases and 1,451 controls	79-item FFQ about diet in the previous 2 yrs	2.7 mg/day versus 1.4 mg/day	1.43 (0.90–2.26)
Potischman et al. (1999) [37]	Population-based case–control study in the U.S., 1990–1992	568 cases and 1,451 controls	100-item FFQ about diet in the past yr	≥400 IU/day versus none	0.98 (0.8–1.2)
Rossi et al. (2009) [38]	Hospital-based case–control study in Italy, 1991–1994	2,569 cases and 2,588 controls (987 premenopausal and 1,579 postmenopausal)	78-item FFQ related to diet in the previous 2 yrs	>190 IU/day versus <60 IU/day	0.76 (0.58–1.00)
Simard et al. (1991) [39]	Nested case–control study within the Canadian National Breast Screening Study, 1981–1983	108 breast cancer cases and 322 controls	24-hour dietary journal	>200 IU/day versus <50 IU/day	2.79 (0.85–9.14)
Frazier et al. (2004) [41]	Nurse Health Study II retrospective cohort, 1989–1998	47,355 women	131-item FFQ about diet during high school	>591 IU/day versus <159. 6 IU/day	0.92 (0.66–1.27)
John et al. (1999) [33]	NHANES I prospective cohort, 1974–1992	5,009 white women	In-person interview with 24-hr dietary recall	≥200 IU/day versus <100 IU/day	0.85 (0.59–1.24)
Lin et al. (2007) [42]	Women's Health Study prospective cohort, 1995–2004	10,578 premenopausal and 20,909 postmenopausal women	131-item FFQ at baseline	≥548 IU/day versus <162 IU/day	Premenopausal, 0.65 (0.42–1.00); postmenopausal, 1.30 (0.97–1.73)
McCullough et al. (2005) [43]	Cancer Prevention Study II Nutrition prospective cohort, 1992–2001	68,567 postmenopausal women	68-item FFQ at baseline	>700 IU/day versus ≤100 IU/day	0.95 (0.81–1.13)
Robien et al. (2007) [44]	Iowa Women's Health prospective cohort, 1986–2004	34,321 postmenopausal women	127-item FFQ at baseline	≥800 IU/day versus <400 IU/day	0.89 (0.77–1.03)
Shin et al. (2002) [45]	Nurses' Health Study prospective cohort, 1980–1996	88,691 (both pre- and postmenopausal women)	61-item FFQ, later expanded to 130-item FFQ	>500 IU/day versus ≤150 IU/day	Premenopausal, 0.72 (0.55–0.94); postmenopausal, 0.94 (0.80–1.10)

Abbreviations: CI, confidence interval; FFQ, food frequency questionnaire; NHANES, National Health and Nutrition Examination Survey; OR, odds ratio.

IGFs [47, 48]. Because circulating levels of IGF-1 and IGF-binding protein 3 decline with increasing age [49], the interactions between IGF pathways and vitamin D are likely to be stronger for premenopausal women than for postmenopausal women.

Serum 25(OH)D Level

Eight case–control studies have examined the relationship between serum levels of 25(OH)D and breast cancer risk (Table 2) [50–57]. Five of those studies showed a statistically significant lower risk and one reported a trend toward a lower breast

Table 2. Studies of serum 25(OH)D levels and breast cancer risk

Study	Description	*n* cases/controls	Comparison (ng/mL)	OR (95% CI)
Abbas et al. (2008) [50]	Population-based case–control study in Germany, 2002–2005	1,394/1,365 (postmenopausal women only)	≥30 versus <12	0.31 (0.24–0.42)
Abbas et al. (2009) [51]	Population-based case–control study in Germany, 1992–1995	285/595 (premenopausal women only)	≥24 versus <12	0.45 (0.29–0.70)
Bertone-Johnson (2007) [52]	Nested case–control study within the Nurses' Health Study cohort, 1989–1990	701/724	≥40 versus ≤20	0.73 (0.49–1.07)
Chlebowski et al. (2008) [53]	Nested case–control study within the Women's Health Initiative clinical trial, 1995–2005	1,067/1,067	≤13 versus ≥27	1.22 (0.89–1.67)
Crew et al. (2009) [54]	Population-based case–control study in New York, 1996–1997	1,026/1,075	≥40 versus ≤20	0.56 (0.41–0.78)
Engel et al. (2010) [55]	Nested case–control study within the French E3N cohort, 1993–1995	636/1,272	>27 versus <19.8	0.73 (0.55–0.96)
Freedman et al. (2008) [56]	Nested case–control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, 1993–2001	1,005/1,005 (postmenopausal women only)	≥33.7 versus <18.3	1.04 (0.75–1.45)
Lowe (2005) [57]	Hospital-based case–control study in the U.K., 1996–2003	179/179	<20 versus >60	5.83 (2.31–14.7)

Abbreviations: CI, confidence interval; OR, odds ratio.

cancer risk with higher serum levels of 25(OH)D [50–52, 54, 55, 57]. The pooled OR for the highest level of circulating 25(OH)D compared with the lowest quantile was 0.55 (95% CI, 0.38–0.80) [58].

Most of the studies included both pre- and postmenopausal women and did not differentiate by menopausal status in the analysis. An exception is the study by Crew et al. [54] in which serum 25(OH)D levels were obtained from 1,026 cases and 1,075 controls. In that study, women with 25(OH)D levels >40 ng/mL were less likely to develop breast cancer than women with levels <20 ng/mL (OR, 0.56; 95% CI, 0.41–0.78), but the inverse association was limited to postmenopausal women [54]. The strengths of the study are the population-based sampling of controls and the large sample size, which allowed stratification of data by potential modifiers. One of the limitations of the study is bias in subject selection because response rates were lower among controls than among cases, notably for women aged >75 years. Patients who consented to provide blood samples differed from those who did not in several factors such as ethnicity, alcohol use, and the use of hormone replacement therapy [59]. The higher breast cancer risk associated with vitamin D deficiency in postmenopausal women might seem contrary to the previously mentioned hypothesis of a stronger interaction between IGF pathways and vitamin D in premenopausal women. However, IGF-related hormones can be influenced by other factors, such as obesity, especially after menopause [60]. Therefore, measurement of body mass index (BMI), circulating levels of IGF-1 and IGF-binding protein 3, and vitamin D levels are necessary to clarify this interaction further.

This finding was consistent with another large populated-based case–control study from Germany that included only postmenopausal women [50]. They observed an ~70% lower breast cancer risk in women with serum 25(OH)D levels ≥30 ng/mL than in those with levels <12 ng/mL ($p < .0001$) [50]. However, this association was not found in another study of postmenopausal women nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial [56]. Variability in study results may be partially explained by differences in the study populations and the assays used for 25(OH)D measurement.

Only two prospective studies have examined the relationship between circulating levels of 25(OH)D and breast cancer mortality [61, 62]. Data from the third NHANES did show a lower breast cancer mortality rate among women with serum 25(OH)D levels of 20–32 ng/mL than among those with values <20 ng/mL (OR, 0.28; 95% CI, 0.08–0.93) [61]. However, the number of breast cancer deaths in the study ($n = 8$) was very small, and the data are based on a single measurement

of 25(OH)D up to 12 years before cancer death [61]; therefore, these results must be interpreted with caution.

In another study of 512 women with early-stage breast cancer, serum 25(OH)D levels at the time of diagnosis were shown to be significant predictors of both distant disease-free and overall survival [62]. On multivariate analysis, women with deficient levels of 25(OH)D (<20 ng/mL) had significantly worse distant disease-free survival (hazard ratio [HR], 1.94; 95% CI, 1.16–3.25) and overall survival (HR, 1.71; 95% CI, 1.02–2.86) compared to women with sufficient levels (≥30 ng/mL) [62]. This was the first study showing that vitamin D may influence breast cancer prognosis. Replication in a larger dataset, however, is necessary to confirm this association.

African Americans with breast cancer have poorer survival outcomes than whites, even after adjustment for stage, treatment, and other prognostic factors [63, 64]. Possible explanations for this ethnic disparity include a difference in tumor biology and access to care [63, 64]. Africans Americans in general have 25%–50% lower circulating vitamin D levels than whites [65]. Data from the third NHANES showed that the mean serum 25(OH)D levels among white, Hispanic, and African-American women were 30 ng/mL, 23 ng/mL, and 18 ng/mL, respectively ($p < .0001$) [65]. A recent study presented at the American Association for Cancer Research conference reported that African-American women with breast cancer were more likely to have vitamin D deficiency (<20 ng/mL) than white women (60% versus 15%; $p < .0001$), and women with vitamin D deficiency were eight times more likely to have locally advanced or metastatic disease than women with normal vitamin D level (OR, 8.6; 95% CI, 1.8–41.2) [66]. Lower breast cancer survival rates in African-American women might partially be explained by lower serum 25(OH)D levels.

Vitamin D–Related Polymorphisms

VDR Gene Polymorphisms

VDR gene polymorphisms may influence receptor affinity and binding to nuclear DNA, RNA transcription, and protein synthesis [67, 68]. In the white population, >25 polymorphisms of *VDR* have been identified [69]. Frequently studied variants include a start codon polymorphism (*Fok1*) in exon 2 (*f* allele), which results in the production of a longer protein that is transcriptionally less active [68, 70]. Several other polymorphisms at the 3′ end of the *VDR* gene, including *Bsm1*, *Taq1*, and *Apa1*, occur in strong linkage disequilibrium and are linked with a poly(A) microsatellite repeat [68–70]. To date, several epidemiological studies have investigated *VDR* polymorphisms in relation to breast cancer risk. The *f* allele of *Fok1* was associated with a higher risk for breast cancer in one study [68]. Another study found a significant association between breast cancer risk and *Bsm1* polymorphism, with an OR for the *bb* versus *BB* genotype >2.3 [67]. This finding has been replicated in some studies in different ethnic populations, but not in others [68, 70–73]. Although these findings are intriguing, the underlying basis for any association between *VDR* polymorphism and breast cancer risk remains to be elucidated.

Vitamin D–Binding Protein

Another key factor in vitamin D metabolism is the vitamin D–binding protein gene known as the group specific component (*GC*). The vitamin D–binding protein GC facilitates vitamin D actions by carrying vitamin D metabolites to various sites of action, and polymorphic vitamin D–binding proteins differ in their affinity for $1,25(OH)_2D$ [74]. Two common vitamin D–binding protein gene single nucleotide polymorphisms (SNPs), *rs4588* and *rs7041*, have been examined in relation to vitamin D concentrations and breast cancer risk [75, 76]. The first study found no significant association between either *rs7041* or *rs4588* and breast cancer risk [76]. The second study found that the combined polymorphisms (*rs7041* and *rs4588*) were associated with a lower breast cancer risk among postmenopausal women [75].

CYP24A1

CYP24A1 initiates the degradation of $1,25(OH)_2D$. Only one study has investigated one CYP24A1 SNP, *rs2296241*, and reported no increased risk of breast cancer among postmenopausal women with this SNP [76].

Vitamin D and Breast Cancer—Clinical Trials

There are two randomized trials of vitamin D_3 supplementation and cancer outcomes in postmenopausal women, with conflicting results. In the Women's Health Initiative, there was no reduction in risk of breast cancer in women randomly assigned to take calcium (1,000 mg) and vitamin D_3 (400 IU) daily, versus a placebo [77]. However, the dose of vitamin D administered in that study might have been too low to produce a protective effect. In addition, women were allowed to start vitamin D supplements of up to 1,000 IU/day during the trial [77]. In another trial of ~1,200 postmenopausal women randomized to receive 1,100 IU of vitamin D_3 and calcium, calcium alone, or placebo, a 60% lower risk for cancer of all types, including breast cancer, was observed after 4 years of supplementations with vitamin D_3 and calcium ($p = .013$) [78]. The number of breast cancer events, however, was small ($n = 19$); therefore, this result must be interpreted with caution.

There are several small ongoing chemoprevention studies looking at the effects of high doses of vitamin D_3 (20,000 IU or 30,000 IU once every week) in both pre- and postmenopausal women at high risk for developing breast cancer. Refice et al. [79] recently presented data on 20 high-risk premenopausal women receiving 1 year of vitamin D_3 (20,000 IU or 30,000 IU weekly) and showed that high doses of vitamin D were able to increase serum 25(OH)D to >40 mg/mL without any evidence of vitamin D toxicity. In addition, a large phase II study sponsored by the Southwest Oncology Group examining the effects of high doses of vitamin D_3 (20,000 IU once every week) in premenopausal women at high risk for breast cancer will open soon.

Defining Vitamin D Deficiency

Clinicians should be cautious when interpreting data on serum 25(OH)D levels. Multiple methodologies for 25(OH)D measurement exist, including radioimmunoassay (RIA), chemilu-

minescence protein-binding assay, and liquid chromatography with mass spectrometry [80]. The RIA and chemiluminescence protein-binding assays are most commonly used to determine vitamin D status. However, these methods lack standardization, and concerns have been raised about the degree of variability among assays and among laboratories, even when using the same assay [81–83]. In addition, seasonal variation in serum 25(OH)D is well known and has led some researchers to suggest collecting multiple blood samples across seasons for accurate 25(OH)D assessment [2].

Classifying a level of serum 25(OH)D as deficient also depends on the level that is defined as normal. According to the World Health Organization, levels <10 ng/mL and <20 ng/mL are considered deficient and insufficient, respectively [84]. In 2010, the International Osteoporosis Foundation recommended a target serum level of 25(OH)D of 30 ng/mL in all elderly persons [85]. In contrast, a recent Institute of Medicine (IOM) report suggested that 25(OH)D levels <20 ng/mL were indicative of vitamin D deficiency [86]. Although there is no consensus on the optimal serum levels of 25(OH)D, vitamin D deficiency is defined by most experts as a 25(OH)D level <20 ng/mL [2, 86].

In the U.S., ~35% of healthy young adults are vitamin D deficient [87]. More than half of Hispanic and African-American adolescents in Boston [88] and 48% of white preadolescent girls in Maine had deficient serum vitamin D levels [89]. In addition, ~40% of African-American women aged 15–49 years had 25(OH)D levels <15 ng/mL [87]. Similar rates of vitamin D deficiency have been reported among women with breast cancer [62, 90], and those who are nonwhite or have a BMI >25 kg/m^2 have a higher risk for deficiency [91].

Many physicians continue to feel that the serum 25(OH)D level for optimal bone health should be >30 ng/mL, which is the level required to maximize intestinal calcium absorption and prevent secondary hyperparathyroidism and its effects on the skeleton; vitamin D levels >20 ng/mL but <30 ng/mL are considered insufficient [2, 92]. Data from observational studies have suggested that the optimal level of 25(OH)D for breast cancer prevention is probably 40–60 ng/mL [54, 93].

A baseline 25(OH)D level should be obtained in breast cancer patients who are starting therapy that could impact bone mineralization, such as premenopausal women starting adjuvant chemotherapy or postmenopausal women beginning aromatase inhibitor therapy. Estrogen can upregulate both the 1-α-hydroxylase enzyme, which is required for generation of 1,25(OH)$_2$D from circulating 25(OH)D, and increase levels of VDR [94, 95]. Therefore, depletion of estrogen may unmask a subclinical vitamin D deficiency, which also may increase the severity of joint symptoms experienced by women on an aromatase inhibitor.

Optimal Dosing of Vitamin D

The IOM recommended daily intake of 600 IU and 800 IU of vitamin D$_3$ for adults aged <70 years and >70 years, respectively [86]. This was an increase from their previous recommendation in which dietary allowances of vitamin D were 200 IU, 400 IU, and 600 IU daily for adults aged <50 years, 50–70 years, and >70 years, respectively [86]. These recommendations presume some sunlight exposure and vitamin D intake from food, but are not adequate to treat vitamin D deficiency.

In general, for every 100 IU (2.5 μg) of vitamin D$_3$, the serum 25(OH)D level increases by ~1.0 ng/mL [96, 97]. The largest increments are seen in patients with the lowest starting 25(OH)D levels, but the increment declines as the 25(OH)D concentration increases to >40 ng/mL [98]. Multiple dosing regimens are effective in treating vitamin D deficiency, including 600 IU vitamin D daily, 4,200 IU weekly, and 18,000 IU monthly [99]. The dosing frequency appears to be less important than the cumulative amount [100]. It is common clinical practice to treat patients with vitamin D deficiency with 50,000 IU vitamin D once per week for 8 weeks followed by maintenance with 800–1,400 IU vitamin D daily thereafter [2, 101]. However, variability in serum vitamin D in response to oral intake is frequently observed and might be a result of other factors, such as sunlight exposure, BMI, and dietary intake [2]. Some experts have suggested that an average of 2,000–3,000 IU vitamin D intake per day from sun exposure, food, and supplements is needed to maintain an adequate vitamin D level for bone health [98].

Data on the impact of vitamin D supplementation in patients already diagnosed with breast cancer are limited. In a prospective study looking at vitamin D deficiency in woman with breast cancer receiving 400 IU vitamin D$_3$ daily for 1 year, the serum 25(OH)D level increased by <3 ng/mL [102]. That study suggested that a dose of 400 IU was inadequate in breast cancer patients, even to maintain bone health, and was probably too low for anticancer effects. Another study reported the safety and efficacy of vitamin D supplementation using 50,000 IU once per week in postmenopausal women with breast cancer initiating adjuvant letrozole [90]. All women achieved a level >40 ng/mL with 6 weeks of treatment, and there were no cases of hypercalcemia or renal stones [90]. In addition, disability from joint symptoms was better in women whose 25(OH)D levels were above versus below the median of 66 ng/mL [90].

Vitamin D intake is generally well tolerated. Although the IOM did not significantly increase the daily vitamin D requirement for adults, it did double the upper limit of vitamin D intake from 2,000 IU to 4,000 IU [86]. Hypercalcemia caused by vitamin D intoxication is rarely seen when serum levels of 25(OH)D are <150 ng/mL [103]. Most reports suggest that the toxicity threshold is 10,000–40,000 IU of vitamin D per day [104]. The optimal circulating level of 25(OH)D for reducing breast cancer risk or the risk for recurrence of breast cancer has yet to be defined, but simply targeting 25(OH)D levels known to be safe and optimal for bone health (30–50 ng/mL) might be a good start.

Conclusion

Vitamin D is important in many physiologic processes. Vitamin D is predominantly obtained through UVB radiation, and deficiency as a result of low sunlight is not easily corrected by dietary intake alone in the absence of supplementation. Though the relationship between vitamin D and breast cancer

remains unclear, a growing body of research currently supports vitamin D deficiency as a risk factor for breast cancer. Well-designed, randomized clinical trials are needed to further address whether or not vitamin D plays a role in breast cancer development, risk of recurrence, and survival in women with early-stage breast cancer. In the absence of further data, it is reasonable to aim for vitamin D levels >30 ng/mL in all patients diagnosed with breast cancer. Further research is also needed to determine the amount of vitamin D necessary to achieve a protective benefit against breast cancer.

Author Contributions

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