

This Provisional PDF corresponds to the article as it appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

Circulating levels of 25-hydroxyvitamin D and risk of breast cancer: a nested case-control study

Breast Cancer Research 2013, **15**:R15 doi:10.1186/bcr3390

Stephanie Scarmo (stephanie.scarmo@nyumc.org)
Yelena Afanasyeva (yelena.afanasyeva@nyumc.org)
Per Lenner (per.lenner@onkologi.umu.se)
Karen Koenig (karen.koenig@nyumc.org)
Ronald Horst (ron.horst@heartlandassays.com)
Tess Clendenen (tess.clendenen@nyumc.org)
Alan Arslan (alan.arslan@nyumc.org)
Yu Chen (yu.chen@nyumc.org)
Goran Hallmans (goran.hallmans@nutrires.umu.se)
Eva Lundin (eva.lundin@medbio.umu.se)
Sabina Rinaldi (rinaldi@iarc.fr)
Paolo Toniolo (paolo.toniolo@nyumc.org)
Roy Shore (shore@rerf.or.jp)
Anne Zeleniuch-Jacquotte (anne.jacquotte@nyumc.org)

ISSN 1465-5411

Article type Research article

Submission date 28 September 2012

Acceptance date 22 February 2013

Publication date 26 February 2013

Article URL <http://breast-cancer-research.com/content/15/1/R15>

This peer-reviewed article can be downloaded, printed and distributed freely for any purposes (see copyright notice below).

Articles in *Breast Cancer Research* are listed in PubMed and archived at PubMed Central.

For information about publishing your research in *Breast Cancer Research* go to

<http://breast-cancer-research.com/authors/instructions/>

© 2013 Scarmo et al.

This is an open access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Circulating levels of 25-hydroxyvitamin D and risk of breast cancer: a nested case-control study

Stephanie Scarmo¹, Yelena Afanasyeva¹, Per Lenner², Karen Koenig^{1,3}, Ronald Horst⁴, Tess Clendenen¹, Alan Arslan^{1,3,5}, Yu Chen^{1,3}, Göran Hallmans⁶, Eva Lundin⁷, Sabina Rinaldi⁸, Paolo Toniolo^{1,3,5}, Roy Shore^{1,9} and Anne Zeleniuch-Jacquotte^{1,3*}

¹Department of Population Health, New York University School of Medicine, 650 First Avenue, New York, NY 10016, USA.

²Department of Radiation Sciences, Oncology, Umeå University, S-90185, Umeå, Sweden.

³New York University Cancer Institute, New York University School of Medicine, 530 First Avenue, New York, NY 10016, USA.

⁴Heartland Assays Inc., 2711 South Loop Drive, Ames, Iowa, USA.

⁵Department of Obstetrics and Gynecology, New York University School of Medicine, 550 First Avenue, New York, NYU 10016, USA.

⁶Department of Public Health and Clinical Medicine/Nutritional Research, Umeå University, SE-901 87, Umeå, Sweden.

⁷Department of Medical Biosciences/Pathology, Umeå University, SE-901 87, Umeå, Sweden.

⁸International Agency for Research on Cancer, 150, Course Albert Thomas, 69372 Lyon Cedex 08, France.

⁹Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732-0815, Japan.

*Corresponding author: Anne Zeleniuch-Jacquotte, anne.jacquotte@nyumc.org

Abstract

Introduction: Experimental evidence suggests a protective role for circulating 25-hydroxyvitamin D (25(OH)D) in breast cancer development, but the results of epidemiological studies have been inconsistent.

Methods: We conducted a case-control study nested within two prospective cohorts, the New York University Women's Health Study and the Northern Sweden Mammary Screening Cohort. Blood samples were collected at enrollment, and women were followed up for breast cancer ascertainment. A total of 1,585 incident breast cancer cases were individually-matched to 2,940 controls. Of these subjects, 678 cases and 1,208 controls contributed two repeat blood samples, at least one year apart. Circulating levels of 25(OH)D were measured, and multivariate odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using conditional logistic regression.

Results: No association was observed between circulating levels of 25(OH)D and overall breast cancer risk (multivariate-adjusted model OR = 0.94, 95% CI = 0.76-1.16 for the highest vs. lowest quintile, $p_{\text{trend}}=0.30$). The temporal reliability of 25(OH)D measured in repeat blood samples was high (intraclass correlation coefficients for season-adjusted 25(OH)D > 0.70). An inverse association between 25(OH)D levels and breast cancer risk was observed among women who were ≤ 45 years of age (OR_{Q5-Q1} = 0.48, 95% CI = 0.30-0.79, $p_{\text{trend}}=0.01$) or premenopausal at enrollment (OR_{Q5-Q1} = 0.67, 95% CI = 0.48-0.92, $p_{\text{trend}}=0.03$).

Conclusions: Circulating 25(OH)D levels were not associated with breast cancer risk overall, although we could not exclude the possibility of a protective effect in younger women. Recommendations regarding vitamin D supplementation should be based on considerations other than breast cancer prevention.

Introduction

Experimental studies support a role for vitamin D in reducing the risk of breast cancer [1, 2]. Vitamin D, which is obtained from both dietary sources (food and supplements) and exposure to type B ultraviolet (UVB) radiation, undergoes two hydroxylation steps before becoming biologically active [3]. 25-hydroxyvitamin D [25(OH)D], produced in the liver from the first hydroxylation, is the precursor of the biologically active form, 1,25(OH)₂D, which is produced in the kidney as well as in target tissues, including the breast [4]. Circulating 25(OH)D is considered the best marker of vitamin D status because it reflects vitamin D obtained from both diet/supplements and sun exposure [5] and has a longer half-life than 1,25(OH)₂D [6]. Vitamin D acts by binding to the vitamin D receptor (VDR), which is expressed in mammary tissue. The VDR controls the expression of genes regulating cell proliferation, differentiation, and apoptosis [1, 7].

The results of epidemiologic studies examining the association between circulating 25(OH)D levels and breast cancer risk have been inconsistent. Seven prospective studies reported no association overall [8-14], while three reported a significant or marginally significant inverse association [15-17]. Some significant findings emerged from the results of subgroup analyses, although the subgroups of women for whom these associations were observed were not consistent across studies. The Nurses' Health Study observed a stronger protective effect of plasma 25(OH)D on breast cancer risk for women ages 60 years and older [16], while the French E3N cohort observed a stronger effect in women <53 years of age at enrollment [15]. In the Nurses' Health Study II, which consists primarily of premenopausal women, no association was observed overall between plasma 25(OH)D levels and breast cancer risk and a positive, rather than inverse, association was observed among women who were overweight or obese (BMI ≥ 25 kg/m²) [13].

Recent reviews have concluded that there is insufficient evidence to recommend vitamin D supplementation for the prevention of breast cancer, but that additional research in humans is needed [3, 18, 19]. The purpose of our study was to examine the association between prediagnostic circulating levels of circulating 25(OH)D and breast cancer risk in a case-control study nested within two prospective cohorts, the New York University Women's Health Study (NYUWHS) and the Northern Sweden Mammary Screening Cohort (NSMSC). In addition to being the largest prospective study to date, a unique feature of this study was the availability of two 25(OH)D measurements from blood samples donated a minimum of one year apart for a large proportion of the study subjects, which allowed us to estimate with good precision the temporal reliability of 25(OH)D, and factors that affected it, in the two cohorts.

Materials and methods

Study population

Descriptions of the NYUWHS and the NSMSC have been provided previously [20, 21]. Briefly, the NYUWHS cohort enrolled 14,274 healthy women, aged 34-65, at a mammography screening clinic in New York City between 1985 and 1991 and the NSMSC enrolled, between 1995 and 2006, approximately 28,800 women, aged 40-69, who are participating in a population-based breast cancer screening program in Västerbotten County. After obtaining written informed consent from all study participants, information on demographic and anthropometric variables, medical and reproductive history, family history of breast cancer, and lifestyle factors including diet was collected through baseline and/or subsequent questionnaires. Venous blood was collected at enrollment, processed according to standard procedures by the respective cohorts (serum for NYUWHS and plasma for

NSMSC), and stored at -80°C. Additional blood samples were collected from women who returned for screening. This study was approved by the Institutional Review Board of the New York University School of Medicine, the Regional Ethical Committee of the University of Umeå, Sweden, and the Swedish Data Inspection Board.

Case ascertainment and control selection

For the NYUWHS, incident cases of invasive breast cancer were identified by mailed questionnaires or follow-up telephone interviews every 2-4 years after 1991, supplemented by linkages to state cancer registries in New York, New Jersey, and Florida, and the U.S. National Death Index. Medical records were reviewed to confirm self-reported cases. Using a capture-recapture analysis, we estimated that combining active and cancer registry-based follow-up resulted in a breast cancer ascertainment rate of 95%. [22]. For the NSMSC, annual linkages to the Swedish National Cancer Registry were used to identify incident cases of breast cancer in the cohort. As of January 1, 2007 for the NYUWHS and January 1, 2010 for the NSMSC, a total of 1,645 incident cases of invasive breast cancer had been identified (909 in the NYUWHS and 736 in the NSMSC). In the NYUWHS, 16 cases (3%) were excluded because they had a low serum balance. In the NSMSC, 44 cases (6%) were excluded for the following reasons: 26 had low plasma balance, 13 had their plasma reserved because of a subsequent diagnosis of a rarer cancer or other disease, 3 had insufficient volume for laboratory measurement, and 2 had both matched controls excluded for one of the reasons above. The present study included a total of 1,585 incident breast cancer cases (893 from the NYUWHS and 692 from the NSMSC).

Each case was matched to two controls who were selected from the respective cohort using incidence-density sampling. Matching factors included age at enrollment in the study (± 6 months), date of enrollment/first blood donation (NYUWHS: ± 3 months; NSMSC: ± 1

month) and number (1, 2+) and dates of subsequent blood donations. For the NYUWHS, matching factors also included menopausal status (pre- or post-menopausal) at enrollment and race/ethnicity (Caucasian, African American, other, or unknown). The vast majority of women in the NSMSC were Caucasian. Initially, women were not matched on menopausal status in this cohort; however, 88% of the cases had at least one control matching on this factor (pre- and peri-menopausal combined, or postmenopausal). A total of 2,940 controls were included in the final analysis (1,642 in NYUWHS and 1,298 in NSMSC).

For 678 matched sets (413 in NYUWHS and 265 in NSMSC), two blood samples were analyzed for 25(OH)D. For participants who had donated more than two blood samples, the first and last samples collected before the relevant case's diagnosis were selected.

Laboratory methods

Circulating 25(OH)D was measured by Heartland Assays, Inc. (Ames, IA) using a direct, competitive chemiluminescence immunoassay using the DiaSorin LIAISON platform (DiaSorin, Inc., Stillwater, Minnesota) [23]. The assay, which is appropriate for either serum or plasma, is cospecific for 25-hydroxyvitamin D₃ and 25-hydroxyvitamin D₂. All samples, including repeat samples, from a case and her matched controls were analyzed together in the same laboratory batch to minimize laboratory variability. Laboratory personnel were blinded to case-control status of the study samples. Samples from quality-control pools (6% of total samples) were masked and inserted randomly in the batches. The intra- and inter-batch coefficients of variation (CVs), were 9.5% and 11.4% respectively for NYUWHS, and 7.4% and 9.0% respectively for NSMSC.

Estrone was measured by double-antibody radioimmunoassay (RIA) with reagents from Diagnostic System Laboratories (Webster, TX) at the Laboratory for Hormone Analyses

at IARC, France for postmenopausal women who were not using hormone replacement therapy (HRT). Intra- and inter-batch CVs were 6.7% and 12.6%, respectively [24, 25].

Statistical analysis

We examined the temporal reliability of circulating 25(OH)D using the intraclass correlation coefficient (ICC). In addition to the overall ICC, we calculated ICCs according to time (years) between sample donations and according to season, for each cohort separately.

Conditional logistic regression was used to estimate odds ratios and 95% CIs for the associations of subject characteristics and circulating 25(OH)D with risk of breast cancer. We conducted analyses separately for each cohort, as well as combining them. Because there was no evidence of cohort heterogeneity, most results are presented for the combined cohorts. 25(OH)D concentrations were log₂-transformed to reduce departure from the normal distribution, and included in the model in three ways. First, we computed season-adjusted residuals to take into account the known variations of 25(OH)D with season [3]. For each 25(OH)D measurement, the residual was the difference between the observed 25(OH)D value and the value predicted for this day of the year, which was obtained using a nonparametric local regression (Proc LOESS, SAS) with 25(OH)D as the dependent variable and day of the year of blood donation as the independent variable [26]. This regression model was run separately for each cohort using all available 25(OH)D measurements, i.e. including repeat samples. We conducted analyses using cohort-specific season-adjusted quintiles based on the distribution of the residuals in controls. Second, we ran analyses with season-adjusted residuals on the continuous scale. All analyses based on residuals were conducted using first (i.e. baseline) samples only. Finally, we examined the association of circulating 25(OH)D with breast cancer risk using pre-specified categories of 25(OH)D levels, which were defined using cut-points recommended by the Institute of Medicine (<50 [inadequate], 50-74

[adequate], and 75+ nmol/L [adequate to high]) [3]. These analyses were conducted separately for “winter” and “summer,” which were defined by examining the unadjusted 25(OH)D levels in controls within each cohort. Winter included the months of January to April, when mean levels were low (48.1-51.2 nmol/L), and summer the months of July to September, when mean levels were high (62.0-68.0 nmol/L). There was little variation in mean level from month to month within each of these two seasons. Subjects who had measurements in both winter and summer were included in both season-specific analyses to increase the sample size. Because there was no difference in the main study findings between conditional logistic regression models and unconditional models adjusting for the matching factors, and because using conditional regression resulted in the loss of matched sets with samples collected in different seasons for the case and her controls, unconditional logistic regression was used for the season-specific analyses. In analyses by quintiles and pre-specified categories, tests for trend were performed using an ordered categorical variable. Tests for heterogeneity were carried out by comparing models including interaction terms to models excluding them or using Cochran’s Q statistic.

In each of the two cohorts, multivariate linear regression analyses were conducted among the controls to explore associations of potential confounders with 25(OH)D. BMI was found to be a negative predictor of 25(OH)D, while multivitamin supplement use and past use of HRT were positive predictors in both cohorts. Caucasian race and physical activity were also positive predictors of 25(OH)D in the NYUWHS and alcohol consumption was a negative predictor in the NSMSC. Covariates in the final multivariate models included the following known breast cancer risk factors: age at menarche (continuous), age at first birth/parity (≤ 20 , 21-15, 26-30, >30 years, nulliparous), family history of breast cancer (no, yes), body mass index (BMI; continuous), past HRT use (never, ever), and alcohol consumption. It is debatable whether to control for outdoor physical activity and multivitamin

use, which have been associated with higher levels of circulating 25(OH)D [27, 28], since these variables may influence breast cancer risk through their effect on 25(OH)D levels. However, these factors may affect breast cancer risk through other mechanisms [29], in which case they could act as possible confounders in analyses of 25(OH)D and breast cancer risk. Therefore, in addition to models adjusting for the factors listed above, we present results also adjusting for physical activity and multivitamin use (no, yes). In the NYUWHS, physical activity was expressed as MET-hours/week from walking and vigorous exercise, and women were classified into tertiles. In the NSMSC, women were classified as inactive, moderately active, and active by combining data on physical activity at work and frequency of walking, biking, and exercising. Baseline data were used for all variables except HRT in the NYUWHS, which represented use up to date of diagnosis (or index date for controls).

We performed multiple imputation using fully conditional specification [30] for the following covariates with missing data: alcohol consumption (23%), physical activity (23%), multivitamin use (18%), HRT use (6%), and age at menarche, parity, age at first full-term pregnancy, body mass index (all with $\leq 2\%$ missing data). We compared analyses using all subjects and imputed data to analyses using only subjects with no missing data (complete case method). Because results from both analyses were similar, we only present the analyses including all subjects and imputed data.

We conducted stratified analyses using conditional logistic regression for the following variables: age at enrollment, lag-time between blood donation and diagnosis and estrogen receptor (ER) status. In order not to lose the matched sets where a case and her controls were discordant, unconditional logistic regression, adjusted for cohort and age at blood sampling, was performed for the following variables: menopausal status, BMI, circulating estrone levels (for postmenopausal women only), and IGF-I levels. Tertiles were

used for the IGF-1 analysis because of the limited number of women for whom IGF-1 had been measured (193 cases and 269 controls from the NYUWHS only). Finally, we performed an analysis limited to Caucasians (90% of subjects). All significance testing was two-sided, and $p < 0.05$ was considered statistically significant.

Results

Descriptive statistics for the breast cancer cases and their matched controls are presented in Table 1. Mean age at enrollment was 54 years for both cases and controls. Cases were diagnosed an average of 8.7 years after blood donation. Established risk factors for breast cancer occurred more commonly in cases, including younger age at menarche, nulliparity, older age at first full-term pregnancy, and having a first-degree family history of breast cancer. Cases were more likely to report having used HRT. BMI was significantly different between cases and controls in postmenopausal women, among whom a greater proportion of cases were overweight and obese. Among the 77% of cases for which receptor status was known, 78% of tumors were ER-positive.

For women who donated more than one blood sample, the average time between sample donations was 2.1 years in the NYUWHS and 4.4 years in the NSMSC. Overall, the temporal reliability of 25(OH)D was good (ICC = 0.65 [95% CI: 0.61, 0.69] for NSMSC and 0.78 [95% CI: 0.76-0.80] for NYUWHS), and it improved for NSMSC when season-adjusted residuals were used (ICC = 0.71 [95% CI: 0.67, 0.74]) (Table 2). The ICC for the NYUWHS was not changed by seasonal adjustment because women in the NYUWHS generally returned to the screening center and donated a blood sample at the same time each year. We observed that the ICC decreased as time increased between sample donations, although this trend did not appear to extend beyond the first 8 years. For the NSMSC, the season-adjusted ICC was

0.56 (95% CI = 0.42-0.68) for samples collected 5 to 8 years apart (N = 113 subjects) and 0.63 (95% CI = 0.51-0.74) for samples collected between 8 and 11.7 years (N = 106 subjects). For both cohorts, the ICC was substantially lower when one sample was donated in the winter, and the other one in the summer months (ICC= 0.47, 95% CI = 0.29-0.61 for NSMSC, N=92; ICC= 0.66, 95% CI = 0.50-0.77 for the NYUWHS, N=68).

Table 3 reports odds ratios and 95% CIs for breast cancer risk according to season-adjusted quintiles of 25(OH)D. There was no association between circulating vitamin D and breast cancer risk overall (adjusted model $OR_{Q5-Q1} = 0.94$, 95% CI = 0.76-1.16, $p_{trend}=0.30$) or within either cohort. Results with 25(OH)D on the continuous scale were similar. Adjusting for physical activity and multivitamin use, in addition to the other confounders, did not materially affect the results ($OR_{Q5-Q1} = 0.94$, 95% CI = 0.76-1.16, $p_{trend} = 0.27$).

In analyses using pre-specified categories of circulating 25(OH)D (not adjusted for season), we observed no association with breast cancer risk for samples taken either in the winter, when more than half of the subjects had levels below 50 nmol/L, or in the summer, when more of the subjects had levels >75 nmol/L (Table 4). A suggestive protective effect was observed for women in the NYUWHS who donated blood in the summer months ($OR = 0.69$, 95% CI = 0.45-1.07, $p_{trend}=0.10$ for concentrations ≥ 75 vs. < 50 nmol/L), but no such association was observed in the NSMSC.

Table 5 shows the results of subgroup analyses. Higher circulating 25(OH)D was associated with a decreased risk of breast cancer among women who were premenopausal at blood donation ($OR_{Q5-Q1} = 0.67$, 95% CI = 0.48-0.92, $p_{trend}=0.03$), but not among those who were postmenopausal ($OR_{Q5-Q1} = 1.21$, 95% CI = 0.92-1.58, $p_{trend}=0.67$; $p_{interaction}=0.05$). A similar protective effect was observed for women who were ≤ 45 years of age at blood donation ($OR_{Q5-Q1} = 0.48$, 95% CI = 0.30-0.79, $p_{trend}=0.01$; $p_{interaction} = 0.08$). There was no

evidence of effect modification by ER status of the tumor, lag-time between blood sampling and diagnosis, BMI, or circulating estrone levels. Results of the analysis limited to Caucasians were similar to those of the analysis including all subjects.

When we examined the association of 25(OH)D with breast cancer risk by IGF-1 levels at baseline, the odds ratio for the third tertile was 0.62 (95% CI = 0.30-1.28) in the below-median stratum and 0.79 (95% CI = 0.39-1.62) in the above median stratum. The test for interaction between IGF-1 and 25(OH)D on the continuous scale was not statistically significant ($p = 0.61$).

Discussion

In this case-control study nested within two cohorts, we did not observe an association between circulating 25(OH)D and breast cancer risk overall. We observed an inverse association between 25(OH)D and breast cancer risk in the subgroups of women who were ≤ 45 yrs of age or premenopausal at blood donation, although the test for interaction was significant only for menopausal status. Because of substantial overlap, we were not able to sort out whether younger age or premenopausal status was driving the association.

Epidemiologic studies on vitamin D and breast cancer risk have been reviewed recently [31, 32]. Traditional case-control studies [33-39] with blood samples collected after breast cancer diagnosis have found inverse associations between circulating 25(OH)D and breast cancer risk. However, changes in lifestyle, particularly physical activity, following breast cancer diagnosis and treatment may affect circulating 25(OH)D, and associations observed in these studies may therefore not reflect pre-diagnosis associations [32]. Among the ten prospective studies published to date, eight reported no association overall [8-14], one

reported a marginally significant inverse association [16], and one reported a significant inverse association [15].

Regarding younger and/or premenopausal women, data from prospective studies are more limited since eligibility in some of the largest studies was restricted to older women [8-10]. Among the five prospective studies that reported results in younger and/or premenopausal women, four reported no association [11-13, 40]. In the largest of the four studies, the Nurses' Health Study II, which collected blood in relatively young (age range, 32-54), mostly premenopausal women, a large number of whom were still premenopausal at diagnosis (294 cases), the multivariate-adjusted odds ratio associated with the top quintile of 25(OH)D was 1.19 (95% CI = 0.77-1.84, $p_{\text{trend}} = 0.51$). The French E3N cohort, though, reported a significant inverse association in women who were younger (<53 years of age) at blood donation, results consistent with ours, and also observed a non-significant protective association in the smaller subgroup of women who were premenopausal at diagnosis. The investigators suggested that vitamin D may act by inhibiting the tumor growth-stimulating effects of IGF-1 [15]. Because IGF-1 levels decrease with age, a stronger anticarcinogenic effect of vitamin D would be expected in younger/premenopausal women. However, our analysis stratifying directly by IGF-1 level did not support this hypothesis, although the sample size was limited.

A protective effect of vitamin D on breast cancer would also be expected to be stronger in premenopausal women if vitamin D acts by inhibiting estrogen-stimulated breast cell proliferation [41], since estrogen levels are much higher before menopause. However, we found no evidence that the effect of vitamin D varies according to estrone levels in postmenopausal women, in spite of the strong positive association between estrone and breast cancer risk in our study. Moreover, 25(OH)D was not associated with either ER-positive or

ER-negative breast cancer, which is consistent with results from other prospective studies of 25(OH)D that found no difference by ER status [10, 13].

Too few subjects had very high concentrations of 25(OH)D for us to be able to examine the association of concentrations ≥ 100 nmol/L with breast cancer risk. The lack of dose response in the <50 to ≥ 75 nmol/L range (table 4) suggests, though, that a true association would have to be of the threshold type, a hypothesis for which there is little biological support. A linear dose-response association at levels ≤ 75 nmol/L has been observed for colorectal cancer, the one cancer site for which there is consistent evidence of a protective effect of vitamin D [42].

Several factors that are associated with breast cancer risk are also associated with circulating 25(OH)D and could therefore confound the 25(OH)D-breast cancer association [43]. Dark skin, higher BMI, and lower physical activity have been repeatedly found to be associated with lower levels of 25(OH)D, while associations between 25(OH)D and current use of HRT, vitamin supplements, and alcohol have been less consistent [28, 43, 44]. The importance of taking into account these lifestyle factors was demonstrated in the Women's Health Initiative (WHI) study, where a significant inverse association of 25(OH)D with breast cancer risk was attenuated and became non-significant after adjusting for BMI and physical activity [43]. In our study, we matched on race/ethnicity (a surrogate for dark skin) and also conducted analyses limited to Caucasians, which gave results similar to the analyses including all races. As shown in tables 3, 4 and 5, adjusting for BMI, HRT use, physical activity and multivitamin use had very little effect on the odds ratios. Residual confounding is possible, particularly by physical activity, for which we classified women in 3 categories and data were missing for 11% in the NYUWHS and 38% in the NSMSC. However, an analysis limited to the subjects for whom physical activity was available showed very similar results,

as did an analysis adjusting for MET-hours/week as a continuous variable in the NYUWHS (data not shown). Another potential source of confounding is breast cancer screening frequency, as more frequent screening visits could result in earlier diagnosis of breast cancer, or correlate with other health-conscious behaviors leading to higher 25(OH)D status. However, because blood donations occurred at the time of mammographic screening visits, number of blood donations can be considered a proxy for mammographic screening frequency. We matched on number of blood donations in the NYUWHS, while in the NSMSC, although we did not match on this variable, 59% of cases had at least one control who matched exactly on the number of blood donations, and 89% of cases had at least one control who matched within ± 1 blood donation. We therefore believe that confounding by screening history is unlikely in our study.

We used residuals obtained by local regression to take into account seasonal variation. This method has been used only rarely in studies of 25(OH)D [45], although it has been used in epidemiologic analyses of other biomarkers with temporal variation, for instance hormones known to vary during pregnancy [46, 47]. In our study, using the residual method, the exposure value for each woman was the difference between the absolute level observed for this woman and the projected mean of 25(OH)D for this day of the year (reference day). A positive residual indicated that a woman had a higher than average level at this time of the year, while a negative residual indicated a lower than average level. The projected mean was calculated using all samples collected in the same cohort on the reference day, as well as samples taken on neighboring days, with progressively decreasing weights given to samples collected further away from the reference day. This method, therefore, seems well suited to take into account the gradual changes observed during the shoulder seasons when levels progressively increase (spring) or decrease (fall).

Strengths of this study include its prospective design, inclusion of two cohorts with different diet and sun exposure, and large sample size. This is also the first study to include repeat blood samples on a large number of women. The repeat samples enabled us to assess temporal reproducibility and gave an indication of the potential impact of ignoring seasonal variation when studying the association of circulating vitamin D with disease risk. The lower ICCs observed when samples were collected in different seasons, compared to the same season, highlight the importance of taking season into account in the study design and/or analysis, as other studies have concluded [48]. The ICC of 0.63 for samples collected 8.0 to 11.7 years apart in the NSMSC compares well with the ICCs of other biomarkers that have been linked to breast cancer risk, such as postmenopausal sex hormones. However, the ICC decreased with increasing time between blood donations, although this trend did not seem to extend beyond the first 8 years. This observation underlines that a single measurement of 25(OH)D is an imperfect reflection of vitamin D status over the long time period during which breast cancer develops. Thus, the association of vitamin D status with breast cancer risk may have been underestimated due to random error in measurement of the true exposure of interest, i.e. the long-term average level of 25(OH)D. Another limitation of our study is the relatively few subjects with very high levels of circulating 25(OH)D (≥ 100 nmol/L).

Conclusions

This large prospective study does not support a relationship between circulating 25(OH)D and risk of breast cancer, except possibly in younger women. These results add to a growing body of evidence from prospective studies and randomized trials that suggests higher vitamin D levels do not reduce breast cancer risk. Recommendations regarding vitamin D

supplementation should be based on considerations other than breast cancer prevention, such as bone health.

Abbreviations

25(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CI, confidence interval; CV, coefficient of variation; ER, estrogen receptor; HRT, hormone replacement therapy; ICC, intraclass correlation coefficient; MET, metabolic equivalent of task; MSC, Mammary Screening Cohort; NYUWHS, New York University Women's Health Study; OR, odds ratio; VDR, vitamin D receptor.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

SS interpreted the data and drafted the manuscript; YA performed the statistical analysis and critically reviewed the manuscript; PL critically reviewed the manuscript; KK conceived of the study design, interpreted the data, and critically reviewed the manuscript; RH performed the 25(OH)D assay and critically reviewed the manuscript; TC interpreted the data and critically reviewed the manuscript; AA critically reviewed the manuscript; YC critically reviewed the manuscript; GH critically reviewed the manuscript; EL critically reviewed the manuscript; SR performed the estrone analyses and critically reviewed the manuscript, PT

critically reviewed the manuscript, RS critically reviewed the manuscript, AZJ secured funding, conceived of the study design, interpreted the data, and drafted the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This work was supported by National Cancer Institute grants R01 CA098661 and P30 CA016087 and Center grant ES000260 from the National Institute of Environmental Health Sciences.

References

1. Welsh J: **Vitamin D and breast cancer: insights from animal models.** *Am J Clin Nutr* 2004, **80**:1721S-1724S.
2. Bertone-Johnson ER: **Vitamin D and breast cancer.** *Ann Epidemiol* 2009, **19**:462-467.
3. Institute of Medicine; Food and Nutrition Board: **Dietary Reference Intakes for Calcium and Vitamin D.** Edited by Ross AC, Taylor CL, Yaktine AL, Del Valle HB. Washington, DC; 2010.
4. Friedrich M, Diesing D, Cordes T, Fischer D, Becker S, Chen TC, Flanagan JN, Tangpricha V, Gherson I, Holick MF, Reichrath J: **Analysis of 25-hydroxyvitamin D3-1alpha-hydroxylase in normal and malignant breast tissue.** *Anticancer Res* 2006, **26**:2615-2620.

5. Davis CD: **Vitamin D and cancer: current dilemmas and future research needs.** *Am J Clin Nutr* 2008, **88**:565S-569S.
6. Prentice A, Goldberg GR, Schoenmakers I: **Vitamin D across the lifecycle: physiology and biomarkers.** *Am J Clin Nutr* 2008, **88**:500S-506S.
7. 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**:2425S-2433S.
8. Chlebowski RT, Johnson KC, Kooperberg C, Pettinger M, Wactawski-Wende J, Rohan T, Rossouw J, Lane D, O'Sullivan MJ, Yasmeen S, Hiatt RA, Shikany JM, Vitolins M, Khandekar J, Hubbell FA; Women's Health Initiative Investigators: **Calcium plus vitamin d supplementation and the risk of breast cancer.** *J Natl Cancer Inst* 2008, **100**:1581-1591.
9. Freedman DM, Chang SC, Falk RT, Purdue MP, Huang WY, McCarty CA, Hollis BW, Graubard BI, Berg CD, Ziegler RG: **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**:889-894.
10. McCullough ML, Stevens VL, Patel R, Jacobs EJ, Bain EB, Horst RL, Gapstur SM, Thun MJ, Calle EE: **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.
11. Agborsangaya CB, Surcel HM, Toriola AT, Pukkala E, Parkkila S, Tuohimaa P, Lukanova A, Lehtinen M: **Serum 25-hydroxyvitamin D at pregnancy and risk of breast cancer in a prospective study.** *Eur J Cancer* 2010, **46**:467-470.

12. 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**:2159-2168.
13. 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.
14. Amir E, Cecchini RS, Ganz PA, Costantino JP, Beddows S, Hood N, Goodwin PJ: **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**:1077-1088.
15. Engel P, Fagherazzi G, Boutten A, Dupre T, Mesrine S, Boutron-Ruault MC, Clavel-Chapelon F: **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**:2341-2350.
16. Bertone-Johnson ER, Chen WY, Holick MF, Hollis BW, Colditz GA, Willett WC, Hankinson SE: **Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer.** *Cancer Epidemiol Biomarkers Prev* 2005, **14**:1991-1997.
17. Rejnmark L, Tietze A, Vestergaard P, Buhl L, Lehbrink M, Heickendorff L, Mosekilde L: **Reduced prediagnostic 25-hydroxyvitamin D levels in women with breast cancer: a nested case-control study.** *Cancer Epidemiol Biomarkers Prev* 2009, **18**:2655-2660.
18. Chung M, Lee J, Terasawa T, Lau J, Trikalinos TA: **Vitamin D With or Without Calcium Supplementation for Prevention of Cancer and Fractures: An Updated**

Meta-analysis for the U.S. Preventive Services Task Force. *Ann Intern Med* 2011, **155**:827-838.

19. International Agency for Research on Cancer: **Vitamin D and Cancer.** *IARC Working Group Reports*. Vol. 5. Lyon: International Agency for Research on Cancer; 2008.
20. Toniolo PG, Pasternack BS, Shore RE, Sonnenschein E, Koenig KL, Rosenberg C, Strax P, Strax S: **Endogenous hormones and breast cancer: a prospective cohort study.** *Breast Cancer Res Treat* 1991, **18 Suppl 1**:S23-26.
21. Hallmans G, Agren A, Johansson G, Johansson A, Stegmayr B, Jansson JH, Lindahl B, Rolandsson O, Söderberg S, Nilsson M, Johansson I, Weinehall L: **Cardiovascular disease and diabetes in the Northern Sweden Health and Disease Study Cohort - evaluation of risk factors and their interactions.** *Scand J Public Health Suppl* 2003, **61**:18-24.
22. Kato I, Toniolo P, Koenig KL, Kahn A, Schymura M, Zeleniuch-Jacquotte A: **Comparison of active and cancer registry-based follow-up for breast cancer in a prospective cohort study.** *Am J Epidemiol* 1999, **149**:372-378.
23. Wagner D, Hanwell HE, Vieth R: **An evaluation of automated methods for measurement of serum 25-hydroxyvitamin D.** *Clin Biochem* 2009, **42**:1549-1556.
24. Zeleniuch-Jacquotte A, Shore RE, Koenig KL, Akhmedkhanov A, Afanasyeva Y, Kato I, Kim MY, Rinaldi S, Kaaks R, Toniolo P: **Postmenopausal levels of oestrogen, androgen, and SHBG and breast cancer: long-term results of a prospective study.** *Br J Cancer* 2004, **90**:153-159.

25. Rinaldi S, Déchaud H, Biessy C, Morin-Raverot V, Toniolo P, Zeleniuch-Jacquotte A, Akhmedkhanov A, Shore RE, Secreto G, Ciampi A, Riboli E, Kaaks R: **Reliability and validity of commercially available, direct radioimmunoassays for measurement of blood androgens and estrogens in postmenopausal women.** *Cancer Epidemiol Biomarkers Prev* 2001, **10**:757-765.
26. Borkowf CB, Albert PS, Abnet CC: **Using lowess to remove systematic trends over time in predictor variables prior to logistic regression with quantile categories.** *Stat Med* 2003, **22**:1477-1493.
27. Brock K, Huang WY, Fraser DR, Ke L, Tseng M, Stolzenberg-Solomon R, Peters U, Ahn J, Purdue M, Mason RS, McCarty C, Ziegler RG, Graubard B: **Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women.** *J Steroid Biochem Mol Biol* 2010, **121**(1-2):462-466.
28. McCullough ML, Weinstein SJ, Freedman DM, Helzlsouer K, Flanders WD, Koenig K, Kolonel L, Laden F, Le Marchand L, Purdue M, Snyder K, Stevens VL, Stolzenberg-Solomon R, Virtamo J, Yang G, Yu K, Zheng W, Albanes D, Ashby J, Bertrand K, Cai H, Chen Y, Gallicchio L, Giovannucci E, Jacobs EJ, Hankinson SE, Hartge P, Hartmuller V, Harvey C, Hayes RB *et al.*: **Correlates of circulating 25-hydroxyvitamin D: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.** *Am J Epidemiol* 2010, **172**:21-35.
29. Winzer BM, Whiteman DC, Reeves MM, Paratz JD: **Physical activity and cancer prevention: a systematic review of clinical trials.** *Cancer Causes Control* 2011, **22**:811-826.

30. van Buuren S: **Multiple imputation of discrete and continuous data by fully conditional specification.** *Stat Methods Med Res* 2007, **16**:219-242.
31. Chen P, Hu P, Xie D, Qin Y, Wang F, Wang H: **Meta-analysis of vitamin D, calcium and the prevention of breast cancer.** *Breast Cancer Res Treat* 2010, **121**:469-477.
32. Chlebowski RT: **Vitamin D and breast cancer: interpreting current evidence.** *Breast Cancer Res* 2011, **13**:217.
33. Janowsky EC, Lester GE, Weinberg CR: **Association between low levels of 1,25-dihydroxyvitamin D and breast cancer risk.** *Public Health Nutr* 1999, **2**:283-291.
34. Abbas S, Linseisen J, Slinger T, Kropp S, Mutschelknauss E, Flesch-Janys D, Chang-Claude J: **Serum 25-hydroxyvitamin D and risk of post-menopausal breast cancer--results of a large case-control study.** *Carcinogenesis* 2008, **29**:93-99.
35. Abbas S, Chang-Claude J, Linseisen J: **Plasma 25-hydroxyvitamin D and premenopausal breast cancer risk in a German case-control study.** *Int J Cancer* 2009, **124**:250-255.
36. Crew KD, Gammon MD, Steck SE, Hershman DL, Cremers S, Dworakowski E, Shane E, Terry MB, Desai M, Teitelbaum SL, Neugut AI, Santella RM: **Association between plasma 25-hydroxyvitamin D and breast cancer risk.** *Cancer Prev Res (Phila)* 2009, **2**:598-604.
37. Lowe LC, Guy M, Mansi JL, Peckitt C, Bliss J, Wilson RG, Colston KW: **Plasma 25-hydroxy vitamin D concentrations, vitamin D receptor genotype and breast cancer risk in a UK Caucasian population.** *Eur J Cancer* 2005, **41**:1164-1169.

38. Dorgan J, Stanczyk F, Kahle L, Brinton L: **Prospective case-control study of premenopausal serum estradiol and testosterone levels and breast cancer risk.** *Breast Cancer Research* 2010, **12**:R98.
39. Fedirko V, Torres-Mejia G, Ortega-Olvera C, Biessy C, Angeles-Llerenas A, Lazcano-Ponce E, Saldana-Quiroz VA, Romieu I: **Serum 25-hydroxyvitamin D and risk of breast cancer: results of a large population-based case-control study in Mexican women.** *Cancer Causes Control* 2012.
40. Bertone-Johnson ER, Chen WY, Holick MF: **Plasma 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D and risk of breast cancer.** *Cancer, Epidemiology Biomarkers and Prevention* 2005, **14**:1991-1997.
41. Welsh J, Wietzke J, Zinser G, Smyczek S, Romu S, Tribble E, Welsh J, Byrne B, Narvaez C: **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.
42. Zhang X, Giovannucci E: **Calcium, vitamin D and colorectal cancer chemoprevention.** *Best Pract Res Clin Gastroenterol* 2011, **25**:485-494.
43. Neuhouwer ML, Manson JE, Millen A, Pettinger M, Margolis K, Jacobs ET, Shikany JM, Vitolins M, Adams-Campbell L, Liu S, LeBlanc E, Johnson KC, Wactawski-Wende J: **The influence of health and lifestyle characteristics on the relation of serum 25-hydroxyvitamin D with risk of colorectal and breast cancer in postmenopausal women.** *Am J Epidemiol* 2012, **175**:673-684.
44. Millen AE, Wactawski-Wende J, Pettinger M, Melamed ML, Tyllavsky FA, Liu S, Robbins J, LaCroix AZ, LeBoff MS, Jackson RD: **Predictors of serum 25-hydroxyvitamin D concentrations among postmenopausal women: the Women's**

- Health Initiative Calcium plus Vitamin D clinical trial.** *Am J Clin Nutr* 2010, **91**:1324-1335.
45. Gallicchio L, Helzlsouer KJ, Chow WH, Freedman DM, Hankinson SE, Hartge P, Hartmuller V, Harvey C, Hayes RB, Horst RL, Koenig KL, Kolonel LN, Laden F, McCullough ML, Parisi D, Purdue MP, Shu XO, Snyder K, Stolzenberg-Solomon RZ, Tworoger SS, Varanasi A, Virtamo J, Wilkens LR, Xiang YB, Yu K, Zeleniuch-Jacquotte A, Zheng W, Abnet CC, Albanes D *et al.*: **Circulating 25-hydroxyvitamin D and the risk of rarer cancers: Design and methods of the Cohort Consortium Vitamin D Pooling Project of Rarer Cancers.** *Am J Epidemiol* 2010, **172**:10-20.
46. Lukanova A, Andersson R, Wulff M, Zeleniuch-Jacquotte A, Grankvist K, Dossus L, Afanasyeva Y, Johansson R, Arslan AA, Lenner P, Wadell G, Hallmans G, Toniolo P, Lundin E: **Human chorionic gonadotropin and alpha-fetoprotein concentrations in pregnancy and maternal risk of breast cancer: a nested case-control study.** *Am J Epidemiol* 2008, **168**:1284-1291.
47. Richardson BE, Hulka BS, David Peck JL, Hughes CL, van den Berg BJ, Christianson RE, Calvin JA: **Levels of maternal serum alpha-fetoprotein (AFP) in pregnant women and subsequent breast cancer risk.** *Am J Epidemiol* 1998, **148**:719-727.
48. Jorde R, Sneve M, Hutchinson M, Emaus N, Figenschau Y, Grimnes G: **Tracking of serum 25-hydroxyvitamin D levels during 14 years in a population-based study and during 12 months in an intervention study.** *Am J Epidemiol* 2010, **171**:903-908.

Table 1. Characteristics of breast cancer cases and matched controls.

Characteristic	Case subjects N (%)	Control subjects N (%)	p-value ¹
Age at enrollment (years)			
≤45	301 (19%)	575 (20%)	Matched
46-54	557 (35%)	1,005 (34%)	
≥55	727 (46%)	1,360 (46%)	
Age at diagnosis (years)			
≤55	393 (25%)		
56-64	511 (32%)		
≥65	681 (43%)		
Race			
Caucasian	1,370 (90%)	2,484 (90%)	Matched
African-American	94 (6%)	163 (6%)	
Other	51 (4%)	118 (4%)	
Missing	70	175	
Education			
Some high school or less	288 (25%)	582 (26%)	0.09
Completed high school	415 (36%)	820 (37%)	
College or higher	439 (38%)	801 (36%)	
Missing	443	737	
Menopausal status at enrollment			
Pre- or peri-menopausal	637 (40%)	1,134 (39%)	Matched ²
Postmenopausal	948 (60%)	1,806 (61%)	
Age at menarche (years)			
< 12	285 (18%)	477 (17%)	0.005
12	397 (26%)	670 (23%)	
13	439 (28%)	803 (28%)	
>13	438 (28%)	926 (32%)	
Missing	26	64	
Nulliparous	327 (21%)	530 (18%)	0.029

Missing	46	63	
Age at first full-term pregnancy (years)			
≤20	171 (14%)	409 (18%)	0.006
21 - 25	523 (44%)	1,016 (44%)	
26 – 30	318 (27%)	596 (26%)	
>30	172 (15%)	281 (12%)	
Missing	28	45	
Ever user of oral contraceptives	677 (46%)	1219 (45%)	0.45
Missing	121	209	
Ever user of hormone replacement therapy	445 (30%)	685 (25%)	<0.001
Missing	99	176	
First-degree family history of breast cancer	299 (19%)	437 (15%)	0.0005
Body mass index (kg/m ²)			
Pre- and perimenopausal			
<20.0	53 (8%)	104 (9%)	0.24
20.0 – 24.9	339 (54%)	614 (55%)	
≥25.0	237 (38%)	409 (36%)	
Missing	8	7	
Postmenopausal			
<20.0	31 (3%)	78 (4%)	0.0009
20.0 – 24.9	396 (43%)	848 (48%)	
≥25.0	498 (54%)	835 (48%)	
Missing	23	45	
Multivitamin user	515 (41%)	956(39%)	0.49
Missing	332	509	
Ever smoker	642 (50%)	1,264 (52%)	0.52
Missing	299	524	
Alcohol (drinks/ day)			
0	501 (43%)	928 (41%)	0.80
< 1	552 (47%)	1,161 (51%)	
≥ 1	123 (10%)	187 (8%)	
Missing	409	664	

Physical activity			
NYUWHS, MET-hours per week			
<5.5	249 (31%)	412 (28%)	0.12
5.5 – 21.5	271 (34%)	506 (35%)	
≥21.5	274 (35%)	548 (37%)	
Missing	99	176	
MSC, activity level			
Inactive	55 (14%)	107 (13%)	0.84
Moderately active	145 (37%)	305 (37%)	
Active	192 (49%)	408 (50%)	
Missing	300	478	
Dietary vit D, IU/day (median; 10 th -90 th percentile)	141 (55, 272)	145 (55, 275)	0.60
Missing	340	526	
Circulating 25(OH)D, nmol/L (median;10-90 th percentile)	53.0 (31.2,81.0)	54.2 (30.7, 82.6)	0.27

¹ p-value from conditional logistic regression (p-value from trend test for ordered categories). ² Menopausal status (pre- or postmenopausal) was a matching factor for all sets in the NYUWHS. For NSMSC, women were not initially matched on this factor, and 88% of the cases had at least one control matched on menopausal status (pre- and peri-menopausal combined, or postmenopausal).

Table 2. Intraclass correlation coefficients (ICCs) by time between sample donation (years) and by season (NSMSC and NYUWHS).

	NSMSC		NYUWHS	
	N of subjects	ICC (95% CI)	N of subjects	ICC (95% CI)
All ¹	700	0.65 (0.61, 0.69)	1168	0.78 (0.76, 0.80)
All ²	700	0.71 (0.67, 0.74)	1168	0.78 (0.76, 0.80)
Time between samples ² (years)				
≤ 1			393	0.80 (0.76, 0.83)
>1 – 2	146	0.79 (0.71, 0.84)	325	0.81 (0.77, 0.84)
>2 – 3	139	0.81 (0.75, 0.86)	208	0.80 (0.75, 0.84)
>3 – 5	196	0.71 (0.63, 0.73)	214	0.74 (0.67, 0.79)
>5 – 8	113	0.56 (0.42, 0.68)		
>8 -11.7	106	0.63 (0.51, 0.74)		
Seasons ¹				
Both samples in winter	148	0.74 (0.66, 0.81)	266	0.78 (0.72, 0.82)
Both samples in summer	53	0.79 (0.67, 0.88)	210	0.81 (0.76, 0.85)
One sample in winter, one in summer	92	0.47 (0.29, 0.61)	68	0.66 (0.50, 0.77)

¹ Log₂-transformed 25(OH)D values. ² Season-adjusted 25(OH)D residual values.

Table 3. Odds ratios and 95% CIs for breast cancer risk according to season-adjusted circulating levels of 25(OH)D (by quintiles and as a continuous variable).

	Quintiles					p-trend	Continuous	
	1	2	3	4	5		OR (95% CI) ¹	p-value
Both cohorts								
Cases/controls	311/589	362/588	309/588	315/588	288/587			
Unadjusted model	1.0 (ref)	1.18 (0.97, 1.43)	0.99 (0.81, 1.21)	1.02 (0.84, 1.24)	0.93 (0.76, 1.14)	0.18	0.94 (0.84, 1.06)	0.31
Adjusted model ²	1.0 (ref)	1.16 (0.95, 1.42)	1.00 (0.81, 1.22)	1.04 (0.85, 1.27)	0.94 (0.76, 1.16)	0.30	0.95 (0.84, 1.08)	0.44
Adjusted model ³	1.0 (ref)	1.16 (0.95, 1.42)	0.99 (0.81, 1.21)	1.03 (0.84, 1.27)	0.94 (0.76, 1.16)	0.27	0.95 (0.84, 1.07)	0.40
NYUWHS								
Cases/controls	191/329	190/328	176/329	170/328	166/328			
Unadjusted model	1.0 (ref)	0.99 (0.76, 1.29)	0.92 (0.71, 1.20)	0.89 (0.69, 1.16)	0.86 (0.66, 1.12)	0.18	0.91 (0.79, 1.05)	0.21
Adjusted model ²	1.0 (ref)	0.98 (0.75, 1.28)	0.94 (0.72, 1.23)	0.92 (0.70, 1.21)	0.88 (0.67, 1.16)	0.31	0.93 (0.80, 1.08)	0.34
Adjusted model ³	1.0 (ref)	1.00 (0.76, 1.31)	0.95 (0.73, 1.24)	0.93 (0.71, 1.22)	0.90 (0.68, 1.19)	0.37	0.94 (0.81, 1.09)	0.42
NSMSC								
Cases/controls	120/260	172/260	133/259	145/260	122/259			
Unadjusted model	1.0 (ref)	1.47 (1.09, 1.97)	1.10 (0.81, 1.49)	1.22 (0.90, 1.64)	1.03 (0.75, 1.40)	0.62	1.00 (0.82, 1.23)	0.99
Adjusted model ²	1.0 (ref)	1.48 (1.08, 2.01)	1.09 (0.79, 1.49)	1.26 (0.92, 1.72)	1.05 (0.76, 1.46)	0.74	1.02 (0.82, 1.26)	0.89
Adjusted model ³	1.0 (ref)	1.46 (1.07, 1.99)	1.08 (0.79, 1.48)	1.24 (0.91, 1.70)	1.04 (0.75, 1.45)	0.71	1.01 (0.81, 1.26)	0.94

¹ Odds ratio for a doubling in 25(OH)D level. ² Adjusted for age at menarche (continuous), age at first birth/parity (≤ 20 , 21-25, 26-30, > 30

years, nulliparous), family history of breast cancer (no, yes), body mass index (continuous), HRT use (never, ever), and alcohol consumption

(continuous). ³ Adjusted for physical activity and multivitamin use in addition to variables in footnote ¹.

Table 4. Odds ratios and 95% CIs for breast cancer risk according to pre-specified categories of circulating 25(OH)D concentration by season¹.

		Pre-specified categories, nmol/L				Continuous ²	p-value
		<50	50-74	75+	p-trend		
Winter (Jan, Feb, Mar, Apr)							
Both cohorts³	Cases/controls	391/704	244/472	65/116			
	OR (95% CI)	1.0 (ref)	0.94 (0.77, 1.15)	1.06 (0.76, 1.48)	0.95	0.97 (0.82, 1.15)	0.74
NYUWHS	Cases/controls	193/331	105/217	35/65			
	OR (95% CI)	1.0 (ref)	0.82 (0.61, 1.10)	0.91 (0.58, 1.44)	0.35	0.90 (0.73, 1.13)	0.37
NSMSC	Cases/controls	198/373	139/255	30/51			
	OR (95% CI)	1.0 (ref)	1.10 (0.83, 1.46)	1.25 (0.76, 2.06)	0.34	1.11 (0.85, 1.47)	0.44
Summer (Jul, Aug, Sep)							
Both cohorts³	Cases/controls	113/186	217/348	99/212			
	OR (95% CI)	1.0 (ref)	1.02 (0.75, 1.38)	0.76 (0.53, 1.09)	0.14	0.82 (0.64, 1.05)	0.12
NYUWHS	Cases/controls	77/121	125/190	67/148			
	OR (95% CI)	1.0 (ref)	1.07 (0.73, 1.56)	0.69 (0.45, 1.07)	0.10	0.80 (0.60, 1.07)	0.14
NSMSC	Cases/controls	36/65	92/158	32/64			
	OR (95% CI)	1.0 (ref)	0.99 (0.59, 1.67)	0.89 (0.46, 1.70)	0.72	0.88 (0.52, 1.47)	0.61

¹ Adjusted for age at menarche (continuous), family history of breast cancer (no, yes), age at first birth/parity (≤ 20 , 21-25, 26-30, > 30 years, nulliparous), body mass index (continuous), HRT use (never, ever), and alcohol consumption (continuous). ² Odds ratio for a doubling in 25(OH)D level. ³ Adjusted for cohort in addition to all factors in footnote¹.

Table 5. Stratified odds ratios and 95% CIs for breast cancer risk according to quintiles of season-adjusted residual values of circulating 25(OH)D concentration at enrollment.

		Quintiles					p-trend	p-het ¹
		1	2	3	4	5		
Caucasians²	Cases/Controls	209/399	298/449	270/469	282/473	244/471	0.46	0.31
	OR (95% CI)	1.00	1.28 (1.01, 1.62)	1.10 (0.87, 1.38)	1.17 (0.92, 1.48)	0.97 (0.76, 1.24)		
Age at enrollment²								0.08
≤ 45	Cases/Controls	72/101	54/103	62/103	61/110	52/136	0.01	
	OR (95% CI)	1.00	0.67 (0.42, 1.07)	0.78 (0.49, 1.25)	0.73 (0.46, 1.14)	0.48 (0.30, 0.79)		
45-54	Cases/Controls	118/220	116/220	97/201	117/209	109/186	0.73	
	OR (95% CI)	1.00	0.92 (0.65, 1.29)	0.82 (0.58,1.17)	0.99 (0.70, 1.40)	1.04 (0.73, 1.48)		
≥55	Cases/Controls	121/268	192/265	150/284	137/269	127/265	0.96	
	OR (95% CI)	1.00	1.76 (1.30, 2.38)	1.28 (0.94, 1.73)	1.28 (0.94, 1.75)	1.20 (0.87, 1.66)		
Lag time to diagnosis²								0.30
≤ 8 yrs	Cases/Controls	123/261	174/240	144/258	140/253	141/252	0.99	
	OR (95% CI)	1.00	1.62 (1.19, 2.22)	1.23 (0.90, 1.69)	1.26 (0.92, 1.73)	1.18 (0.85, 1.63)		
> 8 yrs	Cases/Controls	188/328	188/348	165/330	175/335	157/335	0.20	
	OR (95% CI)	1.00	0.92 (0.71, 1.20)	0.86 (0.66, 1.13)	0.91 (0.70, 1.19)	0.82 (0.62, 1.08)		
ER status²								0.79
ER+	Cases/Controls	173/355	219/347	178/358	200/355	78/352	0.79	
	OR (95% CI)	1.00	1.31 (1.00, 1.70)	1.04 (0.80, 1.35)	1.22 (0.93, 1.58)	1.10 (0.83, 1.44)		
ER-	Cases/Controls	53/99	60/99	56/111	49/93	54/95	0.85	
	OR (95% CI)	1.00	1.03 (0.63, 1.70)	0.99 (0.60, 1.64)	1.00 (0.61, 1.66)	1.08 (0.64, 1.85)		
Menopausal status³								0.05
Premenopausal	Cases/Controls	150/229	128/228	122/218	123/221	114/238	0.03	
	OR (95% CI)	1.00	0.80 (0.59, 1.09)	0.79 (0.58, 1.08)	0.80 (0.58, 1.09)	0.67 (0.48, 0.92)		
Postmenopausal	Cases/Controls	161/360	234/360	187/370	192/367	174/349	0.67	
	OR (95% CI)	1.00	1.48 (1.15, 1.90)	1.16 (0.89, 1.50)	1.22 (0.94, 1.58)	1.21 (0.92, 1.58)		
BMI (kg/m²)³								0.56
< 25	Cases/Controls	115/245	153/312	164/323	195/361	192/403		

25+	OR (95% CI)	1.00	1.01 (0.75, 1.36)	1.05 (0.78, 1.40)	1.10 (0.83, 1.47)	0.97 (0.73, 1.29)	0.99	0.41
	Cases/Controls	187/331	201/267	142/260	115/215	90/171		
Estrone (pg/ml) ^{3,4} < 23.25	OR (95% CI)	1.00	1.31 (1.01, 1.70)	0.93 (0.70, 1.22)	0.91 (0.68, 1.22)	0.93 (0.68, 1.27)	0.16	
	Cases/Controls	32/90	42/85	40/94	44/97	33/102		
23.25 – 32.45	OR (95% CI)	1.00	1.38 (0.79, 2.41)	1.19 (0.68, 2.09)	1.36 (0.78, 2.36)	0.95 (0.53, 1.70)	0.83	
	Cases/Controls	43/83	59/82	48/91	53/81	47/74		
> 32.45	OR (95% CI)	1.00	1.40 (0.84, 2.33)	1.03 (0.61, 1.73)	1.33 (0.78, 2.25)	1.29 (0.75, 2.22)	0.49	
	Cases/Controls	48/83	80/94	56/80	37/82	41/52		
	OR (95% CI)	1.00	1.70 (1.05, 2.76)	1.36 (0.81, 2.27)	0.87 (0.50, 1.50)	1.66 (0.94, 2.95)	0.82	

¹ Cochran's Q statistic was used to test for heterogeneity according to lagtime and ER status. ² Adjusted for age at menarche (continuous), family history of breast cancer (yes, no), age at first birth/parity (≤ 20 , 21-25, 26-30, > 30 years, nulliparous), body mass index (continuous), HRT use (ever, never), and alcohol consumption (continuous). ³ Unconditional logistic regression analyses adjusted for cohort and age at sampling in addition to all factors in footnote². ⁴ Measured in postmenopausal women only.