

# Determinants of circulating 25-hydroxyvitamin D concentration and its association with musculoskeletal health in midlife: Findings from the Hertfordshire Cohort Study

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## ABSTRACT

**Introduction:** Several studies have reported the importance of vitamin D status to musculoskeletal health in populations of older adults. Here we report relationships between circulating serum 25(OH)D and musculoskeletal health in a community cohort of UK adults in midlife and investigate whether environmental (dietary intake, use of supplements) and/or genetic factors (4 SNPs previously related to vitamin D status) play more significant roles in determining vitamin D status in this population.

**Methods:** Participants were recruited from the Hertfordshire Cohort Study, an established longitudinal cohort study of community dwelling adults and were seen at baseline and follow up 9–12 years later. Lumbar spine and total femur BMD were measured at baseline using a Hologic QDR 4500 instrument. Osteoarthritis (OA) was defined by radiographs of the knees graded according to Kellgren & Lawrence at both time points. Serum 25(OH)D concentrations were measured using a DiaSorin Liaison chemiluminescent assay. Genotyping of 4 SNPs previously associated with 25(OH)D values were assessed: (rs12785878 (DHCR7), rs10741657 (CYP2R1) and rs6013897 (CYP24A1)) and a fourth SNP (rs4588), described as “a near-perfect proxy (i.e. substitute) for rs2282679 on the GC gene”.

**Results:** 820 subjects (397 men, 423 women) participated at baseline, and 339 of these 820 subjects (164 men; 175 women) participated in a follow up study of OA progression. The median (IQR) age of participants at baseline was 64.0 (61.8–66.5) and 65.5 (63.3–67.6) for men and women respectively. Median circulating levels of 25(OH)D were 44.6 (35.0–63.0) nmol/L and 41.3 (29.8–53.5) nmol/L in men and women respectively. Circulating 25(OH)D was strongly associated with season of blood testing ( $p < 0.001$ ). The greatest variance in a model of vitamin D status that included the four SNPs measured, season, and whether participants reported taking vitamin D supplements was explained by season of assay (17.9% men; 15.8% women). Higher femoral neck BMD was observed in men with higher baseline vitamin D status, after adjustment for age, season, BMI, smoker status, alcohol consumption, physical activity and social class ( $p = 0.01$ ). Associations between 25(OH)D and BMD in women were not statistically significant in this population. There were no associations between circulating 25(OH)D and radiographic knee OA at either time point after adjustment for confounders and for duration of follow-up.

**Conclusion:** Circulating 25(OH)D levels were generally lower than is recommended in community dwelling adults in midlife, with marked seasonal variation observed, but relationships with reported vitamin D supplementation were weaker. Circulating 25(OH)D was directly associated with hip BMD in men but relationships with BMD in women and radiographic OA were not seen in this sample.

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## 1. Introduction

Vitamin D is an essential nutrient that plays a critical role in supporting musculoskeletal health as it is essential to the regulation of calcium and phosphorus in the human body [1,2]. Prolonged and severe vitamin D deficiency is associated with various adverse musculoskeletal outcomes such as rickets in children, and osteomalacia and osteoporosis in adults [1,2]. Moreover, low levels of circulating 25-hydroxyvitamin D (25(OH)D) have been associated with knee osteoarthritis (OA) progression [3], while epidemiological studies have suggested that higher serum 25(OH)D levels are associated with greater bone mineral density (BMD) [4].

In recent years, scientific and clinical evidence has suggested that vitamin D deficiency and insufficiency represent a major public health issue for the global population due to its high prevalence, particularly in older adults [5–15]. Dark or covered skin, liberal use of sun protection, being overweight or obese, indoor lifestyles and older age are all well-established risk factors for vitamin D deficiency [12]. A recent study also suggested the importance of genetic factors to the contribution of cholecalciferol supplementation to circulating 25-hydroxyvitamin D levels [16]. As stated above, vitamin D insufficiency is common, though the threshold taken to define this varies in different guidance; a recent study found that 13% of European individuals, irrespective of age group, ethnicity, and latitude of study populations, had serum 25(OH)D concentrations <30 nmol/L on average throughout the year, and that this prevalence rose to 40.4% when a threshold of <50 nmol/L, as used in most countries other than the UK, was taken to define deficiency [17]. Seasonal variation in 25(OH)D has been reported previously, with some studies suggesting this may be affected by comorbidity [18].

Most existing studies on serum 25(OH)D concentration and musculoskeletal health have reported findings in populations of older adults [8–10]. In the present study we report circulating levels of serum 25(OH)D and musculoskeletal health, as assessed by bone mineral density (BMD) and radiographic osteoarthritis (OA), in a lesser studied phase of the lifecourse, a community cohort of adults in midlife, and investigate whether environmental (dietary intake, use of supplements and exposure to sunlight) and/or genetic factors had the greater role in determining vitamin D status. We undertook this analysis as intervention in the midlife may allow intervention prior to the stage when the musculoskeletal sequelae of low 25(OH)D levels – osteoporosis and osteoarthritis – become common.

## 2. Material and methods

Participants were recruited from the Hertfordshire Cohort Study, an established longitudinal cohort study of community-dwelling men and women born between 1931 and 9 in Hertfordshire and originally recruited to study the relationship between growth in infancy and the subsequent risk of adult diseases [19,20]. At baseline in 1998–2004, participants were visited in their homes where questionnaires were administered, and height and weight measured, and attended a research clinic, where fasting blood samples were collected, dual X-ray absorptiometry (DXA) scans performed, and radiographic OA of the knee assessed.

Individuals taking drugs known to alter bone metabolism (such as bisphosphonates) were excluded from the study. The baseline nurse-administered questionnaire collected information on socioeconomic status and lifestyle, including alcohol intake, smoking status, physical activity, and diet (assessed by means of a food frequency questionnaire (FFQ), including an assessment of dietary calcium and vitamin D intake). The FFQ included 129 foods and food groups and was used to assess an average frequency of consumption of the listed foods over a 3-month period preceding the interview. Nutrient intakes were calculated by multiplying the frequency of consumption of a portion of each food by its nutrient content according to the UK national food composition

database or manufacturers' composition data. Use of dietary supplements over the preceding 3 months was also recorded. Nutrient intakes from dietary supplements were calculated using the frequency and dose reported by the participant and manufacturers' composition data.

Bone mineral content (BMC), bone area and BMD were measured upon baseline recruitment by DXA at the lumbar spine and proximal femur (i.e. femoral neck, total hip, intertrochanteric and trochanteric regions, and Ward's triangle) using a Hologic QDR 4500 instrument, with measurement precision error (expressed as 'coefficient of variation') of 1.55% for lumbar spine BMD, (1.45% for total femur and 1.83% for femoral neck BMD). Similar DXA scan data were collected from twenty-five volunteers (not part of the study) who underwent two scans in one day to obtain precision measurements for the scanner. Short-term (two month) precision error for the QDR 4500 was less than 1% for both sites (hip and lumbar spine). Antero-posterior (AP) and lateral knee radiographs were taken of both knees and graded for OA by rheumatologists based on K&L scores at baseline and follow up [21,22]. Surgically replaced knees were excluded from this study.

Fasting baseline blood samples were taken and serum 25(OH)D concentrations measured using a DiaSorin Liaison automated chemiluminescent assay. The batch precision for high and low total serum 25(OH)D concentration was 10–12% and 12–15%, respectively, and all assay 'runs' complied with the standards set by the international DEQAS Vitamin D External Quality Assessment Scheme (<http://www.deqas.org>).

Blood samples were available from baseline for genetic analysis. We considered 4 common single-nucleotide polymorphisms (SNPs) in genes related to vitamin D metabolism and serum 25(OH)D that had previously been suggested to be important in determining vitamin D concentration [17,23]. Genotyping was undertaken by LGC Genomics (Hoddeston, UK) using KASP-competitive allele-specific polymerase chain reaction. We assessed the relationships between three SNPs (rs12785878 (DHCR7), rs10741657 (CYP2R1) and rs6013897 (CYP24A1)) and circulating 25(OH)D concentration. We also assessed the relationship with a fourth SNP (rs4588), described on SNPedia (<http://www.snpedia.com/index.php/SNPedia>) as "a near-perfect proxy (i.e. substitute) for rs2282679 on the GC gene".

## 3. Statistical analysis

Study participants' characteristics were summarised using means and standard deviations (SD) or medians and interquartile ranges (IQR) for continuous variables, and numbers and percentages for qualitative (categorical) variables. The circulating 25-hydroxyvitamin D values and DXA outcomes were transformed using the Fisher-Yates rank-based inverse normal transformation to create z-scores.

Multiple linear regression was used to examine the association between the four SNPs and circulating vitamin D using an additive model. The additive model expresses the change in outcome with each additional common allele. Models were initially adjusted for season of blood collection only and then further adjusted for vitamin D intake (diet and supplements), age, Body Mass Index (BMI), social class, smoker status, alcohol consumption, physical activity and calcium intake; models among women were additionally adjusted for years since menopause and HRT use. Mutually adjusted associations between vitamin D intake from food and whether or not participants were taking vitamin D supplements in relation to circulating vitamin D were examined using linear regression after adjustment for season and then additionally adjusted for the set of adjustments stated above. Circulating vitamin D in relation to BMD measures were examined using multiple linear regression with adjustment for season of blood collection, age, BMI, smoker status, alcohol consumption, physical activity and social class; circulating vitamin D in relation to radiographic OA at baseline and follow-up were examined using logistic regression with the same adjustments (OA outcomes at follow-up were additionally adjusted for follow-up time). Analysis of variance (ANOVA) was implemented to calculate the

proportion of variance in circulating vitamin D explained by the following: season of blood collection; each of the four SNPs; and whether participants were taking a vitamin D supplement.

Seasons were defined according to the UK Meteorological Office recommendations ([www.metoffice.gov.uk](http://www.metoffice.gov.uk)) as winter (December to February), spring (March to May), summer (June to August), and autumn (September to November).

Analyses were based on the sample of 820 participants who had underwent knee x-rays at baseline and who had values on circulating vitamin D. Results from the regression analyses are presented as regression coefficients ( $\beta$ ) and 95% confidence intervals (CI). All analyses were performed among men and women separately using Stata v14.2 (StataCorp, College Station, TX) and  $p < 0.05$  was considered statistically significant.

#### 4. Results

Table 1 shows the characteristics of study participants at baseline. We recruited 820 subjects (397 men, 423 were women). The median (IQR) age of participants was 64.0 (61.8–66.5) years for men and 65.5 (63.3–67.6) years for women at baseline. Differences were observed in physical activity time, alcohol consumption, and smoking habits: men had higher physical activity scores than women (mean (SD): 63.6 (14.8) for men, 61.2 (15.1) for women), consumed more alcohol per week than women (median (IQR): 9.5 (2.3–21.0) units/week for men, 1.5 (0.0–5.0) units/week for women), and were more prone to be or have been a smoker than women, with 31.2% of them reporting to have never smoked, whereas 62.1% of women said they had never smoked. These smoking differences and reports of alcohol consumption were statistically significant, and similar to those observed in other comparable

cohorts, though the differences were more pronounced in our sample than has been reported nationally in the UK [24].

As expected, BMI and 25(OH)D were negatively correlated in this cohort among men (Spearman correlation: -0.067) and women (Spearman correlation: -0.069). For this reason, we also repeated our analyses having restricted to participants with BMI values in the normal ( $18.5 \leq \text{BMI} < 25$ ) and overweight ( $25 \leq \text{BMI}$ ) ranges. In these restricted groups, associations were similar to those shown here but some associations were no longer statistically significant, possibly due to the smaller number of observations (data not shown).

Median (IQR) circulating levels of 25(OH)D were 44.6 (35.0–63.0) nmol/L and 41.3 (29.8–53.5) nmol/L in men and women respectively, and the difference was statistically significant ( $p < 0.001$ ). As expected, men had higher lumbar spine, femoral neck, and total femoral BMD than women (all  $p < 0.001$ ), while more women (46.8%) than men (36.3%) reported taking a vitamin D supplement/medication ( $p = 0.002$ ). 12.1% men and 17.0% women were found to have a serum 25 (OH) concentration  $< 25$  nmol/L and 45.1% men and 47.0% women had 25 (OH) concentrations between 40 and 75 nmol/L.

The use of vitamin D supplements was related to higher levels of circulating 25(OH)D in both men ( $\beta$  0.16, 95% CI -0.05 – 0.37 z-score,  $p = 0.141$ ) and women ( $\beta$  0.16, 95% CI -0.01 – 0.34 z-score,  $p = 0.068$ ) but these associations were generally weak and not statistically significant. Significant associations were found in both sexes between higher levels of circulating 25(OH)D and blood collections made in summer (men:  $\beta$  1.05, 95% CI 0.77–1.33 z-score,  $p < 0.001$ ; women:  $\beta$  0.89, 95% CI 0.62–1.16 z-score,  $p < 0.001$ ) and autumn (men:  $\beta$  0.83, 95% CI 0.57–1.09 z-score,  $p < 0.001$ ; women:  $\beta$  0.73, 95% CI 0.51–0.94 z-score,  $p < 0.001$ ) compared to in winter. Circulating 25(OH)D levels according to sex and season of blood testing are shown in Fig. 1; among both sexes,

**Table 1**  
Baseline characteristic of participants.

	Men			Women		
	N	Median	IQR	N	Median	IQR
Age (yrs)	397	64.0	61.8–66.5	423	65.5	63.3–67.6
Follow-up time (yrs)	182	11.7	11.3–12.0	195	10.1	9.7–10.4
	<b>N</b>	<b>Mean</b>	<b>SD</b>	<b>N</b>	<b>Mean</b>	<b>SD</b>
Activity score	397	63.6	14.8	423	61.2	15.1
Height (cm)	397	174	6.7	423	161	5.8
	<b>N</b>	<b>Median</b>	<b>IQR</b>	<b>N</b>	<b>Median</b>	<b>IQR</b>
Weight (kg)	397	81.0	74.0–88.5	423	68.5	60.5–79.5
BMI (kg/m <sup>2</sup> )	397	26.6	24.4–28.7	423	26.3	23.6–29.9
Alcohol consumption (units/week)	397	9.5	2.3–21.0	423	1.5	0.0–5.0
	<b>Total N</b>	<b>N</b>	<b>%</b>	<b>Total N</b>	<b>N</b>	<b>%</b>
Smoker status	397			422		
Never		124	31.2		262	62.1
Ex		208	52.4		121	28.7
Current		65	16.4		39	9.2
Social class	377			423		
I–IIINM		158	41.9		157	37.1
IIIM–V		219	58.1		266	62.9
	<b>N</b>	<b>Median</b>	<b>IQR</b>	<b>N</b>	<b>Median</b>	<b>IQR</b>
Circulating 25(OH)D (nmol/L)	397	44.6	35.0–63.0	423	41.3	29.8–53.5
	<b>N</b>	<b>Mean</b>	<b>SD</b>	<b>N</b>	<b>Mean</b>	<b>SD</b>
Lumbar spine BMD (g/cm <sup>2</sup> )	396	1.08	0.16	422	0.96	0.17
Femoral neck BMD (g/cm <sup>2</sup> )	394	0.85	0.12	421	0.76	0.12
Total femoral BMD (g/cm <sup>2</sup> )	394	1.04	0.13	421	0.89	0.13
	<b>Total N</b>	<b>N</b>	<b>%</b>	<b>Total N</b>	<b>N</b>	<b>%</b>
Radiographic knee OA	397	169	42.6	423	164	38.8
Radiographic knee OA at follow up	166	83	50.0	178	103	57.9
Clinical knee OA at follow up	176	19	10.8	191	36	18.8

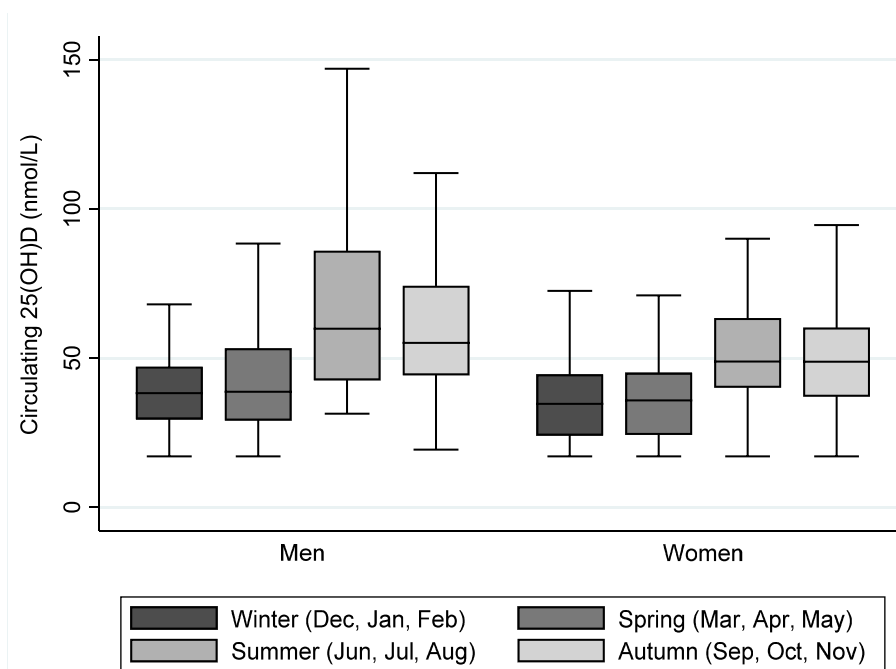


Fig. 1. Circulating 25(OH)D levels according to sex and season of blood testing.

the highest levels were observed during summer and autumn. This pattern was also observed among the group of participants taking vitamin D supplements (Fig. 2). Table 2 shows the mutually adjusted relationships between 25(OH)D concentration and vitamin D intake from food and whether participants were taking vitamin D supplements after adjustment for season. Weaker relationships were seen with reported vitamin D supplement use than with dietary intake.

The distribution of alleles within the SNPs of interest were similar between men and women. In both genders, per additional T allele of rs12785878 (*DHCR7*) was associated with increased circulating 25(OH)D values (men:  $\beta = 0.20$  z-score; 95% CI, 0.05 to 0.35;  $P = 0.008$ ,

women:  $\beta = 0.14$  z-score; 95% CI, 0.01 to 0.28;  $P = 0.040$ ), after adjustment for season of 25(OH)D measurement. Per additional C allele of rs4588, (*GC*) was associated with increased 25(OH)D values in men only ( $\beta = 0.14$  z-score; 95% CI, 0.01 to 0.29;  $P = 0.044$ ), after adjustment for season of measurement. These associations persisted in multivariate analyses after adjustment for season of blood collection, age, BMI, social class, smoker status, alcohol consumption, activity, dietary calcium intake, dietary vitamin D intake, years since the menopause and status of HRT use in women. For both men and women, there were no statistically significant associations between the SNPs at rs10741657 (*CYP2R1*) and rs6013897 (*CYP24A1*) and circulating 25

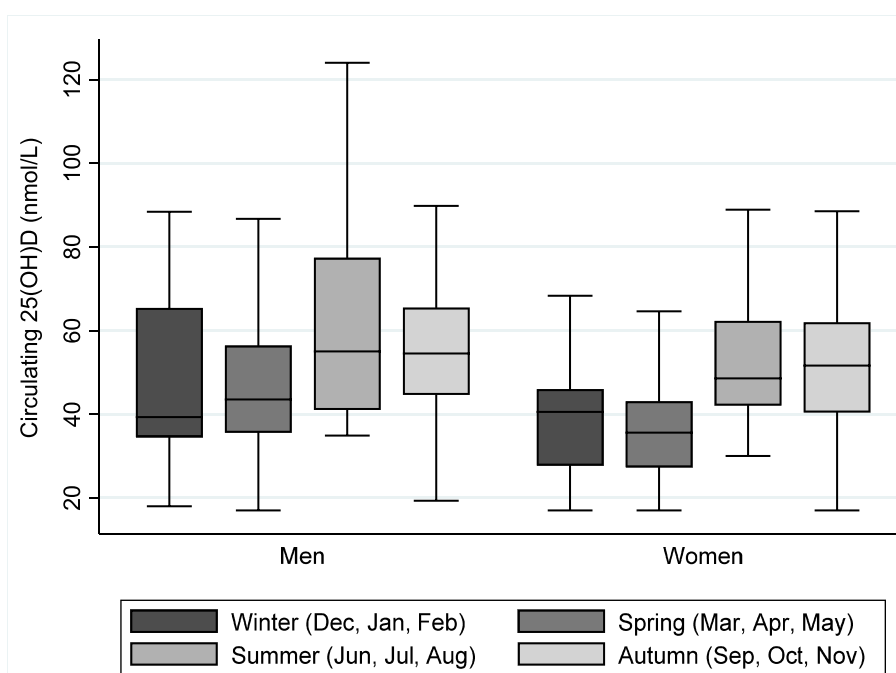


Fig. 2. Circulating 25(OH)D levels among participants taking vitamin D supplements/medications according to sex and season of blood testing.

**Table 2**

Mutually-adjusted relationships between circulating 25(OH)D (z-score) and vitamin D dietary intake and supplement use, after adjustment for season of measurement and other confounders.

	Men				Women			
	N	Regression coefficient	95% CI	p-value	N	Regression coefficient	95% CI	p-value
<i>Adjusted for season only</i>	397				423			
Vit D intake from food only (µg/day)		0.066	(0.021, 0.111)	0.004		0.081	(0.028, 0.135)	0.003
Taking a vit D supplement/medication		0.185	(-0.003, 0.374)	0.054		0.161	(0.001, 0.322)	0.049
<i>Adjusted for season and other confounders<sup>a</sup></i>	377				419			
Vit D intake from food only (µg/day)		0.067	(0.018, 0.116)	0.007		0.077	(0.021, 0.133)	0.008
Taking a vit D supplement/medication		0.166	(-0.031, 0.362)	0.098		0.119	(-0.047, 0.285)	0.158

<sup>a</sup> Adjusted for season of blood collection, age, BMI, social class, smoker status, alcohol consumption, activity score, dietary calcium intake, and years since menopause and HRT use in women.

(OH)D concentrations, either before or after adjustment for the confounders listed above.

We next considered the percentage variance explained by each of the measured vitamin D SNPs, vitamin D supplement use, and season in the determination of vitamin D status (Table 3). Season of blood collection explained 17.9% and 15.8% of the variation in circulating 25(OH)D levels among men and women respectively; proportion of variance explained by each of the other predictors (individual SNPs and taking vitamin D supplements) was less than 2% among both men and women. Season of blood collection was also highly significant ( $p < 0.001$ ) in these sex-specific mutually adjusted models.

Finally, we considered relationships between 25(OH)D level and musculoskeletal outcomes in our cohort. Men with higher baseline 25 (OH)D had higher femoral neck BMD ( $\beta$  0.11, 95% CI 0.02–0.20 z-score,  $p < 0.02$ ), following adjustment for age, season, BMI, smoker status, alcohol consumption, physical activity time, and social class (Fig. 3) though relationships were not significant in women.

Of the 820 subjects (397 men, 423 women) who participated at baseline, 339 subjects participated in a follow up study of OA progression (164 men; 175 women), allowing us to consider relationships between radiological OA progression. There were no associations between circulating 25(OH)D values and radiographic OA at either time point in either sex after adjustment for confounders and for duration of follow-up (data not shown).

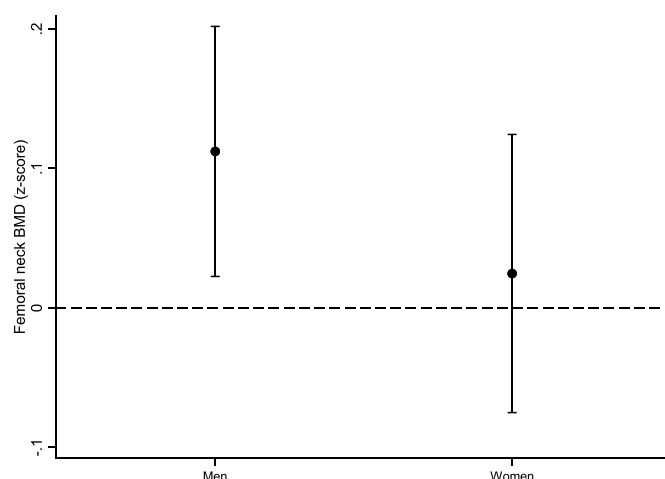
## 5. Discussion

In this study we were interested to consider the factors that contributed to circulating 25(OH)D concentration, and the associations of those concentrations with musculoskeletal health, in late middle age in a UK cohort of community dwelling adults. We found that season of blood sampling was a strong associate of 25(OH)D after adjusting for other factors. While we did not find relationships between radiographic knee OA and circulating vitamin D concentration in this population, we did find evidence of positive relationships between circulating 25(OH)D concentration and BMD in men. Although men in our sample had statistically significantly higher 25(OH) D levels than women (at the 5% significance level) we feel that these may have little clinical relevance,

**Table 3**

Percentage variance in multivariate model explained by vitamin D SNPs, vitamin D supplements, and season.

	Men	Women
	%	%
rs12785878 (DHCR7)	1.87	0.99
rs10741657 (CYP2R1)	0.28	0.18
rs6013897 (CYP24A1)	0.23	0.52
rs4588 (GC)	0.88	1.00
Taking a vit D supplement or medication	0.68	0.45
Season of blood collection	17.87	15.84



**Fig. 3.** Circulating 25(OH)D (z-score) as an explanatory variable for femoral neck BMD (z-score).

but rather we note the low median 25(OH) levels in both sexes. Importantly, despite a relatively high proportion of our population reporting use of vitamin D supplements, 25 (OH)D levels remain low, suggesting that it may be important to consider intense replacement regimens, or documented compliance in individuals at higher risk [25]. Guidelines on vitamin D supplementation have been published since the baseline of this study [26]. Of interest, a higher proportion of women reported use of vitamin D supplements than men, but still recorded lower median 25 (OH)D levels. Although the explanation for this remains unclear, it may reflect compliance issues, or reduced sun exposure in women relative to men [27]. While many studies have reported associations between circulating vitamin D concentration and bone health [28], these are typically in women, and often report associations in insufficient versus replete individuals. Recent publications have highlighted the heterogeneity of vitamin D supplementation regimens and variations in compliance with supplementation, as low as 40% in some studies [29,30].

We observed relationships between 25(OH)D and hip but not spine BMD in men. Lumbar spine BMD is known to be affected by degenerative change in later life [31], and therefore may reflect OA as well as BMD at this site, making it a less reliable site to assess relationships. However, the lack of association between 25(OH)D level and hip BMD in women is interesting. While we adjusted for use of HRT and current age, age at menopause has been shown to be associated with BMD [32]. In this sample, years lived since menopause may vary considerably, and could represent an explanation for the lack of associations seen in women.

In this observational study we did not find associations between circulating 25(OH)D concentration and radiological knee OA at baseline or follow-up. These findings chime with other studies which include



randomised controlled trials. Following initial positive reports that vitamin D status may be associated with the risk of knee OA, a systematic review which included a total of 1599 patients with osteoarthritis of the knee [33] concluded that while vitamin D supplements can improve WOMAC pain and function in patients with knee OA, there was a lack of strong evidence that vitamin D supplementation can prevent structural progression in patients with knee OA.

In this study we also considered associations between genetic and environmental factors in determination of circulating 25(OH)D concentrations. We studied 4 common SNPs in vitamin D metabolism. The contribution of genetic factors to circulating vitamin D concentration has been previously established [34] in a previous genome-wide association study (GWAS), which recruited participants from the Hertfordshire Cohort Study (HCS) and 14 other European-descent cohorts; a number of single-nucleotide polymorphisms (SNPs) in or near genes encoding key components of the vitamin D metabolism pathway (including *DHCR7* (encoding 7-dehydrocholesterol (7-DHC) reductase), *CYP2R1* (encoding 25-hydroxylase), *CYP24A1* (encoding 24-hydroxylase), and *GC* (encoding vitamin D binding protein (VDP)) [33]), were demonstrated to be associated with serum 25(OH)D values. Our findings that *DHCR7* is associated with circulating 25(OH)D levels is biologically plausible as the *DHCR7* gene encodes 7-DHC reductase, which converts 7-DHC back to cholesterol, thereby reducing the availability of 7-DHC for conversion to pre-vitamin D. The association between SNPs in *GC* and circulating 25(OH)D levels makes sense physiologically as *GC* has also been associated with serum vitamin D binding protein (VDP) concentrations, with carriers of the low-frequency C allele having reduced concentrations of VDP and 25(OH)D [34]. Furthermore, SNPs in *GC* have also been associated with the binding affinities of 25(OH)D to VDP [35]. Our findings, therefore suggest that there may be gender-specific associations between vitamin D-related genetic variants and their impact on circulating vitamin D levels. Our results suggest that SNPs in *GC* may act in combination with gender-specific factors such as sex hormone profile to modulate circulating 25(OH)D levels. In contrast to the published GWAS in our cohort no association was found between circulating 25(OH)D levels and SNPs rs10741657 (*CYP2R1*) and rs6013897 (*CYP24A1*), possibly reflecting limited power or different ethnic composition of our cohort.

Our study has both strengths and limitations. The population was exclusively European Caucasian. Importantly no information was provided regarding the regimen (amount and duration) of vitamin D supplementation. Furthermore, we have no information regarding physical activity spent indoors or outdoors. This may be relevant, as time spent outdoors might infer higher sun exposure and may partly explain the higher 25 (OH)D levels seen in men, where higher physical activity levels were noted. We do not know if supplementation followed current guidelines, specifically with regard to form (vitamin D2 or D3), nor do we have information regarding compliance. A final significant limitation is the relatively modest size of our cohort with regard to the genetic analyses we performed, thus potentially reducing our power to identify significant associations. We did, however, find biologically plausible associations, consistent with previous studies.

## 6. Conclusions

In conclusion, in this community dwelling cohort of Caucasian UK adults in midlife, median 25 (OH)D levels fell below commonly recommended levels, and seasonal fluctuations in vitamin D status were observed, as has been demonstrated elsewhere. We observed stronger associations between circulating 25(OH)D and dietary vitamin D intake than reported use of vitamin D supplementation, though we have no information regarding duration or dose of supplementation, highlighting the need to consider whether dosage and compliance with supplementation is adequate. Sexual dimorphism was noted in relationships between circulating 25(OH)D and the assessed SNPs. Circulating 25 (OH)D values were associated with hip BMD in men. Lack

of associations between hip BMD and 25(OH)D in women may reflect the close timing to accelerated perimenopausal bone loss, which masks impact of other environmental factors. Radiological OA was not associated with 25(OH)D in this cohort, consistent with other studies that have suggested vitamin D may play a greater role in OA pain than structural deterioration.

## Consent to publish

Not applicable.

## Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Ethics approval and consent to participate

Ethical approval for the study was granted by the Hertfordshire Research Ethics Committee (reference number EC9824) and all participants provided informed consent.

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## Authors' contributions

Gregorio Bevilacqua and Elaine Dennison prepared the first draft of the paper. Cyrus Cooper is guarantor. Elaine M Dennison designed the study and oversaw data collection. BJ Boucher and K Noonan provided data on serum 25(OH)D. Karen A Jameson was responsible for statistical analysis of the data. All authors reviewed the paper critically for intellectual content and approved the final version.

## Declaration of competing interest

Michael A Clynes has received support for attending conferences from UCB, Pfizer and Eli Lilly. Professor Cyrus Cooper has received lecture fees and honoraria from Amgen, Danone, Eli Lilly, GSK, Kyowa Kirin, Medtronic, Merck, Nestlé, Novartis, Pfizer, Roche, Servier, Shire, Takeda and UCB outside of the submitted work. Professor Elaine Dennison has received speaker honoraria from UCB, Pfizer, Lilly and Viatrix. Gregorio Bevilacqua, Faidra Laskou, Karen A Jameson, Barbara Boucher and Kate Noonan have no relevant interests to declare.

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