Greater seasonal cycling of 25-hydroxyvitamin D is associated with increased parathyroid hormone and bone resorption

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Mini abstract (45 words max)

This analysis assessed whether seasonal change in 25-hydroxyvitamin D concentration was associated with bone resorption, as evidenced by serum parathyroid hormone and c-telopeptide concentrations. The main finding was that increased seasonal fluctuation in 25-hydroxyvitamin D was associated with increased levels of parathyroid hormone and c-telopeptide.

Abstract

Purpose

It is established that adequate 25-hydroxyvitamin D (25(OH)D, vitamin D) concentration is required for healthy bone mineralisation. It is unknown whether seasonal fluctuations in 25(OH)D also impact on bone health. If large seasonal fluctuations in 25(OH)D were associated with increased bone resorption this would suggest a detriment to bone health. Therefore, this analysis assessed whether there is an association between seasonal variation in 25(OH)D and bone resorption.

Methods

The participants were n=279 Caucasian and n=88 South Asian women (mean (± SD) age 48.2y (14.4) who participated in the longitudinal D-FINES (Diet, Food Intake, Nutrition and Exposure to the Sun in Southern England) study (2006-2007). The main outcomes were serum 25(OH)D, serum parathyroid hormone (sPTH) and serum C-telopeptide of collagen (sCTX), sampled once per season for each participant.

Results

Non-linear mixed modelling showed the (amplitude/mesor) ratio for seasonal change in log 25(OH)D to be predictive of log sPTH(estimate=0.057, 95% CI (0.051, 0.063), $p < 0.0001$) Therefore, individuals with a higher seasonal change in log 25(OH)D, adjusted for overall log 25(OH)D concentration, showed increased levels of log sPTH. There was a corresponding significant ability to predict the range of seasonal change in log25(OH)D through the level of sCTX. Here the corresponding parameter statistics were: (estimate=0.528, 95% CI (0.418, 0.638), p=<0.0001).

Conclusions

These findings suggest a possible detriment to bone health via increased levels of sPTH and sCTX in individuals with a larger seasonal change in 25(OH)D concentration. Further larger cohort studies are required to further investigate these preliminary findings.

Key words 25-hydroxyvitamin D, bone resorption, c-telopeptide, parathyroid hormone, circannual rhythm, seasonal variation

Introduction

Globally, it has been shown that 25-hydroxyvitamin D (25(OH)D, vitamin D) concentration decreases with increasing geographical latitude [1]. Poor 25(OH)D status in Western societies has been associated with increased risk of chronic diseases such as osteoporosis, heart disease, cancer and diabetes as well as infectious and autoimmune diseases [2]. Due to their high Northern latitude, the prevalence of vitamin D deficiency has been shown to be high in individuals living in Europe [3] and Canada [4]. The seasonal variability in UVB radiation at higher latitudes also leads to noticeable seasonal variation in serum 25(OH)D concentration in individuals in these countries [5, 6]. Indeed, these seasonal differences are large compared to that of rural dwelling humans living closer to the equator [7].

The situation is further complicated by the inter-individual variation in seasonal serum 25(OH)D within populations [8, 9]. Some individuals show far larger changes in serum 25(OH)D concentration than others across seasons. The reasons for these individual differences are not clear, but differences in sun exposure behaviour [8, 9], ethnicity [8-10], and clothing style[8] may be responsible. Recent work in premenopausal UK women has shown that intra individual (e.g. seasonal) factors are as important as inter-individual factors in determining vitamin D status [8]. The few studies that have investigated seasonal changes in 25(OH)D concentration have found that South Asians [8-10] and older people from all ethnic groups [11, 12] show less pronounced seasonal variation in their 25(OH)D concentration than other population sub-groups including younger adults and Caucasians.

Large seasonal changes in 25(OH)D concentration may have consequences for the activity of the hydroxylase enzymes that control vitamin D metabolism. These enzymes include 1-hydroxylase (CYP 27B1), which catalyses the conversion of the substrate 25(OH)D to 1,25dihydroxyvitamin D [$1,25(OH)_2D$] and 24-hydroxylase (CYP24A1) which catalyses 25(OH)D to 24,25dihydroxyvitamin D [$24,25(OH)_2D$] and 1,25(OH)2D to 1,24,25trihydroxyvitamin D [$1,24,25(OH)_3D$]. The activity of the 1-hydroxylase enzyme is readily affected by changes in its 25(OH)D substrate. This is because,

unlike many other enzymes, it is working well below its Michaelis-Menten constant (Km) at physiological concentrations of 25(OH)D. Therefore, large seasonal fluctuations in 25(OH)D substrate could cause large changes in the activity of the 1-hydroxylase enzyme [13]. In addition, theoretically, a long term decline in levels over the course of the year will not allow the desired level of 1,25(OH) $_2$D to be achieved until the decline finishes [14]. This suggests that individuals with large seasonal change in 25(OH)D concentration may have sub-optimal 1,25$(OH)_2$D concentration for much of the year. In support of this, a recent study assessing seasonal changes in serum 25(OH)D and 1,25$(OH)_2$D concentrations in a Norwegian population (62^oN) suggests that, at least in some individuals, circulating 1,25$(OH)_2$D concentration does fluctuate by season [15], and mirrors fluctuation in 25(OH)D concentration [12]. It must be borne in mind that the level of 1-hydroxylase enzyme is also important in determining 1,25 $(OH)_2$D concentration. Indeed, this enzyme can be up regulated in the kidney, which leads to increases 1,25 $(OH)D_2D$ concentration in the plasma, but not other tissues. Thus, 1,25 $(OH)D_2D$ status may vary between plasma and other tissues.

There is no evidence to date as to whether regular large seasonal changes in 25(OH)D concentration have any effect on health. There has been some suggestion of potential harm, however, based on findings of increased risk of prostate and pancreatic cancers [16, 17] and findings of increased mortality [18] in individuals with high vitamin D status. It has been proposed that these detrimental effects could be due to seasonal changes in 25(OH)D rather than high 25(OH)D itself [14]. This is because individuals with high serum 25(OH)D concentrations tend to be those who show the most seasonal change in 25(OH)D. They are therefore potentially susceptible to the detrimental perturbations in the activity of the hydroxylase enzymes described above. This intriguing hypothesis proposed by Vieth [14] to explain the increased cancer risk begs the question as to whether seasonal fluctuation or 'cycling' of 25(OH)D could also be detrimental to other aspects of health. Indeed, a recent study suggested flares in the autoimmune disease systemic lupus erythematosis (SLE) may be precipitated by large changes in vitamin D status [19]. This finding suggests the effects of seasonal

changes in 25(OH)D may have more widespread implications for health than just cancer risk. It is unknown whether these large seasonal fluctuations in 25(OH)D may have an impact on bone health.

The paracrine and autocrine effects of $1,25(OH)_2D$, produced locally in bone cells by 1-hydroxylase from 25(OH)D, have been recently elucidated [20, 21]. However, not enough is currently known about hydroxylase enzyme activity in bone cells to assess whether fluctuations in the 25(OH)D substrate would have any detriment on their ability to produce $1,25(OH)_2D$ in the correct quantities. Indeed, in bone cells the 1-hydroxylase and 24-hydroxylase have been found to be positively coupled, unlike in kidney cells where they are inversely coupled [22].

This paracrine and autocrine vitamin D activity is important for many bone cell processes, including mineralisation [23] and regulating osteoclast differentiation and activity [24]. It is unknown whether seasonal fluctuations in 25(OH)D concentration could cause adverse perturbations in this regulation, and thus be detrimental to bone health. Some studies show that bone markers show seasonal variation [25], but other studies do not [26]. It is unknown whether people showing a larger change in 25(OH)D over the course of a year show increased bone turnover in comparison to those with a smaller change in 25(OH)D. This study aimed to assess whether there is an association between bone resorption and the amount of seasonal change in 25(OH)D concentration. It was hypothesised that individuals showing a high degree of seasonal cycling of 25(OH)D would show increased bone resorption, as evidenced by both increased serum c-telopeptide [sCTX] and serum parathyroid hormone [sPTH] concentration.

Methods

Study Design

Data from *n* =367 women (South Asian, *n* =88 and Caucasian, *n* =279) who took part in the 2006-2007 D-FINES (Vitamin **D**, **F**ood **I**ntake, **N**utrition and **E**xposure to **S**unlight in Southern England) study [8] were analysed. Only participants who had no diagnosis of any disorder of calcium homeostasis, who were not peri-menopausal, or who were not currently taking any medication likely to affect bone, calcium or vitamin D metabolism were included in the study. Women who had been taking vitamin D supplements or cod liver oil supplements were excluded or asked to refrain from their use 3 months before and during the 12 months of the study. Further details of subject recruitment and D-FINES study background information can be found in Darling et al (2012) [8].

During D-FINES, subjects had blood taken between 0800 and 1000 hours in four seasons (summer, autumn, winter and spring) for determination of 25(OH)D and sPTH concentration. Each participant visited once in each seasonal period, thus the actual visit date varied by participant. The summer visit period spanned June to August 2006 whilst the autumn visit spanned September to November 2006 The winter visit was from December 2006 to February 2007 and the spring visit was from March to May 2007. The original study design for the D-FINES data was to allow comparisons between vitamin D status between seasons and ethnic-menopausal groups, rather than to assess seasonal change in detail over the course of the year. Thus for this subsequent analysis, where assessment of seasonal change was required in more detail, the actual visit date rather than season was used for each measurement and the data pooled.

In a subgroup of *n* = 65 women (South Asian, *n* = 30 and Caucasian, *n* = 35) (randomly selected from all the women who had successfully attended all four visits) blood samples were also assessed for the bone resorption marker serum c-telopeptide (sCTX). In accordance with the ethical standards laid down in the 1964 Declaration of Helsinki, ethical reviews were obtained from relevant Research

158 Ethics Committees (National Health Service NHS REC 06/Q1909/1, and University of Surrey
159 EC/2006/19/SBMS). Written, informed consent was obtained from all participants.

160 **Biochemical Measurements**

161 Serum CTX was measured using an electrochemiluminescent immunoassay (Roche cobas e411
162 automated analyser) at the University of Sheffield (Metabolic Bone Centre, Northern General
163 Hospital, Sheffield, UK). Intra-assay CV was: 5.7% (n = 12, mean 0.19 ng/mL). Inter assay CV was:
164 Level 1 QC: 2.1% (n = 9, mean 0.30 ng/mL); Level 2 QC: 3.6% (n = 9, mean 0.70 ng/mL); Level 3
165 QC: 6.6% (n = 9, mean 2.86 ng/mL). Serum 25(OH)D and sPTH were measured by the Vitamin D
166 Research Group, University of Manchester as described in detail previously [8]. The laboratory
167 participates successfully in the Vitamin D quality assurance scheme (DEQAS) and is accredited to
168 Quality Measurement Standards ISO 9001:2008 and ISO 13485:2003) [8]. Briefly, serum 25(OH)D
169 was measured using the manual IDS enzyme immunoassay (Immunodiagnostic Systems Ltd, Boldon,
170 Tyne and Wear, UK) [8]. Manufacturer's reference ranges were 19-58 ng/mL (48-144 nmol/L) but
171 vary with season; sensitivity 2 ng/mL (5 nmol/L); intra- and inter-assay coefficients of variation 6%
172 and 7%, respectively (manufacturer's values). Serum intact parathyroid hormone (PTH) was
173 measured using the OCTEIA immunoenzymometric assay (Immunodiagnostic Systems Ltd, Boldon,
174 Tyne and Wear, UK). The normal adult reference range is 0.8-3.9 pmol/L; sensitivity 0.06 pmol/L;
175 intra- and inter-assay CV 4% and 6%, respectively (manufacturer's values) [8].

176 **Non-Linear Mixed Modelling Analysis**

177 A non-linear mixed modelling approach was used to assess the hypothesis that individuals with a high
178 degree of seasonal cycling of 25(OH)D would show increased bone resorption, as evidenced by
179 increased serum c-telopeptide [sCTX] and serum parathyroid hormone [sPTH] concentration. The
180 25(OH)D data and the sPTH data were not normally distributed so 25(OH)D and sPTH were
181 logarithmically transformed. The data for sCTX were normally distributed, as assessed by the
182 Kolmogorov-Smirnov test, so were not log transformed. Measurements for sPTH, sCTX and

25(OH)D were approximately equally spaced over a year with precise visit dates used in the analysis, rather than month or season. Demographic data were drawn from baseline data only.

As potential confounders, at all times, BMI and ethnic-menopausal group were included in the model. It was important to control for ethnicity and menopausal status as these two factors are also known to be associated with differences in vitamin D status and vitamin D metabolism. The four ethnic-menopausal subject groups in our dataset were postmenopausal Caucasian, premenopausal Caucasian, postmenopausal South Asian and premenopausal South Asian and were entered into the model as 3 dummy variables, statistically contrasting the first group (postmenopausal Caucasian) with the remainder. BMI was entered into the model as it is known to be associated with overall 25(OH)D [17, 27], and seasonal change in 25(OH)D [12].

The modelling procedure was as follows: To investigate constants of proportionality with seasonal fluctuation in serum 25(OH)D for the first dependent variable (sPTH), the data were analysed for all the participants who had a complete set of 4 data points for sPTH and log 25(OH)D, as well as baseline data for BMI and ethnic/menopausal group. This was a total of n =200 women (n=96,n=65, n=21 and n=18 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively). The procedure followed for the sCTX analysis was analogous to that for sPTH (see above).The equivalent data in this analysis were for n= 60 women (n=15, n=18, n=15 and n=12 respectively in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians).

The model was used to assess whether log sPTH concentration, corrected for confounding effects as described above, was proportional to the level of log25(OH)D as well as to the amplitude of seasonal variation in log 25(OH)D divided by the mesor log 25(OH)D. It was important to adjust the amplitude by the mean log 25(OH)D concentration (mesor), in order to control for the confounding effects of overall mean 25(OH)D concentration. The individual participant's four data points for log 25(OH)D were modelled as a mean level specific to that participant, to which was added a sine wave of

amplitude and angular off-set both also specific for that participant, as well as a random normally distributed error term. The two participant-specific variables, mean level and angular offset were modelled as mixed random effects with unstructured variance-covariance matrix.

The sPTH data were simultaneously regressed as sets of four within participant repeated measures (with unstructured variance covariance matrix, also encompassing the effects of the above mentioned two participant-specific variables) against the independent variables: level of 25(OH)D, ratio of amplitude to mean of log 25(OH)D (i.e. amplitude/mesor), ethnicity and menopausal status category and BMI. The whole procedure was repeated for sCTX as the dependent variable.

The non-linear mixed modelling analysis was conducted using the NLMIXED procedure, of the SAS (SAS Institute, Cary, NC, USA) software suite. Regression parameters significantly different from zero within the limits of the conventional 95% confidence interval were deemed statistically significant. Baseline participant statistics were analysed using PASW Statistics, Release Version 18.0.0 (SPSS Inc., 2009, Chicago IL).

Results

Participant Characteristics

Results are presented as mean (SD). Table 1 shows the baseline characteristics of the cohort (n=367) the participants were drawn from, including 25(OH)D, sPTH and sCTX concentration in each season and anthropometric information. The women had a mean BMI of 26.3Kg/m^2 (5.1), thus were classified as overweight. They also had a mean age of 48.2 (14.4) years and a dietary calcium intake of 833(308) mg/d. Mean 25(OH)D concentration ranged from 39.4-58.4nmol/L, depending on season. Concurrently, the ranges of median values for sPTH and mean values for sCTX concentrations by season were 2.8-3.0pmol/L and 0.33-0.35ng/mL respectively.

Tables 2 and 3 show the same information, but for the subsets of the cohort who were included in the sPTH and sCTX analyses due to having complete data for all relevant variables(n=200, sPTH; n=60, sCTX). As can be seen from comparing table 1 (entire cohort) with that of table 2 (sPTH analysis) and table 3 (sCTX analysis), the women included in the sPTH and sCTX analyses were representative of the entire cohort. They had similar age (48.2 (14.4) vs. 50.6(12.9) vs. 47.7(12.4)y), BMI (26.4 (5.1) vs. 26.2 (4.7) vs. 26.0 (4.1) Kg/m^2) and dietary calcium intake (833(308) vs. 862(329) vs. 857(417) mg/d) to that of the original cohort. Also, for the sPTH analysis, mean 25(OH)D (59.2-38.1 nmol/L vs. 58.4-38.3nmol/L; see tables 1-2 for confidence intervals) and median sPTH concentrations (2.8-3.0 pmol/L vs. 2.8-3.0pmol/L) were similar to that of the whole cohort. For the sCTX analysis, mean 25(OH)D was slightly lower (47.8-33.9nmol/L vs. 58.4-38.4nmol/L; see tables 1 and 3 for confidence intervals) and median sPTH the same (2.8-3.0 pmol/L) between the participants in the regression model and the whole cohort.This result for 25(OH)D was likely due to a more even split of South Asian and Caucasian women in the sCTX analysis. This is in contrast to the sPTH analysis whereby there were a higher number of Caucasians than South Asians.

TABLE 1 ABOUT HERE

246 TABLE 2 ABOUT HERE

247 TABLE 3 ABOUT HERE

248 **Non-Linear Mixed Modelling**

249 The regression analysis is summarised in Table 4. Table 4 includes the effect sizes for the main
250 model parameters, here defined as the absolute value of the quotient of the estimated value and the
251 standard error. Thus defined, the effect size for a parameter is only an indication of how significantly
252 different from 0 the value of the parameter is, i.e. it is an indication of how necessary it is to include,
253 as opposed to excluding, that parameter in the model. However, apart from identifying the importance
254 of including the parameter in the model, the effect size conveys no other information about the
255 functioning of the model.

256

257 TABLE 4 ABOUT HERE

258

259 **sPTH and sCTX Analysis**

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261 For log sPTH, the regression coefficient (and SE) for the amplitude/mesor ratio of 25(OH)D were
262 0.057 (0.003) with a 95% confidence interval (0.051, 0.063); $p<0.0001$. The effect size was 19.0,
263 which means that the estimated value for that parameter was 19 standard errors of the estimate
264 removed from 0. This shows a significant positive relationship, after adjustment for confounders
265 (BMI and ethnic/menopausal group), and indicates that the amplitude/mesor parameter for 25(OH)D
266 was a significant predictor of log sPTH concentration. For sPTH the regression coefficient (SE) for
267 the level of 25(OH)D was -0.018 (0.001) with a 95% confidence interval of (-0.020, -0.016);

p<0.0001. The effect size was 18.0, marginally smaller than for the coefficient referred to immediately above.

For sCTX, the regression coefficient for amplitude/mesor ratio of 25(OH)D had an estimated value of 0.528 (95% confidence interval 0.418, 0.638; p=<0.0001) which was also statistically significant so that conclusions analogous to the above follow. The effect size was 9.3, which means that the estimated value for that parameter is 9.3 standard errors of the estimate removed from 0.

For sCTX the regression coefficient (SE) for the level of 25(OH)D was -0.105 (0.014) with a 95% confidence interval of (-0.132,- 0.078); p<0.0001. The effect size was 7.5, marginally smaller than that for the coefficient referred to immediately above.

Post-hoc Power Considerations

One of the objectives of the study was to investigate the relationship between sPTH and the seasonal variation in serum 25(OH)D and the study results show power in excess of 99.9% for this aim, adjusting for confounding effects.Another of the objectives of the study was to investigate the relationship between sCTX and the seasonal variation in serum 25(OH)D and the study results also show power in excess of 99.9% for this aim, adjusting for confounding effects.

Discussion

This is the first study, to the authors' knowledge, that has examined the association between seasonal change in 25(OH)D and a marker of bone resorption. A significant positive relationship was observed between the seasonal change in 25(OH)D and sPTH, supporting our original hypothesis suggesting that those individuals with a higher seasonal change in 25(OH)D had a higher sPTH. There was also a statistically significant association between seasonal change in 25(OH)D and bone resorption, as measured by sCTX, so that similar conclusions to the above are applicable.

The above findings suggest that the higher sPTH seen with increased seasonal change in 25(OH)D may translate into alterations in bone resorption. Indeed, the result for sCTX are not surprising. A concomitant increase in sCTX would be predicted due to the increased bone resorption implicated by increased sPTH levels. The trends observed for sPTH and sCTX in the current study lend support to Vieth's hypothesis[14] that large seasonal changes in 25(OH)D might be associated with some adverse health outcomes,. Indeed, in this study, for both sPTH and sCTX, seasonal fluctuation (as expressed by the amplitude/mesor ratio) had an (albeit marginally) larger predictive ability in explaining sPTH and sCTX than did the average concentration of 25(OH)D (as assessed by respective coefficient effect sizes). Thus, in this dataset, seasonal variation in 25(OH)D status had a marginally statistically more significant impact on sPTH and sCTX concentration than did overall 25(OH)D concentration.

It is important to know if seasonal cycling of 25(OH)D is detrimental to health, in order to inform supplementation advice for vitamin D. Specifically, it raises the question of whether year round supplementation of vitamin D, or winter only supplementation should be recommended. The clinical and public health implications of this study are the suggestion that wintertime only supplementation may be beneficial in order to blunt the rhythm of 25(OH)D, keeping 25(OH)D levels consistent throughout the year. In addition, it is essential to understand seasonal variation in 25(OH)D to assist in the interpretation of some of the adverse effects reported in the literature in regard to high serum

concentrations of 25(OH)D. Specifically, it is crucial to separate the effects of high levels of 25(OH)D *per se* from those of seasonal variation in order to establish guidelines for optimal 25(OH)D concentrations, which remain a topic of ongoing debate in the vitamin D field. Findings from the current study suggest that seasonal variation, as well as the overall concentration, of 25(OH)D needs to be considered when assessing optimal vitamin D status.

A limitation of the study findings is that they are generalisable only to Caucasian and South Asian women, and may not be generalisable to other ethnic groups due to potential differences in vitamin D metabolism that may affect seasonal changes in 25(OH)D, sPTH and sCTX. A larger sample size for bone markers will be even more informative to clarify the relationship between seasonal fluctuation in 25(OH)D and bone resorption.

In future work, it will be important to assess markers of bone formation as well as resorption as overall bone turnover is important for bone health, not just bone resorption. It is possible that an increase in sPTH may trigger increased bone formation, so may not necessarily be detrimental to bone health. Measurement of bone formation as well as bone resorption is required to investigate further whether an increase in sPTH is likely to be harmful in the longer term. It would also be useful in longitudinal research studies to assess whether structural changes in bone are associated with seasonal changes in 25(OH)D, in order to determine possible chronic effects on bone health. Indeed, even if seasonal fluctuation in 25(OH)D is detrimental to the activity of the bone vitamin D hydroxylase enzymes, there could still be physiological adaptation to this in the long term.

Conclusions

This study shows that greater seasonal cycling of 25(OH)D is associated with increased sPTH concentration and with increased bone resorption. In terms of public health, this finding suggests vitamin D supplements should not necessarily be taken all year round and there may be justification for 'blunting' the rhythm of 25(OH)D concentration over the course of the year via wintertime only supplementation. Furthermore, it suggests seasonal variation in 25(OH)D, as well as overall concentration, should be considered when making recommendations as to optimal concentrations of 25(OH)D for health.

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Dedication

This paper is dedicated to Mr John Pheasant, Practice Manager at Thornton Heath Medical Centre, London who helped with the study recruitment and who sadly died in 2008.

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Tables

Table 1. Characteristics of participants in D-FINES cohort (n=367)≠

	N	Mean	SD	Lower 95% CI	Upper 95% CI
Age (years)	367	48.2	14.4	19.98	76.42
Body Mass Index (BMI) (kg/m^2)	365	26.4	5.1	16.40	36.40
Weight (kg)	365	69.6	12.7	44.71	94.49
Height (m)	365	1.6	0.1	1.40	1.80
Dietary calcium (mg)±	286	833	308	229.32	1436.68
Summer 25(OH)D (nmol/L)	346	58.4	27.1	5.28	111.52
Autumn 25(OH)D (nmol/L)	281	51.1	24.7	2.69	99.51
Winter 25(OH)D (nmol/L)	253	38.4	18.0	3.12	73.68
Spring 25(OH)D nmol/L	248	42.7	22.0	-0.42	85.82
Summer sCTX ng/mL	65	0.34	0.16	0.03	0.65
Autumn sCTX ng/mL	65	0.34	0.15	0.05	0.63
Winter sCTX ng/mL	65	0.33	0.15	0.04	0.62
Spring sCTX ng/mL	65	0.35	0.16	0.04	0.66
	N	Median	25^{th*}	75^{th*}	IQR
Summer sPTH pmol/L	345	2.8	2.0	3.6	1.6
Autumn sPTH pmol/L	291	2.8	2.0	3.8	1.8
Winter sPTH pmol/L	244	3.0	2.1	3.8	1.7
Spring sPTH pmol/L	258	2.8	2.0	3.6	1.6

sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D; n=number of participants with measurement, ≠ *n*=144,*n*=135, *n*=42 and *n*=46 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively. ±Dietary calcium was assessed

using four-day photograph assisted diet diaries (as previously validated in the EPIC cohort)*percentile, IQR=interquartile range

Table 2. Characteristics of participants- (n=200) in the sPTH analysis≠

	N	Mean	SD	Lower 95% CI	Upper 95% CI
Age (years)	200	50.6	12.9	25.32	75.88
Body mass index (BMI) (Kg/m^2)	200	26.2	4.7	16.99	35.41
Weight (kg)	200	68.8	12.0	45.28	92.32
Height (m)	200	1.62	0.06	1.50	1.74
Dietary calcium (mg)	186	862	329	217.16	1506.84
Summer 25(OH)D (nmol/L)	200	59.2	27.7	4.91	113.49
Autumn 25(OH)D (nmol/L)	200	50.7	24.3	3.07	98.33
Winter 25(OH)D nmol/L	200	38.1	17.5	3.80	72.40
Spring 25(OH)D nmol/L	200	43.1	22.5	-1.00	87.20
Summer sCTX ng/mL	59	0.34	0.16	0.03	0.65
Autumn sCTX ng/mL	59	0.34	0.16	0.03	0.65
Winter sCTX ng/mL	59	0.33	0.16	0.02	0.64
Spring sCTX ng/mL	59	0.36	0.17	0.03	0.69
	N	Median	25^{th*}	75^{th*}	IQR
Summer sPTH pmol/L	200	2.90	2.00	3.70	1.7
Autumn sPTH pmol/L	200	2.80	2.00	3.70	1.7
Winter sPTH pmol/L	200	3.00	2.10	3.80	1.7
Spring sPTH pmol/L	200	2.80	2.00	3.60	1.6

sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D; summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D, n=number of participants with measurements

≠ n=96,n=65, n=21 and n=18 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively. ±Dietary calcium was assessed using four-day photograph assisted diet diaries (as previously validated in the EPIC cohort), * percentile, IQR=interquartile range

446 **Table 3. Characteristics of participants- (n=60) in the sCTX analysis≠**

	N	Mean	SD	Lower 95% CI	Upper 95% CI
Age (years)	60	47.7	12.4	23.40	72.00
Body mass index (BMI) (kg/m^2)	60	26.0	4.1	17.96	34.04
Weight (kg)	60	66.5	10.1	46.70	86.30
Height (m)	60	1.60	0.06	1.48	1.72
Dietary calcium (mg)±	52	857	417	39.68	1674.32
Summer 25(OH)D (nmol/L)	60	47.8	25.3	-1.79	97.39
Autumn 25(OH)D nmol/L	60	41.2	25.3	-8.39	90.79
Winter 25(OH)D nmol/L	60	33.9	20.4	-6.08	73.88
Spring 25(OH)D nmol/L	60	36.9	20.9	-4.06	77.86
Summer sCTX ng/mL	60	0.34	0.16	0.03	0.65
Autumn sCTX ng/mL	60	0.34	0.16	0.03	0.65
Winter sCTX ng/mL	60	0.33	0.16	0.02	0.64
Spring sCTX ng/mL	60	0.35	0.17	0.02	0.68
	N	Median	25^{th*}	75^{th*}	IQR
Summer sPTH pmol/L	60	3.10	2.10	3.88	1.78
Autumn sPTH pmol/L	60	3.10	2.40	3.98	1.58
Winter sPTH pmol/L	59	3.20	2.30	4.40	2.10
Spring sPTH pmol/L	60	3.20	1.95	4.00	2.05

447 sPTH=serum parathyroid hormone; sCTX=serum C-telopeptide of collagen; 25(OH)D=serum 25-hydroxyvitamin D;
448 summer to winter 25(OH)D ratio=winter 25(OH)D-summer 25(OH)D; n=number of participants with measurements
449 ≠*n*=15, *n*=18, *n*=15 and *n*=12 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and
450 premenopausal Asians respectively ±Dietary calcium was assessed using four-day photograph assisted diet diaries (as
451 previously validated in the EPIC cohort) *percentile, IQR=interquartile range

Table 4 - Relevant non-linear modelling parameter statistics for sPTH and sCTX

	sPTH n=200≠					sCTX n=60±				
Parameter	Estimate	SE	95% CI	p	Effect size¥	Estimate	SE	95% CI	p	Effect size¥
Indicator (0 1) variable for (PRE C) v (POST	-0.092	0.037	**(-0.165; -0.019)**	0.0123	2.5	-0.205	0.080	(-0.362; -0.048)	0.0109	2.6
Indicator (0 1) variable for (POST A)v(POST	0.511	0.048	**(0.417; 0.605)**	<.0001	10.6	0.181	0.080	(-0.338; -0.024)	0.0242	2.3
Indicator (0 1) variable for (PRE A)v(POST	0.052	0.066	(-0.077; 0.181)	0.4327	0.8	0.070	0.048	(-0.024; 0.164)	0.1406	1.5
BMI (Body mass index) kg/m^2	0.037	0.002	**(0.033; 0.041)**	<.0001	18.5	0.007	0.005	**(-0.003; -0.017)**	**0.1389**	**1.4**
25(OH)D regression coefficient	-0.018	0.001	**(-0.020;-0.016)**	<.0001	18.0	-0.105	0.014	**(-0.132;-0.078)**	**<.0001**	**7.5**
25(OH)D Ratio (amplitude/mesor)	0.057	0.003	**(0.051; 0.063)**	<0.0001	19.0	0.528	0.056	(0.418; 0.638)	<.0001	9.3
-2 log likelihood	**1330.7**					**292.0**				

*POST C= Postmenopausal Caucasian (reference group); PRE C = Premenopausal Caucasian; POST SA = Postmenopausal South Asian, PRE SA= Premenopausal South Asian. ** Body Mass Index. ≠ *n*=96,*n*=65, *n*=21 and *n*=18 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively. ± *n*=15, *n*=18, *n*=15 and *n*=12 in postmenopausal Caucasians, premenopausal Caucasians, postmenopausal Asians and premenopausal Asians respectively

¥Definition of effect sizes: the absolute value of the quotient of the estimated value and the standard error, Thus defined, the effect size for a parameter is only an indication of how significantly different from 0 the value of the parameter is, i.e. it is an indication of how necessary it is to include, as opposed to excluding, that parameter in the model. The conventional 5% significance level is met for a parameter when the effect size for that parameter meets or exceeds a value of 1.96. However, apart from identifying the importance of including the parameter in the model, the effect size conveys no other information about the functioning of the model.

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