Vitamin D – effective solar UV radiation, dietary vitamin D and breast cancer risk

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Abstract

Vitamin D is well known for its important role in calcium and phosphor homeostasis. Recent research suggests that vitamin D also prevent some types of cancers. We studied solar vitamin D effective UV-radiation (VD-dose), dietary vitamin D, sun seeking holidays, use of solarium, frequency of sunburn and breast cancer risk in a large population based cohort study. A total of 41,811 women from the prospective Norwegian Women and Cancer Study, aged 40-70 years at baseline, were followed from 1997/98 – 2007. Dietary vitamin D intake was calculated at baseline. Information on historical VD-dose was used as a proxy for cutaneously obtained vitamin D status. Cox proportional hazards model was used. We adjusted for age, height, BMI, baseline menopausal status, use of hormone replacement therapy, use of oral contraception, alcohol, mother's history of breast cancer, mammography, and parity. During 8.5 years of follow-up, 948 new cases of breast cancer were registered using data from the Norwegian Cancer Registry. We found no significant associations between VD-dose, or vitamin D intake, or sun seeking holidays, or use of solarium, or frequency of sunburn, and breast cancer risk. Relative risks (95% confidence intervals) for highest versus lowest category were 1.17 (0.95 to 1.44), 0.95 (0.75 to 1.21), 1.07 (0.87 to 1.32), 0.93 (0.76 to 1.14) and 1.10 (0.89 to 1.36), respectively. Our results do not support an association between vitamin D status, and breast cancer risk.

Introduction

It has been known since the first half of the 20'th century that sunlight and diet, in particular cod liver oil, have an anti-rachitic effect. In the 1930's, the vitamin D's chemical structure was described, and by this time it was known to be essential for maintaining a healthy skeleton. Later, the role in calcium and phosphor homeostasis was found. As early as in 1941, Apperly[1] reported a possible protective effect of solar radiation on mortality from most cancers known at that time. During the last two decades intensive research has suggested that vitamin D has a preventive effect on some autoimmune diseases[2,3], some forms of cancer[4-8], and a positive effect on cancer survival[9-13]. The exact biological processes involved still remains unclear, but it seem that vitamin D has multiple effects beyond the traditional role in calcium homeostasis. Vitamin D compounds have been demonstrated to alter cellular proliferation through multiple mechanisms, and in particular via effects on cell cycle progression, differentiation and apoptosis[14-17]. Thus, in the last few years there has been a discussion on whether moderate exposure to solar ultraviolet (UV) radiation has a positive overall health effect or not[18]. Sun exposure is an established risk factor for basal cell carcinoma, squamous cell carcinoma and melanoma[19-21].

Since the early 1990's, many different epidemiological studies of the vitamin D – breast cancer relationship have been carried out. In an ecological study in U.S.A. a strong inverse correlation between mean daily solar radiation and breast cancer mortality was found[22]. In the first National Health and Nutrition Examination Survey Epidemiological Follow-up Study (NHANES I), several measures of sunlight exposure were found to reduce risk of breast cancer, but total vitamin D intake was not[23]. Other studies have indicated that breast cancer diagnosis in summer and fall may improve survival rates, as at this time of year the vitamin D status is most likely at the highest[11,13,24]. In both the Cancer Prevention Study II Nutrition cohort (CPS II) and Nurses' Health Study (NHS) cohort, primary analyses on the relation between total vitamin D intake and breast cancer risk showed practically no assiciation[25,26]. However, secondary analyses suggest the possibility that the vitamin D – breast cancer relationship may be modified by other factors, like

tumor characteristics[25] and menopausal status[26]. In the CPS II-study an intake of 300 IU or more was associated with a significant lower risk of only estrogen receptor – positive breast cancer. In the NHS-study, a high intake of vitamin D (≥ 500 IU) was associated with a significant reduced risk of breast cancer in premenopausal women, but not in postmenopausal women.

Breast cancer is one of the most frequent types of cancer, and the World Cancer Research Fund/American Institute for Cancer Research concluded in their review from 2007[27] that a protective effect of vitamin D on breast cancer risk is inconclusive, and further investigation is needed. The International Agency for Research on Cancer's working group on vitamin D and cancer risk have systematically reviewed the epidemiological literature on vitamin D and the risk of colorectal, prostate, and breast cancer, and concluded in their report from 2008[28] that the epidemiological evidence on observational studies suggest a protective effect of vitamin D on breast cancer risk. However, the differences between the studies were large, and new studies on vitamin D and breast cancer risk are warranted.

Contrary to people living at a low latitude where the main source of vitamin D is solar exposure, people living at mid and high latitudes have to partly rely on vitamin D intake through diet and supplements[29-32] during the vitamin D winter[33-35], to avoid hypovitaminosis D. Both vitamin D intake and estimated hours per day of exposure to UV-B radiation significantly predicted plasma levels of vitamin D in blood samples from 309 middle-aged women living above 65°N[30].

Brustad et al.[30,35] found a significant positive association between estimated vitamin D effective solar UV-exposure and vitamin D levels in blood in people living at high latitudes in Norway, although, the generally high dietary intakes of vitamin D, especially in winter, mask largely the effect of seasonal variation in UV-exposure. The estimated UV-exposure predictor was supported by solar UV-radiation measurements at a site close to where the subjects lived, in combination with a questionnaire asking about the subject's outdoors habits and diet. For large cohort studies where people live at a wide geographic range, such as in Norway, estimation of UV-exposure is more complicated since there only exist a limited network of solar UV-radiation

instruments, which is not dense enough to adequately cover the geographic area of interest. There is also a temporal limitation in the UV-measurements as the measurements only go about 10 years back in time. Thus, population based research conducted at higher latitudes on the relationship between vitamin D status and different health outcomes, both intake of vitamin D and vitamin D effective UV-radiation need to be carefully considered.

In the current paper we study breast cancer risk in relation to vitamin-D effective solar radiation (VD-dose) and dietary vitamin D intake in 41,811 Norwegian women from the prospective Norwegian Women and Cancer Study.

Methods

Sample

The national population based cohort study called the Norwegian Women and Cancer Study (NOWAC)[36] was initiated in 1991. The women were randomly selected from the National Central Person Registry. During the years from 1991 to 1997, the first round of questionnaires were sent out to 179,338 women aged 40 – 70 years, from which 102,443 (57%) responded. From 1998 to 2002 the second round of questionnaires was sent to the women who had responded to the first round, from which 80,693 (81%) responded. The questionnaires from both rounds involved six slightly different versions, from which three of them could be used in the current study, as the questions of interest were common, and 49,395 women answered the relevant questions. We excluded 1,889 women (5 deaths, 15 had migrated, and 1869 prevalent cancer cases including 630 breast cancers), and 47,506 women were included in our study.

Diet and Vitamin D

The food frequency (FFQ) part of the questionnaires contained detailed questions on all vitamin D rich foods and enabled estimation of daily vitamin D intake, using the Norwegian food composition table[37]. The main focus of the FFQ was to study relations between diet - and fish in

particular - and health. The vitamin D intake was calculated at baseline, and included cod liver oil supplement. No other supplements were included due to lack of information on their vitamin D content. Daily intake of alcohol (units of beer, wine and spirits) is also estimated from the FFQ. The FFQ and its validity has been described in detail elsewhere[38-41].

UV-radiation and Vitamin D

Based on the action spectrum for the production of vitamin D in human skin from the Commision Internationale de L'eclariage (CIE) report CIE 174[42], we estimated the solar UV-radiation level needed for facial cutaneous production of vitamin D[33]. We have developed a method for estimation of the daily number of vitamin D effective hours (VD-hours) for arbitrary locations on the Earths surface back to 1957[43]. However, the VD-hours variable is only a measure of the duration of UV-radiation above a threshold value, and does not take into account the intensities of vitamin-D effective solar radiation, other than for the start and stop condition of the duration above the threshold[35]. Thus, VD-hours are in this study replaced by the vitamin D effective UV-dose (VD-dose) that estimate total vitamin D effective UV-radiation, and not only the duration of adequately intense UV-radiation.

The VD-dose was calculated using the thoroughly tested FastRT software package[44]. As the two main factors affecting the UV-radiation at the earths surface, are atmospheric ozone and clouds, these physical parameters were the main inputs to the model, and was taken from the ERA-40 archive, "The 40+ year re-analysis archive from the European Centre for Medium-Range Weather forecasts[45]. Other inputs to the model were kept constant at values typical for high latitude areas. Daily VD-dose was estimated by calculating the VD-radiation throughout the day with a time-dependent (one minute resolution) solar zenith angle as input to the model. The ozone and cloud input parameters to the model were kept fixed during the day. The minute-by-minute calculations were integrated over each day, providing daily VD-dose for the period from 1957 – 2002. A full description of the model and its fixed parameter settings may be found in Engelsen and Kylling[44].

Self reported history of places of residence during lifetime was used to give each subject a corresponding VD-dose at baseline. Based on where the subjects have lived the last 20 years, each corresponding calculated VD-dose were summarized, and calculated as a yearly mean value.

All subjects were born some time between 1927 and 1957, and we only have residential VD-dose information for the period 1957-2002. To avoid different exposure periods in life between the subjects as a mixture between childhood and adolescent exposure periods, the 20 year duration was chosen to ensure calculations based on places of residence for everyone from the age not younger than 20. This gave a yearly mean VD-dose number for each subject that were used together with vitamin D intake as an individual proxy for vitamin D status.

A total of 7,576 women had incomplete recording for history of places of residence and VD-dose could not be calculated. As imputation for the missing 20-year mean VD-dose, we used the 20-year mean VD-dose from the place they lived at inclusion. Available national figures from Statistics Norway (http://statbank.ssb.no//statistikkbanken/default_fr.asp?PLanguage=1) on frequency of moving in the period 2000-2008 showed that less than 2% of the women at the age 40-70 moved between municipalities each year. Frequency of moving in this period was very stable, and the assumption that the NOWAC women had a similar moving pattern, seem valid. Moving was probably hardly a concern, as 2% of the 7,576 women without a complete recording for history of places of residence only constitute about 150 subjects.

In addition, the questionnaire recorded information on sun-seeking holidays, use of solarium, and frequency of sunburn, as these variables may contribute to a person's vitamin D status[20,46]. The sun-seeking holidays variable was recorded in 5 categories (never, 1 week/year, 2-3 weeks/year, 4-5 weeks/year, 6+ weeks/year), use of solarium in 6 categories (never, rarely, 1 time/month, 2 times/month, 3-4 times/month, 5+ times/month), and frequency of sunburn in 5 categories (never, 1 time/year, 2-3 times/year, 4-5 times/year, 6+ times/year), for various periods of life. We did not consider information given from the period of life before the age of 20 as we did not have complete information on VD-dose for the subjects who were older than 20 before 1957

(start of VD-dose data). After the age of 20, information was given for the age periods 20-29 years, 30-39 years and 40+ years, or for the age periods 20-45 and 45+ years, depending on which version of the questionnaire that was answered.

Follow-up

Start of follow-up was set to the date of receipt of the returned questionnaire, which was in 1997/98, and the end date was set to the date of diagnosis of breast cancer, or to the date of emigration, or to the date of death, or to the end of follow-up (December 31, 2006). As each citizen of Norway is assigned a unique personal registration number, which was linked to the National Cancer Registry, we could obtain information on cancer data. Information on death and emigration was collected from the Central Population Register of Norway. These registers are considered to be virtually complete.

Other variables

The questionnaire also recorded height (cm) and weight (kg), menstrual situation (regular, irregular, age when it stopped naturally, and uncertain), hormone therapy (HT) use (never, former, current), use (never, ever) of oral contraceptives (OC), mother's history of breast cancer (diagnosed or not), frequency of mammography (never, after every two years, at least every two years), number of births and age at first birth, and hair color (black/dark brown, brown, blond, red). The color of the untanned skin was recorded by comparing the skin with a scale graded from 1 to 10 (1 = very fair, 10 = dark brown)[46].

Statistical analysis

The total vitamin D exposure was based on VD-dose at each of Norway's municipality centers, vitamin D intake, sun-seeking holidays, use of solarium, and frequency of sunburn. The associations between VD-dose following the places of lifetime residence and VD-dose from the

place of residence at inclusion, and between VD-dose and vitamin D intake were estimated by Pearson's correlation coefficient. The latter association was also estimated by linear regression analysis. Cox proportional hazards model, with follow-up time as the time scale, was used to estimate relative risks (RR) with corresponding 95% confidence intervals (95% CI) for breast cancer for quartiles of each of the vitamin D related exposures. VD-dose and vitamin D were characterized at baseline as follows: Yearly VD-dose was categorized into quartiles with low: exposure < 311.8 kJ/m^2, medium-low: 311.8 ≤ exposure < 429.7 kJ/m^2, medium-high: 429.7 ≤ exposure < 463.3 kJ/m^2, and high: exposure ≥ 463.3 kJ/m^2. Vitamin D intake in micrograms pr. day in quartiles with low: 0 ≤ intake < 3.99 µg/d, medium-low: 3.99 ≤ intake < 6.46 µg/d, medium-high: 6.46 ≤ intake < 21.31 µg/d, and high: intake ≥ 12.31 µg/d.

A variable for sun seeking holidays was calculated as the mean number of weeks per year for the last 20 years, in 4 categories as: few: holidays < 1 w/y, medium-few: 1 ≤ holidays < 2 w/y, medium-often: 2 ≤ holidays < 3 w/y, often: holidays ≥ 3 w/y. A variable for solarium use was calculated as the mean number of times per year for the last 20 years in 4 categories as: never, rarely: 0 < solarium use < 3 t/y, medium: 3 ≤ solarium use < 6 t/y, often: solarium use ≥ 6 t/y. A variable for frequency of sunburn was calculated as the mean number of times per year for the last 20 years in 4 categories as: never, rare: 0 < sunburn < 1 t/y, medium: sunburn = 1 t/y, often: sunburn > 1 t/y. The skin color variable was recoded into three categories: fair 1-3, medium: 4-7, dark: 8-10.

Age at entry (from the National Central Person Registry) was treated as continuous in the models. The body mass index (BMI) at entry of study was recoded in 4 categories: underweight: BMI < 20 kg/m^2, normal: 20 ≤ BMI < 25 kg/m^2, overweight: 25 ≤ BMI < 30 kg/m^2, obese: BMI ≥ 30 kg/m^2. Menopausal status was registered on the basis of the participants' answers about their menstrual situation (regular, irregular, stopped, and uncertain). If the menstruations had stopped, they were asked to give the reason why (natural stop, bilateral oophorectomy, hysterectomy, or other reasons) and the age at menopause. Postmenopausal status was given if her periods had

stopped and/or she stated the reason why and/or the age when it stopped. Participants' reporting use of HT or hormone containing intrauterine device, hysterectomy and those who "did not know" were considered postmenopausal if they were 53 years or older and premenopausal if they were younger. This cut-off age is based on the definition used in the Million Women Study[47] and the classifications' validity has been demonstrated in a previous NOWAC publication[48] .We constructed a new variable for parity by combining "age at first birth" and "total number of births": one child, two children, three or more children where the first was born at age < 25, or at age 25 to < 30, or at age ≥ 30. Daily intake of alcohol was registered in standard units and recalculated into intake in grams per day. In the models we used: zero intake (reference), 0 < intake ≤ 5 g/d, intake > 5 g/d. Daily energy intake in kilo Joule was calculated based on the FFQ[39], and included as a continuous variable in the model.

The final model included 10 covariates, all established risk factors[49] for breast cancer (age, menopausal status, HT, OC, mothers' history of breast cancer, mammography, parity, BMI, height and alcohol consumption). Initially, both age adjusted and multivariable analyses were performed, and finally, we performed mutually adjusted multivariable analyses for determination of the possible effects of VD-dose, intake of vitamin D, sun seeking holidays, use of solarium, and frequency of sunburn. There were 5,695 (12.0%) of the participants who had incomplete answers on one or more of the questions related to the exposures and covariates, and all of them were treated as missing and excluded from the analyses. We used R, version 2.1.1, for the statistical analyses.

Results

During almost 8.5 years of follow-up of 41,811 women, 2,643 women were diagnosed with cancer of whom 948 (36%) with breast cancer. The mean age at baseline was 49.6 years (range 41-70) and mean time from baseline until breast cancer diagnosis was 4.5 years.

Mean vitamin D intake at baseline was 9.4 µg/day (range 0-67.3) and the median was 6.5 µg/day, i.e. the distribution was skewed towards the right (figure 2). For those under the age of 60,

58% had an intake below the recommended value of 7.5 µg/day and for those at the age of 60 or older, 62% had an intake below the recommended value of 10 µg/day[50]. For those taking cod liver oil, the mean daily vitamin D intake was up to three times higher than the mean daily vitamin D intake for the study sample. We found no strong association between VD-dose and vitamin D intake (Pearson correlation coefficient -0.03), and the predicted effect was small (-4.8 kJ/m^2 per 10 µg/day of increase in vitamin D intake, 95% CI (-5.9, -3.7)). The geographical distribution (fig. 3) showed a higher vitamin D intake in general above 66˚ N, but also in the most western parts (south of 64˚ N) the intake was above average. In a belt stretching from Trondheim and south towards the areas around Oslo (capitol), the intake is slightly above the average intake. These are also the most densely populated areas of Norway.

Mean VD-dose before imputation of VD-dose data was 391.2 kJ/m^2/y (range 181.2-644.1) and 7,576 missing, and after imputation of VD-dose data it was 388.9 kJ/m^2/y (range 180.1-644.1) and no missing values. These two were significantly different ($P < 0.01$). The correlation coefficient between the VD-dose following the places of residence through lifetime and the VD-dose from the place of residence at inclusion was 0.96. The geographical distribution of the 20 year mean VD-dose is presented in figure 1. The north to south gradient is most distinct, but there is also a clear east to west variation around 60˚N.

Table 1 shows selected characteristics of the women in the study according to quartiles of vitamin D intake and VD-dose. It was a large difference in vitamin D intake between the quartiles, where the mean for the high-intake quartile was 7.7 times the mean for the low-intake quartile. The women in the quartile with the lowest vitamin D intake were also the youngest, had the lowest intake of alcohol, and had the highest BMI. Fewer were postmenopausal and used less HT. Ever use of OC was lowest in this quartile.

The women in the lowest quartile of VD-dose had the highest intake of vitamin D, though relatively small differences in intake were found between the VD-dose quartiles. They traveled only slightly above average on sun seeking holidays, but reported most frequent use of solarium. The

number of weeks of sun seeking holidays was around average for all quartiles, except for the women in the second quartile of the VD-dose variable, who traveled only 1.8 weeks/year, compared to the average of 2.1 weeks/year. Mean frequency of sunburn was on average (0.8 t/y) for all quartiles of both vitamin D intake and VD-dose, except for the lowest quartile of VD-dose (0.6 t/y).

The women in the lowest quartile of VD-dose also were the oldest, had the lowest intake of alcohol, had the highest BMI, were the shortest, and almost half of them were postmenopausal at baseline. In this quartile, there were fewest women who their mother had breast cancer, and fewer had mammography frequently. Fewer of them were nulliparous, and they were more likely to have their first child before the age of 25.

We found a significant positive trend (P_{trend} = 0.007) between VD-dose and breast cancer risk in the age adjusted analysis, while no significant association was found in the multivariable analysis (P_{trend} = 0.21). No significant association was found between dietary vitamin D intake and breast cancer risk, neither in the age adjusted nor the multivariable analysis (P_{trend} was 0.96 and 0.69, respectively) (table 2). No significant association was found between sun seeking holidays, use of solarium, frequency of sunburn, and breast cancer risk in the age adjusted (P_{trend} was 0.71 0.20, and 0.49, respectively) or the multivariable analysis (P_{trend} was 0.55 and 0.55, and 0.95, respectively) (table 2). Mutual adjustments did not change the results for any of the five exposures.

We also performed age adjusted and multivariable analyses for skin color and hair color (as an indicator on pigmentation) in relation to breast cancer risk, but no significant associations were found (P_{trend} was 0.98 and 0.40, respectively). Relative risks (95% confidence intervals) for highest versus lowest category were 0.96 (0.59 to 1.58) and 1.58 (0.90 to 2.75), respectively. Also, the variables gave together over 9000 additional missing values, and were kept out of the final analyses.

Among the established risk factors, age ($p < 0.001$), HT ($P < 0.001$), mothers' history of breast cancer ($P < 0.001$), mammography ($P = 0.02$), and having 3 or more children after the age of 30 ($P = 0.03$) were significantly associated with breast cancer risk (results not shown). The analyses were done with both follow-up time and attained age as time scales, and the results were almost

identical. As the difference between VD-dose before and after imputation was significant, we repeated the analyses without the imputed data and found similar results (results not shown).

Discussion

As we have detailed information on the subjects history of UV-exposure spanning 13 degrees of latitude, their diet, information on sun seeking holidays, use of solarium and frequency of sunburn, we think this cohort study is based on vitamin D status information of relatively large range and high quality.

As Norway spans this large latitudinal range, one would expect people's vitamin D status to vary according to the VD-dose. A study on diet[51] has shown that intake of vitamin D was just slightly higher (not significant) in the north of Norway than in the south, due to dietary traditions. In the current study, the relationship between VD-dose and vitamin D intake was rather weak (e.g. regression showed that an increase in dietary intake from 10 to 20 µg/day gave a decrease in VD-dose of only 4.8 kJ/m^2). By comparison, the difference in yearly VD-dose between Oslo (60˚N) and Tromsø (69˚N) is around 200 kJ/m^2 (figure 1). The weak correlation between VD-dose and vitamin D intake suggests there is no strong relation between where the subjects have lived and their intake of vitamin D. As VD-dose was one of the main exposure variables in this study and the number of missing participants connected to it was quite high, we imputed VD-dose for the missing subjects with incomplete records for places of residence. As the subjects all are older than 40 years, we assumed most of them to be well settled, and expected most of them to have lived at the last place of residence (where they lived at inclusion to the study) for a significant amount of time. We found this to be a reasonable substitute as the correlation between the VD-dose following the places of residence and the VD-dose from the place of residence at inclusion was very high.

The strength of this study is that it takes into account many important variables affecting human blood levels of vitamin D, as a combination of diet and UV-radiation related activities. The FFQ included all dietary sources to vitamin D, and the UV-radiation related questions cover

lifetime related UV-exposure (places of residence), holidays related UV-exposure, use of solarium and information on frequency of sunburns. Previous studies have used latitude as a proxy for UV-exposure[5,52-56]. It can be a rather crude measure for UV-exposure, as it does not take into account other factors directly affecting the vitamin D effective part of the solar spectrum. Both atmospheric ozone content and cloud conditions, which are two main driving variables for UV-radiation doses, are accounted for in the calculation of the VD-dose. Another important aspect is that some people change places of residence during their lifetime. If this is not accounted for with respect to the VD-dose, it might lead to misclassification when assigning the VD-dose variable to the subjects only based on their last place of residence. This seems however not to be a problem in our study because of the high correlation between historical exposure and exposure based on place of residence at inclusion. Another strength of the study is the use of registry data from the Cancer Registry of Norway to identify incident breast cancer cases. This register is considered virtually complete.

The main weakness of the use of the VD-dose variable is that it is a result of radiative transfer model calculations on vitamin D effective UV-radiation as a yearly mean value located at a geographical site. The connection of the variable to the individuals is based on the subjects' corresponding place of residence to the geographical site for which the variable was calculated. As we have no detailed information on how much sun the participants have been exposed to in general, mostly influenced by amount of time spent outdoors, and how much skin that was exposed, it is difficult to quantify the relation between the ambient VD-dose and vitamin D status. The lack of information on the absolute value of the individual solar exposure does not only apply to the VD-dose variable, but will apply equally to any other solar UV exposure variables selected (latitude, cloud cover, etc), as the last stage of exposure is related to every participant's solar exposure habits. On the other hand, as we have quite accurate estimates of the VD-dose for the places of residences for a large study group over the last 20 years, it is most likely that the variation in the vitamin D status for the subjects is reflected in the variation of the VD-dose connected to them.

Looking at the geographical VD-dose distribution (figure 1) and the vitamin D intake

distribution (figure 3), obviously, one would expect the vitamin D status to be significantly higher in the south of Norway as the vitamin D dietary intake is only about 9 % higher in the north (vitamin D intake in lowest vs. highest VD-dose quartile), probably not enough to compensate for the lack of dermal UV-induced vitamin D production. Despite the strong positive VD-dose gradient towards south, there is no reduced breast cancer risk towards the south, rather a slightly increased breast cancer risk (non significant). Other studies from Norway[13,57] and Sweden[58,59] support our findings of no latitudinal association between vitamin D and breast cancer risk. However, these studies show that there is a possible association between survival and season of diagnosis of breast cancer. Diagnoses during summer and fall, assuming a higher blood level of vitamin D in the population, revealed the lowest risk of breast cancer death. To which extent this is due to a higher vitamin D level, or other reasons, remains unclear.

An interesting result is that we found a significant positive association between VD-dose and breast cancer risk in the age-adjusted analysis. However, the RR's attenuated towards unity when we adjusted for known risk factors, suggesting that the known risk factors play a more important role than vitamin D in breast cancer etiology.

Previous studies of vitamin D and breast cancer have found that an improved vitamin D status can be associated with a reduced risk of breast cancer[60-64], while others found no association[59,65,66]. In our study, which can be compared to Kuper et al.[59] with respect to design and location, we did not find any significant association between vitamin D intake or VD-dose, and risk of breast cancer among women living at high latitudes. A recent review of the literature concluded that there could be a modest inverse relationship between blood levels of vitamin D and breast cancer risk[67]. Only a handful of the studies in the review were prospective, and the subjects' vitamin D status was estimated in highly different ways, which can introduce heterogeneity between the studies.

In this study we did not find reduced risk of breast cancer among women who had lived in areas with high VD-dose as compared to those with low VD-dose, nor among women with high

vitamin D intake compared to those with low intake, when adjusting for known risk factors.

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Table 1. Characteristics of the cohort at baseline in 1997/98, n = 47,506

Characteristics	Vitamin D intake				VD-dose			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Cases	238	242	226	242	224	231	250	243
Person year	96077	96022	96274	96243	98704	95434	95193	95345
Mean per quartile								
VD-dose (kJ/m^2/y)	398	391	383	384	251	373	445	486
Vitamin D intake (µg/d)	2.70	5.14	8.80	20.8	9.92	9.08	9.32	9.09
Sun seeking holidays (weeks per year)	2.0	2.1	2.1	2.1	2.2	1.8	2.2	2.1
Use of solarium (times per year)	4.5	4.5	4.4	4.3	4.8	4.4	4.3	4.2
Frequency of sunburn (times per year)	1	1	1	1	1	1	1	1
Age (y)	48.9	49.2	49.6	50.3	52.3	48.7	48.9	48.1
Alcohol intake (g/d)	2.9	3.2	3.4	3.6	2.3	2.8	4.0	3.4
BMI (kg/m^2)	24.8	24.7	24.6	24.0	25.0	24.5	24.2	24.4
Height (cm)	166.0	166.2	166.3	166.4	164.9	166.3	166.9	166.9
Percentage per quartile								
Postmenopausal at baseline	20.4	20.5	21.9	25.5	47.0	14.7	14.5	12.3
Hormone therapy use at baseline	20.2	21.3	22.2	24.1	20.7	21.5	24.3	21.4
Ever use of oral contraception	37.8	38.9	39.8	42.0	47.4	40.2	34.8	36.0
Mothers' history of breast cancer	5.1	5.3	5.0	5.3	4.5	5.4	5.7	5.2
Mammography (frequently)	19.2	19.3	18.7	21.4	13.4	20.2	23.9	21.9
Age at first birth								
Nulliparous	8.7	7.3	8.4	9.5	6.8	7.5	10.8	8.9
<25 y	56.0	57.3	57.8	54.7	67.9	58.0	47.1	52.9
25 – 29 y	25.9	25.9	24.1	24.7	19.1	25.2	29.0	27.1
≥30 y	6.3	6.4	6.5	7.7	5.8	8.6	12.1	9.9

Table 2. Age adjusted, multivariable, and mutually adjusted multivariable relative risk (RR, 95% CI) of breast cancer by quartiles and categories of vitamin D related exposures. N = 41,758.

Exposure	Q	Cases (n)	RR (95% CI) [a]	P [a,d]	RR (95% CI) [b]	P [b,d]	RR (95% CI) [c]	P [c,d]
VD-dose, kJ/m^2	1	193 (10 077)	1.00		1.00		1.00	
	2	205 (10 543)	1.18 (0.96 – 1.44)		1.08 (0.88 – 1.33)		1.10 (0.89 – 1.35)	
	3	225 (10 566)	1.29 (1.06 – 1.57)		1.07 (0.87 – 1.32)		1.08 (0.88 – 1.34)	
	4	221 (10 572)	1.30 (1.06 – 1.58)	0.007	1.17 (0.94 – 1.43)	0.21	1.17 (0.95 – 1.44)	0.18
Vitamin D intake, µg/day	1	199 (10 206)	1.00		1.00		1.00	
	2	219 (10 565)	1.05 (0.87 – 1.27)		1.08 (0.89 – 1.32)		1.09 (0.89 – 1.32)	
	3	208 (10 516)	0.99 (0.81 – 1.20)		1.05 (0.85 – 1.30)		1.06 (0.86 – 1.31)	
	4	218 (10 471)	1.02 (0.84 – 1.23)	0.96	1.06 (0.86 – 1.31)	0.69	1.07 (0.87 – 1.32)	0.63
Sun seeking holidays, weeks/yr	1[e]	222 (11 146)	1.00		1.00		1.00	
	2	203 (10 100)	1.07 (0.89 – 1.30)		1.01 (0.83 – 1.22)		1.00 (0.82 – 1.22)	
	3	212 (10 510)	1.07 (0.89 – 1.29)		0.99 (0.82 – 1.20)		0.98 (0.81 – 1.19)	
	4	207 (10 002)	1.04 (0.86 – 1.25)	0.71	0.94 (0.77 – 1.15)	0.55	0.93 (0.76 – 1.14)	0.46
Use of solarium, times/yr	1[e]	281 (14 230)	1.00		1.00		1.00	
	2	181 (8 843)	1.03 (0.85 – 1.24)		0.99 (0.82 – 1.19)		1.00 (0.82 – 1.20)	
	3	236 (11 896)	1.06 (0.89 – 1.26)		1.01 (0.85 – 1.21)		1.04 (0.86 – 1.24)	
	4	146 (6 789)	1.15 (0.94 – 1.40)	0.20	1.07 (0.87 – 1.32)	0.55	1.10 (0.89 – 1.36)	0.39
Freq. of sunburn, times/yr	1[e]	201 (10 150)	1.00		1.00		1.00	
	2	267 (12 623)	1.10 (0.92 – 1.33)		1.03 (0.85 – 1.23)		1.02 (0.85 – 1.23)	
	3	265 (13 217)	1.12 (0.93 – 1.34)		1.05 (0.87 – 1.27)		1.05 (0.87 – 1.27)	
	4	111 (5 768)	1.06 (0.84 – 1.33)	0.49	0.96 (0.76 – 1.21)	0.95	0.95 (0.75 – 1.21)	0.93

[a] Age adjusted

[b] Adjusted for age at entry, BMI, height, menopausal status, hormone therapy use, use of oral contraceptives, mothers' history of breast cancer, frequency of mammography, combined parity and age at first birth, daily intake of alcohol.

[c] Mutually adjusted, multivariable as for [b].

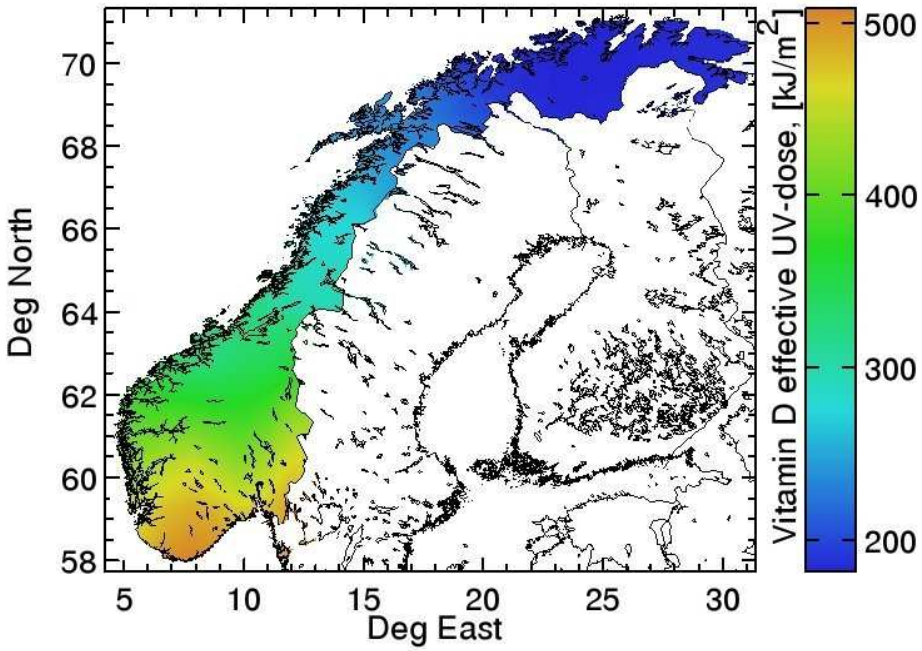
[d] P-value for trend. Calculated using the categorical variable as continuous in the model.

[e] Categorized.

Fig 1. **Yearly mean vitamin D effective UV-dose, mean 1979 – 1998**. All three main driving forces for vitamin D effective UV-radiation (total atmospheric ozone, cloudiness and solar zenith angle) are accounted for in the estimations.

Fig 2. **Distribution of vitamin D intake.** Among the increased numbers of participants having an intake of 26 – 30 µg/day are many of them taking cod liver oil during wintertime.

Fig 3. **Daily vitamin D intake.** Geographical distribution of vitamin D intake at inclusion of the study as a mean over each municipality. Values from 10 µg/day and above are given the same color code.



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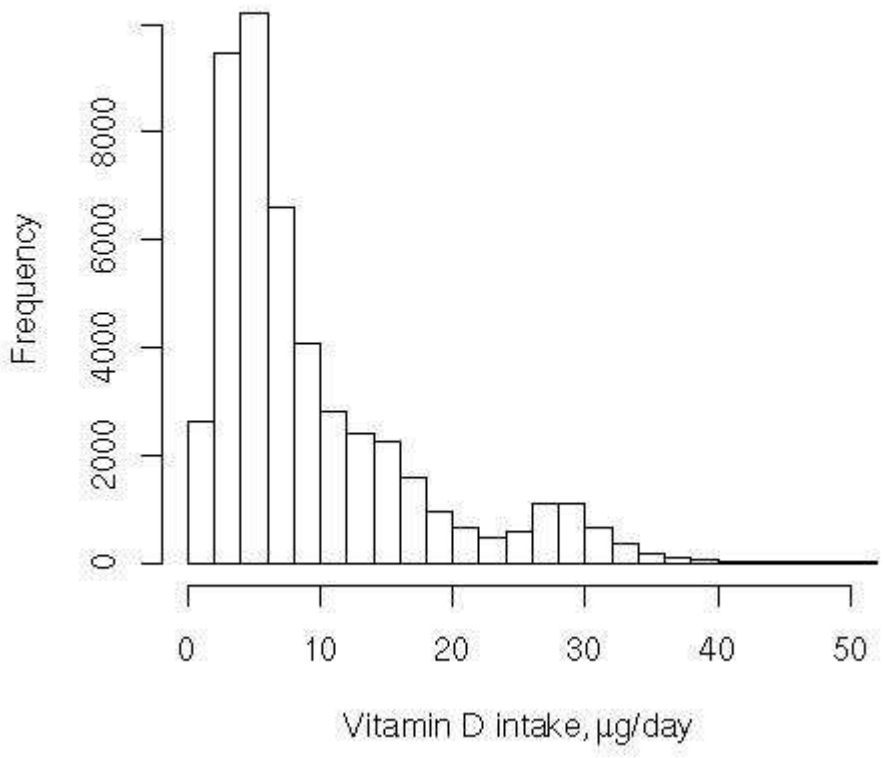

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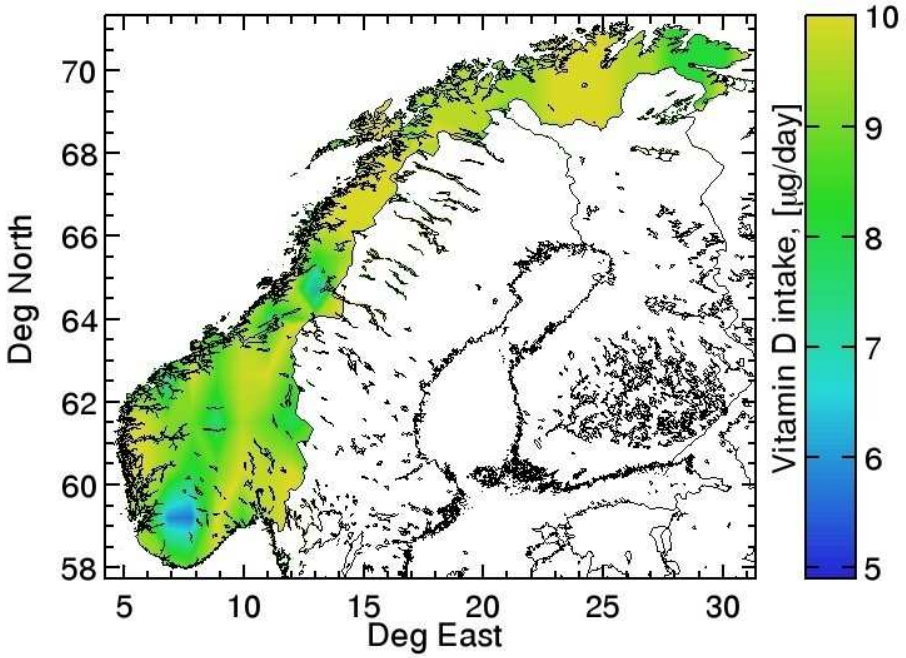

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