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Serum 25-hydroxyvitamin D and risk of postmenopausal breast cancer - results of a large case-control study

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Abstract

Various studies suggest that vitamin D may reduce breast cancer risk. Most studies assessed the effects of dietary intake only, although endogenous production is an important source of vitamin D. Therefore, the measurement of serum 25-hydroxyvitamin D [25(OH)D] better indicates overall vitamin D status.

To assess the association of 25(OH)D serum concentrations with postmenopausal breast cancer risk, we used a population-based case-control study in Germany, which recruited incident breast cancer patients aged 50-74 between 2002 and 2005. Information on sociodemographic and breast cancer risk factors was collected by personal interview. For this analysis, we included 1394 cases and 1365 controls, matched on year of birth and time of blood collection. Conditional logistic regression was used to calculate odds ratios for breast cancer adjusted for potential confounders. Serum 25(OH)D concentration was significantly inversely associated with postmenopausal breast cancer risk. Compared with the lowest category (< 30 nM), odds ratios and 95% confidence intervals [OR (95% CI)] for the higher categories of 25(OH)D (30-45, 45-60, 60-75, ≥ 75 nM) were 0.57 (0.45-0.73), 0.49 (0.38-0.64), 0.43 (0.32-0.57) and 0.31 (0.24-0.42), respectively (p_{trend} <0.0001). Analysis using fractional polynomials indicated a non-linear association. The association was stronger in women never using menopausal hormone therapy compared to past and current users ($p_{interaction}$ <0.0001). Our findings strongly suggest a protective effect for postmenopausal breast cancer through a better vitamin D supply as characterized by serum 25(OH)D measurement, with a stronger inverse association in women with low serum 25(OH)D concentrations (< 50 nM).

Introduction

Epidemiological and experimental studies suggest an inverse association between vitamin D and cancer of different sites, including breast cancer (1-6). Most epidemiological studies have assessed the effects of dietary intake only, although endogenous production after sun exposure is the main source of vitamin D (6). Both vitamin D from diet and endogenous production is converted via two consecutive hydroxylation steps to 25-hydroxyvitamin D [25(OH)D] in the liver and to the biologically active form of vitamin D 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in the kidneys. In addition to its essential role in calcium homeostasis and bone metabolism, 1,25$(OH)_2$D has a wide range of non-classical actions that include induction of cell differentiation, inhibition of cell growth and regulation of apoptosis in normal and malignant cells, including human breast cancer cells (7-10). 1,25$(OH)_2$D exerts its anticarcinogenic actions via the vitamin D receptor (VDR) modulating the transcription of target genes such as p21, p27, c-fos, and c-myc (1).

Although not biologically active, there is overall agreement that 25(OH)D is the appropriate biomarker with which to measure vitamin D-status in humans. In contrast to 1,25$(OH)_2$D, 25(OH)D is not tightly regulated and better reflects vitamin D-stores obtained from both sunlight exposure and ingested vitamin D over longer periods (11-14). Recent studies have demonstrated that conversion to the biologically active form of vitamin D 1,25$(OH)_2$D also occurs in extrarenal tissues such as colon, prostate, and breast (15-17). In addition, a recent cell study reported expression of CYP27B1, the enzyme that converts 25(OH)D to active 1,25$(OH)_2$D, in mammary cells as well as growth inhibition of mammary cells by 25(OH)D, thus linking vitamin D status to breast cancer risk (18).

Data from epidemiologic studies assessing the association between dietary intake of vitamin D and breast cancer risk have been inconclusive (5,19-25). Thus far only four studies have

assessed the relationship between serum vitamin D metabolites [25(OH)D or $1,25(OH)_2D$)] and breast cancer risk (2,4,26,27). Of two small hospital-based case-control studies, one found a significantly inverse association for 25(OH)D and breast cancer risk (4) whereas the other study revealed no association for 25(OH)D but an inverse association for $1,25(OH)_2D$ (27). A case-control study nested in a voluntary health check up showed no association between $1,25(OH)_2D$ and breast cancer risk (26). To date, the largest case-control study including 701 cases nested in the Nurses´ Health Study (NHS) (2), reported a non-significant decrease in breast cancer risk with both higher serum 25(OH)D or $1,25(OH)_2D$ concentrations.

To our knowledge, the present study including 1394 cases is by far the largest population-based case-control study assessing the relationship of serum 25(OH)D concentrations and breast cancer risk in postmenopausal women. Because estrogens are known to increase concentrations of vitamin D binding protein, vitamin D receptor and serum 25(OH)D concentration (28-34), we additionally examined effect modification by risk factors including hormone-related variables such as menopausal hormone therapy (HT) and number of pregnancies.

Material and Methods

Study population and data collection

We used data from a large population-based case-control study (MARIE-study, Mamma Carcinoma Risk factor Investigation) carried out in two regions in Germany, the city of Hamburg and the Rhein-Neckar-Karlsruhe (R-N-K) region. The study was approved by the ethics committees of both the University of Heidelberg and the University of Hamburg and conducted in accordance with the Declaration of Helsinki. All study participants gave informed consent. Cases were eligible if they had a histologically confirmed primary invasive or *in situ* breast cancer diagnosed between 01.01.2001 and 30.09.2005 in Hamburg and

between 01.08.2002 and 31.07.2005 in the R-N-K region. Further inclusion criteria were age between 50–74 and being a resident of one of the study regions. Cases were identified through frequent monitoring of hospital admissions, surgery schedules and pathology records. Clinical and pathological characteristics of the patients were abstracted from hospital and pathology records. Of the 5,970 eligible patients who could be contacted, 3,919 (65.6%) participated, while 2051 (34.4 %) declined participation or did not respond to invitation letters.

Two controls per case were randomly selected from lists of residents provided by population registries and frequency-matched by year of birth and study region to the cases. Of the 17,093 controls who met the inclusion criteria, 7,421 (43.4%) participated, 7,521 (44.0%) refused to participate and 2,151 (12.6%) did not respond.

All participants were interviewed by trained personnel to obtain information on sociodemographic factors, anthropometric measures, data on lifetime HT exposure, including information on timing and duration, type, dose and brand name of HT, and other potential breast cancer risk factors. An alphabetical list with photographic depictions of over 300 HT products prescribed in Germany over the past 35 years was presented to women as a memory aid recalling their HT history. In addition, participants reported their dietary habits during the past 12 months with a self-administered validated 176 items food frequency questionnaire (35). Nutrient intake was calculated with the German food composition table BLS II.3 (Bundesinstitut für Gesundheitlichen Verbraucherschutz und Veterinärmedizin, Berlin, Germany).

Measurement of 25-hydroxyvitamin D

For quantification of 25(OH)D in serum we used the OCTEIA 25-hydroxyvitamin D enzyme immunoassay (IDS, Immundiagnostic Systems Limited, England). Serum samples were stored in aliquots at -80°C until measurement. Samples were analyzed in a single batch between November 2006 and January 2007. The coefficient of variation was 3.4% for

intraassay determination and 7.6% for interassay determination. Recovery was analyzed by spiking a high with a low serum concentration sample (98.7 and 26.3 nM, respectively) and comparing the observed with the expected values. When spiking the high and low concentration samples in a 1:1, 3:1 and 1:3 ratio, recovery was 105%, 99% and 96%, respectively. We measured 235 random samples (8.5%) in duplicate. The average absolute deviation from the mean between two duplicates was 2.2%.

Data analysis

Women were defined as postmenopausal if they reported their last natural menstrual bleeding at least 12 months before the reference date, a bilateral oophorectomy, or cessation of menses due to radiation or chemotherapy. In addition, women above 55 years with unclear menopausal status due to hysterectomy or hormone use were also considered postmenopausal (since the 90th percentile for age at menopause for women with natural menopause was 55 years). Women under age 55 with unclear menopausal status and women who were clearly premenopausal were excluded from the analysis. A total of 3,464 invasive or *in situ* breast cancer cases and 6,657 controls, of which 1,559 cases and 3,008 controls came from the R-N-K region, were classified as postmenopausal. For this analysis we identified 1,400 postmenopausal cases from the R-N-K region for whom we had blood samples (90% of the cases from the study region) and randomly selected 1,400 postmenopausal controls from the same study region with matching on time of blood collection in 4 categories (Jan-March, April-June, July-September, October-December) and year of birth (continuous). After exclusion of haemolytic serum samples our final study population consisted of 1,394 postmenopausal cases and 1,365 postmenopausal controls.

We assessed the association of serum 25(OH)D and postmenopausal breast cancer risk by means of logistic regression with stratification by year of birth (continuous) and time of blood collection (4 categories). Odds ratio estimates and their 95% confidence intervals (CIs) were

calculated assessing 25(OH)D concentration both as continuous (per 10 nM increment) and as categorical variable divided into 5 categories (< 30, 30-45, 45-60, 60-75, ≥ 75 nM). This categorization closely corresponds to the classification into quintiles according to the distribution in the control group. We present odds ratio estimates adjusted for age at menopause (< 47, 47-51, 52-55, ≥ 56 , unknown), BMI (< 22.5, 22.5-25, 25-30, ≥ 30 kg/m^2), education level (low, middle, high), first degree family history of breast cancer (yes, no, unknown), history of benign breast disease (yes, no), number of pregnancies (≥ 28^{th} week) (0, 1, 2, ≥ 3), age at menarche (< 12, 12-14, ≥ 15), breast feeding history (ever, never), total number of mammograms (0, 1-4, 5-9, ≥ 10, unknown), smoking status (never, past, current) and use of menopausal hormone therapy (never, past, current). Women reporting HT use for ≤ 3 months before the reference date were considered "never" users, women with more than 3 months were considered "ever" users. Among the "ever" users women were defined as "current" users of HT if they reported use within 6 months before the reference date; otherwise they were defined as "past" users. Adjustment on type of menopause, type of hormone therapy, age at first birth, alcohol consumption, contraceptive use, physical activity, dietary intake of calcium and dietary intake of vitamin D did not affect the odds ratio estimates substantially and were therefore not included in the fully adjusted models. Test for linear trend was performed using the median values in each category as an ordinal variable. To further examine dose-response-relation and non-linearity of the log odds ratio function for predictive 25(OH)D concentrations, we used the method of fractional polynomials (36). The continuous 25(OH)D variable was entered into the multivariate logistic regression model via a set of defined transformations [x^{-2}, x^{-1}, $x^{-0.5}$, $x^{0.5}$, x^2, x^3 and log(x)], allowing a maximum of two terms (including the untransformed variable) in the model. The function that best fitted the data was selected on the basis of the –2 log likelihood of the respective model. We additionally fitted the multivariate logistic regression models with restricted cubic splines for

25(OH)D concentration treated as a continuous variable (37). We specified 4 knot points at 25(OH)D concentrations of 9.7, 36, 51.5 and 70.7 representing the minimum, the 25th, 50th and 75th percentiles in the control group, respectively. Other knot points were also specified but did not change the shape of the odds ratio function.

We assessed effect modification by testing for multiplicative interaction. We included an interaction term of the continuous variable of interest [25(OH)D] and potential interaction variables in the fully adjusted model and evaluated statistical significance with the likelihood-ratio test. Differences in cases and controls were evaluated with the χ^2-test. All tests were two-sided and considered to be statistically significant if $p \leq 0.05$. All statistical analyses were carried out using the statistical software SAS 9.1 (SAS Institute, Cary, NC).

Results

The mean age of cases and controls was 63.6 and 63.5 years, respectively. Characteristics, including sociodemographic variables and main risk factors for breast cancer, of the 1,394 postmenopausal cases and 1,365 postmenopausal controls are shown in table I. Cases and controls differed significantly with respect to age at menopause, family history of breast cancer in at least one first-degree relative, benign breast disease history, number of pregnancies, breast feeding history, age at menarche, number of mammograms and use of menopausal hormone therapy (Table I).

Median serum concentration of 25(OH)D was 44.9 nM and 51.4 nM in cases and controls, respectively ($p < 0.0001$). We found a significant inverse association between serum concentration of 25(OH)D and risk of postmenopausal breast cancer. Compared with the lowest category (< 30 nM), the odds ratios [OR (95% CI)] for higher serum concentrations (30-45, 45-60, 60-75, and ≥ 75 nM) were 0.57 (0.45-0.73), 0.49 (0.38-0.64), 0.43 (0.32-0.57) and 0.31 (0.24-0.42), respectively ($p_{trend} < 0.0001$) (Table II). We additionally analyzed 25(OH)D as a continuous variable in the multivariate model and found a significantly reduced

risk for postmenopausal breast cancer of 0.88 (0.85-0.91) per 10 nM increment in serum 25(OH)D (Table II). We further examined the shape of the risk function using fractional polynomials and found a non-linear association between the log odds ratio and the 25(OH)D concentration ($p_{non\text{-}linearity}$ = 0.0002). The model with the polynomial $(x+1)^{-0.5}$ best fitted the data. The resulting function, $OR(x) = \exp[10.4*(x+1)^{-0.5}]$, taking the median value of the reference category from the categorical analysis (24.3 nM 25(OH)D in the controls) as the reference, is displayed in figure 1. Our data suggest a more pronounced inverse association between 25(OH)D and breast cancer risk in the lower concentration range (< 50 nM 25(OH)D) and a flattening of the risk function with increasing 25(OH)D concentrations. There was a good fit between the curve and the odds ratio estimates of the categorical analysis. Using restricted cubic spline regression, the shape of the risk function was similar, but model fit was inferior compared to the model using fractional polynomials.

Following previous reports on the interaction between the estrogen- and the vitamin D-endocrine system, we evaluated effect modification by use of hormone therapy. Median serum 25(OH)D concentrations were higher in women currently using HT compared to past or never users. However, these differences were more prominent in cases than in controls (Table III). In women who reported never having used HT, breast cancer risk reduction per 10 nM increment in 25(OH)D was greater [OR (95% CI)= 0.78 (0.73-0.83)] than in past and current hormone users [OR (95% CI)= 0.86 (0.79-0.94) and 0.96 (0.91-1.02), respectively] (Table III). The interaction between 25(OH)D concentration and use of HT was statistically significant ($p_{interaction}$ < 0.0001) and independent of the type of HT used (estrogen-only or combined estrogen-progestin therapy). The effect modification by HT use also did not differ by estrogen receptor (ER) or progesterone receptor (PR) status of the tumor.

The effect of 25(OH)D on breast cancer risk was also modified by number of pregnancies ($p_{interaction}$ < 0.004). The odds ratio (95% CI) for breast cancer per 10 nM increment in

25(OH)D in women with less than two pregnancies was 0.91 (0.87-0.96), while the association was stronger in women with two [0.86 (0.82-0.92)] and three or more pregnancies [0.81 (0.73-0.89] (Table IV).

The association between 25(OH)D concentration and breast cancer risk was not significantly modified by age, smoking status, age at menarche, age at menopause, BMI, alcohol, family history of breast cancer, breast feeding history, history of benign breast disease, physical activity or dietary intake of calcium. In addition, no modification by ER or PR status of the tumor or combinations of both was observed. We further examined the association of serum 25(OH)D and breast cancer risk within strata of dietary vitamin D intake. The association remained inverse and no statistical interaction was observed. In addition, dietary intake was not correlated with serum 25(OH)D (r=0.03, *p*=0.14).

Median difference between time of diagnosis and time of blood collection in cases was 66 days. In a sensitivity analysis we evaluated potential effects of diagnosis or cancer therapy on circulating 25(OH)D concentration by excluding patients close to diagnosis. Compared with the lowest category (< 30 nM), the odds ratios (95% CI) for the highest category (≥ 75 nM) were 0.31 (0.24-0.42) for all cases, and 0.32 (0.23-0.43) and 0.39 (0.26-0.58), respectively, when excluding cases with less than 15 days (26% of all cases) and cases with less than 6 months (63% of all cases) between time of diagnosis and time of blood collection.

Discussion

In this population-based case-control study, the concentration of serum 25(OH)D was significantly inversely associated with the risk of postmenopausal breast cancer. To date, only three studies have assessed the association of breast cancer risk and 25(OH)D (2,4,27). Our findings support the recently reported results from a case-control study nested in the NHS (2). The authors found an inverse, although not significant, association between breast cancer risk

and both 25(OH)D and 1,25$(OH)_2$D metabolites restricted to postmenopausal women only. Lowe et al. also observed a significant risk reduction of breast cancer in the highest category of 25(OH)D compared to the lowest in predominantly postmenopausal women (4). In contrast, Janowsky et al. reported no association of breast cancer with serum 25(OH)D but with 1,25$(OH)_2$D levels (27). However, the authors included both pre- and postmenopausal women and used hospital-based controls, who may have had less sun exposure than population-based controls. This might explain the conflicting results for 25(OH)D and 1,25$(OH)_2$D as less sun exposure has a greater impact on 25(OH)D status than on the more tightly regulated 1,25$(OH)_2$D concentration (14). Recently, a case-control study found lower levels of 1,25$(OH)_2$D but not 25(OH)D in breast cancer patients (38). However, this study was very small and no risk estimates were reported.

Results regarding the association between vitamin D metabolites and breast cancer risk are not easy to compare because of different metabolite levels in the study populations and reference groups used for calculating relative risks. In our study, median serum 25(OH)D concentrations of 44.9 and 51.4 nM for cases and controls, respectively, were comparable to recently published data from the German National Health Survey for women in this age range (39). However, 25(OH)D concentrations were relatively low compared to other studies in the US and the UK reporting mean concentrations of more than 80 nM (2,4). We therefore repeated our analysis calculating odds ratio estimates for breast cancer risk comparing women with concentrations above 100 nM 25(OH)D to women with concentrations below 50 nM 25(OH)D, which is comparable to the categorization in the NHS (2). The observed inverse association was considerably stronger in our study population compared with that found in the NHS [OR (95% CI): 0.44 (0.30-0.65) and 0.73 (0.49-1.07), respectively].

There is ample evidence from cellular and animal studies linking 25(OH)D to breast cancer. This includes the known anticarcinogenic effects of vitamin D with regard to apoptosis, cell

differentiation and proliferation, growth inhibition of human mammary epithelial cells by both $1,25(OH)_2D$ and 25(OH)D, the presence and expression of the VDR and CYP27B1, the enzyme that converts 25(OH)D to active $1,25(OH)_2D$, in mammary cells, and the uptake of the vitamin D binding protein-25(OH)D complex in mammary cells *in vitro* (40). However, little is known about the delivery of 25(OH)D to mammary cells and therefore further investigations into the mechanisms of delivery , uptake and action of 25(OH)D in mammary cells is needed.

Our findings of a nonlinear relationship between 25(OH)D concentrations and the log odds ratio for breast cancer using fractional polynomials are of special interest. In our study population the protective effect was strongest in women with low 25(OH)D concentrations (below 50 nM), and was reduced substantially with higher 25(OH)D concentration. These are important public health findings for women in low 25(OH)D concentration ranges and indicate subgroups where vitamin D supplementation may be most relevant for breast cancer prevention. Due to the low 25(OH)D concentrations in our study population, we were able to provide important additional information to further understand the dose-response relationship of vitamin D and breast cancer risk.

We found a statistically significant modification of the association of breast cancer risk and serum 25(OH)D concentrations with a stronger effect in women never having used HT as compared to HT users. Accordingly, results from the NHS showed some evidence for a stronger effect in HT users, although power was low for their comparisons (2). In women using hormone therapy, taking oral contraceptives, or with increased endogenous estrogen levels, higher concentrations of vitamin D metabolites have been reported (32,33,41,42). The underlying mechanisms include the estrogen-induced activation of renal 1α-hydroxylase, inhibition of 24-vitamin D hydroxylase, and the upregulation of the vitamin D receptor resulting in increased serum concentrations and activity of 25(OH)D and $1,25(OH)_2D$

(33,41,43,44). We also found higher 25(OH)D serum concentrations in women currently using HT compared to past or never users, and this difference was not attributable to differences in age, BMI, physical activity, or other known breast cancer risk factors. Serum 25(OH)D concentrations in women never using HT were significantly lower in cases than in controls ($p < 0.0001$), whereas no significant case-control differences were observed in women currently using HT. We hypothesize that HT may induce an increase in 25(OH)D only below a certain threshold level of 25(OH)D (Median: 37.3 nM and 47.6 nM for cases and controls never using hormone therapy, respectively), resulting in differential effects in cases and controls in our study population and in effect modification by HT on the association of 25(OH)D with breast cancer risk. In line with this hypothesis, use of HT was significantly associated with 25(OH)D using a linear regression model in women with low serum 25(OH)D concentrations (< 50 nM, ß=2.2, p=0.004) but not in women with high serum concentrations (> 50 nM, ß=-2.2, p=0.21). To our knowledge, there is, however, no data biologically supporting this threshold hypothesis.

We also found a stronger effect of 25(OH)D on breast cancer risk in women with increasing number of pregnancies. Parity is known to be associated with changes in sex-hormone level resulting in a potential lower life time estrogen exposure (45,46). Cell studies on the interaction of estrogens, antiestrogens and $1,25(OH)_2D$ in tumor cell lines have been conducted. In cell studies $1,25(OH)_2D$ has been shown to be considerably more anticarcinogenic in combination with antiestrogens (47,48). In addition, in the presence of estradiol $1,25(OH)_2D$ has been shown to stimulate proliferation at low doses and inhibit proliferation at higher doses, whereas in a non-estrogen environment only antiproliferative effects have been observed (48). Therefore, both, the interaction of 25(OH)D with use of hormone therapy and with the number of pregnancies, could be explained by interactions with estrogen metabolism, i.e. a lower life time exposure to estrogens resulting in a potential

stronger anticarcinogenic effect of vitamin D. However, the complex regulation of vitamin D in the human body and its interactions with the estrogen metabolism in breast cancer cells deserves further investigation.

We further analyzed whether the effect of vitamin D on breast cancer differed by receptor status of the tumor and did not find differential effects. Recent data from the NHS suggests an inverse association for ER-/PR-, but not for ER+/PR+ and ER+/PR- tumors, although power was low for their analysis (2). Because the effect of hormone therapy on breast cancer is known to be stronger in receptor positive tumors (49,50), we additionally analyzed potential differences in the modification of the breast cancer-25(OH)D association by hormone use in ER+ or PR+ tumors only. No evidence was found for a stronger effect modification in ER+ or PR+ tumors.

With regard to the contribution of dietary vitamin D to serum 25(OH)D, few foods naturally contain vitamin D (i.e., fish and eggs) and fortification of food with vitamin D as well as high fish consumption is not common in Germany. Therefore, a low contribution of dietary vitamin D to the serum 25(OH)D concentration in our study population seems plausible.

Our study may have methodological limitations due to the retrospective case-control design. We are aware that a cancer diagnosis may change dietary or behavioural habits (less dietary intake of vitamin D, less sun exposure), which may influence 25(OH)D concentrations. Nevertheless, short-term changes in diet or behavioural habits are less likely to influence serum concentration, because the half-life of 25(OH)D is rather long (2-3 weeks), the correlation between dietary vitamin D and serum 25(OH)D in this study was low (r=0.03), and plasma levels of 25(OH)D are fairly consistent over time (51). Although a notable change in 25(OH)D concentration after chemotherapeutic treatment was not observed in two studies (52,53), cancer therapy following diagnosis may affect circulating 25(OH)D concentration.

However, the sensitivity analysis excluding patients < 6 months after diagnosis showed no notable change in the risk estimates.

Selection bias due to the participation rate of cases and controls is also unlikely to have biased our results in terms of 25(OH)D status. Information collected via short questionnaire from subjects who refused participation indicated that the study participants were better educated and reported hormone use more frequently than non-participants. However, these differences between study participants and non-participants answering the short questionnaire only were similarly observed both for cases and controls and therefore unlikely to have biased our results. Unfortunately, no information on 25(OH)D status, diet or vitamin D related variables like outdoor activity was available for the non-participants with short questionnaire information.

Finally, we address the concern of possible measurement errors with respect to 25(OH)D status. Several reports emphasize the measurement variation introduced by different assays and different laboratories (14,54,55). Nevertheless, in the international Vitamin D Quality Assessment Scheme (DEQAS), which monitors the performance of vitamin D assays, the IDS enzyme immunoassay used in our study gave comparable results to the gold standard HPLC method (56). In addition, intra- and interassay variation in 25(OH)D measurement as well as the average absolute deviation from the mean between two duplicate samples were very low in our analysis, reassuring the validity of the biomarker measurement.

In summary, we found a significant inverse association between serum 25(OH)D concentration and postmenopausal breast cancer risk. In addition, the relationship was non-linear, suggesting a stronger effect in women with low 25(OH)D concentrations as compared to women with higher concentrations. Use of hormone therapy and number of pregnancies were identified as modifiers of the association between serum 25(OH)D and breast cancer

risk. The association was stronger in women who had never used hormone therapy compared to women with past or current use and in women with increasing number of pregnancies, however, these findings need confirmation in further studies. Our findings strongly suggest a protective effect for postmenopausal breast cancer risk through a better vitamin D supply as characterized by measurement of serum 25(OH)D.

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Table I. Characteristics and risk factors for postmenopausal breast cancer in cases and matched controls in the study population

	Cases (N=1,394)		Controls (N=1,365)		P^a
	N	%	N	%	
Characteristics					
Age at diagnosis/recruitment (years)					0.84
50 – 54	91	6.5	86	6.3	
55 – 59	300	21.5	272	19.9	
60 – 64	447	32.1	457	33.5	
65 – 69	380	27.3	372	27.3	
≥ 70	176	12.6	178	13.0	
BMI (kg/m^2)					0.75
< 22.5	544	39.0	536	39.3	
22.5 – 25	470	33.7	436	32.0	
25 – 30	322	23.1	333	24.4	
≥ 30	58	4.2	659	4.3	
Missing values			1		
Educational level					0.12
Low	905	64.9	923	67.6	
Middle	299	21.5	290	21.3	
High	190	13.6	152	11.1	
Age at menopause (years)					< 0.01
< 47	146	10.5	210	15.4	
47 – 51	399	28.6	384	28.1	
52 – 55	243	17.4	196	14.4	
≥ 56	63	4.5	65	4.8	
unknown	543	39.0	510	37.3	
First degree family history of breast cancer					0.01
No	1,102	79.1	1118	81.9	
Yes	232	16.6	181	13.3	
Unknown	60	4.3	66	4.8	
Benign breast disease					< 0.01
No	848	61.0	976	72.8	
Yes	542	39.0	383	27.2	
Missing values	4		6		
Number of pregnancies (≥ 28th week)					< 0.01
0	199	14.3	163	11.9	
1	377	27.0	311	22.8	
2	514	36.9	555	40.7	
≥ 3	304	21.8	336	24.6	
Age at menarche (years)					0.04
< 12	126	9.0	120	8.8	
12 – 14	938	67.3	862	63.3	
≥ 15	330	23.7	380	27.9	
Missing values			3		
Ever breast feeding					< 0.01
No	550	39.5	457	33.5	
Yes	844	60.5	908	66.5	
Number of mammograms in total					< 0.01
0	192	13.8	175	12.8	
1 – 4	590	42.3	720	52.8	

5 – 9	344	24.7	317	23.2	
≥ 10	250	17.9	145	10.6	
unknown number	18	1.3	8	0.6	
Use of hormone therapy					< 0.01
Never	524	38.0	595	44.0	
Past	288	20.9	346	25.6	
Current (≤ 6 months)	567	41.1	411	30.4	
Missing values	15		13		
Smoking					0.05
Never	876	62.8	806	59.1	
Past	317	22.8	365	26.7	
Current	201	14.4	194	14.2	
Alcohol consumption (g/day)					0.51
0	247	17.7	222	16.3	
> 0 – 18	977	70.1	983	72.0	
≥ 19	170	12.2	160	11.7	
Physical activity in quintiles (MET; hr/week)[b]					0.41
< 145.3	281	20.3	273	20	
145.3 – 179.3	293	21.1	272	20	
179.4 – 213.1	303	21.8	273	20	
213.2 – 257.0	243	17.5	273	20	
≥ 257.1	268	19.3	272	20	
Missing values	6		2		
Hormonal receptor status of the tumor [c]					
Estrogen receptor					
positive	984	76.4			
negative	304	23.6			
Progesterone receptor					
positive	842	65.4			
negative	445	34.6			
Time of blood collection					0.99
Jan – March	319	22.9	311	22.8	
April – June	292	21.0	286	21.0	
July – September	402	28.8	392	28.7	
October - December	381	27.3	376	27.5	

[a] χ^2 - test.
[b] MET, metabolic equivalents.
[c] data on estrogen receptor and progesterone receptor status was available for 1288 and 1287 invasive tumor cases (in situ tumors excluded), respectively.

Table II. Odds Ratios for postmenopausal breast cancer by serum 25(OH)D concentration

serum 25(OH)D (nM)	Cases N	Cases %	Controls N	Controls %	crude model[a] OR (95% CI)	adjusted model[b] OR (95% CI)
Categorized						
< 30	345	24.8	218	16.0	1	1
30 - 45	354	25.4	327	23.9	0.65 (0.52 - 0.82)	0.57 (0.45 - 0.73)
45 - 60	300	21.5	308	22.6	0.56 (0.44 - 0.71)	0.49 (0.38 - 0.64)
60 - 75	186	13.3	218	16.0	0.49 (0.37 - 0.64)	0.43 (0.32 - 0.57)
≥ 75	209	15.0	294	21.5	0.39 (0.30 - 0.50)	0.31 (0.24 - 0.42)
p_{trend}					< 0.0001	< 0.0001
As continuous variable						
per 10 nM increment	1,394		1,365		0.89 (0.87 - 0.92)	0.88 (0.85 - 0.91)

[a] Conditional logistic regression stratified by time of blood collection and year of birth.

[b] Conditional logistic regression stratified by time of blood collection and year of birth adjusted for age at menopause, first degree family history of breast cancer, history of benign breast disease, number of pregnancies (≥ 28th week), age at menarche, breast feeding history, total number of mammograms, use of hormone therapy, BMI, education level, smoking status; due to missing values 40 observations were not included in the adjusted model.

Table III. Odds Ratios[a] for postmenopausal breast cancer by serum 25(OH)D concentration by use of hormone therapy (HT)

	Never HT use		Past HT use		Current HT use	
serum 25(OH)D (nM)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)	Ca/Co	OR (95% CI)
Median	37.3 / 47.6		45.8 / 53.4		51.5 / 54.0	
Categorized	N		N		N	
< 30	193/125	1	58/46	1	89/44	1
30 - 45	130/149	0.51 (0.36 - 0.74)	82/71	0.62 (0.33 - 1.14)	140/105	0.57 (0.35 - 0.92)
45 - 60	101/123	0.45 (0.30 - 0.68)	59/83	0.36 (0.19 - 0.68)	136/98	0.61 (0.37 - 1.01)
60 - 75	56/81	0.30 (0.19 - 0.49)	48/72	0.35 (0.18 - 0.69)	80/63	0.60 (0.34 - 1.05)
≥ 75	44/117	0.18 (0.11 - 0.30)	41/74	0.25 (0.12 - 0.51)	122/101	0.49 (0.29 - 0.83)
p_{trend}		< 0.0001		0.0001		0.05
As continuous variable[b]						
per 10 nM increment	524/595	0.78 (0.73 - 0.83)	288/346	0.86 (0.79 - 0.94)	567/411	0.96 (0.91 - 1.02)

[a] Conditional logistic regression stratified by time of blood collection and year of birth adjusted for age at menopause, first degree family history of breast cancer, history of benign breast disease, number of pregnancies (≥ 28th week), age at menarche, breast feeding history, total number of mammograms, BMI, education level, smoking status; due to missing values 40 observationswere not included in the model.

[b] $p_{interaction}$: < 0.0001

Table IV: Odds Ratios[a] for postmenopausal breast cancer by serum 25(OH)D by number of pregnancies

	0 - 1 pregnancies		2 pregnancies		≥ 3 pregnancies	
serum 25(OH)D (nM)	Ca/Co	OR (95 % CI)	Ca/Co	OR (95 % CI)	Ca/Co	OR (95 % CI)
Median	45.9 /50.1		45.2 / 53.6		42.3 / 49.7	
Categorized	N		N		N	
< 30	139/76	1	119/82	1	87/60	1
30 - 45	136/119	0.42 (0.27 - 0.65)	134/123	0.72 (0.47 - 1.08)	84/85	0.51 (0.29 - 0.88)
45 - 60	122/107	0.40 (0.26 - 0.63)	104/127	0.50 (0.33 - 0.78)	74/74	0.46 (0.25 - 0.82)
60 - 75	75/70	0.39 (0.24 - 0.65)	76/88	0.51 (0.31 - 0.82)	35/60	0.28 (0.14 - 0.55)
≥ 75	104/102	0.36 (0.22 - 0.58)	81/135	0.31 (0.19 - 0.49)	24/57	0.23 (0.11 - 0.47)
p_{trend}		0.0006		< 0.0001		< 0.0001
As continuous variable[b]	576/474		514/555		304/336	
per 10 nM increment		0.91 (0.87 - 0.96)		0.86 (0.82 - 0.92)		0.81 (0.73 - 0.89)

[a] Conditional logistic regression stratified by time of blood collection and year of birth adjusted for age at menopause, first degree family history of breast cancer, history of benign breast disease, age at menarche, breast feeding history, total number of mammograms, use of hormone therapy, BMI, education level, smoking status; due to missing values 40 observations were not included in the model.

[b] $p_{interaction}$: 0.004

Fig. 1. Odds ratios for postmenopausal breast cancer by 25(OH)D concentrations using fractional polynomial as dose-response analysis. The resulting function OR(x) = $\exp[10.4*(x+1)^{-0.5}]$ is displayed, setting the median of the lowest category from the categorical analysis as reference (24.3 nM 25(OH)D). The solid squares and respective bars represent the odds ratios and 95% confidence intervals of the categorical analysis (30-45, 45-60, 60-75, ≥75 nM). For graphical illustration categorical odds ratio estimates were displayed at the median value in the controls of each category, at 24.3 (reference), 38.0, 52.2, 67.1, 88.7 nM, respectively.