Association between Plasma 25-Hydroxyvitamin D and Breast Cancer Risk

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Abstract Vitamin D has been associated with decreased risk of several cancers. In experimental studies, vitamin D has been shown to inhibit cell proliferation and induce differentiation and apoptosis in normal and malignant breast cells. Using a population-based case-control study on Long Island, New York, we examined the association of breast cancer with plasma 25-hydroxyvitamin D (25-OHD) levels, a measure of vitamin D body stores. In-person interviews and blood specimens were obtained from 1,026 incident breast cancer cases diagnosed in 1996 to 1997 and 1,075 population-based controls. Plasma 25-OHD was measured in batched, archived specimens by Diasorin RIA. The mean (SD) plasma 25-OHD concentration was 27.1 (13.0) and 29.7 (15.1) ng/mL in the cases and controls, respectively (P < 0.0001). Plasma 25-OHD was inversely associated with breast cancer risk in a concentration-dependent fashion (P_trend = 0.002). Compared with women with vitamin D deficiency (25-OHD, <20 ng/mL), levels above 40 ng/mL were associated with decreased breast cancer risk (odds ratio, 0.56; 95% confidence interval, 0.41-0.78). The reduction in risk was greater among postmenopausal women (odds ratio, 0.46; 95% confidence interval, 0.09-0.83), and the effect did not vary according to tumor hormone receptor status. In summary, these results add to a growing body of evidence that adequate vitamin D stores may prevent breast cancer development. Whereas circulating 25-OHD levels of >32 ng/mL are associated with normal bone mineral metabolism, our data suggest that the optimal level for breast cancer prevention is ≥40 ng/mL. Well-designed clinical trials are urgently needed to determine whether vitamin D supplementation is effective for breast cancer chemoprevention.

Breast cancer is the most common cancer among women in the United States. Due to the magnitude of this disease, considerable research effort has been directed toward identifying breast cancer risk factors to target for prevention. However, relatively few modifiable lifestyle and environmental factors have been associated with reduced breast cancer risk. Chemoprevention refers to altering the carcinogenesis process with a drug intervention. The antiestrogens, tamoxifen and raloxifene, are the only drugs that have been approved by the U.S. Food and Drug Administration for breast cancer prevention in high-risk populations. Due to serious toxicities associated with these agents, namely endometrial cancer and thromboembolic disease, they have not gained widespread acceptance in the primary prevention setting. In addition, these antiestrogens do not lower the incidence of more aggressive estrogen receptor (ER)-negative breast cancers, which account for about one third of all breast tumors and are associated with a poorer prognosis compared with ER-positive cancer.

Vitamin D is a fat-soluble vitamin that regulates calcium and bone homeostasis but also has diverse biologicaleffects relevant to carcinogenesis. Modest amounts of vitamin D come from dietary sources such as fortified dairy products and cereals, fatty fish, and supplements. However, the majority of
vitamin D, up to 90%, is produced naturally in the body when UVB light hits a precursor molecule in the skin. Vitamin D then undergoes a series of hydroxylation steps in the liver to yield 25-hydroxyvitamin D (25-OHD), the major circulating metabolite, and in the kidney to produce 1,25-dihydroxyvitamin D \([1,25-(OH)_2D]\), the most biologically active form. 1,25-(OH)_2D exerts its effects in tissues traditionally linked with mineral metabolism, such as bone, kidney, and intestine, by binding to the vitamin D receptor. In addition, extrarenal vitamin D activation occurs in diverse target tissues, such as the colon, prostate, and breast, which express the activating enzyme (1α-hydroxylase) and the vitamin D receptor, to locally regulate cell turnover (1, 2). Activated vitamin D exerts its antitumor effects via the vitamin D receptor to form a nuclear receptor-ligand complex, which regulates the expression of target genes such as \(p21\), \(p27\), \(c-fos\), and \(c-myc\) (2).

Ecological studies have associated increased solar UVB irradiation with lower breast cancer incidence and mortality (3–5). Numerous preclinical studies have shown that 1,25-(OH)_2D inhibits cell proliferation, induces differentiation and apoptosis, and has antiangiogenesis effects in normal and malignant breast cells (6–9). Several epidemiologic studies evaluating the association between vitamin D and breast cancer risk have yielded inconsistent results (10). Some of these studies assessed the effects of dietary and supplemental intake of parent vitamin D. However, endogenous production through sunlight exposure is the major source of vitamin D in the body. Measurement of the circulating concentration of 25-OHD provides an integrated measure of vitamin D from all sources—diet, supplements, and sunlight exposure—and is considered the best indicator of vitamin D body stores (11, 12).

The Long Island Breast Cancer Study Project was undertaken specifically to investigate environmental factors associated with breast cancer risk (13). We used samples and data previously collected in the Long Island Breast Cancer Study Project to examine the hypothesis that higher plasma 25-OHD levels are associated with decreased breast cancer risk.

**Materials and Methods**

**Study population**

Subjects of the Long Island Breast Cancer Study Project are from a population-based case-control study conducted on Long Island, New York (13). Breast cancer cases were composed of women over age 20 y who were residents of Nassau and Suffolk counties, spoke English, and were newly diagnosed with \(in situ\) or invasive breast cancer between August 1, 1996 and July 31, 1997. For full details of case ascertainment, see the description of the parent study (13). Population-based controls were identified by random digit dialing at the subject’s home, and laboratory analyses using blood samples to measure plasma 25-OHD concentration (13). As part of the structured questionnaire, \(^{10}\) respondents were asked about their medical history, reproductive history, family history of cancer, body size changes, dietary factors, recreational physical activities, cigarette smoking and alcohol consumption, occupational and residential history, and demographic characteristics (13).

**Statistical methods**

We assessed the association of plasma 25-OHD and breast cancer risk by means of logistic regression with adjustment for potential confounders. First, a test for global significance was conducted using the Wald \(\chi^2\) test. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using plasma 25-OHD concentration both as a continuous variable (in 5 ng/mL increments) and categorical variable divided into four categories (<20, 20-29, 30-39, and ≥40 ng/mL). Vitamin D deficiency has been variably defined in the literature, but 25-OHD levels above 20 to 32 ng/mL (50-80 nmol/L) are considered optimal for bone health (15, 16). In our analysis, we defined vitamin D deficiency as plasma 25-OHD of <20 ng/mL. For simplicity and more model flexibility, we categorized by quintiles according to the distribution in the controls.

All models were adjusted for age at reference as a continuous variable, defined as age at diagnosis for cases and age at identification for controls. We examined potential confounding by the following covariates as continuous variables [age at menarche, age at first pregnancy, parity, body mass index (BMI)] or categorical variables [race (White/Non-White), first-degree family history of breast cancer, history of benign breast disease, menopausal status, ever use of hormone replacement therapy, physical activity (average number of hours of recreational activity per week by quartile), and season of blood collection (January-March, April-June, July-September, October-December)]. Tests for linear trend were done using each category as an ordinal variable.

Effect modification by menopausal status was examined by including multiplicative interaction terms in the logistic regression model (17). Menopausal status was based on self-reported information collected during the baseline interview, including date of last menstrual period and prior hysterectomy or bilateral oophorectomy. Postmenopausal status was defined as last menstrual period at least 6 mo from the reference date or removal of both ovaries. If a subject had a hysterectomy without removal of both ovaries before her last menstrual period, menopausal status was initially classified as unknown and then reclassified based on the subject’s age at reference.

**Measurement of plasma 25-OHD**

Plasma samples were stored in aliquots at −80°C until measurement. For quantification of 25-OHD in plasma, we used the Diasorin RIA method. This assay measures two distinct forms of 25-OHD: cotaneously derived vitamin D\(_3\) (cholecalciferol) and vitamin D\(_2\) (ergocalciferol) derived from supplements or fortified foods (14). Samples were analyzed between September 2007 and December 2007 using a total of eight lots of the assay. Interassay accuracy and precision for quality controls were +4% and 15% at 17 ng/mL, respectively. At 48 ng/mL, interassay accuracy and precision were +7% and 18%, and at 57 ng/mL, these were +15% and 15%, respectively. Cases and controls were assayed in each batch and laboratory personnel were blinded to case-control status.

**Data collection**

Exposure information comes from two sources—the parent study questionnaire, which was administered by trained interviewers in the subject’s home, and laboratory analyses using blood samples to measure plasma 25-OHD concentration (13). As part of the structured questionnaire, \(^{10}\) respondents were asked about their medical history, reproductive history, family history of cancer, body size changes, dietary factors, recreational physical activities, cigarette smoking and alcohol consumption, occupational and residential history, and demographic characteristics (13).

(13). Differences in risk estimates by hormone receptor status were examined using polytomous logistic regression (17). These models categorized the dependent variable into four groups based on ER and progesterone receptor (PR) positivity, negativity, or unknown status: ER+/PR+, ER−/PR−, ER+PR unknown, and controls. We also examined the associations with the cases restricted to women with invasive breast cancer and those who had not yet initiated chemotherapy by the time of the blood collection.

All tests were two sided and considered to be statistically significant if P value was <0.05. All statistical analyses were conducted using the SAS version 9.1 (SAS Institute).

Results

Baseline characteristics including demographic information and breast cancer risk factor data for the cases and controls are shown in Table 1. The mean age of cases and controls was 58.6 and 56.1 years, respectively. Over 90% of participants were White and about two-thirds were postmenopausal. Of the 1,026 breast cancer cases, 846 (82%) were invasive and 180 (18%) were in situ. The mean plasma 25-OHD concentration was 27.1 and 29.7 ng/mL in the cases and controls, respectively (P < 0.0001).

Vitamin D deficiency, defined as plasma 25-OHD of <20 ng/mL, was relatively common among the controls (28%). Table 2 shows the prevalence of vitamin D deficiency among the controls, based on factors known to influence circulating vitamin D levels. Rates of vitamin D deficiency did not differ substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantially by age. Non-Whites had twice the rate of Whites for vitamin D deficiency. Increasing BMI was associated with a substantial

Table 1. Baseline characteristics of the subset of breast cancer cases and controls with plasma 25-OHD levels, Long Island Breast Cancer Study Project (1996-1997)

<table>
<thead>
<tr>
<th>Baseline Characteristics</th>
<th>Cases (n = 1,026)</th>
<th>Controls (n = 1,075)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>58.6 (12.5)</td>
<td>56.1 (12.5)</td>
</tr>
<tr>
<td>Age at menarche (y)</td>
<td>12.6 (1.5)</td>
<td>12.5 (1.6)</td>
</tr>
<tr>
<td>Parity*</td>
<td>2.4 (1.6)</td>
<td>2.5 (1.6)</td>
</tr>
<tr>
<td>Age at first birth (y)*</td>
<td>25.4 (4.6)</td>
<td>25.1 (4.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.6 (5.6)</td>
<td>26.3 (5.8)</td>
</tr>
<tr>
<td>Plasma 25-OHD (ng/mL)</td>
<td>27.1 (13.0)</td>
<td>29.7 (15.1)</td>
</tr>
<tr>
<td>White</td>
<td>94.4</td>
<td>92.4</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>67.5</td>
<td>64.6</td>
</tr>
<tr>
<td>First-degree family history of breast cancer</td>
<td>18.5</td>
<td>13.8</td>
</tr>
<tr>
<td>History of benign breast disease</td>
<td>19.3</td>
<td>13.7</td>
</tr>
<tr>
<td>Ever breastfeeding</td>
<td>34.2</td>
<td>36.2</td>
</tr>
<tr>
<td>Ever hormone replacement therapy</td>
<td>29.7</td>
<td>27.5</td>
</tr>
</tbody>
</table>

*Among parous women (n = 890 cases and 955 controls).
In this large population-based case-control study, vitamin D status as measured by plasma 25-OHD levels was inversely associated with breast cancer risk in a concentration-dependent fashion. Women with circulating 25-OHD above 40 ng/mL had approximately a 40% reduction in breast cancer risk compared with those who were vitamin D deficient. We found that the risk reduction was more pronounced among postmenopausal women but was similar for hormone receptor–positive and hormone receptor–negative tumors. These results add to a growing body of literature supporting a protective effect of vitamin D on breast cancer risk, particularly among postmenopausal women.

Several epidemiologic studies examining the association between dietary and supplemental intake of vitamin D and breast cancer risk have yielded inconclusive results (10). However, dietary intake of vitamin D is not a complete measure of vitamin D status and is subject to inaccuracies in recall of dietary intake. Circulating 25-OHD correlates with exogenous intake and endogenous production of vitamin D, is the substrate for conversion to 1,25-(OH)₂D in target tissues, and may be the limiting factor in 1,25-(OH)₂D synthesis, particularly in extrarenal tissues (18).

Several studies have examined the association between endogenous 25-OHD levels and breast cancer risk (Table 6). Of two small hospital-based case-control studies, one found a significant inverse association for 25-OHD and breast cancer risk (19), and the other study found an inverse association for 1,25-(OH)₂D but not 25-OHD (20). These studies were limited by small sample sizes and the use of hospital-based controls. Our results are consistent with the recently reported findings from a large population-based case-control study from Germany (21), which showed that postmenopausal women with serum 25-OHD concentrations of ≥75 nmol/L (≥30 ng/mL) had about a 70% reduction in breast cancer risk compared with those with <30 nmol/L (<12 ng/mL). Although statistically insignificant, an inverse association between plasma 25-OHD and 1,25-(OH)₂D and breast cancer risk was observed in the case-control study nested in the Nurses’ Health Study (22). Another prospective study of postmenopausal women nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial did not observe an association between circulating 25-OHD or 1,25-(OH)₂D and breast cancer risk (23). Variability in study results may be partially explained by differences in the study populations and the assays used for 25-OHD measurement. In addition, vitamin D seems to be more effective in combating cancer near the time of detection (24, 25), which may explain why studies based on 25-OHD levels from stored sera several years before cancer diagnosis often do not find an association. All of the published studies on endogenous 25-OHD concentrations in relation to breast cancer risk have used single measurements of 25-OHD and do not take into account changes in vitamin D levels over time.

Plasma 25-OHD is a useful biomarker for measuring an individual’s recent exposure to environmental sources of vitamin D but may not correlate with lifetime patterns of sun exposure. The data suggest that higher vitamin D status is associated with a reduced risk of breast cancer, particularly among postmenopausal women. This finding is consistent with other studies and supports the hypothesis that vitamin D may play a protective role in breast cancer prevention.

### Table 2. Prevalence of vitamin D deficiency (plasma 25-OHD, <20 ng/mL) by age, race, BMI, and season of blood draw among controls

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Vitamin D deficiency n (%)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>29 (29)</td>
<td>0.480</td>
</tr>
<tr>
<td>40-50</td>
<td>92 (32)</td>
<td></td>
</tr>
<tr>
<td>51-60</td>
<td>79 (27)</td>
<td></td>
</tr>
<tr>
<td>61-70</td>
<td>58 (25)</td>
<td></td>
</tr>
<tr>
<td>&gt;70</td>
<td>44 (29)</td>
<td></td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>262 (26)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non-White</td>
<td>40 (49)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;25</td>
<td>121 (22)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>25-30</td>
<td>101 (32)</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>80 (37)</td>
<td></td>
</tr>
<tr>
<td>Season of blood draw</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Jan-Mar</td>
<td>99 (38)</td>
<td></td>
</tr>
<tr>
<td>Apr-Jun</td>
<td>99 (34)</td>
<td></td>
</tr>
<tr>
<td>July-Sept</td>
<td>26 (12)</td>
<td></td>
</tr>
<tr>
<td>Oct-Dec</td>
<td>78 (26)</td>
<td></td>
</tr>
</tbody>
</table>

*Based on χ² test.

### Table 3. ORs and 95% CIs for breast cancer risk by plasma 25-OHD levels

<table>
<thead>
<tr>
<th>Plasma 25-OHD (ng/mL)</th>
<th>Cases n (%)</th>
<th>Controls n (%)</th>
<th>Age-adjusted model</th>
<th>Adjusted model*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma 25-OHD (ng/mL)</td>
<td>Cases n (%)</td>
<td>Controls n (%)</td>
<td>Age-adjusted model</td>
<td>Adjusted model*</td>
</tr>
<tr>
<td>&lt;20</td>
<td>342 (33)</td>
<td>302 (28)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>20-29</td>
<td>274 (27)</td>
<td>287 (27)</td>
<td>0.84 (0.67-1.05)</td>
<td>0.80 (0.62-1.04)</td>
</tr>
<tr>
<td>30-39</td>
<td>287 (26)</td>
<td>295 (27)</td>
<td>0.87 (0.69-1.08)</td>
<td>0.83 (0.64-1.07)</td>
</tr>
<tr>
<td>≥40</td>
<td>123 (12)</td>
<td>191 (16)</td>
<td>0.59 (0.44-0.77)</td>
<td>0.56 (0.41-0.78)</td>
</tr>
<tr>
<td>OR trend</td>
<td></td>
<td></td>
<td>0.87 (0.80-0.95)</td>
<td>0.86 (0.78-0.94)</td>
</tr>
<tr>
<td>As continuous variable per 5 ng/mL increment</td>
<td>1,026</td>
<td>1,075</td>
<td>0.94 (0.91-0.97)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Adjusted for age, race, age of menarche, age of first birth, parity, breastfeeding history, menopausal status, use of hormone replacement therapy, first-degree family history of breast cancer, history of benign breast disease, BMI, physical activity, and season of blood draw.
exposure or dietary intake. However, the half-life of 25-OHD in the circulation is about 2 months and blood levels are fairly consistent over time (26, 27). It is unclear whether plasma 25-OHD varies following a cancer diagnosis and during cancer treatments. After a breast cancer diagnosis, dietary and behavioral changes such as decreased dietary intake of vitamin D or sunlight exposure may occur, which may alter plasma 25-OHD levels. Results of a large nested case-control study showed that higher prediagnostic plasma 25-OHD levels were associated with a trend toward decreasing breast cancer risk (22). In addition, three prospective cohort studies on dietary/supplemental intake of vitamin D and breast cancer risk supported an inverse association (28–30), whereas one did not (31). However, no studies to date have offered a comprehensive assessment of all sources of vitamin D in relation to cancer risk at levels of vitamin D exposure that may be needed for breast cancer prevention.

As for the effects of cancer treatments on plasma 25-OHD levels, a notable change in 25-OHD concentration after chemotherapy was not observed in two studies (32, 33) or in our own study of premenopausal women undergoing adjuvant chemotherapy for early-stage breast cancer (34). When we restricted our analysis to cases who had not received chemotherapy before blood collection, our results did not change.

Calcium may also have anticancer properties. Vitamin D and calcium are metabolically interrelated and highly correlated dietary factors that may influence breast cancer development through a variety of mechanisms (31, 35). A population-based case-control study from Germany examined the independent and joint effects of dietary vitamin D and calcium on premenopausal breast cancer risk (36). In this study, breast cancer risk was inversely associated with vitamin D but not calcium intake (36).

Prior studies have suggested that the association between vitamin D and breast cancer risk may be stronger for premenopausal women compared with postmenopausal women. One cohort study found a reduced risk of breast cancer in association with vitamin D intake among premenopausal women, but no reduction in breast cancer risk in postmenopausal women (28). In the Women’s Health Study, higher intake of vitamin D was moderately associated with a lower risk of breast cancer among premenopausal women, but not postmenopausal women (29). Similarly, the Cancer Prevention Study II Nutrition Cohort observed no association of breast cancer with total vitamin D intake among postmenopausal women (31). However, these studies did not include evaluation of circulating 25-OHD levels. We observed a significant inverse association between plasma 25-OHD levels and breast cancer risk among postmenopausal women but not premenopausal women. Our results may be due to the smaller sample size of the premenopausal group. Compared with postmenopausal women with vitamin D deficiency, those with plasma 25-OHD levels of ≥ 40 ng/mL had about a 50% reduction in breast cancer risk. Our results are consistent with the findings of Abbas et al. (21), which showed a protective effect of vitamin D for postmenopausal breast cancer.

We found a significant inverse association of vitamin D levels with breast cancer regardless of hormone receptor status. Recent data from the Nurses’ Health Study suggested an

| Table 4. Multivariate-adjusted ORs and 95% CIs for breast cancer risk by plasma 25-OHD levels (ng/mL) stratified by menopausal status |
|-----------------|----------------|------------------|------------------|------------------|
| Menopausal Status | Cases | Controls | OR (95% CI)* |
|------------------|----------------|------------------|------------------|------------------|
| Premenopausal    |                |                  |                  |                  |
| <20              | 98             | 117              | 1.00             |                  |
| 20-29            | 79             | 82               | 1.07 (0.65-1.49) |                  |
| 30-39            | 101            | 98               | 1.20 (0.81-1.59) |                  |
| ≥ 40             | 48             | 67               | 0.83 (0.36-1.30) |                  |
| P_trend          |                |                  | 0.821            |                  |
| As continuous variable per 5 ng/mL increment | 326 | 364 | 0.97 (0.92-1.02) |
| Postmenopausal   |                |                  |                  |                  |
| <20              | 232            | 173              | 1.00             |                  |
| 20-29            | 190            | 196              | 0.70 (0.41-0.99) |                  |
| 30-39            | 180            | 183              | 0.70 (0.40-1.00) |                  |
| ≥ 40             | 74             | 113              | 0.46 (0.09-0.83) |                  |
| P_trend          |                |                  | <0.001           |                  |
| As continuous variable per 5 ng/mL increment | 676 | 665 | 0.92 (0.89-0.96) |

*Adjusted for age, race, BMI, and season of blood draw.

<p>| Table 5. Association between plasma 25-OHD levels (ng/mL) and breast cancer risk by tumor ER and PR status |
|-----------------|----------------|------------------|------------------|
| Plasma 25-OHD (ng/mL) | Controls (n = 538) | ER+ and/or PR+ (n = 538) | ER-/PR- (n = 131) | ER/PR unknown (n = 355) |</p>
<table>
<thead>
<tr>
<th>No.</th>
<th>OR (95% CI)</th>
<th>No.</th>
<th>OR (95% CI)</th>
<th>No.</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20</td>
<td>302</td>
<td>175</td>
<td>1.00</td>
<td>49</td>
<td>1.00</td>
</tr>
<tr>
<td>20-29</td>
<td>287</td>
<td>131</td>
<td>0.86 (0.72-1.00)</td>
<td>33</td>
<td>0.83 (0.59-1.07)</td>
</tr>
<tr>
<td>30-39</td>
<td>295</td>
<td>163</td>
<td>0.95 (0.81-1.09)</td>
<td>34</td>
<td>0.82 (0.58-1.06)</td>
</tr>
<tr>
<td>≥ 40</td>
<td>191</td>
<td>69</td>
<td>0.78 (0.60-0.96)</td>
<td>15</td>
<td>0.67 (0.35-0.99)</td>
</tr>
</tbody>
</table>

NOTE: Adjusted for age, race, BMI, and season of blood draw.
inverse association for ER−/PR−, but not ER+ and/or PR+ tu-
mors (22). The Iowa Women’s Health Study also found a
stronger protective effect of vitamin D supplement use among
women with breast cancers that were negative rather than
positive for ER or PR status (30). Our results suggest that
vitamin D may have anticancer effects that are independent
of the ER pathway, and that targeting the vitamin D pathway
may be a useful preventive strategy for ER-negative, as well as
ER-positive breast cancers.

Our study has several strengths, including the population-
based sampling of controls, a large sample size that allowed
us to stratify the data by potential effect modifiers, and the di-
rect assessment of vitamin D status by an assay that measures
both dietary sources of vitamin D (ergocalciferol) and endoge-
nous vitamin D (cholecalciferol) synthesized in the skin. Vi-
tamin D assays were completed in a short period of time by the
same laboratory and were shown to have high precision. Po-
tential sources of bias in our retrospective case-control design
include subject selection and measurement error on plasma
25-OHD concentration. In terms of subject selection, response
rates were lower among controls compared with cases, espe-
cially among women over the age of 75 years (13). Therefore,
these results may not be generalizable to older women. In
addition, blood donors differed from those who did not donate
blood on a number of factors (13). However, all models includ-
ed the frequency matching factor age at reference, and adjust-
ment for other known breast cancer risk factors did not appreciably change the effect estimates, and were therefore
not included in our final models. Unlike questionnaire ex-
posure data, measurement of circulating 25-OHD levels is not
subject to recall bias, although laboratory error is possible.
However, this measurement error in plasma 25-OHD is unlik-
ely to differ by case-control status given that all laboratory spe-
cimens were labeled with a random number (and cannot be
linked back to the subject by the laboratory personnel); thus,
this potential source of error cannot explain the observed asso-
ciations between 25-OHD and breast cancer risk.

Clinical trials of vitamin D supplementation suggest a ben-
etit in the prevention setting. In the Women’s Health Initiative,
over 36,000 postmenopausal women were randomized to
1,000 mg of calcium carbonate and 400 IU of vitamin D3 or
matching placebo for 7 years (37). Although breast cancer in-
cidence did not differ between the two groups, personal sup-
plementation with vitamin D (up to 1,000 IU per day) was
allowed, which may have dampened the ability to differenti-
ate between the active and control arms. In a subgroup anal-
ysis of women who did not report additional personal supple-
ment use (n = 19,115), there was a significant decrease in
breast cancer incidence with calcium and vitamin D com-
pared with placebo (hazard ratio, 0.82; 95% CI, 0.70-0.97).
Another intervention trial of calcium and 1,100 IU of vitamin
D3 daily for 4 years in postmenopausal women found a 60%
reduction in overall cancer incidence compared with placebo
(24). However, the number of cancer events was small and the
follow-up was only 4 years. In addition, neither this trial nor
the Women’s Health Initiative can distinguish between the
effects of calcium and vitamin D.

In the United States, the Dietary Reference Intake of vitamin
D is 200, 400, and 600 IU daily for adults ages <50, 50 to 70,
and >70 years, respectively (38). Oral daily intake of 1,000 IU of vi-
tamin D increases circulating 25-OHD levels by about 10 ng/
ml (39). Given the high prevalence of vitamin D deficiency in
the general population, to raise plasma 25-OHD levels above
40 ng/ml, the putative target level for breast cancer risk reduc-
tion, women would have to consume about 4,000 IU daily,
which exceeds the National Academy of Sciences upper limit
of 2,000 IU/day (38). In the time since this upper safety limit
was set in 1997, accumulating evidence in trials of healthy
adults suggests that doses well above those recommended cur-
cently are safe (40). However, controversy in this field re-
mains.

In summary, our study adds to a growing body of evidence
that adequate vitamin D stores may prevent the development
of breast cancer. The protective effect of vitamin D was stronger
among postmenopausal women, but was similar for hormone
receptor–positive and hormone receptor–negative tumors.
These results represent an important public health finding, as
women with low circulating 25-OHD levels may benefit from
vitamin D supplementation for breast cancer risk reduction.
Whereas circulating 25-OHD levels of >32 ng/mL are associ-
ated with normal mineral metabolism, data from our study and
others (41) suggest that the optimal level for breast cancer pre-
vention is ≥40 ng/mL. Future studies should include repeated
measures of 25-OHD to determine whether changes over time
are linked to cancer risk. In addition, well-designed clinical
trials are necessary to determine whether vitamin D supple-
mentation is effective for breast cancer chemoprevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Table 6. Endogenous 25-OHD levels and breast cancer risk

<table>
<thead>
<tr>
<th>Study</th>
<th>Study Design</th>
<th>No. cases/controls</th>
<th>Comparison*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lowe et al., 2005 (19)</td>
<td>Hospital-based case-control</td>
<td>179/179</td>
<td>25-OHD (ng/mL) &gt;20 vs &gt;60</td>
<td>5.83 (2.31-14.7)</td>
</tr>
<tr>
<td>Abbas et al., 2008 (21)</td>
<td>Population-based case-control</td>
<td>1,394/1,365</td>
<td>25-OHD (ng/mL) ≥30 vs &lt;12</td>
<td>0.31 (0.24-0.42)</td>
</tr>
<tr>
<td>Crew et al.</td>
<td>Population-based case-control</td>
<td>1,026/1,075</td>
<td>25-OHD (ng/mL) ≥40 vs &lt;20</td>
<td>0.56 (0.41-0.78)</td>
</tr>
<tr>
<td>Bertone-Johnson et al., 2005 (22)</td>
<td>Nested case-control</td>
<td>701/724</td>
<td>25-OHD (ng/mL) ≥40 vs ≥20†</td>
<td>0.73 (0.49-1.07)</td>
</tr>
<tr>
<td>Freedman et al., 2008 (23)</td>
<td>Nested case-control</td>
<td>1,005/1,005</td>
<td>25-OHD (ng/mL) ≥33.7 vs &lt;18.3</td>
<td>1.04 (0.75-1.45)</td>
</tr>
</tbody>
</table>

*For circulating concentration of 25-OHD, 1 ng/mL = 2.5 nmol/L.
†For this study, breast cancer risk was analyzed by quintiles of plasma 25-OHD. Quintile cut points differed in the three batches of plasma 25-OHD measurements.
References

Association between Plasma 25-Hydroxyvitamin D and Breast Cancer Risk

Katherine D. Crew, Marilie D. Gammon, Susan E. Steck, et al.


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