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The seasonal importance of serum 25-hydroxyvitamin D for bone mineral density in older women

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Abstract. Michaëlsson K, Wolk A, Byberg L, Mitchell A, Mallmin H, Melhus H (Department of Surgical Sciences, Uppsala University, Uppsala; Institute of Environmental Medicine, Karolinska Institutet, Stockholm; and Department of Medical Sciences, Uppsala University, Uppsala, Sweden). The seasonal importance of serum 25-hydroxyvitamin D for bone mineral density in older women. *J Intern Med* 2017; **281**: 167–178.

Background. The impact of season when determining a serum 25-hydroxyvitamin D (S-25OHD) cut-off level for optimal bone health is unknown.

Objective. To investigate the relative importance of S-25OHD for bone mineral density (BMD) by season.

Methods. A subcohort of 5002 Swedish women (mean age 68 years), randomly selected from a large population-based longitudinal cohort study with repeat dietary and lifestyle information, was enrolled during 2003–2009 for a clinical examination, which included dual-energy X-ray absorptiometry and collection of fasting blood samples. Categories of vitamin D status were determined by S-25OHD (measured by HPLC-MS/MS).

Results. In samples collected during summer, we found a gradual increase in BMD of the total hip up to a S-25OHD level of 40 nmol L^{-1} (6% of the cohort). In women with S-25OHD concentrations below 30 nmol L^{-1} during summer, adjusted BMD was 11% lower [95% confidence interval (CI) 3–19] and in those with S-25OHD levels of 30–40 nmol L^{-1} BMD was 6% lower (95% CI 1–11), compared with women with S-25OHD levels above 80 nmol L^{-1}. Low S-25OHD concentrations during summer (<30 nmol L^{-1}) were also associated with higher adjusted relative risk of osteoporosis (4.9; 95% CI 2.9–8.4) compared with concentrations above 80 nmol L^{-1}. By contrast, no differences in mean BMD values between categories of S-25OHD were found during winter.

Conclusions. Summer concentrations of S-25OHD appear to be the most useful to predict BMD, whereas winter levels have limited value. To determine a S-25OHD cut-off level for vitamin D deficiency, it may be necessary to take into account the season of blood collection.

Keywords: 25-hydroxyvitamin D, bone mineral density, season, vitamin D, vitamin D intake.

Introduction

Vitamin D stimulates enhanced intestinal calcium absorption and renal calcium conservation to maintain adequate levels of ionized calcium in serum. During periods of vitamin D deficiency, it is thought that bone resorption increases as a result of reduced active calcium absorption and the bone mineral density (BMD) therefore decreases [1].

The major source of vitamin D is synthesis in the skin induced by ultraviolet (UV)-B radiation from sunlight. The radiation strength depends on the season and latitude, and UV-B radiation at high latitudes is insufficient for the production of vitamin D during the winter season [2]. Therefore, considerable variation in vitamin D status between seasons would be expected in individuals residing at high latitudes.

Serum 25-hydroxyvitamin D (S-25OHD) is the generally accepted indicator of vitamin D status. Seasonal differences of 15–35 nmol L^{-1} between the winter nadir and the summer zenith have been reported [3–13]. This seasonality is not only seen at high latitudes [9] but also in temperate climates [7, 10], suggesting that season is more important than latitude [7]. With such large fluctuations, it is somewhat surprising that season is rarely taken into account in studies of vitamin D and health outcomes.

As stated in a report on dietary requirements for calcium and vitamin D from the Institute of Medicine (IOM) [1], there is an urgent clinical and public health need for consensus cut-off values for S-25OHD inadequacy. An important question is whether summer or winter concentrations of S-25OHD for the determination of sufficiency levels are of greatest significance for BMD. We hypothesized that season of blood draw may be important when interpreting S-25OHD levels for the definition of such cut-off values. At Swedish latitudes (55°–69°N), UV-B radiation is very low from October to March, leaving the population at risk of low vitamin D levels during a large part of the year and making this a suitable setting to investigate seasonal effects on BMD. We therefore measured S-25OHD by the gold standard method and investigated the association between S-25OHD concentration and BMD during different seasons in a large population-based cohort of Swedish women.

Methods

Study sample

The Swedish Mammography Cohort (SMC) is a population-based cohort in central Sweden (latitude, 60°N) [14, 15]. All women born between 1914 and 1948 living in Uppsala County (n = 48 517) and all women born between 1917 and 1948 living in Västmanland County (n = 41 786) were asked to respond to a comprehensive food frequency questionnaire (FFQ) when invited to a mammography screening (1987–1990). Completed questionnaires were obtained from 66 651 (74%) individuals; after exclusions, 61 433 women remained in the cohort [14, 15]. In 1997, a second expanded questionnaire was sent to all eligible participants and 38 984 (70%) responded. Between November 2003 and October 2009, we invited a randomly selected subcohort of the SMC [SMC Clinical (SMCC)], living in the city of Uppsala or the surrounding area, to undergo dual-energy X-ray absorptiometry [(DXA) bone, fat and lean mass] and height and weight measurement, and to provide biological samples. Blood samples were collected in the morning following an overnight fast. The samples were protected from light and spun in a refrigerated centrifuge, frozen in multiple tubes and stored at −80 °C until analysis. A third questionnaire on diet and lifestyle factors was also completed 1–3 months before a clinical examination. The participation rate was 65%, and the SMCC consists of 5022 women (see flow chart in Appendix S1).

BMD

BMD at the total hip, femoral neck and lumbar spine (L1–L4) and of the total body was measured using dual-energy DXA with the same equipment (Lunar Prodigy; GE Medical Systems, Madison, WI, USA) throughout the study. Osteoporosis was defined as a BMD of ≥2.5 SD below the mean of US White female reference populations aged 20–40 years at the total hip, femoral neck or lumbar spine. The short-term precision measurement error, based on duplicate measurements with repositioning according to recommendations from the International Society for Clinical Densitometry, varied between 0.8% and 1.5% depending on the measurement site. The long-term coefficient of variation was <1% for a spine phantom [16].

Determination of S-25OHD

In 2012, levels of $25OHD_2$ and $25OHD_3$ in serum were determined by high-performance liquid chromatography (HPLC), performed with a 1260/1290 liquid chromatograph (Agilent Technologies, Palo Alta, CA, USA) interfaced by atmospheric pressure chemical ionization (APCI) to a 6420 Triple Quad LC-MS/MS (Agilent Technologies) operated in multiple reaction monitoring mode, at Vitas, Oslo, Norway (www.vitas.no). The method is linear from at least 5–400 nmol L^{-1}, with 95% recovery, and the limit of detection is 1–4 nmol L^{-1}. Coefficients of variation for interassay analyses were found to be between 3% and 6%. Samples from 5002 of 5022 women in the SMCC were analysed at the same time. Total 25OHD was determined as the sum of $25OHD_3$ and $25OHD_2$, with the latter detected in only 12% of the samples which contained on average 4.5 nmol L^{-1} $25OHD_2$. The method has a high accuracy (95%) compared with all laboratory trimmed mean (ALTM) spiked samples from the Vitamin D External Quality Assessment (DEQAS) scheme and has been standardized against serum provided by the US National Institute of Standards and Technology [17].

Other measurements

Plasma levels of parathyroid hormone (PTH), calcium, creatinine, cystatin C and ALAT, and serum albumin, beta CrossLaps and osteocalcin were analysed using routine methods as described in the Appendix S1. Nutrient intakes were estimated using data from the FFQs, and other lifestyle information was categorized as previously described [14, 15]. Details can be found

in the Appendix S1. Average daily UV radiation [18] data in Sweden for the period 2003–2009 were retrieved from the Swedish Meteorological and Hydrological Institute.

Statistical analysis

The seasonal change in UV radiation dose was plotted against the seasonal change in total S-25OHD. The association between six predefined categories of total S-25OHD (<30, 30–<40, 40–<50, 50–<60, 60–<80 and ≥80 nmol L^{-1}) and adjusted percentage differences in BMD values by season (winter, December–February; spring, March–May; summer, June–August; autumn, September–November) was estimated (PROC GLM, SAS 9.4; SAS Institute, Cary, NC, USA). We used a modified Poisson regression approach [19] with robust error variance (PROC GENMOD, SAS 9.4) to assess relative risks of osteoporosis with 95% confidence intervals (CIs) in categories of vitamin D status by season. Two models were considered: an age-adjusted model and a multivariable model including leisure time physical exercise (<1, 1, 2–3, 4–5 and >5 h per week), total fat mass (continuous), total lean muscle mass (continuous), body height (continuous), current smoking status, vitamin D supplementation (because use might be related to a general health-seeking behaviour and lower risk of frailty) [20], bisphosphonate use (current use versus no use), ever use of postmenopausal oestrogen-replacement therapy (ever versus never use), previous hip fracture (yes versus no), previous fracture of any type (yes versus no), weighted Charlson's comorbidity index, plasma cystatin C, plasma creatinine, energy intake and dietary calcium intake (all continuous). Additional adjustment for educational level (three categories), marital status (living alone), nulliparity, use of calcium supplementation, and menopausal age and estimated glomerular filtration rate (eGFR) (both continuous) did not substantially affect our estimates. Moreover, adjustment for propensity scores [21] estimated from ordinal logistic regression (PROC logistic, SAS 9.4) including the covariates described in the primary multivariable model revealed similar estimates to those obtained after adjustment for individual variables. Finally, we considered whether three categories of total (dietary and supplements) vitamin D intake (<5, 5–7.5 and >7.5 μg day^{-1}) and four categories of plasma PTH concentration (<5, 5–<6, 6–<6.9 and ≥6.9 pmol L^{-1}) modified the association between S-25OHD and BMD by season. Sensitivity analyses were performed by excluding women with an eGFR (based on both plasma creatinine and cystatin C) [22] below 50 mL min^{-1}, vitamin D supplement or bisphosphonate users, or women with serum calcium levels above 2.5 mmol L^{-1} and PTH levels higher than 6.9 pmol L^{-1} (the upper normal level).

Results

Characteristics of the women by vitamin D status are shown in Table 1. The mean age of the women was 68 years (range 55–86 years). Women with low S-25OHD levels had on average higher body mass index (BMI), higher fat mass, higher PTH levels, lower leisure time physical activity and less frequent vitamin D and calcium supplement use. Otherwise, covariates were distributed approximately equally between categories of S-25OHD.

Mean S-25OHD was 58 nmol L^{-1}, and 5%, 34% and 83% of the women had serum concentrations below 30, 50 and 75 nmol L^{-1}, respectively (Fig. S1). The values were, however, highly dependent on season of blood collection (Fig. 1) and followed the variations in average UV-B radiation dose, with a lag period of 1–2 months. The highest values were found in samples collected in August and the lowest in February to March, with 28% (15 nmol L^{-1}) higher values in late summer.

In samples collected during summer, compared with the reference S-25OHD level of >80 nmol L^{-1}, we found a gradual increase in BMD of the total hip up to 40 nmol L^{-1} S-25OHD (Fig. 2), as well as a similar pattern at the femoral neck (Fig. S2), total body (Fig. S3) and lumbar spine (Fig. S4). Furthermore, during summer, compared with the reference S-25OHD level of >80 nmol L^{-1}, BMD at the total hip was 11% lower (95% CI 3–19) in women with S-25OHD below 30 nmol L^{-1} (n = 13) and 6% (95% CI 1–11) lower amongst those with S-25OHD levels between 30 and 40 nmol L^{-1} (n = 36). Compared with women who had higher S-25OHD concentrations during the summer, those with concentrations <40 nmol L^{-1} had a higher level of education and reported lower physical activity and calcium supplement use and a higher frequency of current smoking and nulliparity (see Table S1). As expected, they also had on average higher serum PTH concentrations, whereas other biomarkers or the comorbidity index did not differ between groups (see Table S1).

By contrast, no differences in mean BMD values between categories of S-25OHD were found during

Table 1 *Characteristics of the Swedish Mammography Cohort Clinical by total serum 25-hydroxyvitamin D (S-25OHD) categories*

		Categories of total S-25OHD											
		<30 nmol L^{-1}		30–<40 nmol L^{-1}		40–<50 nmol L^{-1}		50–<60 nmol L^{-1}		60–<80 nmol L^{-1}		≥80 nmol L^{-1}	
		n = 245 (4.9%)		*n* = 520 (10.4%)		*n* = 946 (18.9%)		*n* = 1103 (22.1%)		*n* = 1629 (32.6%)		*n* = 559 (11.2%)	
Variable	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Age	Years	68.0	7.1	67.9	6.9	68.0	6.9	67.5	6.7	67.4	6.6	67.4	6.7
Height	cm	162.5	7.0	163.6	6.2	163.3	6.1	163.5	5.8	163.8	6.1	163.9	6.3
Body mass index	kg m^{-2}	27.2	5.4	27.0	4.7	26.8	4.5	26.2	4.3	25.4	3.8	24.4	3.5
Total fat mass	g	28635	9862	28977	9088	28363	8745	27305	8681	25885	8134	23698	7851
Total lean mass	g	39617	4957	39645	4445	39803	4627	39605	4429	39247	4234	38840	4115
Total bone mass	g	2249	375	2346	407	2340	374	2333	366	2321	373	2281	397
S-25OHD	nmol L^{-1}	24.3	4.5	36.0	2.8	45.1	2.9	55.0	2.9	68.7	5.7	90.7	12.5
P-PTH (intact)	pmol L^{-1}	6.1	2.4	5.7	2.1	5.3	1.9	5.1	1.7	4.8	1.6	4.6	2.1
P-Phosphate	mmol L^{-1}	1.14	0.15	1.15	0.15	1.16	0.29	1.15	0.13	1.16	0.13	1.16	0.13
eGFR	mL min^{-1}	82.8	15.7	80.2	15.6	81.3	15.4	81.7	14.6	81.4	14.8	79.5	16.6
P-Cystatin C	mg L^{-1}	0.93	0.22	0.95	0.23	0.94	0.35	0.92	0.20	0.93	0.23	0.96	0.28
P-Creatinine	μmol L^{-1}	65.7	13.2	69.5	15.6	69.4	17.7	69.7	11.7	71.0	16.5	74.2	17.3
P-Calcium	mmol L^{-1}	2.30	0.11	2.31	0.11	2.31	0.11	2.31	0.11	2.31	0.11	2.31	0.11
S-Albumin	g L^{-1}	42.2	2.6	42.2	2.6	42.0	4.0	42.9	3.5	42.8	2.3	42.1	2.7
P-ALAT	μkat L^{-1}	0.24	0.13	0.24	0.17	0.25	0.33	0.23	0.13	0.23	0.14	0.23	0.14
S-CrossLaps	ng L^{-1}	454	184	468	201	461	194	457	185	460	190	465	202
S-Osteocalcin	μg L^{-1}	25.1	9.3	25.9	9.7	25.0	8.6	25.0	8.6	24.8	8.7	24.9	9.5
Charlson's comorbidity index	Points	0.29	0.71	0.23	0.60	0.25	0.63	0.19	0.59	0.20	0.58	0.20	0.60
Age at menopause	Years	49.7	4.9	50.0	4.7	49.8	4.5	49.9	4.2	50.3	4.3	50.1	4.2
Dietary intake													
Energy	kcal day^{-1}	1694	546	1772	573	1765	512	1802	535	1820	547	1784	513
Calcium	mg day^{-1}	1063	420	1100	383	1121	414	1110	400	1130	387	1120	378
Vitamin D	μg day^{-1}	5.0	2.4	5.7	3.6	5.8	2.7	5.8	2.4	6.0	2.6	5.9	2.5
Alcohol (ethanol)	g day^{-1}	5.7	8.3	5.5	7.6	5.3	6.1	6.1	6.5	6.6	6.6	7.7	9.8

Table 1 (*Continued*)

		Categories of total S-25OHD											
		<30 nmol L^{-1}		30–<40 nmol L^{-1}		40–<50 nmol L^{-1}		50–<60 nmol L^{-1}		60–<80 nmol L^{-1}		≥80 nmol L^{-1}	
		n = 245 (4.9%)		*n* = 520 (10.4%)		*n* = 946 (18.9%)		*n* = 1103 (22.1%)		*n* = 1629 (32.6%)		*n* = 559 (11.2%)	
Variable	Unit	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Retinol	μg day^{-1}	768	606	789	689	834	731	829	584	809	547	775	480
Phosphorus	mg day^{-1}	1469	564	1536	557	1557	525	1554	536	1588	541	1561	524
Potassium	mg day^{-1}	3182	1178	3343	1129	3375	1031	3410	1060	3504	1114	3444	1041
		n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
Any prevalent fracture		39	16	87	17	167	18	194	18	292	18	115	21
Leisure time physical activity													
<1 h per week		52	21	105	20	161	17	189	17	246	15	73	13
1 h per week		34	14	86	17	187	20	212	19	283	17	87	16
2–3 h per week		115	47	217	42	408	43	459	42	684	42	244	44
4–5 h per week		27	11	62	12	93	10	136	12	216	13	82	15
>5 h per week		17	7	50	10	97	10	107	10	200	12	73	13
Ever use of postmenopausal oestrogen		121	49	306	59	552	58	670	61	1050	65	379	68
Living alone		64	26	124	24	197	21	208	19	281	17	98	18
Nulliparity		37	15	68	13	114	12	114	10	152	9	61	11
Education													
<10 years		123	50	274	53	550	58	596	54	873	54	282	51
10–12 years		21	9	54	10	80	9	102	9	126	8	59	11
>12 years		101	41	192	37	316	33	405	37	630	39	218	39
Current smoker		47	19	64	12	80	9	105	10	115	7	37	7
Vitamin D supplement use		6	3	24	5	57	6	104	9	201	12	95	17
Calcium supplement use		5	2	23	4	49	5	104	9	283	17	141	25
Bisphosphonate use		0	0	1	0	2	0	11	1	27	2	22	4

P, plasma; S, serum; eGFR, estimated glomerular filtration rate; PTH, parathyroid hormone; ALAT, alanin aminotransferase.

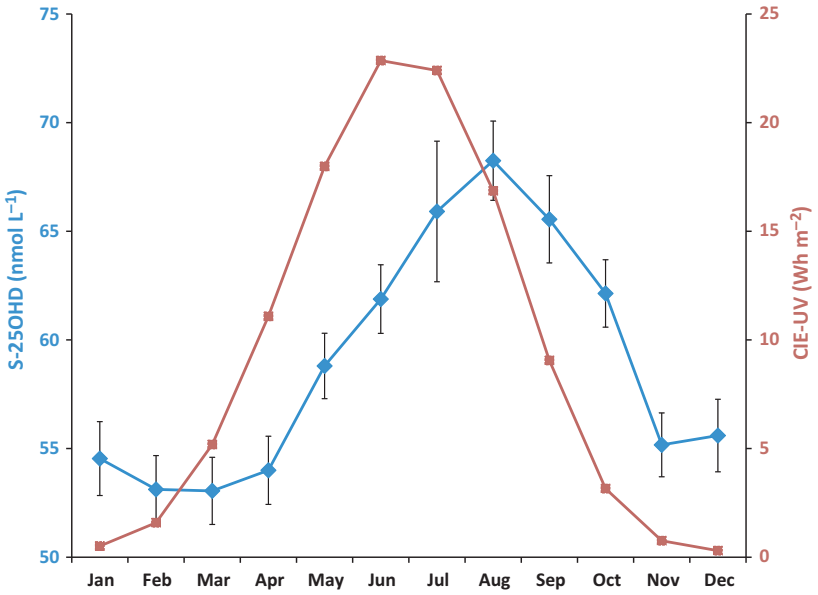

Fig. 1 *Seasonal variation in serum 25-hydroxyvitamin D (S-25OHD) concentration and average monthly ultraviolet (UV)-B radiation in the period 2003–2009 as measured by CIE action (Commission Internationale de l'Éclairage) spectrum per square metre [18], to mimic the erythemal effect of UV radiation.*

winter. Thus, women with the lowest levels of S-25OHD (<30 nmol L^{-1}) had similar BMD values compared to those with >80 nmol L^{-1} S-25OHD (Figs 2 and S2–S4). Even women with S-25OHD concentrations below 20 nmol L^{-1} ($n = 12$) had BMD values similar to those of individuals with levels of >80 nmol L^{-1} (mean difference in BMD −0.7%; 95% CI −8.7–7.3) during the winter season. Low S-25OHD concentrations (<30 nmol L^{-1}) during the spring and autumn were also related to lower BMD at the total hip (and the other BMD sites; Figs S2–S4). The results remained essentially unaltered after exclusion of subjects with low eGFR, vitamin D supplement and bisphosphonate users, those with high plasma calcium and PTH levels and women younger than 70 years of age (data not shown). To exclude the possibility that the association between summer vitamin D deficiency and low BMD simply represents the selection of a different cohort, we also compared women with S-25OHD concentrations <40 and ≥40 nmol L^{-1} during winter (Table S2). Similar to those women with S-25OHD levels <40 nmol L^{-1} during summer, there were no differences in comorbidity or biomarkers other than PTH. Moreover, also in concordance with women who had low S-25OHD concentration during summer, the frequency of current smoking was higher and the reported calcium supplement use lower compared to those with 25OHD ≥40 nmol L^{-1}. In addition, BMI, fat mass and energy intake were higher in women with S-25OHD <40 nmol L^{-1}. We also compared women with lowest levels of S-25OHD (<30 nmol L^{-1}) in summer and in winter (Table S3). The only statistically significant difference between these two subgroups was a lower mean age amongst those with low concentrations during summer.

Relative risks of osteoporosis by vitamin D status and by season are shown in Fig. 3. In accordance with mean BMD values, low S-25OHD levels during summer (<30 nmol L^{-1}) were associated with higher adjusted relative risk of osteoporosis (4.9; 95% CI 2.9–8.4) compared with levels above 80 nmol L^{-1}. During summer, no excess risk of osteoporosis was found with levels above 30 nmol L^{-1}. Low S-25OHD concentrations (<30 nmol L^{-1}) during winter, spring and autumn were not related to a higher risk of osteoporosis although there was a weak tendency towards an increase in risk for both spring and autumn values.

We also examined whether the observed associations were dependent on vitamin D intake. In contrast to women with S-25OHD levels <30 or 30–40 nmol L^{-1} during summer and intakes <5 or 5–7.5 μg day^{-1}, those with an intake >7.5 μg day^{-1} did not seem to have on average lower BMD values at the total hip, but the subgroups are based on small sample sizes (Fig. S5).

Approximately 4% of the variance in PTH was explained by S-25OHD (correlation coefficient 0.2, irrespective of season and multiple adjustments).

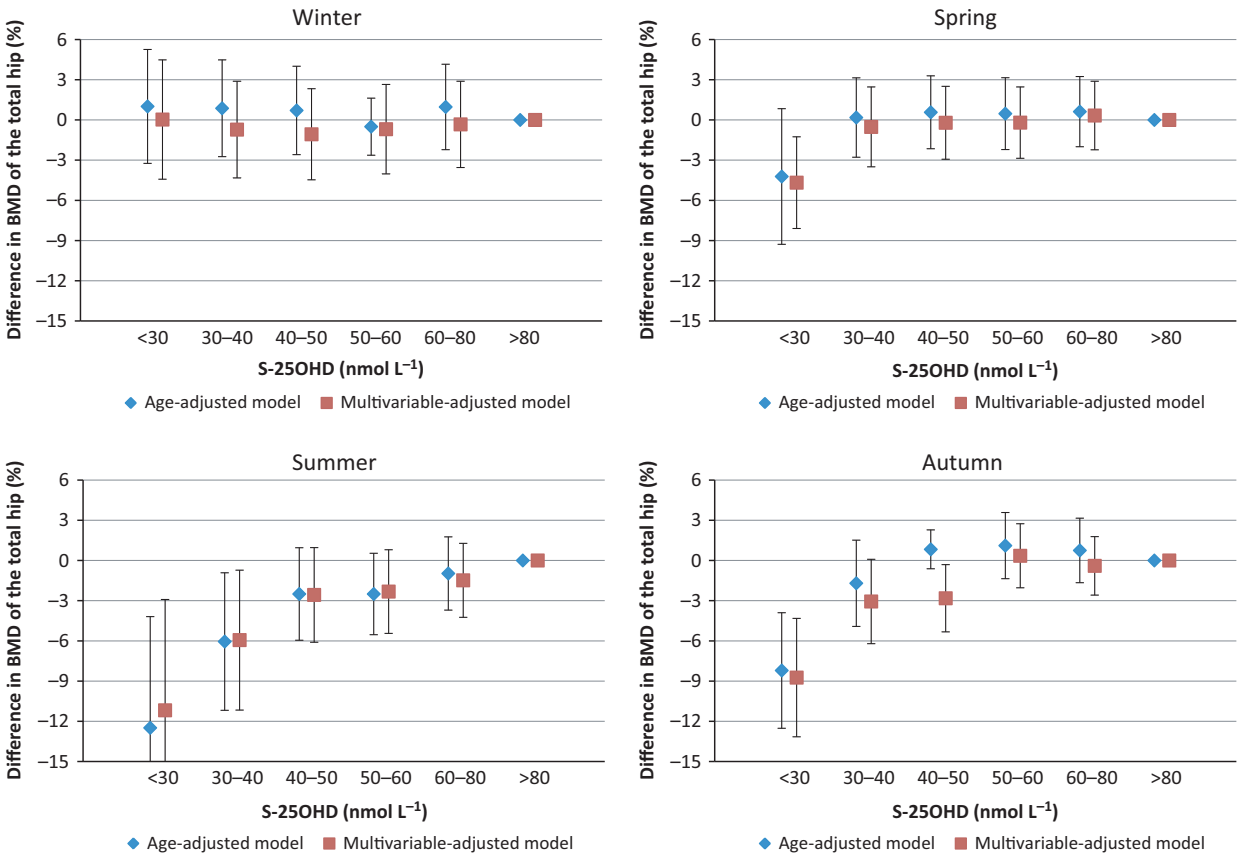

Fig. 2 *Age-adjusted (dark grey diamonds) and multivariable-adjusted (light grey squares) differences in bone mineral density (BMD) at the total hip within different groups of serum 25-hydroxyvitamin D (S-25OHD) levels, using >80 nmol L^{-1} as the reference, during the four seasons. The error bars denote 95% confidence intervals. The multivariable model was adjusted for age, leisure time physical exercise, total fat mass, total lean muscle mass, body height, current smoking, vitamin D supplementation, bisphosphonate use, ever use of postmenopausal oestrogen-replacement therapy, weighted Charlson's comorbidity index, previous hip fracture, previous fracture of any type, plasma cystatin C, plasma creatinine, energy intake and dietary calcium intake. The season-specific numbers and proportions of women in each S-25OHD category were as follows: during winter: <30 nmol L^{-1}, n = 71 (6.0%); 30–40 nmol L^{-1}, n = 171 (14.5%); 40–50 nmol L^{-1}, n = 250 (21.2%); 50–60 nmol L^{-1}, n = 259 (22.0%); 60–80 nmol L^{-1}, n = 344 (29.2%); >80 nmol L^{-1}, n = 82 (7.0%); during spring: <30 nmol L^{-1}, n = 116 (7.3%); 30–40 nmol L^{-1}, n = 204 (12.8%); 40–50 nmol L^{-1}, n = 329 (20.7%); 50–60 nmol L^{-1}, n = 357 (22.4%); 60–80 nmol L^{-1}, n = 437 (27.5%); >80 nmol L^{-1}, n = 148 (9.3%); during summer: <30 nmol L^{-1}, n = 13 (1.6%); 30–40 nmol L^{-1}, n = 36 (4.3%); 40–50 nmol L^{-1}, n = 112 (13.3%); 50–60 nmol L^{-1}, n = 188 (22.4%); 60–80 nmol L^{-1}, n = 347 (41.3%); >80 nmol L^{-1}, n = 144 (17.1%); during autumn: <30 nmol L^{-1}, n = 45 (3.2%); 30–40 nmol L^{-1}, n = 109 (7.8%); 40–50 nmol L^{-1}, n = 255 (18.3%); 50–60 nmol L^{-1}, n = 299 (21.5%); 60–80 nmol L^{-1}, n = 501 (35.9%); >80 nmol L^{-1}, n = 185 (13.3%).*

Importantly, we found no seasonal variation in mean PTH levels (winter: 5.1 pmol L^{-1}, 95% CI 5.0–5.2; spring: 5.2 pmol L^{-1}, 95% CI 5.1–5.3; summer: 5.0 pmol L^{-1}, 95% CI 4.9–5.2; autumn: 5.1 pmol L^{-1}, 95% CI 5.0–5.2). Moreover, we found no clear added value for the prediction of BMD of the combined measurement of levels of S-25OHD and plasma PTH in four categories (Fig. S6). Women with PTH concentrations higher than 6.9 pmol L^{-1} and S-25OHD concentrations lower than 30 nmol L^{-1} during winter had similar BMD values compared to women with PTH concentrations below 5 pmol L^{-1} and S-25OHD above 80 nmol L^{-1}.

Finally, we observed no clear trend, irrespective of season, in the association between S-25OHD levels and bone turnover markers (CrossLaps and osteocalcin) (Figs S7 and S8).

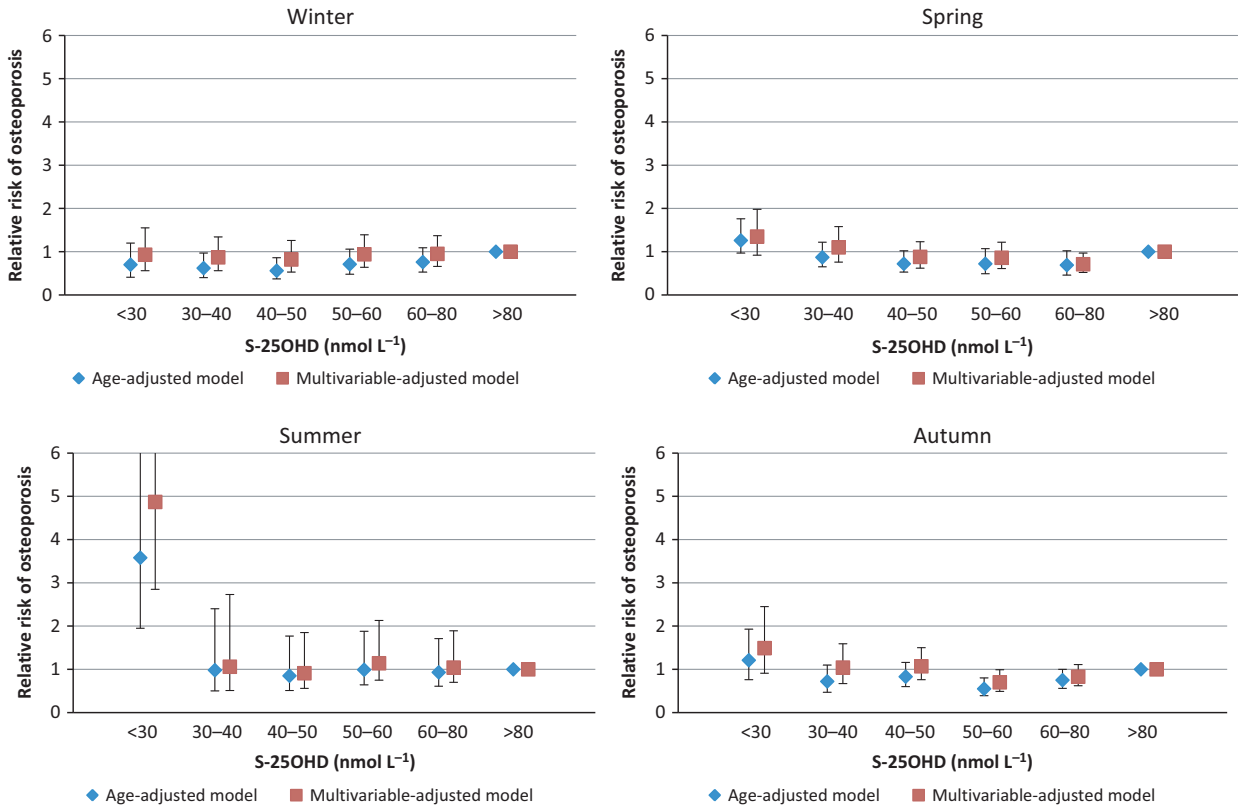

Fig. 3 *Age-adjusted (dark grey diamonds) and multivariable-adjusted (light grey squares) relative risk of osteoporosis (T-score < −2.5 at the total hip, femoral neck or lumbar spine) in relation to serum 25-hydroxyvitamin D (S-25OHD) concentration and season, with >80 nmol L^{-1} as the reference. The error bars denote 95% confidence intervals. The multivariable model was adjusted for age, leisure time physical exercise, total fat mass, total lean muscle mass, body height, current smoking, vitamin D supplementation, bisphosphonate use, ever use of postmenopausal oestrogen-replacement therapy, weighted Charlson's comorbidity index, previous hip fracture, previous fracture of any type, plasma cystatin C, plasma creatinine, energy intake and dietary calcium intake. The number of women with osteoporosis in each S-25OHD concentration category was as follows: during winter: <30 nmol L^{-1}, n = 16; 30–40 nmol L^{-1}, n = 29; 40–50 nmol L^{-1}, n = 43; 50–60 nmol L^{-1}, n = 52; 60–80 nmol L^{-1}, n = 80; >80 nmol L^{-1}, n = 25; during spring: <30 nmol L^{-1}, n = 35; 30–40 nmol L^{-1}, n = 45; 40–50 nmol L^{-1}, n = 59; 50–60 nmol L^{-1}, n = 63; 60–80 nmol L^{-1}, n = 71; >80 nmol L^{-1}, n = 40; during summer: <30 nmol L^{-1}, n = 7; 30–40 nmol L^{-1}, n = 7; 40–50 nmol L^{-1}, n = 22; 50–60 nmol L^{-1}, n = 38; 60–80 nmol L^{-1}, n = 62; >80 nmol L^{-1}, n = 26; during autumn: <30 nmol L^{-1}, n = 18; 30–40 nmol L^{-1}, n = 21; 40–50 nmol L^{-1}, n = 58; 50–60 nmol L^{-1}, n = 48; 60–80 nmol L^{-1}, n = 98; >80 nmol L^{-1}, n = 46.*

Discussion

We found considerable seasonal variation in the importance of S-25OHD concentration on bone health. Our results support a cut-off value of 40–50 nmol L^{-1} for optimal bone health [1] if the patient's blood sample is collected in the summer, whilst women with low S-25OHD levels during winter were not, on average, at higher risk of osteoporosis. The patterns for the association between S-25OHD and BMD in spring and autumn were midway between the patterns seen during summer and winter, suggesting a dynamic process throughout the year. Only 6% of the women failed to reach S-25OHD levels >40 nmol L^{-1} during summer indicating that summer activities and sun habits are adequate for optimal bone health for the vast majority of this population. In general, these individuals will also have low S-25OHD concentrations during the dark season but the predictive ability of a low winter value will be masked by the higher proportion (21%) of women with S-25OHD levels <40 nmol L^{-1} during winter. Those with both low summer and winter values

will be difficult to identify using winter values alone.

Previously, using longitudinal assessment of S-25OHD concentrations in 100 women from the SMCC, we showed that avoiding direct sun exposure was independently associated with 30 nmol L^{-1} lower S-25OHD and having a sensitive skin type with a further 19 nmol L^{-1} lower S-25OHD concentrations during summer [11]. In the present analysis, we also demonstrated that lifestyle habits may contribute to S-25OHD concentrations and found no indication that those who had low S-25OHD concentrations in the summer were especially frail individuals with derangements in biomarkers other than those belonging to the vitamin D axis.

The dietary reference intakes for vitamin D and calcium recommended by the IOM [1] were mainly based on calcium homeostasis and bone health. Average requirements for vitamin D intake for older (>70 years) individuals with minimal sun exposure were estimated to be 10 μg day^{-1} and the recommended dietary allowance to be 20 μg day^{-1}. As a comparison, despite mandatory vitamin D food fortification of low-fat dairy products and margarine in Sweden, only 1% of our female population reached a dietary intake level higher than 10 μg day^{-1} [15]. Amongst women with low summer S-25OHD levels (<40 nmol L^{-1}), we observed low BMD only in those with a dietary intake of ≤7.5 μg day^{-1}, that is only 5% of the population seems to gain a potential benefit from an increase in vitamin D intake. Nowadays, half of all middle-aged and older North American women take vitamin D supplements [23, 24], and an increasing trend in their use has been reported [25]. Since the publication of the IOM report, much more evidence regarding the efficacy of vitamin D supplementation has been published. Indeed, although vitamin D supplementation has dramatic effects on BMD in patients with osteomalacia, it does not lead to improved BMD in elderly women in general; clear improvements have not even been found in those with S-25OHD levels <50 nmol L^{-1} [26]. Comparison between studies is, however, hampered by different degrees of bias in the measurement of S-25OHD [27, 28] and few have used LC-MS/MS, which is considered the gold standard method [28]. The main skeletal effect of vitamin D is indirect, by increasing intestinal calcium absorption, but recent randomized controlled trials (RCTs) revealed no clear improvement in calcium absorption with increasing doses of vitamin D in young or in older women [29, 30]. The lack of threshold level of S-25OHD for calcium absorption in these trials suggests that active transport of calcium is already maximal at very low levels (below approximately 12.5–25 nmol L^{-1}) [30]. However, the association between S-25OHD and BMD may not simply be determined by a low threshold level, but may be a dynamic process where time is an important factor. For example, if it is assumed that calcium absorption is reduced at a S-25OHD concentration of 10 nmol L^{-1}, then a concentration of 25 nmol L^{-1} in February would not affect calcium absorption. However, if S-25OHD concentration was 10 nmol L^{-1} in February and increased in April–May, calcium absorption would be reduced for about 2–3 months. This could be compared to another potential scenario in which the S-25OHD level of 25 nmol L^{-1} in August falls to 10 nmol L^{-1} in November, that is decreased calcium absorption for 5–6 months leading to more substantial and sustained bone mineral loss. This may explain the lack of effect seen with vitamin D supplementation on BMD in many trials, that is the participants have not been selected based on inadequate vitamin D status during the sunny season [26]. Can vitamin D have no direct positive effect on bone? In animal studies, selective knockout of the vitamin D receptor (VDR) in osteoblasts leads to higher BMD, indicating that the primary biological role of the VDR is to regulate calcium concentrations and not to maintain bone health [31].

Given that 25OHD has a half-life of 2–4 weeks [32], that there is little or no cutaneous vitamin D synthesis between October and March and that the dietary intake is far below the recommended levels of 15 μg (51–70 years) and 20 μg (>70 years) per day [1], vitamin D deficiency could be expected in most individuals by late winter. Obviously this is not the case, and in a recent systematic review [33], it was demonstrated that many healthy adults in different populations can maintain adequate S-25OHD levels despite negligible UV exposure for several months of the year. Therefore, a key question is, how long do vitamin D stores last? Although adipose tissue contains substantial amounts of vitamin D [34], very little is known about the storage capacity and how the storage and mobilization processes are regulated. Based on the analysis of vitamin D in fat biopsies from subjects living in northern Norway (latitude 70°) who had participated in an RCT for 3–5 years, Didriksen

et al. [35] recently estimated that the stores of vitamin D in subcutaneous fat tissue were equivalent to 90 daily doses of 10 μg in the placebo group and 328 daily doses of 20 μg in the group taking vitamin D (oral dose of 500 μg per week). The observations of a clear positive association between vitamin D concentration in fat and serum and a linear increase in circulating 25OHD levels as a function of adiposity volume loss indicate bioavailability of cholecalciferol in fat stores during periods of insufficiency [32, 35–38]. Together, these results may help explain why individuals living at high latitudes do not have extremely low S-25OHD levels at the end of the winter season. Combined with our findings that BMD values are better predicted from summer S-25OHD levels, we suggest that vitamin D stores can last through the winter if enough time is spent in the sun during the summer. If the critical S-25OHD level of about 40–50 nmol L^{-1} is not attained during the summer season, the fat stores may not suffice through the winter and therefore the dietary intake may become of greater importance.

During chronic vitamin D deficiency (e.g. in kidney disease or gastrointestinal malabsorption), bone metabolism is affected as a result of reduced calcium absorption. This leads to increased PTH secretion, which stimulates the release of mineral from the bone. Much emphasis has therefore been placed on serum PTH levels when trying to define vitamin D insufficiency, that is to identify a threshold of S-25OHD below which secondary hyperparathyroidism occurs [39, 40]. However, and as pointed out in the IOM report, a critical question remains whether physiological regulations in PTH concentrations are directly harmful to bone and, if so, at what level [1]. Furthermore, in the present study, we observed an inverse relationship between the levels of S-25OHD and PTH irrespective of season, although no seasonal influence on PTH concentrations was detected. Importantly, BMD was not lower in women with S-25OHD <30 nmol L^{-1} and PTH above the upper normal limit (6.9 pmol L^{-1}) during winter compared with women with PTH <5 pmol L^{-1} and S-25OHD >80 nmol L^{-1}.

The strengths of our study include the size of the cohort, the population-based design, a clinically relevant outcome with short-term stability, the use of S-25OHD measured by a gold standard method with high accuracy, inclusion of a large number of covariates and the use of repeated and validated FFQs. Because of the unique personal identification number assigned to all Swedish residents, in combination with the availability of national healthcare registers, we are able to ascertain all comorbidities. Our results can be applied to Swedish middle-aged and elderly women, a population with a high incidence of osteoporotic fractures.

Several limitations of our study should be considered when interpreting the results. First, our cross-sectional design may be considered a limitation but there is a fairly good correlation (range 0.4–0.8) between individual S-25OHD concentrations measured several years apart [41–43], and the annual bone turnover rate is on average only 10%, with even less turnover in cortical bone predominant at the femoral neck region [44] measured by DXA. This is in contrast to the more rapid turnover of bone turnover markers and PTH. A limitation common to cross-sectional observational studies is that they may preclude conclusions regarding causality. Secondly, our results may not apply to populations of different ethnic origin or to men. Finally, our estimates were adjusted for several important covariates, but residual confounding still remains a possible limitation.

We conclude that summer levels of S-25OHD seem to be more suitable for assessing bone mineral status, whereas winter concentrations provide limited value. Thus, to determine a S-25OHD cut-off level for vitamin D deficiency, it may be necessary to take into account the season of blood collection.

Conflict of interest statement

No financial support or other benefits from commercial sources have been received, and none of the authors has any financial interests that could create potential conflict of interest.

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Authors' contributions

KM and HM contributed to concept and design. KM, AW and HM contributed to data collection. KM performed data analysis. KM and HM performed drafting of the manuscript. All authors contributed

to the interpretation of the data, and revision of the manuscript, and read and approved the final manuscript. KM takes responsibility for the integrity of the data analysis.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cumulative distribution of serum 25-hydroxyvitamin D concentrations in the Swedish Mammography Cohort Clinical.

Figure S2. Age-adjusted (dark grey diamond) and multivariable adjusted (light grey square) bone mineral density (BMD) differences at the femoral neck in relation serum 25-hydroxyvitamin D concentration and season.

Figure S3 Age-adjusted (dark grey diamond) and multivariable adjusted (light grey square) bone mineral density (BMD) differences of the total body in relation serum 25-hydroxyvitamin D concentration and season.

Figure S4. Age-adjusted (dark grey diamond) and multivariable adjusted (light grey square) bone mineral density (BMD) differences at the lumbar spine L1-L4 in relation serum 25-hydroxyvitamin D concentration and season.

Figure S5. Multivariable adjusted multivariable adjusted bone mineral differences at the total hip in relation serum 25-hydroxyvitamin D concentration, season and total vitamin D intake.

Figure S6. Multivariable adjusted multivariable adjusted bone mineral differences at total hip in relation serum 25-hydroxyvitamin D concentration, season and plasma PTH concentration.

Figure S7. Age-adjusted (dark grey diamond) and multivariable adjusted (light grey square) differences in serum CrossLaps concentrations in relation serum 25-hydroxyvitamin D concentration and season.

Figure S8. Age-adjusted (dark grey diamond) and multivariable adjusted (light grey square) differences in serum osteocalcin concentrations in relation serum 25-hydroxyvitamin D concentration and season.

Table S1. Summer value characteristics of the Swedish Mammography Cohort Clinical by lower (<40 nmol L^{-1}) and higher (>40 nmol L^{-1}) serum 25-OH-D concentrations.

Table S2. Winter value characteristics of the Swedish Mammography Cohort Clinical by lower (<40 nmol L^{-1}) and higher (>40 nmol/L^{-1}) serum 25-OH-D concentrations.

Table S3. Characteristics of the women in the Swedish Mammography Cohort Clinical with serum 25-OH-D concentrations <30 nmol L^{-1} in summer and winter, respectively.

Appendix S1. Flow chart for the Swedish Mammography Cohort (SMC) and the Swedish Mammography Cohort Clinical (SMCC).

Appendix S2. Methods.■

