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Reduced Prediagnostic 25-Hydroxyvitamin D Levels in Women with Breast Cancer: A Nested Case-Control Study

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Abstract

Vitamin D status may affect risk of cancer. In a cross-sectional study with a nested case-control analysis, we determined whether risk of breast cancer is associated with prediagnostic plasma 25-hydroxyvitamin D (25OHD) levels and the effects of lifestyle characteristics known to influence vitamin D status on risk of breast cancer. We studied women without a prior history of breast cancer referred to a diagnostic mammography examination (n = 2,465). Cases were women diagnosed with an incident breast cancer (n = 142). Controls were women not diagnosed with a breast cancer matched to cases on age, menopausal status, and time of year of blood sampling (n = 420). Characteristics of cases and controls were assessed by a self-administrated questionnaire. Blood samples were collected prior to the diagnostic mammography examination. Cases had lower plasma 25OHD levels than controls. Compared with the lowest tertile of 25OHD levels, risk of breast cancer was significantly reduced among women in the highest tertile (relative risk, 0.52; 95% confidence interval, 0.32-0.85). Risk estimates were similar in women with an estrogen receptor–positive and estrogen receptor–negative breast cancer. Use of vitamin D supplements, sunbathing frequency, and fish intake was associated with 25OHD levels, but did not affect the risk of breast cancer. Accordingly, risk of breast cancer was inversely associated with 25OHD levels. Randomized controlled trials are warranted in order to assess whether a causal relationship exists. (Cancer Epidemiol Biomarkers Prev 2009;18(10):2655–60)

Introduction

According to recent studies, vitamin D may exert anticarcinogenic effects. The hormonally active form of vitamin D is 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Binding of 1,25$(OH)_2$D to the vitamin D receptor has been shown to increase differentiation and decrease proliferation in different cell types, including breast and breast cancer cells (1).

The primary source of vitamin D is endogenously synthesized vitamin D_3 (cholecalciferol) in response to exposure of the skin surface to type B UV (UVB) radiation. In addition, a minor part (10-20%) of the vitamin D body stores comes from a limited number of dietary sources rich in vitamin D, especially fatty fish. After being synthesized in the skin or absorbed from the intestine, vitamin D is metabolized in the liver to 25-hydroxyvitamin (25OHD). Until recently, 25OHD was considered to be a substrate for only the renal 1α-hydroxylase, whereby circulating 1,25$(OH)_2$D was formed. However, the 1α-hydroxylase is now known to be expressed in several nonrenal cell lines for which circulating levels of 25OHD serves as a substrate (1, 2). Accordingly, vitamin D status as determined by plasma 25OHD levels may be of importance to the function of different cells and may influence the risk of cancer.

Although discrepant results have been reported, an inverse association has been shown by some investigators between UVB exposure and risk of breast cancer (3-7), as well as between vitamin D intake and breast cancer risk (6, 8). In a few studies, vitamin D status as determined by plasma 25OHD levels have been studied in relation to breast cancer risk. In some (9, 10) but not all (11, 12) studies, lower 25OHD levels have been found in women with known breast cancer compared with healthy controls. Only in few case-control studies, nested within established cohorts of women, has the risk of breast cancer been studied in relation to prediagnostic 25OHD levels. In an analysis from the Nurses' Health Study cohort, women with baseline 25OHD levels in the highest quintile had a borderline significantly reduced risk of breast cancer compared with women in the lowest quintile (13), whereas no associations between prediagnostic 25OHD levels and risk of developing breast cancer was found in an analysis from the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (14). Moreover, in a re-analysis of data from the Women's Health Initiative clinical trial, breast cancer incidence did not differ between women randomized to a daily dose of 400 IU of vitamin D plus 1,000 mg of calcium and women randomized to placebo for 7 years (12).

We now report the results of a study initiated in 2003 specifically designed to assess whether breast cancer is associated with prediagnostic plasma 25OHD levels and whether different lifestyle characteristics known to influence vitamin D status affects the risk of breast cancer.

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Subjects and Methods

Between May 1, 2003 and July 1, 2007, 2,555 women referred to a diagnostic mammography examination were included in our study. We collected data prospectively, as all women prior to the mammography were asked to donate a blood sample and fill in a self-administered questionnaire on lifestyle characteristics associated with vitamin D status and breast cancer risk. The study was conducted according to the Declaration of Helsinki II. Each individual gave written informed consent. The study was approved by the regional Ethical Committee (Aarhus County #:2002/0169) and the National Danish Data Protection Agency.

Identification of Incident Breast Cancer Cases. Incident breast cancers were defined as all invasive or *in situ* breast cancers observed in the follow-up period, i.e., after the mammography examination. For all women included, we reviewed the results of the mammography examinations. In women with a mammogram showing signs of a breast cancer, we reviewed pathology records in order to confirm the diagnosis and abstracted pathologic characteristics of the cancer. Only women, in whom the pathologic examination verified the presence of a breast cancer, were considered as breast cancer cases. In order to assure that all incident breast cancer cases were identified, we also retrieved information on studied subjects from The Danish National Hospital Discharge Register and the Danish Cancer Register, as detailed below.

Identification of Controls. For each incident breast cancer case, we randomly selected three women without an incident breast cancer matched on age (± 2 y), menopausal state, and time of year of blood sampling (± 2 mo). However, in a few of the very old women ($n = 4$) with an incident breast cancer, it was only possible to identify one to two matched control subjects, based on the abovementioned criteria. In order to assure that the controls did not have a breast cancer history, we retrieved information on all studied subjects ($N = 2,555$) from the Danish Cancer Register and the National Hospital Discharge Register. In the Danish Cancer Register, cancers are classified according to a modified version of the seventh revision of the International Classification of Diseases (ICD) and data are available on all cancer cases diagnosed in Denmark between 1943 and 2003 (15). The Danish National Hospital Discharge Register covers all contacts to the hospitals. The register was founded in 1977 and includes information on discharge diagnoses and date of discharge assigned exclusively by the physician at discharge according to the Danish versions of the International Classification of Diseases (v. 8) until the end of 1993, and to the International Classification of Diseases (v. 10), from 1994. The register has nationwide coverage of public hospitals with an almost 100% completeness of recordings and a high precision of diagnoses (16). Using these two registers, we identified all women, within our study group, who had a diagnosis of breast cancer between January 1943 and April 2008. Using this approach, we identified and excluded 93 women from our study group because they had been previously diagnosed with a breast cancer, leaving 2,465 women for further analyses. The database retrieval did not identify additional women with an incident breast cancer, who had not been identified through our review of medical records. However, four of the women identified as cases due to a histologically verified breast cancer, did not have a cancer diagnosis in any of the registers, but of course they were still considered as incident cancer cases in our analysis.

Self-Administered Questionnaire. Prior to the mammography examination, the studied subjects were asked to fill in a questionnaire on indices related to vitamin D status and breast cancer risk (Table 1). We asked studied subjects whether they had a family history of breast cancer (mother and/or sister), whether they had passed menopause, and if so, their age at last menstrual bleeding, and whether they had used postmenopausal hormone replacement therapy (HRT). In addition, we asked studied subjects on sun-seeking behavior, on use of vitamin D supplements, including use of multivitamin pills, and on use calcium supplements. Moreover, we asked subjects on their consumption of fish, fruits, and dairy products. Total daily calcium intake was assessed according to reported dietary intake of milk, cheese, milk products, and use of calcium supplements (17). Importantly, in Denmark, dairy products are not fortified with vitamin D. Finally, we asked studied subjects to report their height, weight, alcohol consumption and whether they were smoking.

Biochemistry. Prior to the mammography, we collected a blood sample from each participant. Samples were divided in aliquots and stored immediately at −80°C until analysis. At the end of the study, plasma 25OHD levels were analyzed by isotope dilution liquid chromatography-tandem mass spectrometry by a method adapted

Table 1. Characteristics of cases and controls

	Controls ($n = 420$)	Cases ($n = 142$)	*P*
Matched variables*			
Age (y)†	58 (29-87)	58 (29-86)	0.66
Postmenopausal, no. (%)	277 (65.5%)	94 (65.7%)	0.84
Unmatched variables			
Body weight (kg)	69.2 ± 13.1	69.1 ± 14.2	0.97
Body mass index (kg/m^2)	24.9 ± 4.5	24.9 ± 5.2	0.85
Daily calcium intake (mg)	1,161 ± 470	1,117 ± 396	0.33
Current smoking, *N* (%)	79 (20.3%)	32 (23.2%)	0.47
Units of alcohol per week, no. of subjects (%)			0.25
<1	95 (22.3%)	37 (26.1%)	
1-7	200 (46.9%)	55 (38.7%)	
8-14	86 (20.2%)	37 (26.1%)	
>14	45 (10.6%)	13 (9.2%)	
Pieces of fresh fruit per week, no. of subjects (%)			0.62
≤7	177 (41.5%)	65 (45.5%)	
8-14	149 (34.9%)	44 (30.8%)	
≥15	101 (23.7%)	34 (23.8%)	
Age at menarche, range (y)	13.5 (10-18)	13.5 (10-19)	0.94
Nulliparas, no. (%)	44 (10.6%)	13 (9.3%)	0.66
No. of childbirths (range)‡	2.3 (1-10)	2.3 (1-6)	0.99
Age at first childbirth, range (y)‡	24.9 (15-40)	24.8 (16-38)	0.74
Age at last menstrual bleeding, range (y)§	49.4 (28-65)	50.5 (40-60)	0.07
Ever use of postmenopausal HRT, no. (%)§	156 (58.4%)	49 (54.4%)	0.78
A family history of breast cancer, no. (%)	126 (30.1%)	18 (12.8%)	<0.01

*In addition, cases and controls were matched on time of year of blood sampling.
†Mean with range.
‡Excluding nulliparas.
§Only postmenopausal women.

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from Maunsell et al. (18). Mean coefficients of variation for $25OHD_3$ were 6.4% and 9.1% at levels of 66.5 and 21.1 nmol/L, and for 25OHD2, the coefficients of variation were 8.8% and 9.4% at levels of 41.2 and 25.3 nmol/L, respectively. Estrogen receptor (ER) status was assessed by standard methods at the Department of Pathology, Aarhus University Hospital (Aarhus, Denmark) using immunohistochemistry on tumor sections from fixed tissue, with anti-ER from Neomarker (clone SP1) until 2005, and then from Ventana (clone SP1) until 2007.

Statistics. In order to assess whether our questionnaire was able to detect differences in plasma 25OHD levels according to indices known to affect vitamin D status, we used the entire study population (n = 2,462) to test differences in 25OHD levels between users and nonusers of vitamin D supplements, different groups of sunbathing habits, and different categories of fish consumption.

We assessed differences between groups using χ^2 tests for categorical variables and a two-sample t test or Mann-Whitney U test for continuous variables, as appropriate, after testing for normal distributions. Similarly, tests for several independent samples were done using the One-way ANOVA or the Kruskal-Wallis H test as appropriate. In our case-control analyses, we used logistic regression analyses to assess relative risk (RR) with 95% confidence intervals (95% CI) of breast cancer in association with measured indices of vitamin D status. Due to the design of our study, the odds ratio derived from logistic regression directly estimates the incidence rate ratio and, therefore, the relative risk (19). We checked the statistical models by the Hosmer and Lemeshow's goodness-of-fit test and only models without a significant lack of fit were accepted. All statistical analyses were done using the Statistical Package for Social Sciences (SPSS 14.0) for Windows.

Results

Among the 2,462 women without a prior diagnosis of breast cancer, 142 (5.8%) women were diagnosed with an incident breast cancer. The women with an incident breast cancer were well matched with 420 control subjects, as shown in Table 1. Body weight, smoking habits, and intake of calcium, fresh fruits, and alcohol did not differ between cases and controls. Moreover, age at menarche did not differ, and a similar percentage of cases and controls were nulliparas. Among women who had given birth to a child, average number of childbirths and age at first childbirth did not differ between cases and controls. However, a family history of breast cancer was reported more frequently by women in the control group (30.1%) than by women with an incident breast cancer (12.8%). Among postmenopausal women, cases had a borderline significant higher age at last menstrual bleeding compared with controls (P = 0.07), but use of HRT did not differ between cases and controls (Table 1).

Biochemical Vitamin D Status and Breast Cancer Risk. Plasma 25OHD levels were significantly (P < 0.01) lower in cases (69 ± 23 nmol/L) than in controls (76 ± 28 nmol/L). Table 2 shows the risk of breast cancer according to tertiles of plasma 25OHD levels. Women in the highest tertile had a 48% reduced risk of breast cancer compared with women in the lowest tertile (odds ratio, 0.52; 95% CI, 0.32-0.85, P < 0.01). Stratification by menopausal status showed lower 25OHD levels in cases compared with controls in premenopausal (64 ± 27 versus 75 ± 29 nmol/L, P < 0.05) as well as in postmenopausal (72 ± 21 versus 77 ± 28 nmol/L, P = 0.03) women. In both premenopausal and postmenopausal women, risk of breast cancer was reduced in the highest compared with the lowest tertile of plasma 25OHD levels, although the association only reached statistical significance in the group of premenopausal women (Table 2).

Table 2. Risk of breast cancer according to tertiles of 25OHD levels

	RR (95% CI)	Menopausal status	
		Premenopausal RR (95% CI)	Postmenopausal RR (95% CI)
25OHD tertiles (nmol/L)			
<60	Reference	Reference	Reference
60-84	0.94 (0.59-1.47)	0.59 (0.26-1.33)	1.20 (0.67-2.16)
>84	0.52 (0.32-0.85)*	0.38 (0.15-0.97)*	0.71 (0.38-1.30)

*P < 0.05.

ER Status. ER status was assessed in 130 (92%) of our cases, and 96 (74%) had an ER-positive breast cancer. Compared with the controls, plasma 25OHD levels were lower in women with an ER-positive breast cancer (70 ± 22 versus 77 ± 27 nmol/L, P = 0.02), as well as in women with an ER-negative (64 ± 24 versus 78 ± 33 nmol/L, P < 0.03) breast cancer. 25OHD levels did not differ significantly between women with an ER-positive and ER-negative breast cancer (P = 0.15). Among women with an ER-positive breast cancer, increased 25OHD levels were associated with a decreased risk of breast cancer. Thus, compared with women in the lowest tertile, risk of breast cancer was reduced in the mid-tertile (RR, 0.88; 95% CI, 0.52-1.49) and in the highest tertile (RR, 0.42; 95% CI, 0.23-0.78) of 25OHD levels. Similarly, we found a borderline significant trend (P = 0.09) towards reduced risk of breast cancer according to plasma 25OHD tertiles in women with an ER-negative breast cancer. Compared with the lowest tertile, risk estimates were reduced in the mid-tertile (RR, 0.62; 95% CI, 0.25-1.56) and highest tertile (RR, 0.42; 95% CI, 0.16-1.16) of 25OHD levels among women with an ER-negative breast cancer.

Use of Vitamin D Supplements, Vitamin D Status, and Risk of Breast Cancer. Users of vitamin D supplements had significantly (P < 0.01) higher plasma 25OHD levels (79 ± 25 nmol/L) compared with nonusers (69 ± 29 nmol/L). However, vitamin D supplements were used by an almost equal number of cases (58.5%) and controls (61.0%), and use of vitamin D supplements was not associated with risk of breast cancer (odds ratio, 0.90; 95% CI, 0.61-1.32).

Sunbathing Habits, Vitamin D Status, and Risk of Breast Cancer. Women reporting that they rarely sunbathe had significantly lower (P < 0.01) plasma 25OHD levels than women reporting that they sometimes or often sunbathe (Table 3). Stratification by time of year of blood sampling showed that the effect of sunbathing on 25OHD levels was more pronounced in women in whom blood samples had been collected from May to October than

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Table 3. 25OHD according to sunbathing habits (mean ± SD)

	All year		Stratification according to time of year of blood collection			
			Summertime*		Wintertime†	
	N	25OHD (nmol/L)	*N*	25OHD (nmol/L)	*N*	25OHD (nmol/L)
Rarely	322	60 ± 28	157	68 ± 27	165	54 ± 27
Sometimes	1,511	74 ± 28	656	83 ± 26	855	66 ± 28
Often	597	80 ± 31	273	92 ± 33	324	70 ± 27
P-trend		<0.01		<0.01		<0.01

*Summertime was defined as samples collected from May to October.
†Wintertime was defined as samples collected from November to April.

in women in whom samples were collected during wintertime (Table 3). However, reported sunbathing habits did not differ to any major degree between cases and controls (Table 4). Accordingly, risk of breast cancer was not associated with current sunbathing habits (Table 4). Neither did prior sunbathing habits, i.e., how often studied subjects went sunbathing 15 to 20 years ago, influence risk of breast cancer (Table 4). Stratification by menopausal state did not show significant associations in either premenopausal or postmenopausal women (data not shown).

Fish Intake, Vitamin D Status, and Risk of Breast Cancer. We asked studied subjects how often they ate fish as a cold or a warm dish. As shown in Table 5, women reporting that they never have fish as a cold dish had lower plasma 25OHD levels than women having fish as a cold dish weekly or monthly (*P*-trend < 0.01). A similar relationship was found for fish as a warm dish (*P*-trend = 0.03; Table 4). Moreover, women reporting that they never ate fish (n = 98) as either a cold or a warm dish had slightly (P = 0.08) lower 25OHD levels (69 ± 32 nmol/L) than women reporting fish intake (plasma 25OHD, 74 ± 29 nmol/L). However, fish intake as either a cold or a warm dish did not influence risk of breast cancer (Table 6). Neither did prior intake of fish, i.e., how often studied subjects consumed fish 15 to 20 years ago, influence risk of breast cancer (Table 6).

Additional Measures. Daily calcium intake did not influence the risk of breast cancer, i.e., compared with the lowest tertile of calcium intake (<800 mg/d), risk of breast cancer in the mid-tertile (800-1,250 mg/d) and highest tertile (>1,250 mg/d) was 0.94 (95% CI, 0.57-1.56) and 1.03 (95% CI, 0.64-1.66), respectively.

Nor did categories of alcohol intake or smoking affect the risk of breast cancer (data not shown). A similar number of women were diagnosed with a breast cancer during summertime (n = 68) as during wintertime (n = 74, P = 0.85).

Discussion

In this case-control study, nested within a large group of women referred for a diagnostic mammography examination, we found significantly lower plasma 25OHD levels in women diagnosed with an incident breast cancer compared with women without a breast cancer. Thus, in blood samples obtained prior to the mammography examination, a 25OHD level in the highest tertile was associated with an almost 50% reduced risk of being diagnosed with a breast cancer compared with a 25OHD level in the lowest tertile. Apparently, this effect is independent of ER status. Despite our findings of an association between plasma 25OHD levels and use of vitamin D supplements, sunbathing habits, and frequency of fish consumption, our analyses did not show any association between these indices and the risk of breast cancer.

Our study is the first investigation specifically designed to assess associations between breast cancer risk and prediagnostic plasma 25OHD levels. Prediagnostic 25OHD levels have been related to risk of breast cancer in only a few prior studies (12-14). In a case-control study nested within the Nurses' Health Study cohort, plasma levels of vitamin D metabolites were measured in blood samples obtained up to 82 months prior to the diagnosis of a breast cancer (n = 701) and compared with plasma levels in breast cancer–free controls (n = 724; ref. 13). Cases and controls were matched on several indices, including age (±2 years), menopausal status, and month of blood collection. Similar to our results, the study showed lower 25OHD levels in cases than in controls. In the study, breast cancer risk in women in the highest quintile of 25OHD levels was borderline significantly reduced by 27% (RR, 0.73; 95% CI, 0.49-1.07; *P*-trend = 0.06) compared with those in the lowest quintile. In contrast, in a cohort of women ages 55 to 74 years, included in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial, plasma 25OHD levels in cases, as measured on average 3.9 years prior to the diagnosis of breast cancer, did not differ from

Table 4. Risk of breast cancer according to current and prior (15-20 y ago) sunbathing habits

	Current sunbathing habits			Prior sunbathing habits		
	Controls No. (%)	Cases No. (%)	RR (95% CI)	Controls No. (%)	Cases No. (%)	RR (95% CI)
Sunbathing habits						
Rarely	60 (15)	17 (12)	Reference	48 (12)	21 (15)	Reference
Sometimes	249 (60)	89 (65)	1.26 (0.70-2.28)	270 (66)	88 (64)	0.75 (0.43-1.31)
Often	106 (25)	32 (23)	1.07 (0.55-2.08)	90 (22)	29 (21)	0.74 (0.38-1.43)
P-trend			0.97			0.43

NOTE: Number of cases and controls in each sunbathing category and RR with 95% CI.

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Table 5. 25OHD levels according to frequency of fish consumption as either a cold or a warm dish (mean ± SD)

	Fish as a cold dish		Fish as a warm dish	
	N	25OHD (nmol/L)	*N*	25OHD (nmol/L)
Never	212	69 ± 31	273	71 ± 34
Monthly	755	72 ± 29	1,036	73 ± 29
Weekly	1,462	75 ± 29	1,120	75 ± 28
P-trend		<0.01		0.03

levels in a matched control group and risk of breast cancer was not associated with vitamin D status (14).

In a few prior studies, plasma 25OHD levels between women already diagnosed with a breast cancer and healthy controls showing either no difference in (11) or an inverse association between plasma 25OHD levels and breast cancer risk (9, 10) have been compared. As measurements of 25OHD levels in these studies were done at variable times after the study subjects were diagnosed with a breast cancer, the interpretation of these studies is hampered by the possible influence of changes in lifestyle as a result of being diagnosed with a severe disease. Nevertheless, in a pooled analysis including two of the abovementioned studies (10, 13), risk of breast cancer decreased dose-dependently with increasing 25OHD levels (20).

In our study, we assessed indices known to affect vitamin D status, i.e., sunbathing habits, use of vitamin D supplements, and fish intake. Our study showed significant dose-response relationships between measured indices and plasma 25OHD levels. Accordingly, with the questionnaire used, we were able to detect the influence of different lifestyle characteristics on vitamin D status. However, despite significant dose-response relationships between measured indices of vitamin D status and 25OHD levels, none of the lifestyle characteristics were associated with risk of breast cancer. Similarly, discrepant results have been reported in previous studies (21). In a recent meta-analysis by our group, we found no overall association between the amount of vitamin D intake and risk of breast cancer, although our analysis showed a trend towards a decreased breast cancer risk in subjects with a high (≥400 IU/d) vitamin D intake (22). Accordingly, the lack of effect of vitamin D supplements on risk of breast cancer in the Women's Health Initiative study, might at least in part be due to the dose investigated, as women in this study received a daily dose of only 400 IU of cholecalciferol (12).

Although several ecological studies (23-25) have shown an inverse association between UVB exposure and risk of breast cancer, there is only limited evidence from clinical studies on the effects of sun exposure on risk of breast cancer (5, 6). It is probably difficult to assess the contribution of a single measure such as sun exposure, fish intake, etc., to overall risk of breast cancer due to the fact that vitamin D status in the individual subject is the sum of multiple lifestyle characteristics and that the contribution of each characteristic may vary between and within subjects over time (26). As plasma 25OHD levels are the combined result of multiple indices known to influence vitamin D status, plasma levels of 25OHD are the most applicable measure of a possible relation between vitamin D status and cancer risk.

In the interpretation of our results, the procedure for recruiting participants and the study design should be taken into consideration. Our finding of a higher frequency of a family history of breast cancer in controls compared with cases might be explained by the fact that we recruited women who were referred to a diagnostic mammography examination. It is likely that women with a family history of breast cancer are more concerned about a lump or other breast-related symptoms and therefore may be referred more often to a mammography than women without a family history of breast cancer. Symptoms may be more severe and therefore breast cancer might be more likely in women without a family history referred for a mammography screening. In a case-control analysis, nested within a cross-sectional design, this might cause a positive family history of breast cancer seem less likely in cases than in controls. A similar mechanism might explain the lack of an association between risk of breast cancer and use of HRT, i.e., most women know that use of HRT increases the risk of breast cancer, and therefore users of HRT may more often/less strictly be referred to a mammography examination compared with nonusers.

The major strengths of our study is that we specifically designed this investigation to assess prediagnostic plasma 25OHD levels and indices related to vitamin D status and risk of breast cancer. In contrast to some previous studies (13), we analyzed all samples using the same 25OHD assay; applying the liquid chromatography-tandem mass spectrometry methods which is today considered the gold standard for 25OHD measurements. We assessed the status of our cases very carefully, but we cannot exclude the presence of breast cancer among the controls that were not diagnosed at the mammography examination. However, if breast cancer is associated with low plasma 25OHD levels, the presence of undiagnosed cases in the control group will tend to underestimate the calculated RR. An additional limitation to our results is the lack of assessment of physical activity in the studied subjects, as physical activity may affect vitamin D status and has been associated with risk of breast cancer (27). In Denmark, herrings (which are rich in vitamin D) are often eaten as a cold fish dish; whereas less oily fish with lower vitamin D content often are eaten as warn dishes. Therefore, in our questionnaire, we asked specifically how often fish was eaten as either a cold or a warm dish. Nevertheless, according to our results, both cold and warm fish dishes increased vitamin D levels to

Table 6. Risk of breast cancer according to current or prior (15-20 y ago) fish intake as either a cold or a warm dish

	Fish intake as a cold dish, RR (95% CI)	Fish intake as a warm dish, RR (95% CI)
Current frequency		
Never	Reference	Reference
Monthly	0.94 (0.44-2.00)	0.68 (0.35-1.35)
Weekly	1.16 (0.58-2.31)	1.06 (0.55-2.02)
P-trend	0.43	0.25
Prior frequency		
Never	Reference	Reference
Monthly	0.96 (0.50-1.85)	1.34 (0.74-2.41)
Weekly	1.25 (0.62-2.51)	1.55 (0.62-3.88)
P-trend	0.35	0.29

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a similar extent. Vitamin D supplements were used by ~60% of our studied subjects who had 25OHD levels which were on average 10 nmol/L higher than those not using vitamin D supplements. As most vitamin D supplements contains 10 μg (400 IU) of vitamin D, our findings are in good agreement with previous studies showing that 25OHD levels increased by ~1 nmol/L for every 1 μg (40 IU) of vitamin D ingested (28). Due to the nature of our data, our results do not allow for definite causal conclusions, i.e., whether low 25OHD levels cause breast cancer or are the result of the disease. As breast cancer is a disease that develops over several years, a major assumption for a causal inverse relationship between 25OHD levels and risk of breast cancer is that 25OHD levels, in the individual subject, are at a steady level for a longer time period. Studies are needed on whether a single measurement of a plasma 25OHD level is a sensitive indicator of a person's long-term vitamin D status (29). In addition, further studies should focus on the potential effects of polymorphisms in genes related to the effects of vitamin D, such as vitamin D–binding protein (*DBP*), vitamin D receptor (*VDR*), 25-hydroxylase (*CYP27A1*), 1-hydroxylase (*CYP27B1*), and 24-hydroxylase (*CYP24*). As the effects of vitamin D may—at least in part—be modified by polymorphisms in these genes, important interactions might exist between such polymorphisms, 25OHD levels, and risk of cancer (21).

In conclusion, risk of breast cancer is inversely associated with prediagnostic plasma 25OHD levels. Randomized trials on the effects of sufficient vitamin D supplements on cancer risk should be done in order to establish whether a causal relationship exists.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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