

# Serum Levels of Vitamin D Metabolites and Breast Cancer Risk in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial

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## Abstract

Experimental and epidemiologic studies suggest that vitamin D metabolites (1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D] and its precursor 25-hydroxyvitamin D [25(OH)D]) may reduce breast cancer risk. We examined subsequent breast cancer risk related to serum levels of these metabolites. In a cohort of women ages 55 to 74 years, who donated blood at baseline (1993-2001) in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, we identified 1,005 incident breast cancer cases during follow-up through 2005 (mean time between blood draw and diagnosis, 3.9 years). Noncases ( $n = 1,005$ ) were frequency matched to the cases based on age and year of entry. Sample weights that accounted for unequal probabilities of selecting cases and non-cases were applied to make inferences that reflected the entire Prostate, Lung, Colorectal, and Ovarian cohort. Using Cox proportional hazards modeling, we comput-

ed breast cancer relative risks (RR) and 95% confidence intervals (95% CI) by quintile for each metabolite. The RR of breast cancer for the highest quintile of 25(OH)D concentration versus the lowest was 1.04 (95% CI, 0.75-1.45;  $P_{\text{trend}} = 0.81$ ). Similarly, the breast cancer RR for the highest quintile of 1,25(OH)<sub>2</sub>D compared with the lowest was 1.23 (95% CI, 0.91-1.68;  $P_{\text{trend}} = 0.14$ ). Excluding the first 2 years of follow-up did not materially alter these estimates. There was also no evidence of inverse risk in older women ( $\geq 60$  years) versus younger women ( $< 60$  years). In this prospective study of postmenopausal women, we did not observe an inverse association between circulating 25(OH)D or 1,25(OH)<sub>2</sub>D and breast cancer risk, although we cannot exclude an association in younger women or with long-term or earlier exposure. (Cancer Epidemiol Biomarkers Prev 2008;17(4):889-94)

## Introduction

In recent years, there has been considerable interest in whether vitamin D inhibits breast cancer development (1, 2). Experimental studies have shown that vitamin D promotes cell differentiation and retards or terminates proliferation of breast cancer cells (3, 4). Several animal studies have also shown that carcinogen-exposed rats fed vitamin D or its analogues have fewer and later mammary tumors (4, 5). The anticarcinogenic potential of vitamin D is attributed to the active or hormonal form of vitamin D, 1,25-dihydroxyvitamin D [1,25(OH)<sub>2</sub>D], which is produced in the kidneys from the metabolite, 25-hydroxyvitamin D [25(OH)D]. Experimental studies indicate that 1,25(OH)<sub>2</sub>D can also be synthesized from

25(OH)D in other tissues, possibly including breast tissue (6, 7). Regulatory mechanisms closely maintain circulating levels of the biologically active metabolite 1,25(OH)<sub>2</sub>D, whereas the precursor 25(OH)D provides an indicator of vitamin D status from UV and, to a much lesser extent, dietary sources. Thus, because circulating 25(OH) may be related to levels of 1,25(OH)<sub>2</sub>D in breast tissue and circulating 1,25(OH)<sub>2</sub>D is homeostatically controlled, circulating 25(OH)D is thought to be potentially relevant to breast carcinogenesis.

Epidemiologic studies of vitamin D and breast cancer also suggest a possible protective association (8). A few studies (9-12), but not all (1, 13), have found an inverse association between breast cancer and indicators of high sun exposure, which is generally the principal source of 25(OH)D. Studies of vitamin D intake and breast cancer have been inconsistent, with some, but not all, showing inverse associations (8). To our knowledge, only two studies have examined the association between circulating vitamin D metabolites and subsequent breast cancer risk. One small study ( $n = 96$  cases) found no relationship between breast cancer and 1,25(OH)<sub>2</sub>D but did not examine associations with 25(OH)D (14). The other, larger study ( $n = 701$  cases) found high levels of both metabolites to be nonsignificantly associated with a lower risk of breast cancer

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overall, although a statistically significant inverse trend for 25(OH)D was observed in women ages  $\geq 60$  years at blood collection (15).

To better understand the role of vitamin D in breast cancer, we report here the results of a cohort analysis designed to evaluate whether vitamin D, as measured prediagnostically in serum, is associated with risk of breast cancer. We examined both 25(OH)D and 1,25(OH)<sub>2</sub>D because 25(OH)D is considered indicative of an individual's vitamin D status, and 1,25(OH)<sub>2</sub>D, although homeostatically controlled in the blood, is regarded as the active metabolite.

## Materials and Methods

**Study Design and Population.** The current study is nested in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (16), a multisite randomized clinical trial designed to evaluate the efficacy of screening for these four cancers in reducing specific causes of cancer incidence and mortality. The Trial, which recruited 154,952 participants, randomly assigned participants to a screening arm and a nonscreening arm. Subjects for the current study were drawn from the 38,660 female participants randomized to the screening arm. Details of the trial have been described elsewhere (17, 18). Briefly, women ages 55 to 74 years were recruited between November 1993 and July 2001 in 10 U.S. centers. Potential participants were excluded from the trial if they had a history of one of the four PLCO cancers, had a recent history of screening procedures for one of the cancers, or were undergoing treatment for any cancer (other than nonmelanoma skin cancer). Study subjects provided written informed consent, and the study was approved by the institutional review boards of the U.S. National Cancer Institute and the 10 screening centers.

At randomization, all participants were asked to complete a self-administered baseline questionnaire that included questions on demographic factors, medical history, and health-related behaviors. Individuals who were randomized to the screening arm underwent periodic screening tests, completed a food frequency questionnaire at baseline designed to characterize usual dietary intake over the past year, and donated blood for use in etiologic studies.

Incident breast cancer cases were ascertained through self-report in an annual health survey, linkage to state cancer registries, death certificates, physician reports, and next-of-kin reports (for deceased participants). A total of 92% of the ascertained breast cancer cases were confirmed through review of medical records. The results from analyses excluding self-reported but unconfirmed cases did not differ materially from the overall results; therefore, all ascertained cases were retained in the analyses presented.

**Study Sample.** Eligible cases were all participants who provided baseline blood samples and background questionnaires, had not reported a history of breast cancer at baseline, and were diagnosed with an incident breast cancer between the date of the baseline blood collection (1993-2001) and June 30, 2005. Noncases were selected from participants who provided blood samples

at baseline but had not reported a breast cancer as of June 30, 2005. Noncases were frequency matched to cases by randomly subsampling noncases from each of four age groups (55-59, 60-64, 65-69, and 70-74 years) and two serum collection periods (before September 30, 1997, September 30, 1997 or after, split at the midpoint of the collection period), so that sample sizes in each of these categories matched those of the cases. There were a total of 1,005 cases and 1,005 noncases, with one less case for the metabolite, 1,25(OH)<sub>2</sub>D, because one sample was not assayed for this metabolite. Sample weights that accounted for the unequal rates of selecting cases and noncases (19) were applied to make inferences that reflected the entire PLCO sample. A weight of one was assigned to cases and weights that were the inverse of the sampling fractions from each of the eight sampling categories were assigned to noncases.

**Vitamin D Measurement.** Serum specimens were analyzed for levels of 25(OH)D and 1,25(OH)<sub>2</sub>D using RIA in the laboratory of Dr. Bruce Hollis at the University of South Carolina (20). Laboratory personnel were blinded as to case status. Twelve blinded quality-control samples, composed of duplicates from each of six preselected individuals, were randomly ordered in each of 14 batches, which were assayed over 4 months in 2006. Cases and noncases were assayed in each batch. The total coefficient of variation was 8.2% for 25(OH)D and 12.8% for 1,25(OH)<sub>2</sub>D.

**Assessment of Covariates.** We examined numerous factors that could potentially confound the relationship between the vitamin D metabolites and breast cancer. These included period of blood donation (before September 30, 1997, September 30, 1997 or after); season of blood draw (June-August, September-November, December-February, March-May); month of blood draw (12 categories by month); hour of blood draw (four categories in 3-h intervals, 6 a.m.-6 p.m.); and several characteristics identified at baseline, that is, menopausal status (postmenopausal versus perimenopausal and unknown); hormonal replacement therapy status (never, former, current); body mass index (BMI) at baseline [calculated as weight (kg)/height (m) squared: <25, 25 to <30,  $\geq 30$  kg/m<sup>2</sup>]; BMI at age 18 to 20 (<25, 25 to <30,  $\geq 30$  years); age at menarche (<11, 12-13,  $\geq 14$  years); age at menopause (<50, 50-54,  $\geq 55$  years); smoking status (never, former, current); family history of breast cancer (0,  $\geq 1$  family members); a reported personal history of benign or fibrocystic breast disease (yes, no); reported recent (in last 3 years) mammography (no, once, more than once); a variable combining parity and age at first birth (nulliparous; <25 years, 1-4 children; 25-29 years, 1-4 children;  $\geq 30$  years, 1-4 children; <25 years,  $\geq 5$  children;  $\geq 25$  years,  $\geq 5$  children); and race (White, Black, Hispanic, Asian American, Pacific Islander, American Indian, other). We used the food frequency questionnaire to assess dietary intake of calcium (median split), total intake of calcium from diet and supplements (<1,000, 1,000 to <2,000,  $\geq 2,000$  mg/d) (both calcium variables residually adjusted for energy), and alcohol intake (nondrinker, drinker). Missing values were also included as a separate category for the covariates. As noted below, age during the follow-up period was used as the timeline and thus treated as a continuous variable.

**Statistical Analysis.** Our main analysis was based on 25(OH)D and 1,25(OH)<sub>2</sub>D stratified by quintiles that were based on the distribution in noncases. Using Cox proportional hazards modeling with sample weighting, we computed relative risks (RR) with 95% confidence intervals (95% CI) for breast cancer incidence by quintile of each metabolite. The initial (simple) analysis included period of blood draw and season of blood collection (four seasons: December-February, March-May, June-August, and September-November), with age as the timeline. More detailed multivariate models were also fit, with additional adjustment for BMI at age 18 to 20, age at menarche, age at menopause, hormone replacement therapy use, history of benign breast disease, family history of breast cancer, combined parity and age at first birth, smoking status, alcohol intake, and total calcium intake, which were largely comparable with the covariates in the other prospective study of both vitamin D metabolites and breast cancer (15). Further adjustment for month of blood collection, hour of blood collection, BMI at PLCO baseline, race, recent mammography, and dietary calcium intake were not included because they did not materially change the risks.

We examined the breast cancer and vitamin D association in the cohort and within strata defined by age group (<60, ≥60 years); smoking status (never, ever); residential region based on ranges of UV radiation levels obtained from Robertson-Berger (R-B) meters located in states in which screening centers are located (21): low sun (R-B ≤ 105), moderate sun (R-B = 113-133), and high sun (R-B ≥ 154); baseline BMI level (<25, ≥25 kg/m<sup>2</sup>); hormone therapy (current, former/never user); family history of breast cancer (≥1, no family member); total dietary intake of calcium (split at median); and multivitamin supplement use (ever, never). When strata analysis involved three strata (that is, region), tertiles were used to accommodate the smaller case numbers. To explore effect modification with season of blood draw, we did stratified analyses and evaluated multiplicative interaction by creating product terms.

We also calculated RR separately for case subgroups defined based on histology (ductal carcinoma *International Classification of Diseases for Oncology, Second Edition* code C50-8500, lobular carcinoma code C50-8520) and behavior (invasive, behavior code = 3; *in situ* cancers, behavior code = 2). To address concerns that levels of vitamin D metabolites may be affected by early, preclinical disease, we analyzed the relationship between breast cancer and the two metabolites after excluding the first 2 years of follow-up.

We assessed the sensitivity of our findings to different analytic approaches. We used season-specific quintiles (December-February, March-May, June-August, and September-November), in which we identified the first quintile of measurements for each season and combined them to constitute a first quintile for the study and then identified the second quintile for each season and combined them to constitute a second quintile and so on, for all five quintiles. Then, risk was assessed based on this combined quintile categorization. We also estimated RRs for serum levels of 25(OH)D and 1,25(OH)<sub>2</sub>D below compared with above 15 ng/mL (22) and 26 pg/mL (23), respectively, because levels less than these cut points are generally considered below clinically normal (in the context of non-cancer-related outcomes) and other

studies of circulating vitamin D metabolites and cancer-related outcomes have assessed risk associated with these cut points (24, 25). Lastly, we examined risk associated with the ratio of 1,25(OH)<sub>2</sub>D to 25(OH)D to explore the hypothesis that this ratio reflects the efficiency of transforming 25(OH)D to hormonal 1,25(OH)<sub>2</sub>D and therefore may be related to risk.

All statistical tests using Cox proportional hazards analysis were based on the Wald *F* test (26). The data were analyzed using the SUDAAN software program (26), a family of statistical procedures for analysis of weighted data. Trend tests were based on assigning the median value to each quintile and modeling the resulting variable as continuous. All statistical tests were two sided ( $\alpha = 0.05$ ).

## Results

Of the 1,005 breast cancer cases, 73% were classified as invasive, 19% as *in situ*, and 8% unclassified as to behavior. Cancers included 62% ductal carcinoma and 12% lobular carcinoma and the remainder cancers are other histologic types and unknown. The mean time between baseline blood draw and breast cancer diagnosis was 3.9 years (range, 0.6-10.8) and the mean age at diagnosis was 66.5 years. At entry to the study, 99% of the women were postmenopausal and ~90% were Caucasian.

Mean values of 25(OH)D and 1,25(OH)<sub>2</sub>D were similar between cases (26.7 ng/mL and 38.6 pg/mL, respectively) and the weighted sample (26.2 ng/mL and 37.7 pg/mL). Serum concentrations of 25(OH)D below 15 ng/mL, typically considered below normal, characterized 8.9% of cases and 11.0% of the cohort (based on the weighted sample), whereas 11.2% of cases and 13.2% of the cohort (based on the weighted sample) had measured levels of 1,25(OH)<sub>2</sub>D levels considered below normal (<26 pg/mL).

As shown in Table 1, at baseline, women with high levels of circulating 25(OH)D typically had lower baseline BMI, engaged in more physical activity, had greater total calcium intake, and were more likely to live in the south, be White, report benign breast disease, use hormone therapy, and use supplements with vitamin D. Women with high levels of circulating 1,25(OH)<sub>2</sub>D generally had lower baseline BMI, earlier age at menopause, lower likelihood of benign breast disease, and earlier age at first childbirth.

In the simple analysis of 25(OH)D by quintile, which was adjusted for study design matching factors and season of blood draw, there was no association with breast cancer risk (Table 2). After adjusting for additional factors, the pattern remained the same. After excluding the first 2 years of follow-up, there was also no association with breast cancer risk. It is noteworthy, however, that quintiles 3 to 5 were associated with nonsignificantly elevated risks compared with the lowest quintile. The multivariate risk associated with 25(OH)D levels below 15 ng/mL compared with higher levels was 0.81 (95% CI, 0.59-1.12).

The analysis of 1,25(OH)<sub>2</sub>D that was adjusted only for study design matching factors and season of blood draw showed a pattern of increasing risk ( $P_{\text{trend}} = 0.04$ ) (Table 2). When the regression model was adjusted for additional factors, the trend was still positive but no longer statistically significant. There was also no

**Table 1. Characteristics of cohort (based on the weighted sample) at baseline blood collection (1993-2001) in women in PLCO study by quintile of vitamin D metabolite**

Characteristic	25(OH)D*			1,25(OH)D <sup>†</sup>		
	Q1	Q3	Q5	Q1	Q3	Q5
No. controls	200	196	202	201	202	202
Mean						
25(OH)D (ng/mL)	13.6	25.8	39.2	24.4	26.9	27.5
1,25(OH) <sub>2</sub> D (pg/mL)	36.5	38.4	39.6	23.7	36.2	55.1
Age (y) at serum collection	62.3	62.2	61.8	63.2	61.8	62.2
BMI (at 18-20 y; kg/m <sup>2</sup> )	21.4	21.1	21.0	21.7	21.3	20.6
BMI (at baseline; kg/m <sup>2</sup> )	28.9	27.3	25.4	28.1	27.4	25.8
No. live births	3.1	3.5	3.0	3.3	3.1	3.3
Physical activity, vigorous (h/wk)	2.2	2.7	3.1	2.6	2.8	2.7
Alcohol (g/d) <sup>‡</sup>	6.2	4.4	5.9	5.2	4.7	5.8
Total calcium <sup>‡</sup> (g/d)	1.1	1.5	1.7	1.5	1.4	1.4
Percentage <sup>§</sup>						
Residence at study entry <sup>  </sup>						
Low sun	46.1	39.3	38.7	42.7	46.1	45.8
Moderate sun	40.8	43.4	41.7	43.0	39.8	34.5
High sun	13.2	17.3	19.6	14.2	14.1	19.6
Race/ethnicity (White)	81.9	89.9	94.0	92.8	92.9	85.5
Education (≥1 y college)	52.3	52.6	56.4	55.8	60.4	59.5
Ever smoker	47.0	40.2	37.0	39.2	44.5	42.4
Family history of breast cancer (first-degree relative/half-sister)	18.1	18.5	15.6	16.2	17.2	12.4
History of benign breast disease	15.8	29.7	27.0	31.3	23.4	19.9
Age at menarche (≤11 y)	20.0	18.7	25.2	18.0	22.2	22.4
Age at first childbirth (y)						
Nulliparous	10.9	7.0	7.4	8.4	9.9	9.1
<25	67.2	71.3	65.7	62.3	68.6	65.1
25-29	14.6	16.1	20.5	19.4	15.4	21.8
≥30	6.5	5.6	6.4	9.5	6.2	3.6
Age at menopause (<50 y)	49.5	47.0	47.0	51.1	50.4	46.2
Oral contraceptive use	52.6	61.2	58.0	51.4	62.6	56.8
Hormone therapy use (at baseline)	63.1	75.9	78.1	68.7	69.4	76.9
Season of serum collection						
Dec-Feb	29.1	19.6	11.6	22.0	22.3	19.3
Mar-May	34.4	22.2	18.4	24.1	20.3	28.1
Jun-Aug	13.8	28.7	32.6	29.9	25.6	22.6
Sep-Nov	22.6	29.5	37.4	24.1	31.8	30.1
Supplement with vitamin D use <sup>¶</sup>	25.2	60.3	64.6	50.5	50.0	53.1

\*Quintiles (<18.3, 18.3 to <23.5, 23.5 to <28.3, 28.3 to <33.7, ≥33.7 ng/mL).

†Quintiles (<28.2, 28.2 to <33.5, 33.5 to <39, 39 to <46.3, ≥46.3 pg/mL).

‡Adjusted for total energy intake.

§Percentages do not always add up to 100% because of rounding or missing data.

||Residential regions based on ranges of UV radiation levels obtained from annual R-B meters in states in which screening centers are located: low sun (R-B ≤105, Detroit, MI; Minneapolis, MN; Marshfield, WI), moderate sun (R-B = 113-134, Pittsburgh, PA; St. Louis, MO; Denver, CO; Washington, DC; Salt Lake City, UT), and high sun (R-B ≥154 Birmingham, AL; Honolulu, HI; ref. 21).

¶Includes multivitamin supplements, vitamin D supplements, or cod liver oil; missing are treated as not taking supplements.

statistically significant trend after excluding the first 2 years of follow-up after blood collection, although risk continued to be elevated in quintiles 2 to 5 compared with the lowest quintile. The multivariate risk associated with levels of 1,25(OH)<sub>2</sub>D below 26 pg/mL compared with higher levels was 0.82 (95% CI, 0.62-1.10).

When we included both 25(OH)D and 1,25(OH)<sub>2</sub>D in the model, the risk estimates did not change much, which was not unexpected given the low correlation between the two metabolites ( $r^2 = 0.12$ ). When we classified quintiles based on the vitamin D metabolite distribution within each season of blood draw and combined quintiles across seasons, the pattern of association for both metabolites was similar to that presented in Table 2. We also found no association with the ratio of 1,25(OH)<sub>2</sub>D to 25(OH)D and risk.

We observed no evidence of an inverse association between the vitamin D metabolites and breast cancer risk within strata defined by age group, smoking status,

baseline BMI, region of residence, hormone therapy, family history of breast cancer, dietary calcium intake, multivitamin supplement use or for those with ductal carcinoma, lobular carcinoma, or invasive or *in situ* cancer. In addition, there was no interaction between vitamin D metabolite and breast cancer risk by season of blood draw.

## Discussion

To our knowledge, this is the largest study of prediagnostic circulating 25(OH)D and 1,25(OH)<sub>2</sub>D in relation to breast cancer risk conducted to date. We found no association between either metabolite in the study population, when the first 2 years of follow-up after blood collection were excluded, or in older women (≥60 years), a group showing a protective association with 25(OH)D in the study by Bertone-Johnson et al. (15). In

our study, risks were not elevated in those with vitamin D metabolite levels typically considered below normal.

Experimental studies have shown a variety of anti-cancer effects of 1,25(OH)<sub>2</sub>D (27), but the scant epidemiologic literature do not corroborate these findings. Consistent with our findings, one of two breast cancer studies using prediagnostic blood by Hiatt et al. (14) found no association with circulating 1,25(OH)<sub>2</sub>D. The other, by Bertone-Johnson et al. (15), found a nonsignificantly lower risk in the highest quintiles of circulating 1,25(OH)<sub>2</sub>D, but no trend ( $P_{\text{trend}} = 0.39$ ), and similar mean levels in cases and controls. Although a small clinic-based study of 1,25(OH)<sub>2</sub>D (28) in blood collected at or after breast cancer diagnosis found lower levels in White cases than in White controls, it found higher levels in Black cases than in Black controls, and the retrospective design limits inferences about causality. Moreover, most prospective studies of other cancers have not found inverse associations with prediagnostic 1,25(OH)<sub>2</sub>D (24, 29-31). Our null findings for 1,25(OH)<sub>2</sub>D are not necessarily unexpected, because circulating 1,25(OH)<sub>2</sub>D is homeostatically controlled and may not reflect localized levels in the breast.

The null findings on 25(OH)D are more noteworthy. They are inconsistent with the inverse associations found in the study by Bertone-Johnson et al. on circulating 25(OH)D, as well as hypotheses and inferences generated by several ecologic/ecologic-type analyses (9, 10), and other studies of vitamin D or surrogates for vitamin D exposure and breast cancer (11, 12). Overall, however, the epidemiologic data on breast cancer risk are relatively sparse, and some studies (using geographic residence as the exposure or breast density as the outcome) have not supported an association (13, 32). One possible explanation for our findings, of course, is that breast cancer is not related to 25(OH)D exposure.

It is also possible that the elapsed time between blood collection and diagnosis (mean, 3.9 years) in our study may have been too short to detect an association, particularly if long-term or early exposure is most relevant. Ecologic analyses may reflect long-term exposure, and a recent case-control study (12) suggests that adolescent sun exposure may be particularly relevant, although postdiagnostic recollections of adolescent sun exposure may be subject to substantial misclassification. In addition, our study relied on a single measure of 25(OH)D, which may not reflect long-term status even in adulthood. It is reassuring, however, that a recent cohort study measured 25(OH)D concentrations at two times that were 3 years apart and found a Pearson correlation coefficient of 0.70 between samples (<0.0001; ref. 24). In any case, the resulting misclassification would likely attenuate any risk relationship, but notably we found no indication of even a weakly protective relationship. A further possibility is that 1,25(OH)<sub>2</sub>D in breast tissue is anticarcinogenic, but neither circulating 25(OH)D nor 1,25(OH)<sub>2</sub>D is a good surrogate for the relevant cellular exposure.

Our study has many, notable strengths. It is a very large study, with more than 1,000 cases and 1,000 noncases. Blood samples were collected prediagnostically, and all assays were completed in a short period of time by the same laboratory and were shown to have high precision. One of the metabolites, 25(OH)D, integrates all sources of vitamin D from diet, supplements, and sunlight and is considered indicative of clinical vitamin D status. The finding that 11% of the cohort (weighted sample) had 25(OH)D levels below 15 mg/mL is similar to results reported for U.S. women in the nationally representative National Health and Nutrition Examination Survey III (range, 10-15% for ages 60-79 presented by region/season; ref. 33) and lends some external validity to our findings.

**Table 2. RRs and 95% CI by quintile of serum 25(OH)D and 1,25(OH)<sub>2</sub>D**

	Cases	Entire follow-up period		Excluding first 2 y of follow-up
		Simple RR* (95% CI)	Multivariate RR <sup>†</sup> (95% CI)	Multivariate RR <sup>†</sup> (95% CI)
<b>25(OH)D</b>				
Quintile <sup>‡</sup>				
1	172	1.0	1.0	1.0
2	188	1.09 (0.82-1.45)	1.02 (0.75-1.41)	1.04 (0.74-1.48)
3	244	1.43 (1.08-1.89)	1.36 (0.99-1.87)	1.25 (0.88-1.77)
4	205	1.20 (0.90-1.60)	1.13 (0.82-1.55)	1.12 (0.78-1.59)
5	196	1.15 (0.86-1.53)	1.04 (0.75-1.45)	1.04 (0.72-1.51)
$P_{\text{trend}}^{\S}$		0.33	0.81	0.82
<b>1,25(OH)<sub>2</sub>D</b>				
Quintile <sup>  </sup>				
1	166	1.0	1.0	1.0
2	186	1.22 (0.91-1.63)	1.22 (0.90-1.66)	1.19 (0.85-1.68)
3	206	1.25 (0.94-1.66)	1.28 (0.94-1.73)	1.31 (0.94-1.83)
4	234	1.46 (1.10-1.93)	1.47 (1.09-1.98)	1.45 (1.04-2.01)
5	212	1.32 (0.99-1.75)	1.23 (0.91-1.68)	1.27 (0.90-1.78)
$P_{\text{trend}}^{\S}$		0.04	0.14	0.14

\*Adjusted for study design matching factor [period of blood draw (before September 30, 1997, September 30, 1997 or after) and age (as the timeline)] and season of serum collection (December-February, March-May, June-July, August-September).

<sup>†</sup>Additionally adjusted for BMI (ages 18-20), age at menarche, age at menopause, hormone replacement therapy use, benign breast disease, family history of breast cancer, combination of parity and age at first birth, smoking status, daily alcohol intake, and daily dietary calcium intake.

<sup>‡</sup><18.3, 18.3 to <23.5, 23.5 to <28.3, 28.3 to <33.7, and ≥33.7 ng/mL.

<sup>§</sup>Calculated based on assigning a median value to each category of measured values and modeling the resulting variable as continuous.

<sup>||</sup><28.2, 28.2 to <33.5, 33.5 to <39, 39 to <46.3, and ≥46.3 pg/mL.

In conclusion, we did not find that higher, prediagnostic levels of either vitamin D metabolite were associated with lower risk of postmenopausal breast cancer. We, however, cannot exclude an association in younger, premenopausal women or with long-term or earlier exposure. The relationship between breast cancer and circulating 25(OH)D should be explored in other large populations, including those with younger women, with multiple measurements, and with measurements at different intervals before diagnosis. Prospective studies of long-term sun exposure could also be valuable in clarifying the role of vitamin D in breast cancer risk, although there are certainly substantial difficulties in estimating such exposures. Lastly, investigations of genetic polymorphisms within the vitamin D pathway may yield additional insight as to whether vitamin D influences breast cancer risk.

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### References

- Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). *Cancer Causes Control* 2005; 16:83–95.
- Garland CF, Garland FC, Gorham ED, et al. The role of vitamin D in cancer prevention. *Am J Public Health* 2006;96:252–61.
- Welsh J, Wietzke JA, Zinser GM, Byrne B, Smith K, Narvaez CJ. Vitamin D-3 receptor as a target for breast cancer prevention. *J Nutr* 2003;133:2425–33S.
- Colston KW, Hansen CM. Mechanisms implicated in the growth regulatory effects of vitamin D in breast cancer. *Endocr Relat Cancer* 2002;9:45–59.
- Welsh J. Vitamin D and breast cancer: insights from animal models. *Am J Clin Nutr* 2004;80:1721–4S.
- Segersten U, Holm PK, Bjorklund P, et al. 25-Hydroxyvitamin D<sub>3</sub> 1 $\alpha$ -hydroxylase expression in breast cancer and use of non-1 $\alpha$ -hydroxylated vitamin D analogue. *Breast Cancer Res* 2005;7:R980–6.
- Friedrich M, Rafi L, Mitschele T, Tilgen W, Schmidt W, Reichrath J. Analysis of the vitamin D system in cervical carcinomas, breast cancer and ovarian cancer. *Recent Results Cancer Res* 2003;164: 239–46.
- Cui Y, Rohan TE. Vitamin D, calcium, and breast cancer risk: a review. *Cancer Epidemiol Biomarkers Prev* 2006;15:1427–37.
- Freedman DM, Dosemeci M, McGlynn K. Sunlight and mortality from breast, ovarian, colon, prostate, and non-melanoma skin cancer: a composite death certificate based case-control study. *Occup Environ Med* 2002;59:257–62.
- Gorham ED, Garland FC, Garland CF. Sunlight and breast cancer incidence in the USSR. *Int J Epidemiol* 1990;19:820–4.
- John EM, Schwartz GG, Dreon DM, Koo J. Vitamin D and breast cancer risk: the NHANES I epidemiologic follow-up study, 1971-to 1992. *National Health and Nutrition Examination Survey. Cancer Epidemiol Biomarkers Prev* 1999;8:399–406.
- Knight JA, Lesosky M, Barnett H, Raboud JM, Vieth R. Vitamin D and reduced risk of breast cancer: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev* 2007;16:422–9.
- Laden F, Spiegelman D, Neas LM, et al. Geographic variation in breast cancer incidence rates in a cohort of U.S. women 1. *J Natl Cancer Inst* 1997;89:1373–8.
- Hiatt RA, Krieger N, Lobaugh B, Drezner MK, Vogelmann JH, Orentreich N. Prediagnostic serum vitamin D and breast cancer. *J Natl Cancer Inst* 1998;90:461–3.
- Bertone-Johnson ER, Chen WY, Holick MF, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:1991–7.
- Gohagan JK, Prorok PC, Hayes RB, Kramer BS. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial of the National Cancer Institute: history, organization, and status 1. *Control Clin Trials* 2000;21:251–72S.
- Prorok PC, Andriole GL, Bresalier RS, et al. Design of the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial 1. *Control Clin Trials* 2000;21:273–309S.
- Hayes RB, Sigurdson A, Moore L, et al. Methods for etiologic and early marker investigations in the PLCO trial. *Mutat Res* 2005;592: 147–54.
- Korn EL, Graubard BI. *Analysis of health surveys*. New York: Wiley; 1999. p. 160–5.
- Hollis BW. Quantitation of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D by radioimmunoassay using radioiodinated tracers 1. *Methods Enzymol* 1997;282:174–86.
- Scotto J, Fears TR, Fraumeni JF Jr. Solar radiation. In: D. Schottenfeld, editor. *Cancer epidemiology and prevention*. Oxford: Oxford University Press; 1996. p. 355–72.
- Gloth FM III, Tobin JD, Sherman SS, Hollis BW. Is the recommended daily allowance for vitamin D too low for the homebound elderly? *J Am Geriatr Soc* 1991;39:137–41.
- Thomas C. *Taber's cyclopedic medical dictionary*. Philadelphia: FA Davis Co.; 1989.
- Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. *Cancer Causes Control* 2004;15:255–65.
- Platz EA, Hankinson SE, Hollis BW, et al. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and adenomatous polyps of the distal colorectum. *Cancer Epidemiol Biomarkers Prev* 2000;9:1059–65.
- Research Triangle Institute. SUDAAN language manual, release 9.0. Research Triangle Park (NC): Research Triangle Institute; 2004.
- Townsend K, Evans KN, Campbell MJ, Colston KW, Adams JS, Hewison M. Biological actions of extra-renal 25-hydroxyvitamin D-1 $\alpha$ -hydroxylase and implications for chemoprevention and treatment. *J Steroid Biochem Mol Biol* 2005;97:103–9.
- Janowsky EC, Lester GE, Weinberg CR, et al. Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk. *Public Health Nutr* 1999;2:283–91.
- Feskanich D, Ma J, Fuchs CS, et al. Plasma vitamin D metabolites and risk of colorectal cancer in women. *Cancer Epidemiol Biomarkers Prev* 2004;13:1502–8.
- Jacobs ET, Giuliano AR, Martinez ME, Hollis BW, Reid ME, Marshall JR. Plasma levels of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D and the risk of prostate cancer. *J Steroid Biochem Mol Biol* 2004;89–90:533–7.
- Braun MM, Helzlsouer KJ, Hollis BW, Comstock GW. Colon cancer and serum vitamin D metabolite levels 10-17 years prior to diagnosis. *Am J Epidemiol* 1995;142:608–11.
- Knight JA, Vachon CM, Vierkant RA, Vieth R, Cerhan JR, Sellers TA. No association between 25-hydroxyvitamin D and mammographic density. *Cancer Epidemiol Biomarkers Prev* 2006;15:1988–92.
- Looker AC, Dawson-Hughes B, Calvo MS, Gunter EW, Sahyoun NR. Serum 25-hydroxyvitamin D status of adolescents and adults in two seasonal subpopulations from NHANES III. *Bone* 2002;30:771–7.

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