Cancer Research

Plasma 25-Hydroxyvitamin D and Risk of Breast Cancer in Women Followed over 20 Years

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Abstract

Experimental evidence supports a protective role of 25-hydroxyvitamin D [25(OH)D] in breast carcinogenesis, but epidemiologic evidence is inconsistent. Whether plasma 25(OH)D interacts with breast tumor expression of vitamin D receptor (VDR) and retinoid X receptor-α (RXR) has not been investigated. We conducted a nested case–control study in the Nurses' Health Study, with 1,506 invasive breast cancer cases diagnosed after blood donation in 1989–1990, 417 of whom donated a second sample in 2000–2002. VDR and RXR expression were assessed by immunohistochemical staining of tumor microarrays ($n = 669$ cases). Multivariate relative risks (RR) and 95% confidence intervals (CI) were calculated using conditional logistic regression. Plasma 25(OH)D levels were not associated with breast cancer risk overall [top (≥32.7 ng/mL) vs. bottom (<17.2 ng/mL) quintile RR = 0.87; 95% CI, 0.67–1.13; P trend = 0.21]. 25(OH)D measured in summer (May–October) was significantly inversely associated with risk (top vs. bottom quintile RR = 0.66; 95% CI, 0.46–0.94; P trend = 0.01); winter levels (November–April) were not (RR = 1.10; 95% CI, 0.75–1.60; P trend = 0.64; P interaction = 0.03). 25(OH)D levels were inversely associated with risk of tumors with high expression of stromal nuclear VDR [≥30 ng/mL vs. <30 ng/mL RR (95% CI): VDR ≥ median = 0.67 (0.48–0.93); VDR < median = 0.98 (0.72–1.35), P heterogeneity = 0.12] and significantly stronger for summer measures (P heterogeneity = 0.01). Associations were not significantly different by RXR expression. No overall association was observed between plasma 25(OH)D and breast cancer risk. However, our results suggest women with high, compared with low, plasma 25(OH)D levels in the summer have a reduced breast cancer risk, and plasma 25(OH)D may be inversely associated with risk of tumors expressing high levels of VDR. *Cancer Res; 76(18); 5423–30. ©2016 AACR.*

Introduction

Vitamin D, a steroid hormone, is an essential component of the endocrine system, responsible for calcium level maintenance, and is hypothesized to play a role in many other body systems. The biologically active metabolite, $1,25(OH)_2D$, binds to the vitamin D receptor (VDR), which is expressed in many tissues throughout the body, including normal mammary tissue. Activated VDR forms a heterodimer with the retinoid X receptor (RXR), then binds to vitamin D response elements, resulting in transcriptional activation. Experimental evidence supports a role of vitamin D in normal breast development as well as in the inhibition of breast carcinogenesis. VDR is critical for mammary gland development from puberty to pregnancy and involution (1, 2). VDR-regulated genes and proteins represent key inhibitors of mitogenic signaling (3), and VDR agonist administration in several cancer cell lines results in cell-cycle arrest, differentiation, apoptosis, and autophagy (3). $1,25(OH)_2D$ reduces proliferation and promotes differentiation and apoptosis in breast cancer cell lines (4–6), and inhibits growth of both estrogen receptor $(ER)^+$ and ER^- tumors in animal models (7–9).

While $1,25(OH)_2D$ is tightly regulated to maintain calcium homeostasis, 25(OH)D serves as a pool for biologically active vitamin D and thus is a marker of overall vitamin D status (10, 11). Further, normal mammary cells express the critical enzyme 1α-hydroxylase, encoded by *CYP27B1*, and can therefore metabolize 25(OH)D into $1,25(OH)_2D$ within the breast (3). The association between circulating 25(OH)D and breast cancer has been assessed in several prospective studies with conflicting results. While no association has been observed in many studies (12–20), significant inverse associations were observed in two studies (21, 22), and a suggested inverse association was observed in our prior analysis in the Nurses' Health Study (NHS) with 701 cases (23). Further, although stronger associations among older or postmenopausal women were reported in two meta-analyses (24, 25) and our prior NHS analysis (23), stronger associations among younger women were observed in two individual studies (20, 21). Thus, results to date are inconsistent and it is not clear if there are specific subgroups that may benefit from higher plasma 25(OH)D.

The importance of the timing of exposure and associations with specific tumor subtypes, including by expression of VDR

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and RXR, have not been thoroughly investigated, and may have contributed to the inconsistent results. Therefore, in a case–control study nested within the NHS with more than double the number of cases in our prior publication (23), we examined the association between 25(OH)D and breast cancer risk by using blood samples collected 10 years apart with 20 years of follow-up, and tumor tissue expression of VDR and RXR.

Patients and Methods

Study population

In 1976, 121,701 female, registered nurses, ages 30 to 55 years, were enrolled in NHS. Biennially, participants completed mailed questionnaires on lifestyle, diet, reproductive history, and disease diagnoses. In 1989–1990, 32,826 women ages 43 to 69 years donated blood samples (26). Briefly, each woman arranged to have her blood drawn and shipped overnight with an ice-pack to our laboratory, where it was processed and archived in liquid nitrogen freezers; 97% of samples arrived within 26 hours of collection. In 2000–2002, a second sample was collected, using a similar protocol, from 18,743 of these women, ages 53 to 80 years (27). The follow-up rate among the 32,826 women was 97% in 2010. Information on breast cancer risk factors, including anthropometrics, reproductive history, and diet, was collected from biennial and blood collection questionnaires. The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital (Boston, MA); completion of the self-administered questionnaire and blood collection was considered to imply informed consent.

Case and control selection

Cases had no reported cancer (other than nonmelanoma skin) before blood collection and were diagnosed with invasive breast cancer between the first collection and June 2010. The subset of cases diagnosed from 1998 to 2000 was not included for financial reasons. Overall, 1,506 cases of breast cancer were reported and confirmed by medical record review. Time from blood collection to diagnosis ranged from <1 month to 20 years (median = 7.9 years). One control was matched per case by the following factors (at both collections for those with two blood samples): age (±2 years), menopausal status and menopausal hormone therapy (HT) use at blood collection and diagnosis (premenopausal, postmenopausal/not taking HT, postmenopausal/taking HT, unknown), and month (±1), time of day (±2 hours), and fasting status at blood collection (<10 hours after a meal or unknown, ≥10 hours).

25(OH)D assay

Plasma 25(OH)D was assayed in seven batches in three laboratories. The first two batches were assayed in the laboratory of Dr. Michael Hollick. After extraction with absolute ethanol, the extract was treated with a protein-binding assay with a high affinity for 25(OH)D. The third batch was assayed in the laboratory of Dr. Bruce Hollis by radioimmunoassay with radioiodinated tracers after acetonitrile extraction. The remaining batches were assayed at Heartland Assays Inc. using a chemiluminescence immunoassay (28). Coefficients of variations (CV) from blinded quality control (QC) replicates were 18.1% to 21.8% for the first three batches and 6.5% to 9.4% for the remaining batches. Ten samples from controls in each of the first three batches were rerun in the fourth batch to assess laboratory drift and recalibrate the original batches using linear regression, applying the resulting slope and intercept to the original data (29). Correlation across repeated samples between batches was 0.79. Distribution of season of collection across batches was similar to the distributions for each blood collection (1st blood, 53% summer collections; 2nd blood, 66% summer collections).

Tumor data

Cases with available tumor tissue were included in tissue microarrays (TMA) constructed at the Dana Farber Harvard Cancer Center Tissue Microarray Core Facility in Boston, MA, using methods previously described (30, 31). Briefly, three 0.6 mm diameter cores were obtained from each sample, and inserted into TMA blocks that were subsequently cut into 5-μm paraffin sections prior to immunohistochemical staining. Immunostaining for ER, progesterone receptor (PR), HER2, VDR, and RXR was performed on a Dako Autostainer (Dako Corporation). A study pathologist evaluated invasiveness and histologic grade, as well as read manually the stains for ER, PR, and HER2 (31). For cases with no tumor tissue available, detailed information on case characteristics, including invasiveness, histologic grade, and ER, PR, HER2 status, was extracted from pathology reports. ER and PR were considered positive if ≥1% cells stained for the receptor; HER2 was classified as positive if ≥10% of cells stained for the receptor. We used antibodies for cytoplasmic VDR (mouse monoclonal D-6, sc-13133; Santa Cruz Biotechnology; 1:400 dilution), nuclear VDR (rabbit polyclonal NBP1–19478; Novus Biologicals; 1:500 dilution), and RXR-alpha (rabbit monoclonal D-20, sc-553; Santa Cruz Biotechnology; 1:3,200 dilution). VDR and RXR expression was evaluated by an automated computational image analysis system (Definiens Tissue Studio software); spearman correlations with manual analysis [scored negative, low positive (1%–9% of cells positive), or positive (≥10% of cells positive)] by a study pathologist were calculated for one TMA (n = 120–133 cases). The VDR cytoplasmic stain obscured cellular borders in strongly positive cases making it difficult to accurately segment cells. Therefore, expression was classified by average IHC intensity for epithelial and stromal cells separately, and means were calculated across evaluable cores and categorized as high (≥median) versus low (<median). For epithelial cells, the correlation between Definiens and manual read was moderate ($r = 0.52$); mean IHC intensities, quantified by Definiens, across manually scored categories (negative, low positive, positive) were: 0.21, 0.36, and 0.87. For stromal cells, this stain was challenging for both manual and automated reading of expression, resulting in a lower correlation ($r = 0.10$); average IHC intensity across manually scored categories (negative, low positive, positive) were: 0.11, 0.10, and 0.12. VDR nuclear expression was assessed by percentage of cells that stained positive for epithelial and stromal cells separately, and categorized as high (≥median) versus low (<median). The correlation between Definiens and manual read was moderate ($r = 0.51$ for epithelial; $r = 0.46$ for stromal); mean percent cells positive, quantified by Definiens, across manually scored categories (negative, low positive, positive) were: 0.15, 0.32, and 0.57 for epithelial and 0.21, 0.31, and 0.43 for stromal. When further assessed by ≥10% cells staining positive, the concordance between the manual and automated reads was 87.5% for epithelial and 78.6% for stromal. Similar to VDR cytoplasmic staining in stromal cells, the RXR stain proved challenging for both manual and automated reading of expression, which contributed to a lower correlation ($r = 0.23$); mean percent cells high positive,

quantified by Definiens, across manually scored categories (negative, low positive, positive) were: 0.03, 0.04, and 0.05. Due to poor distinction by cell type, expression was assessed in stromal and epithelial cells combined. Most tumor specimens had widespread RXR expression; therefore, we used staining intensity to identify the percentage of cells that stained high positive and categorized tumors by high (≥median) versus low (<median). Given that neither the RXR nor the VDR cytoplasmic stain in the stroma was well measured, we have included these only as secondary analyses.

Statistical analysis

Included in the analyses were 1,506 distinct cases and 1,506 controls. Of these, 1,502 cases and 1,502 controls had 25(OH)D measures from the first blood collection; 413 cases and 413 controls had two 25(OH)D measures from the first and second collections; 4 cases and 4 controls had only second blood measures. Using all available data, 1,215 cases had 25(OH)D measured <10 years before diagnosis, and 704 cases had 25(OH)D measured ≥10 years before diagnosis.

We calculated the intraclass correlation coefficient (ICC) over 10 years among women with two bloods whose samples were collected in concordant seasons. Quintile cutpoints for 25(OH)D were established among all controls. We also assessed ≥30 ng/mL, deemed "sufficient" by the Endocrine Society (32), and "adequate to high" by the Institute of Medicine (33). Relative risks (RR) and 95% confidence intervals (CI) were calculated from multivariate conditional logistic regression models adjusted for the following breast cancer risk factors: age at menarche, body mass index (BMI) at age 18, weight change from age 18 to blood draw, parity and age at first birth, family history of breast cancer, and history of benign breast disease. Given that outdoor physical activity may serve as a proxy for sun exposure (34), an important source of vitamin D, our main model did not include physical activity; however, we adjusted for it in sensitivity analyses. Tests for trend were conducted by a Wald test on quintiles medians, modeled continuously. We conducted separate analyses for 25(OH)D measured <10 years and ≥10 years before diagnosis, using all available measures (i.e., first and second blood). We also conducted analyses of all available cases and controls, using one 25(OH)D measure, or when available using the average of two measures. In sensitivity analyses, we used a sine-cosine series to standardize 25(OH)D levels across season (35), and defined quintiles among controls. Unconditional logistic regression models, additionally adjusting for matching factors, were used for stratified analyses.

We evaluated if the 25(OH)D levels and breast cancer association varied by follow-up time, season of blood collection, and lifestyle factors, including BMI, alcohol intake, supplemental vitamin D use, HT use, and menopausal status, using likelihood ratio tests. We assessed the associations with 25(OH)D by ER status, and luminal A (ER-positive or PR-positive, HER2-negative, and grade 1 or 2), luminal B (ER-positive or PR-positive and either HER2-positive or HER2-negative and grade 3), and triple-negative tumors (ER-negative, PR-negative, and HER2-negative; ref. 36). We also examined the associations by tumor invasiveness, histologic grade, tumor size, and lymph node status. We investigated if associations differed by VDR and RXR expression. To test whether associations differed by tumor subtype, we used polychotomous logistic regression (37) with a likelihood ratio test comparing a model with separate slopes for the 25(OH)D in each case group with one with a common slope. All *P* values were based on two-sided tests and were considered statistically significant if ≤0.05. Analyses were conducted by SAS version 9.3 (SAS Institute) or STATA version 11.0 (StataCorp).

Results

At the first collection, cases were more likely than controls to be nulliparous and current smokers, and have a history of benign breast disease and family history of breast cancer (Table 1). By the second collection, women were, on average, 10 years older, slightly heavier, and less likely to smoke. Approximately two-thirds of women were postmenopausal at the first blood collection; nearly all women were postmenopausal at the second collection. The 10-year ICC among 238 controls with 2 blood samples collected in concordant seasons was 0.51, 95% CI (0.42–0.60), with a mean increase (SD) of 12% (52%) in 25(OH)D levels from first to second collection.

Among all cases, plasma 25(OH)D levels were not significantly associated with breast cancer risk [top (≥32.7 ng/mL) vs. bottom (<17.5 ng/mL) quintile, all cases RR = 0.87; 95% CI, 0.67–1.13, *P* trend = 0.21; Table 2]. Estimates were similar for cases diagnosed less than 10 years [RR (95% CI) = 0.84 (0.63–1.12)] and 10 to 20 years [0.84 (0.58–1.21)] after blood collection. When

Table 1. Characteristics of breast cancer cases and matched controls at each blood collection in the NHS, mean (SD) or %

	1989–1990 blood draw		2000–2002 blood draw	
	Cases	**Controls**	**Cases**	**Controls**
N	1,502	1,502	417	417
Age at blood draw, y	56.7 (7.1)	56.8 (7.0)	66.6 (6.9)	66.7 (6.8)
BMI at age 18, kg/m^2	21.1 (2.7)	21.3 (3.0)	–[a]	–
BMI at blood draw, kg/m^2	25.5 (4.5)	25.2 (4.7)	26.9 (5.1)	26.4 (5.5)
Age at menarche, y	12.5 (1.6)	12.6 (1.4)	–	–
Nulliparous, %	10.7	8.9	–	–
Age at first birth, y	24.9 (3.4)	24.7 (3.1)	–	–
Postmenopausal, %	67.3	67.1	98.0	98.3
Age at menopause[b], y	49.5 (4.4)	49.1 (4.5)	50.3 (3.9)	49.5 (4.5)
Current smokers, %	13.3	10.9	5.0	4.8
Alcohol consumption, g/d	5.6 (9.6)	5.2 (8.4)	6.4 (10.2)	5.5 (9.0)
History of benign breast disease, %	46.4	37.4	60.9	54.0
Family history of breast cancer, %	15.9	9.9	22.1	13.4
Plasma 25(OH)D, ng/mL [median (10th–90th percentile)]	24.0 (13.5–35.4)	24.9 (13.7–37.8)	25.3 (14.5–37.7)	25.7 (14.5–41.0)

[a]–, variables did not change after 1st blood collection.

[b]Among postmenopausal women with natural menopause or bilateral oophorectomy.

Table 2. Multivariate[a] RRs of breast cancer and 95% CI according to quintile of plasma 25(OH)D (ng/mL) in the NHS, by follow-up period (<10 vs. ≥10 years)

		Q1 <17.5	Q2 17.5–<22.5	Q3 22.5–<26.7	Q4 26.7–<32.7	Q5 ≥32.7	*P* trend
<10 years (1,215 cases)	N (ca/co)	201/198	252/234	271/254	264/267	227/262	
	RR (95% CI)	1.00	1.03 (0.78–1.37)	1.03 (0.78–1.37)	1.00 (0.75–1.33)	0.84 (0.62–1.12)	0.17
≥10 years (704 cases)	N (ca/co)	169/173	161/152	142/117	128/122	104/140	
	RR (95% CI)	1.00	1.13 (0.83–1.55)	1.31 (0.94–1.84)	1.17 (0.82–1.65)	0.84 (0.58–1.21)	0.44
Overall[b] (1,506 cases)	N (ca/co)	280/287	339/316	359/297	304/309	244/297	
	RR (95% CI)	1.00	1.10 (0.86–1.39)	1.17 (0.92–1.50)	1.05 (0.82–1.36)	0.87 (0.67–1.13)	0.21
Winter[c]	N (ca/co)	173/189	182/170	149/137	121/119	87/88	
	RR (95% CI)	1.00	1.19 (0.88–1.60)	1.12 (0.81–1.55)	1.13 (0.81–1.59)	1.10 (0.75–1.60)	0.64
Summer[c]	N (ca/co)	106/95	155/146	189/159	180/190	153/209	
	RR (95% CI)	1.00	0.92 (0.64–1.32)	1.08 (0.76–1.54)	0.85 (0.59–1.21)	0.66 (0.46–0.94)	0.01

[a]Adjusted for age at menarche (continuous), BMI at age 18 (continuous), weight change from age 18 to blood draw (continuous), parity and age at first birth (nulliparous, 1–2 children/<25 years, 1–2 children/≥25 years, ≥3 children/<25 years, ≥3 children/≥25 years), family history of breast cancer (yes/no), history of benign breast disease (yes/no).

[b]Overall values use the 1990 blood collection or the average of 1990 and 2000 blood collections if available and collected in concordant seasons.

[c]Unconditional logistic regression, additionally adjusting for matching factors: fasting status (yes/no), time of blood draw (1 am–8 am, 9 am–noon, 1 pm–midnight), age at blood draw (continuous), menopausal status and postmenopausal hormone therapy use (premenopausal/unknown menopause status, postmenopausal/no hormone use, postmenopausal/current hormone therapy); *P* interaction with season = 0.03.

examined by deciles, women in the highest (vs. lowest) decile had a suggested lower risk of breast cancer [≥37.8 vs. <13.7 ng/mL, RR = 0.69, 95% CI (0.47–1.01), *P* trend = 0.06]. Further adjustment for physical activity, plasma carotenoids, or mammography screening did not alter the results. Results also were similar when we used batch-specific cutpoints or season-standardized 25(OH)D levels (data not shown). The interaction between plasma 25(OH)D levels and season of blood collection on breast cancer risk was significant (*P* interaction = 0.03), with no association observed for samples collected in winter months [November–April; top vs. bottom quintile RR (95% CI) = 1.10 (0.75–1.60), *P* trend = 0.64], and a significant inverse association for samples collected in summer months [May–October; comparable RR = 0.66, 95% CI (0.46–0.94), *P* trend = 0.01)].

Interactions between menopausal status, HT use, alcohol intake, supplemental vitamin D use, BMI, and 25(OH)D levels were not significant (data not shown). Associations were not significantly different by most tumor characteristics, including ER status, histology, nodal involvement, differentiation, molecular subtype, and lethal outcome (Table 3). However, the association was significantly stronger among larger tumors (≥2 vs. <2 cm) both overall [≥30 vs. <30 ng/mL RR (95% CI) ≥2 cm = 0.71 (0.52–0.95); <2 cm = 0.96 (0.79–1.15); *P* heterogeneity = 0.02] and with summer levels [≥2 cm = 0.55 (0.37–0.83); <2 cm = 0.83 (0.66–1.05); *P* heterogeneity = 0.02].

Among 669 cases with tumor VDR and/or RXR expression, we observed no association overall [≥30 vs. <30 ng/mL RR (95% CI) = 0.91 (0.73–1.13)] and a suggestively inverse association with summer levels [0.79 (0.59–1.06); Table 4]. No significant difference was observed by cytoplasmic VDR expression in epithelial cells [25(OH)D ≥ vs. <30 ng/mL, RR (95% CI) ≥median = 0.93 (0.70–1.24); <median = 0.86 (0.63–1.17); *P* heterogeneity = 0.99]. However, suggestive or significantly stronger associations were observed for tumors with high nuclear VDR expression in stromal (but not epithelial) tissue [all blood samples ≥30 ng/mL, ≥median VDR, RR (95% CI) = 0.67 (0.48–0.93) vs. <median VDR = 0.98 (0.72–1.35), *P* heterogeneity = 0.12; summer levels ≥median VDR = 0.55 (0.35–0.85) vs. <median VDR = 1.12 (0.75–1.69), *P* heterogeneity = 0.01]. Similar results were observed when we defined high expression by the top 25th or 10th percentile of expression (data not shown). In secondary analyses, associations with 25(OH)D were not significantly different by tumor RXR expression or cytoplasmic VDR expression in stromal tissue (*P* heterogeneity ≥ 0.21). No significant heterogeneity was observed by combined VDR (nuclear)/RXR expression overall (*P* heterogeneity = 0.29), with suggested inverse associations observed for tumors with high VDR expression, regardless of RXR expression [≥30 ng/mL RR (95% CI) high/high = 0.68 (0.43–1.06); high/low = 0.68 (0.42–1.11)]. VDR expression was not correlated with tumor size (cytoplasmic epithelial, and nuclear, epithelial and stromal, r range −0.02 to −0.12).

Discussion

Although no association was observed overall in this large prospective study of plasma 25(OH)D levels and breast cancer risk, women with relatively high, compared with low, levels in summer months were at reduced risk. Further, an inverse association was apparent for tumors with high levels of nuclear VDR expression in tumor stromal cells.

In our previous analysis of 701 cases within the NHS, we observed a suggestive inverse association between plasma 25(OH)D and breast cancer risk (23), and a significant inverse association was observed in the French E3N study [top vs. bottom tertile RR = 0.73, 95% CI (0.55–0.96), *N* = 636 cases; ref. 21]. In the Multiethnic Cohort Study, an inverse association was observed among Caucasian women [per 10 ng/mL increase RR = 0.66, 95% CI (0.48–0.90), *N* = 147 cases], but not women of other races (22). In this updated NHS analysis, with more than double the number of cases, we did not observe a significant association overall, consistent with many other prospective studies of circulating 25(OH)D and breast cancer risk (12–20), with case numbers ranging from 211 (15) to 1,391 (19). We also did not observe significant differences in exposure either <10 years before diagnosis or 10 to 20 years before diagnosis. However, we observed heterogeneity by season of blood collection, with a significant inverse association observed with summer measures of plasma 25(OH)D.

Vitamin D sources include diet, encompassing both naturally occurring and supplemental sources, and endogenous production as a result of sun exposure. Given the latter, circulating 25(OH)D

Table 3. RR of breast cancer and 95% CI according to plasma 25(OH)D (ng/mL) and tumor subtypes in the NHS

		Overall			Winter levels			Summer levels		
		<30	≥30	*P* het	<30	≥30	*P* het	<30	≥30	*P* het
All cases	*N* (ca/co[a])	1,133/1,099	373/407		576/576	136/127		552/519	231/280	
	RR (95% CI)	1.00	0.90 (0.76–1.08)	–	1.00	1.10 (0.83–1.45)	–	1.00	0.77 (0.62–0.96)	–
ER^+	*N* (cases)	859	282		430	105		426	172	
	RR (95% CI)	1.00	0.89 (0.74–1.08)		1.00	1.14 (0.85–1.54)		1.00	0.74 (0.59–0.94)	
ER^-	*N* (cases)	198	63		104	23		93	40	
	RR (95% CI)	1.00	0.87 (0.63–1.20)	0.87	1.00	0.99 (0.59–1.65)	0.70	1.00	0.81 (0.53–1.22)	0.76
Ductal	*N* (cases)	927	297		468	109		454	184	
	RR (95% CI)	1.00	0.88 (0.73–1.06)		1.00	1.09 (0.81–1.46)		1.00	0.76 (0.60–0.96)	
Lobular	*N* (cases)	139	54		75	17		64	35	
	RR (95% CI)	1.00	1.04 (0.73–1.48)	0.15	1.00	0.96 (0.53–1.73)	0.93	1.00	0.99 (0.63–1.57)	0.19
Well differentiated	*N* (cases)	283	88		151	33		132	54	
	RR (95% CI)	1.00	0.85 (0.65–1.12)		1.00	0.99 (0.64–1.54)		1.00	0.76 (0.53–1.08)	
Moderately differentiated	*N* (cases)	476	152		239	60		233	90	
	RR (95% CI)	1.00	0.86 (0.69–1.07)		1.00	1.17 (0.82–1.67)		1.00	0.71 (0.53–0.95)	
Poorly differentiated	*N* (cases)	221	61		114	22		106	36	
	RR (95% CI)	1.00	0.80 (0.58–1.10)	0.85	1.00	0.96 (0.57–1.61)	0.60	1.00	0.65 (0.43–1.00)	0.75
Node negative	*N* (cases)	858	296		426	108		429	184	
	RR (95% CI)	1.00	0.94 (0.78–1.12)		1.00	1.19 (0.89–1.60)		1.00	0.78 (0.62–0.99)	
Node positive	*N* (cases)	273	77		150	28		121	47	
	RR (95% CI)	1.00	0.79 (0.59–1.06)	0.26	1.00	0.82 (0.51–1.31)	0.18	1.00	0.76 (0.52–1.12)	0.61
Tumor <2 cm	*N* (cases)	818	289		413	100		403	184	
	RR (95% CI)	1.00	0.96 (0.79–1.15)		1.00	1.14 (0.84–1.54)		1.00	0.83 (0.66–1.05)	
Tumor ≥2 cm	*N* (cases)	277	69		143	31		131	37	
	RR (95% CI)	1.00	0.71 (0.52–0.95)	0.02	1.00	1.01 (0.64–1.59)	0.62	1.00	0.55 (0.37–0.83)	0.02
Luminal A	*N* (cases)	443	142		227	56		214	84	
	RR (95% CI)	1.00	0.88 (0.69–1.10)		1.00	1.13 (0.78–1.63)		1.00	0.72 (0.53–0.97)	
Luminal B	*N* (cases)	139	45		71	15		68	28	
	RR (95% CI)	1.00	0.92 (0.63–1.33)		1.00	1.03 (0.56–1.90)		1.00	0.82 (0.51–1.33)	
Triple negative	*N* (cases)	80	19		44	10		35	9	
	RR (95% CI)	1.00	0.68 (0.40–1.16)	0.44	1.00	1.06 (0.50–2.25)	0.21	1.00	0.45 (0.21–0.96)	0.52
Lethal/recurrent	*N* (cases)	200	64		101	26		98	37	
	RR (95% CI)	1.00	0.85 (0.62–1.17)		1.00	1.08 (0.65–1.79)		1.00	0.71 (0.47–1.08)	
Not lethal or recurrent	*N* (cases)	933	309		475	110		454	194	
	RR (95% CI)	1.00	0.91 (0.76–1.09)	0.90	1.00	1.09 (0.81–1.46)	0.70	1.00	0.79 (0.63–1.00)	0.56

NOTE: Adjusted for fasting status (yes/no), time of blood draw (1 am–8 am, 9 am–noon, 1 pm–midnight), age at blood draw (continuous), menopausal status and postmenopausal hormone therapy use (premenopausal/unknown menopause status, postmenopausal/no hormone use, postmenopausal/current hormone therapy), age at menarche (continuous), BMI at age 18 (continuous), weight change from age 18 to blood draw (continuous), parity and age at first birth (nulliparous, 1–2 children/<25 years, 1–2 children/≥25 years, ≥3 children/<25 years, ≥3 children/≥25 years), family history of breast cancer (yes/no), history of benign breast disease (yes/no).

Overall additionally adjusted for season (winter/summer).

[a]Number of controls remains the same in all analyses below.

fluctuates seasonally, as is evident in our controls where mean levels were 22.8 ng/mL in samples collected November through April, and 27.5 ng/mL in samples collected May through October. While reproducibility over time is good for 25(OH)D (20, 38), ICCs are higher when blood samples are collected in concordant seasons (20). Studies of 25(OH)D and breast cancer have addressed this seasonal variation in a number of ways, including adjusting for season in the analysis (12, 22) and standardizing all blood values for season (19, 20, 39). In secondary analyses, several studies have stratified by season (12, 14, 17, 20, 23) or used season-specific cutpoints (12, 14, 16, 17, 23). While many of these studies did not observe significant differences by season (12, 14, 17, 23), summer levels in the NYUWHS study were suggestively inversely associated with breast cancer risk [≥75 nmol/L vs. <50 nmol/L RR (95% CI) = 0.69 (0.45–1.07)], whereas winter levels were not [0.91 (0.58–1.44); ref. 20] similar to what we observed in the current analysis. One possible explanation for the differences in association we observed by season is that women with low 25(OH)D levels in the summer are likely truly low year-round, whereas women with low levels in the winter are more likely to be misclassified if their endogenous production increases with sun exposure in the summer. These seasonal differences and any potential variation by latitude should be explored further in future studies.

A few prior studies have examined the association with 25(OH)D by ER status of breast tumors, with no significant heterogeneity (14, 17, 19, 20, 23), as we observed in the current analysis. To our knowledge, ours is the first study to investigate potential heterogeneity by grade, tumor size, luminal A/B and triple-negative subtypes, and lethal/recurrent outcomes. For most tumor characteristics, we did not observe significant heterogeneity in the overall association. The exception was tumor size, where we

Table 4. RR of breast cancer and 95% CI according to plasma 25(OH)D (ng/mL) and tumor VDR expression in the NHS

		Overall			Winter levels			Summer levels		
		<30	≥30	*P* het	<30	≥30	*P* het	<30	≥30	*P* het
All cases with tissue	*N* (ca/co[a])	505/1,099	164/407		278/576	65/127		226/519	98/280	
	RR (95% CI)	1.00	0.91 (0.73–1.13)	–	1.00	1.08 (0.76–1.52)	–	1.00	0.79 (0.59–1.06)	–
VDR cytoplasmic, epithelial										
High	*N* (cases)	253	84		146	35		107	48	
	RR (95% CI)	1.00	0.93 (0.70–1.24)		1.00	1.01 (0.66–1.57)		1.00	0.85 (0.58–1.25)	
Low	*N* (cases)	220	69		113	26		106	43	
	RR (95% CI)	1.00	0.86 (0.63–1.17)	0.99	1.00	1.13 (0.69–1.85)	0.89	1.00	0.72 (0.48–1.07)	0.69
VDR nuclear, stromal										
High	*N* (cases)	208	52		106	21		102	31	
	RR (95% CI)	1.00	0.67 (0.48–0.93)		1.00	0.90 (0.53–1.53)		1.00	0.55 (0.35–0.85)	
Low	*N* (cases)	200	68		123	21		76	47	
	RR (95% CI)	1.00	0.98 (0.72–1.35)	0.12	1.00	0.80 (0.47–1.33)	0.66	1.00	1.12 (0.75–1.69)	0.01
VDR nuclear, epithelial										
High	*N* (cases)	198	56		110	21		87	35	
	RR (95% CI)	1.00	0.79 (0.56–1.10)		1.00	0.87 (0.51–1.48)		1.00	0.76 (0.49–1.18)	
Low	*N* (cases)	188	60		107	17		81	43	
	RR (95% CI)	1.00	0.87 (0.62–1.20)	0.50	1.00	0.72 (0.41–1.27)	0.60	1.00	0.94 (0.62–1.43)	0.31

NOTE: Adjusted for fasting status (yes/no), time of blood draw (1 am–8 am, 9 am–noon, 1 pm–midnight), age at blood draw (continuous), menopausal status and postmenopausal hormone therapy use (premenopausal/unknown menopause status, postmenopausal/no hormone use, postmenopausal/current hormone therapy), age at menarche (continuous), BMI at age 18 (continuous), weight change from age 18 to blood draw (continuous), parity and age at first birth (nulliparous, 1–2 children/<25 years, 1–2 children/≥25 years, ≥3 children/<25 years, ≥3 children/≥25 years), family history of breast cancer (yes/no), history of benign breast disease (yes/no).

Overall additionally adjusted for season (winter/summer).

[a]Number of controls remains the same in all analyses below.

observed an inverse association between plasma 25(OH)D and large tumors. While larger tumor size may represent a more aggressive tumor, we did not consistently observe inverse associations with other markers of more aggressive tumors, and thus this may have been a chance finding.

This is the first study to investigate the association of 25(OH)D levels with breast cancer risk by tumor expression of VDR and RXR. These receptors play an important role in the function of vitamin D as the heterodimer of VDR and RXR, along with the ligand $1,25(OH)_2D$, bind to vitamin D response elements and affect transcriptional activation. Further, they are relevant to breast tissue in particular, as VDR-null mice have excessive mammary gland proliferation and branching as well as impaired apoptosis during puberty, pregnancy, and involution (1, 2). Although we measured these receptors in tumor tissue, we are assuming tumor expression is a proxy for the surrounding normal tissue at the time of cancer development, which is likely a reasonable assumption given these receptors are not typically mutated or overexpressed in breast tumors (40). We hypothesized that higher expression of VDR and RXR would result in a higher likelihood for 25(OH)D to inhibit tumor growth; therefore, higher 25(OH)D levels would be more strongly inversely associated with tumors that expressed VDR and/or RXR. Consistent with our hypothesis, we observed suggestively stronger inverse associations between 25(OH)D levels and breast tumors expressing high levels of nuclear VDR. Although we did not observe significant associations by expression levels of VDR in the cytoplasm of stromal cells or RXR, the difficulties we experienced in reading these stains may have inhibited our ability to detect an important association.

We quantified VDR expression in both the cytoplasm and nuclear compartments. Evidence suggests VDR is present in the cytoplasm, but migrates to the nucleus once activated (41), suggesting that nuclear expression is a marker of active receptors. We also assessed expression in both stromal and epithelial cells, finding stronger inverse associations with 25(OH)D and stromal nuclear VDR expression. Interactions between stromal and epithelial cells are key in the development and maintenance of mammary glands (42), with stromal fibroblasts and adipocytes providing critical growth factor signals (3). Evidence also suggests that vitamin D activity, specifically, in the stromal tissue is important (3). Breast adipocytes express CYP27B1, which generates $1,25(OH)_2D$, allowing adipocyte VDR signaling to contribute to mammary epithelial cell growth regulation (43). Thus, while it may be a chance finding that our results were stronger with stromal, but not epithelial, VDR expression, there is biological rationale that this tissue compartment may be critical in the role vitamin D plays in reducing breast cancer risk.

Our study has several strengths, including a large sample size. It is the first comprehensive assessment of timing of 25(OH)D exposure, with blood samples collected 10 years apart and 20 years of follow-up. It is also the first study to investigate the association by tumor expression of VDR and RXR. Although we cannot eliminate the possibility of residual confounding, the comprehensive information on breast cancer risk factors in the NHS allowed for thorough adjustment for potential confounders. While only one blood sample was available for the majority of women, reproducibility over a 2- to 3-year period in the NHS is very good (38). Further, we reduced measurement error by averaging the values of two blood samples 10 years apart, when available.

The results of this large prospective analysis suggest that overall 25(OH)D levels are not importantly associated with breast cancer. However, our results also suggest that 25(OH)D may be important in some contexts. Our finding of inverse associations when 25(OH)D levels are measured during months with higher average sun exposure, and among the subset of breast cancers with high VDR expression warrants follow-up in additional studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The study sponsors had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the article; or the decision to submit the article for publication.

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References

1. Zinser G, Packman K, Welsh J. Vitamin D(3) receptor ablation alters mammary gland morphogenesis. Development 2002;129:3067–76.
2. Zinser GM, Welsh J. Accelerated mammary gland development during pregnancy and delayed postlactational involution in vitamin D3 receptor null mice. Mol Endocrinol 2004;18:2208–23.
3. Welsh J. Cellular and molecular effects of vitamin D on carcinogenesis. Arch Biochem Biophys 2012;523:107–14.
4. Welsh J, Wietzke JA, Zinser GM, Smyczek S, Romu S, Tribble E, et al. Impact of the Vitamin D3 receptor on growth-regulatory pathways in mammary gland and breast cancer. J Steroid Biochem Mol Biol 2002;83:85–92.
5. Jensen SS, Madsen MW, Lukas J, Binderup L, Bartek J. Inhibitory effects of 1alpha,25-dihydroxyvitamin D(3) on the G(1)-S phase-controlling machinery. Mol Endocrinol 2001;15:1370–80.
6. Colston KW, Berger U, Coombes RC. Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet 1989;1:188–91.
7. Colston KW, Pirianov G, Bramm E, Hamberg KJ, Binderup L. Effects of Seocalcitol (EB1089) on nitrosomethyl urea-induced rat mammary tumors. Breast Cancer Res Treat 2003;80:303–11.
8. Nolan E, Donepudi M, VanWeelden K, Flanagan L, Welsh J. Dissociation of vitamin D3 and anti-estrogen mediated growth regulation in MCF-7 breast cancer cells. Mol Cell Biochem 1998;188:13–20.
9. VanWeelden K, Flanagan L, Binderup L, Tenniswood M, Welsh J. Apoptotic regression of MCF-7 xenografts in nude mice treated with the vitamin D3 analog, EB1089. Endocrinology 1998;139:2102–10.
10. Reichel H, Koeffler HP, Norman AW. The role of the vitamin D endocrine system in health and disease. N Engl J Med 1989;320:980–91.
11. Hollis BW. Assessment of vitamin D nutritional and hormonal status: What to measure and how to do it. Calcif Tissue Int 1996;58:4–5.
12. Freedman DM, Chang SC, Falk RT, Purdue MP, Huang WY, McCarty CA, et al. Serum levels of vitamin D metabolites and breast cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. Cancer Epidemiol Biomarkers Prev 2008;17:889–94.
13. Chlebowski RT, Johnson KC, Kooperberg C, Pettinger M, Wactawski-Wende J, Rohan T, et al. Calcium plus vitamin D supplementation and the risk of breast cancer. J Natl Cancer Inst 2008;100:1581–91.
14. McCullough ML, Stevens VL, Patel R, Jacobs EJ, Bain EB, Horst RL, et al. Serum 25-hydroxyvitamin D concentrations and postmenopausal breast cancer risk: A nested case control study in the Cancer Prevention Study-II Nutrition Cohort. Breast Cancer Res 2009;11:R64.
15. Agborsangaya CB, Surcel HM, Toriola AT, Pukkala E, Parkkila S, Tuohimaa P, et al. Serum 25-hydroxyvitamin D at pregnancy and risk of breast cancer in a prospective study. Eur J Cancer 2010;46:467–70.
16. Almquist M, Bondeson AG, Bondeson L, Malm J, Manjer J. Serum levels of vitamin D, PTH and calcium and breast cancer risk-a prospective nested case-control study. Int J Cancer 2010;127:2159–68.
17. Eliassen AH, Spiegelman D, Hollis BW, Horst RL, Willett WC, Hankinson SE. Plasma 25-hydroxyvitamin D and risk of breast cancer in the Nurses' Health Study II. Breast Cancer Res 2011;13:R50.
18. Amir E, Cecchini RS, Ganz PA, Costantino JP, Beddows S, Hood N, et al. 25-Hydroxy vitamin-D, obesity, and associated variables as predictors of breast cancer risk and tamoxifen benefit in NSABP-P1. Breast Cancer Res Treat 2012;133:1077–88.
19. Kuhn T, Kaaks R, Becker S, Eomois PP, Clavel-Chapelon F, Kvaskoff M, et al. Plasma 25-hydroxyvitamin D and the risk of breast cancer in the European prospective investigation into cancer and nutrition: A nested case-control study. Int J Cancer 2013;133:1689–700.
20. Scarmo S, Afanasyeva Y, Lenner P, Koenig KL, Horst RL, Clendenen TV, et al. Circulating levels of 25-hydroxyvitamin D and risk of breast cancer: A nested case-control study. Breast Cancer Res 2013;15:R15.
21. Engel P, Fagherazzi G, Boutten A, Dupre T, Mesrine S, Boutron-Ruault MC, et al. Serum 25(OH) vitamin D and risk of breast cancer: A nested case-control study from the French E3N cohort. Cancer Epidemiol Biomarkers Prev 2010;19:2341–50.
22. Kim Y, Franke AA, Shvetsov YB, Wilkens LR, Cooney RV, Lurie G, et al. Plasma 25-hydroxyvitamin D3 is associated with decreased risk of postmenopausal breast cancer in whites: A nested case-control study in the multiethnic cohort study. BMC Cancer 2014;14:29.
23. Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, et al. Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer. Cancer Epidemiol Biomarkers Prev 2005;14:1991–7.
24. Bauer SR, Hankinson SE, Bertone-Johnson ER, Ding EL. Plasma vitamin D levels, menopause, and risk of breast cancer: Dose-response meta-analysis of prospective studies. Medicine (Baltimore) 2013;92:123–31.
25. Wang D, Velez de-la-Paz OI, Zhai JX, Liu DW. Serum 25-hydroxyvitamin D and breast cancer risk: A meta-analysis of prospective studies. Tumour Biol 2013;34:3509–17.
26. Hankinson SE, Willett WC, Manson JE, Hunter DJ, Colditz GA, Stampfer MJ, et al. Alcohol, height, and adiposity in relation to estrogen and prolactin levels in postmenopausal women. J Natl Cancer Inst 1995;87:1297–302.
27. Zhang X, Tworoger SS, Eliassen AH, Hankinson SE. Postmenopausal plasma sex hormone levels and breast cancer risk over 20 years of follow-up. Breast Cancer Res Treat 2013;137:883–92.
28. Ersfeld DL, Rao DS, Body JJ, Sackrison JLJr., Miller AB, Parikh N, et al. Analytical and clinical validation of the 25 OH vitamin D assay for the LIAISON automated analyzer. Clin Biochem 2004;37:867–74.
29. Eliassen AH, Hendrickson SJ, Brinton LA, Buring JE, Campos H, Dai Q, et al. Circulating carotenoids and risk of breast cancer: Pooled analysis of eight prospective studies. J Natl Cancer Inst 2012;104:1905–16.

30. Collins LC, Marotti JD, Baer HJ, Tamimi RM. Comparison of estrogen receptor results from pathology reports with results from central laboratory testing. J Natl Cancer Inst 2008;100:218–21.
31. Tamimi RM, Baer HJ, Marotti J, Galan M, Galaburda L, Fu Y, et al. Comparison of molecular phenotypes of ductal carcinoma in situ and invasive breast cancer. Breast Cancer Res 2008; 10:R67.
32. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, et al. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2011;96:1911–30.
33. Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: The National Academies Press, 2011.
34. Bertrand KA, Giovannucci E, Liu Y, Malspeis S, Eliassen AH, Wu K, et al. Determinants of plasma 25-hydroxyvitamin D and development of prediction models in three US cohorts. Br J Nutr 2012;108: 1889–96.
35. Gail MH, Wu J, Wang M, Yaun SS, Cook N, Eliassen AH, et al. Calibration and seasonal adjustment for matched case–control studies of vitamin D and cancer. Statist Med 2016;35:2133–48.
36. Tamimi RM, Colditz GA, Hazra A, Baer HJ, Hankinson SE, Rosner B, et al. Traditional breast cancer risk factors in relation to molecular subtypes of breast cancer. Breast Cancer Res Treat 2012;131: 159–67.
37. Marshall RJ, Chisholm EM. Hypothesis testing in the polychotomous logistic model with an application to detecting gastrointestinal cancer. Stat Med 1985;4:337–44.
38. Kotsopoulos J, Tworoger SS, Campos H, Chung FL, Clevenger CV, Franke AA, et al. Reproducibility of plasma and urine biomarkers among premenopausal and postmenopausal women from the Nurses' Health Studies. Cancer Epidemiol Biomarkers Prev 2010;19:938–46.
39. Ordonez-Mena JM, Schottker B, Haug U, Muller H, Kohrle J, Schomburg L, et al. Serum 25-hydroxyvitamin d and cancer risk in older adults: Results from a large German prospective cohort study. Cancer Epidemiol Biomarkers Prev 2013;22:905–16.
40. Santagata S, Thakkar A, Ergonul A, Wang B, Woo T, Hu R, et al. Taxonomy of breast cancer based on normal cell phenotype predicts outcome. J Clin Invest 2014;124:859–70.
41. Racz A, Barsony J. Hormone-dependent translocation of vitamin D receptors is linked to transactivation. J Biol Chem 1999;274:19352–60.
42. Welsh J, Zinser LN, Mianecki-Morton L, Martin J, Waltz SE, James H, et al. Age-related changes in the epithelial and stromal compartments of the mammary gland in normocalcemic mice lacking the vitamin D3 receptor. PLoS One 2011;6:e16479.
43. Ching S, Kashinkunti S, Niehaus MD, Zinser GM. Mammary adipocytes bioactivate 25-hydroxyvitamin D(3) and signal via vitamin D(3) receptor, modulating mammary epithelial cell growth. J Cell Biochem 2011;112: 3393–405.