25-hydroxyvitamin D is lower in deprived groups, but is not associated with carotid intima media thickness or plaques: results from pSoBid

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Tables: 2

Figures: 1

Words: 3512 including abstract, tables and figures

References: 30

ABSTRACT

Objective

The association of the circulating serum vitamin D metabolite 25-hydroxyvitamin D (25OHD) with atherosclerotic burden is unclear, with previous studies reporting disparate results.

Method

Psychological, social and biological determinants of ill health (pSoBid) is a study of participants aged 35-64 years from Glasgow who live at extremes of the socioeconomic spectrum. Vitamin D deficiency was defined as 25OHD <25nmol/L, as per convention. Cross-sectional associations between circulating 25OHD concentrations and a range of socioeconomic, lifestyle, and biochemistry factors, as well as carotid intima media thickness (cIMT) and plaque presence were assessed in 625 participants.

Results

Geometric mean levels of circulating 25OHD were higher among the least deprived (45.6 nmol/L, 1-SD range 24.4-85.5) versus most deprived (34.2 nmol/L, 1-SD range 16.9-69.2; $p<0.0001$). In the least deprived group 15% were "deficient" in circulating 25OHD versus 30.8% in the most deprived (χ^2 $p<0.0001$). Log 25OHD was 27% lower among smokers ($p<0.0001$), 20% higher among the physically active versus inactive ($p=0.01$), 2% lower per 1kg/m^2 increase in body mass index (BMI) ($p<0.0001$), and showed expected seasonal variation (χ^2 $p<0.0001$). Log 25OHD was 13% lower in the most versus least deprived independent of the aforementioned lifestyle confounding factors ($p=0.03$). Circulating 25OHD concentrations were not associated with atherosclerotic burden in univariable models; cIMT (effect estimate 0.0mm [95% CI -0.02, 0.02]); plaque presence (OR 1.15 [0.85, 1.57]), or in multivariable models.

Conclusion

There is no strong association of 25OHD with cIMT or plaque presence, despite strong evidence 25OHD associates with lifestyle factors and socioeconomic deprivation.

Keywords: Vitamin D; 25-hydoxyvitamin D; atherosclerosis, plaque; intima media thickness; socioeconomic deprivation

INTRODUCTION

It has been widely reported that vitamin D deficiency, as assessed by measuring the major circulating vitamin D precursor 25-hydroxyvitamin D (25OHD), may adversely affect the cardiovascular system [1]. Prospectively, several studies report a link between low circulating 25OHD concentrations and higher risk for incident CVD events [2-4], as well as a range of other acute and chronic diseases. Such research has led to recommendations regarding desirable 25OHD levels, and the requirement for vitamin D supplements in large proportions of the population [5]. As such, routine clinical biochemistry departments locally and elsewhere, are reporting vastly increased clinical demand for 25OHD measurements [6].

We have previously argued, as have others, that observational data may be confounded and subject to reverse causality, these limitations being of particular relevance for 25OHD [6-8]. There is no convincing evidence from randomized trials that vitamin D supplements can reduce CVD risk, despite reported observational associations with risk [9]. To advance the literature, we aimed to investigate the association of circulating 25OHD with measures of atherosclerotic burden, whilst also investigating (and therefore adjusting for) any association of 25OHD with socioeconomic status and related characteristics. Carotid intima media thickness (cIMT) and plaque presence are emerging as potentially strong markers of CVD risk in general populations, with plaque presence is perhaps a stronger predictor of cardiovascular events than cIMT [10]..

The pSoBid (psychological, social and biological determinants of ill health) study comprises a cross section of men and women from either socially deprived or socially advantaged areas in Greater Glasgow, who had cIMT and plaque presence measured. We hypothesised that 25OHD would be lower in more deprived individuals independently of measured lifestyle factors, and

that such associations could at least partially explain a hypothesised association of 25OHD with markers of carotid atherosclerosis.

METHODS

Participants

The recruitment procedure for the pSoBid study has been described in detail previously [11]. Briefly, participants were invited to participate in the study at random (via ten general practice lists) from areas known to be at the extremes of the socioeconomic continuum in Glasgow, Scotland, such that approximately equal numbers of males and females across three age categories (35-44, 45-54, and 55-64 years) would be recruited. Socioeconomic selection was based on Scottish Index for Multiple Deprivation (SIMD) 2004 scores which is a small area-based score of deprivation based on data from public records relating to 38 indicators across 7 domains: income; employment; health; education, skills and training; housing; geographic access; crime [12] (see supplementary data). As an illustration annual household income was <£15,000 among in 60% of the most deprived participants, and >£45,000 among 60% of the least deprived participants. SIMD is currently used for clinical cardiovascular risk assessment in Scotland (the ASSIGN risk score [13]). Those recruited were either most deprived (bottom 5% of SIMD score) or least deprived (top 20% of SIMD). A total of 666 participants recruited between Dec 2005 and May 2007 (616 underwent ultrasound measurements of the carotid artery) [11]. This group comprised 171 males and 171 females in the least deprived group, and 156 males and 168 females in the most deprived group. Only 19 participants (2.9%) were born outside of the United Kingdom or Republic of Ireland. Comparison of participants with non-participants has been reported previously [14]. Participants underwent a routine physical examination (including measurement of blood pressure, body mass index [BMI], and waist hip ratio [WHR]) and lifestyle questionnaire [11]. Dysglycaemia was identified through current

treatment for diabetes, and a fasting measure of plasma glucose [11]. Physical activity was assessed using a validated questionnaire and categorised those who were inactive, moderately inactive, moderately active, and active. The study was reviewed and approved by the Glasgow Royal Infirmary Research Ethics Committee; all participants gave written informed consent.

Carotid intima-media thickness and plaque count

Doppler velocity in right and left internal carotid arteries was recorded in order to identify carotid artery stenosis using an ACUSON Sequoia 512 Ultrasound System with an L7 5-12 MHz linear array broadband transducer (Siemens Medical Solutions, Erlangen, Germany). Images of the distal 1 cm of the common carotid artery, the carotid bulb and the proximal internal carotid artery were recorded on the left and right side, and intima-media thickness of the far wall of the artery determined (using eTrack software). At the six sites, the number of carotid plaques was determined [15], and counted. Plaque was defined as a focal structure encroaching into the arterial lumen of at least 0.5mm or 50% of the surrounding IMT value, or demonstrating a thickness >1.5mm as measured from media-adventitia interface to intima-lumen interface. In order to adjust for unreadable images, total plaque count for each subject was divided by the number of readable images present and multiplied by 6 (the maximum possible number of images per subject) [15]. Nurses performing scans were subject to reproducibility checks as detailed [14]. Reading of the scans was performed off-line by a reader who was blinded to the identity of the participants.

Analytical procedures

Fasting venous blood samples were taken on the same visit as the ultrasound examination. We have a high-throughput method for the measurement of $25OHD_3$ and D_2 using an automated solid-phase extraction (SPE) procedure with liquid chromatography-tandem mass spectrometry

[16]. The lower limit of sensitivity was reported as 7.5nmol/L for $25OHD_2$ and for $25OHD_3$. Within- and between-assay precision was below 10%. Our method is currently in routine clinical use. Results are reported as total 25OHD ($25OHD_2$ + $25OHD_3$). Measurements of 25OHD were made by technicians who did not have access to the prior cIMT or plaque measurements, and were therefore blinded. A total 25OHD of <25nmol/L (10ng/ml) was defined as "deficiency", by convention frequently cited in the literature.

Statistical Analysis

Descriptive statistics are presented as mean (SD) and median (interquartile range, IQR) for continuous variables and count (%) for categorical outcomes. Due to skew, 25OHD is presented as a geometric mean and geometric standard deviation (i.e. the square root of the factor the geometric mean must be divided or multiplied by to give the 95% range). Associations between 25OHD and demographic and socioeconomic factors are presented adjusting for age, sex, and month of participation, and again with further adjustment for BMI, physical activity level and smoking status. Linear regression models were used, with log 25OHD as the response variable. Effect estimates are reported as percentage differences associated with a 10-year increase in age, or for categorical variables, relative to a reference category. Associations between 25OHD and cardiovascular risk factors were assessed using linear regression models with the risk factor (or its logarithm – see Table 2 for details) as the outcome, and the logarithm of 25OHD as a predictor (scaled by the standard deviation (SD) of log 25OHD), with adjustment for age, sex and deprivation group. Effect estimates are reported as the regression coefficient, interpretable as the absolute change in the response (or in the logarithm of the response) associated with a 1-SD increase in log 25OHD. All reported p-values are two sided. No adjustments were made for multiple statistical comparisons, so our p-values must be considered as descriptive measures of the strength of evidence for the observed associations. Significance levels of <5% have been

treated as suggestive of true associations, with smaller p-values representing greater levels of evidence.

As an indication of the power of the study, we had 80% power to detect a difference in 25OHD between those with and without plaques of 17% (or 24% in the least deprived group alone).

Analyses were conducted in S-Plus (TIBCO software) for Windows v8.1.

RESULTS

Baseline characteristics

The baseline characteristics of the 666 study participants by deprivation status have been previously reported [14]. Most classical CVD risk factors, including CRP, were more adverse in the most socioeconomically deprived group ($p<0.0001$ for all) with the exception of blood pressure and total and HDL cholesterol, the latter two variables being slightly elevated in the least deprived group. As a result, LDL:HDL ratio did not differ between the two deprivation groups (mean of 2.37mmol/L in both; $p=0.91$). Mean cIMT was 0.68mm (standard deviation ±0.12) in the least deprived group, and 0.70mm (±0.16) in the most deprived ($p=0.015$). There was at least one plaque present in 43.1% of the least deprived, and 58.3% of the most deprived group, with a mean plaque score of 1.0 in the least deprived group and 1.7 in the most deprived group ($p<0.0001$).

A total of 625 participants (93.8% of included participants) had given plasma samples for which aliquots were sufficient for the LC-MS/MS 25OHD assay. None of the cohort had analytically detectable $25OHD_2$ and 96.5% had detectable $25OHD_3$. The standard deviation of log total 25OHD was 0.68 in the whole population. Geometric mean concentration of 25OHD were

higher among the least deprived (45.6 nmol/L, 1-SD range 24.4-85.5) versus most deprived (34.2 nmol/L, 1-SD range 16.9-69.2; p<0.0001). A total of 141 patients (22.6%) could be defined as being deficient in circulating 25OHD (<25nmol/L), 49 (15%) in the least deprived group and 92 (30.8%) in the most deprived group (χ^2 p<0.0001).

Associations of circulating 25OHD concentrations with seasonal variation

Circulating log 25OHD concentrations were strongly associated with month of participation (blood sampling) across all participants (χ^2 p<0.0001) and in both deprivation groups (Figure 1). Median 25OHD concentrations were lowest in February (33.1nmol/L; IQR 22.0-49.9nmol/L, n=103) and highest in June (70.8nmol/L; IQR 44.7-103.5nmol/L, n=40). There was no evidence of a difference in month of participation by deprivation group (χ^2 p=0.69).

Associations of circulating 25OHD with selected demographic and socioeconomic variables

Log 25OHD concentrations showed no strong associations with either age or sex (Table 1). After adjusting for age, sex, month, physical activity and BMI, current smokers had 27% lower log 25OHD levels than never smokers (p<0.0001). In age, sex, month, BMI and smoking adjustment models, log 25OHD levels showed positive associations with greater physical activity; difference between the extreme active and inactive groups was 20%, p=0.01.

For markers of deprivation, log 25OHD concentrations were 23% lower in the most deprived group in adulthood (SIMD) after adjusting for age, sex and month (p<0.0001). This association of 25OHD with deprivation remained evident after additionally adjusting for BMI, smoking and physical activity (difference 13%; p=0.03). In addition, log 25OHD concentrations were positively and independently associated with annual income (being 16-33% higher in those earning >£25K, compared to those earning <£15K). In contrast, there was no strong evidence of

an association of log 25OHD with either educational attainment or childhood markers of deprivation (including number of siblings, people per room, or leg length) in either model (Table 1).

After adjustment age, sex, deprivation, month of participation, income, smoking status, BMI, and physical activity 25OHD should no strong associations with continuous classical cardiometabolic or inflammatory risk factors (supplementary data).

Associations of 25OHD with markers of atherosclerotic burden

Of the 625 participants with 25OHD data, there were 311 individuals with, and 302 without plaques (12 missing). We related 1 unit increase in log vitamin D to odds ratio (OR) of any plaque presence, and to effect size on the cIMT (Table 2). There was no strong evidence of an association of increasing 25OHD concentrations with risk of plaque presence or cIMT in the whole group in univariable or adjusted models. There was weak evidence that 25OHD may be associated with plaque presence in men only in unvariable models, although there was no evidence that this association was any different between men and women (p=0.13).

DISCUSSION

Circulating 25OHD concentrations were “deficient” in a sizeable proportion (22.6%) of this selected cohort living in Glasgow, but deficiency was more prevalent in socially deprived communities. This association of deprivation with 25OHD was only partially explained by markers of poor health among the deprived. There was no evidence of an association of 25OHD with markers of carotid atherosclerosis (cIMT or plaque presence).

A previous study in 390 consecutive diabetic patients attending a clinic showed that hypovitaminosis D (defined as 25OHD <15nmol/L) is associated with greater cIMT [17]. People with severe or long-standing type 2 diabetes (resulting in diabetic nephropathy) have lower 25OHD than people with diabetes but without complications [18]. We hypothesise that people with more severe diabetes are likely to have reduced physical activity and sunlight exposure, resulting in a confounded associations between 25OHD and diabetes severity/duration and atherosclerosis indices. Indeed, other population based studies have also reported that circulating 25OHD concentrations are not strongly related to cIMT [19-21]. One recent study of 203 older people in North America reports no association of 25OHD with cIMT or plaque count in the whole cohort, but a borderline significant association in those with plaques (for cIMT and maximal plaque thickness measures), although that study did not adjust for measures of deprivation. [22].

Socioeconomic deprivation is associated with risk of a large number of chronic diseases, and mortality, and is associated with CVD risk independent of classical risk factors [23]. Our detailed observations that social deprivation (and related factors such as income) is associated with 25OHD concentrations extends findings from the National Diet and Nutrition Survey cohort, suggesting 25OHD concentrations are lower among UK state benefit recipients than non-recipients [24], and data from the 1958 cohort [25]. Our hypothesis generating observation that 25OHD is lower among deprived groups particularly in winter (but perhaps not summer) requires further study. Clearly, increased background exposure to sunlight during summer may ameliorate differences in 25OHD between deprivation groups which may be more evident in winter months.

Association of 25OHD with social deprivation may be one explanation for the discrepancy between epidemiological study of associations between 25OHD and CVD risk, and thus far, lack of efficacy of vitamin D supplementation in RCTs to prevent chronic or acute disease not related to bone mineral metabolism [8, 26-29]. Further, our results suggest that education may be relatively weakly associated with serum 25OHD, and that other markers of deprivation may be better linked to 25OHD levels. It may be more robust and appropriate to adjust for multiple markers of deprivation simultaneously rather than just one, a procedure shown by others to be relevant to investigating the links of vitamin C to CVD risk [30].

We recognise strengths and limitations in the present study. We measured 25OHD by a routine NHS tandem mass spectrometry method which is calibrated against international standards. Plasma levels of $25OHD_2$ were frequently undetectable, but in our experience this is reflective of what is generally seen in clinical practice, and generally $25OHD_2$ levels mean little in terms of clinical interpretation. Discussion of the generalisability of results in cross-sectional studies is often challenging. Glasgow is broadly similar to many other UK post-industrial cities although in this study only individuals from the two extremes of social deprivation were included. Given that we observe a significant gradient in both 25OHD and atherosclerotic burden between deprivation groups, we would expect to see an association between 25OHD and atherosclerosis if one existed. However, we cannot exclude the possibility that, by design, we do not detect more subtle associations which might be evident in a general population. We do not have detailed dietary or sunlight exposure data to allow dietary intake of vitamin D to be examined as a determinant of vitamin D status in pSoBid. We have not adjusted for ethnicity; of all participants, only 19 (2.9%) participants were born outside the United Kingdom or Ireland. Finally we cannot rule out the possibility that pSoBid was underpowered to detect an association between 25OHD levels and cIMT or carotid plaques in a multivariable model, although our power calculations suggest

any association of 25OHD with atherosclerosis we might have been underpowered to detect would be of questionable clinical significance.

In conclusion, 25OHD concentrations are not strongly associated with either cIMT or plaque presence in the pSoBid study. 25OHD is however associated inversely with several measures of social deprivation independently of the primary determinants of poor health among the deprived (obesity, smoking, and physical inactivity). Therefore future studies relating circulating 25OHD concentrations to risk of chronic illness or death should, wherever possible, adjust for deprivation or income or for multiple markers of deprivation rather than education alone.

Acknowledgments

Sources of funding: This work was supported by the Glasgow Centre for Population Health, a partnership between NHS Greater Glasgow and Clyde, Glasgow City Council and the University of Glasgow, supported by the Scottish Government. 25OHD measurements were funded by a grant from Glasgow Royal Infirmary Research Endowment Fund. PW is supported by BHF fellowship grant FS/10/005/28147

Disclosure: All authors declare no conflict of interest

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Table 1: Associations between 25OHD concentrations (outcome) and selected exposure variables after adjustment for age, sex, month of participation, and (optionally) BMI, smoking status, and physical activity.

Predictor Variable		Relative Effect Estimate (95% CI), p-value	
		Adj. for age, sex, month	Also adj. for BMI, activity and smoking, as appropriate
Adjustment variables			
Age	(+10 years)	1.04 (0.97, 1.11), p=0.25	1.07 (1.00, 1.14), p=0.05
Sex (vs. Female)	Male	0.98 (0.89, 1.09), p=0.72	0.98 (0.88, 1.08), p=0.69
BMI	(+1 kg/m^2)	-	0.98 (0.97, 0.99), p<0.0001
Activity Level (vs. Inactive)	Moderately Inactive	-	1.13 (0.98, 1.31), p=0.10
	Moderately Active		1.12 (0.98, 1.29), p=0.10
	Active		1.20 (1.04, 1.38), p=0.01
Smoking (vs. Never Smokers)	Former Smokers	-	0.99 (0.88, 1.11), p=0.85
	Current Smokers		0.73 (0.64, 0.83), p<0.0001
Adult Markers of Social Deprivation			
Deprivation (vs. Least Deprived)	Most Deprived	0.77 (0.69, 0.85), p<0.0001	0.87 (0.78, 0.98), p=0.03
Annual Income (vs. < 15,000)	16-25,000	1.13 (0.97, 1.32), p=0.13	1.12 (0.95, 1.32), p=0.17
	26-35,000	1.25 (1.03, 1.50), p=0.02	1.16 (0.95, 1.42), p=0.15
	36-45,000	1.53 (1.26, 1.86), p<0.0001	1.33 (1.08, 1.65), p=0.01
	> 45,000	1.35 (1.18, 1.54), p<0.0001	1.17 (1.00, 1.37), p=0.05

Education (vs. ≤ 11 yrs)	12-13 yrs	1.09 (0.94, 1.27), p=0.23	1.00 (0.86, 1.17), p=0.99
	14-16 yrs	1.06 (0.93, 1.21), p=0.38	0.91 (0.79, 1.05), p=0.19
	≥ 17 yrs	1.14 (0.99, 1.31), p=0.06	0.93 (0.80, 1.08), p=0.35
Childhood Markers of Social Deprivation			
Number of Siblings (vs. None)	1-2	1.16 (0.99, 1.35), p=0.06	1.12 (0.96, 1.31), p=0.14
	3	1.27 (1.06, 1.52), p=0.01	1.34 (1.12, 1.60), p=0.002
	≥ 4	1.10 (0.92, 1.33), p=0.29	1.15 (0.95, 1.38), p=0.15
People per Room (vs. ≤ 1)	> 1, ≤ 1.5	1.03 (0.91, 1.17), p=0.62	1.13 (0.99, 1.27), p=0.06
	> 1.5	1.03 (0.91, 1.16), p=0.68	1.13 (0.99, 1.28), p=0.06
Leg Length (vs. ≤ 75cm)	75.1-80cm	1.15 (0.98, 1.34), p=0.08	1.12 (0.96, 1.30), p=0.15
	80.1-85cm	1.15 (0.97, 1.35), p=0.11	1.05 (0.88, 1.24), p=0.61
	> 85cm	1.19 (0.98, 1.43), p=0.07	1.02 (0.84, 1.24), p=0.82

Effects are the relative change in 25OHD for a specified change in continuous variables, or relative to a referent group for categorical variables.

Table 2 Association of 1 unit increase in log 25OHD with plaque presence (odds ratio) and cIMT (effect size)

	Plaque presence	**Odds Ratio**	**95% CI**	**P-value**	**P-interaction**
Univariable	All participants	0.88	(0.75 : 1.03)	0.10	
	Males only	0.75	(0.58 : 0.97)	0.027	0.13
	Females only	0.97	(0.78 : 1.19)	0.75	
	Most deprived	0.80	(0.64 : 1.01)	0.065	0.066
	Least deprived	1.10	(0.86 : 1.40)	0.44	
Multivariable	All participants	0.92	(0.73 : 1.17)	0.51	
	cIMT	**Effect size (mm)**	**95% CI**		
Univariable	All participants	0.000	(-0.011 : 0.012)	0.99	
	Males only	0.008	(-0.010 : 0.025)	0.41	0.26
	Females only	-0.006	(-0.021 : 0.009)	0.45	
	Most deprived	0.000	(-0.016 : 0.017)	0.96	0.77
	Least deprived	0.004	(-0.013 : 0.021)	0.64	
Multivariable	All participants	-0.007	(-0.018 : 0.005)	0.25	

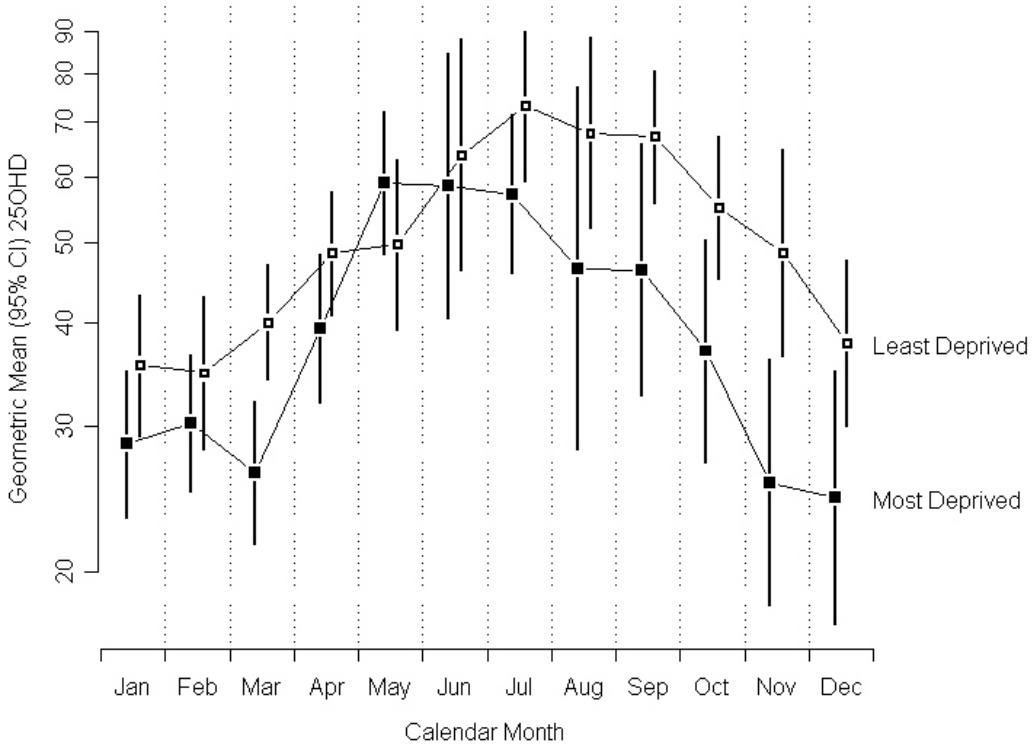
Multivariable adjustment model includes age, sex, readable scans (plaque presence only), HDL and LDL cholesterol, triglycerides, systolic and diastolic blood pressure, hypertension, smoking, fasting glucose, dysglycaemia treatment, physical activity, deprivation, and month of participation.

Interaction tests compare associations in males vs. females and most deprived vs. least deprived respectively

FIGURE LEGENDS

Figure 1

Variation in 25OHD concentrations by month of participation in the two deprivation groups. Point estimates are geometric means, error bars are 95%CI.



- We investigated circulating 25OHD at extremes of deprivation
- 25OHD is not associated with cIMT or plaques in the carotid artery
- 25OHD is strongly associated with certain markers of socioeconomic deprivation
- Observational studies of 25OHD should be cautious in causal inference, and adjust for SES